Critical Trifoliolate Leaf and Petiole Potassium Concentrations during the Reproductive Stages of Soybean

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ABSTRACT

The critical K concentration in soybean [*Glycine max* (L.) Merr.] has been determined only for leaf tissue at the R2 (full bloom) stage. Our research objective was to develop critical K concentrations in soybean for both leaves and petioles across reproductive stages. Fifteen fully-expanded, uppermost trifoliolate leaves with petioles plot^{-1} were collected 7 to 12 times from the V5 to R7 stages in five research trials that evaluated multiple fertilizer-K rates and/or cultivars from different maturity groups (MGs). Both leaf- and petiole-K concentrations, regardless of site-year, cultivar, and fertilizer-K rate, peaked around R2 stage and declined linearly with time at average rates of $-0.198\,\mathrm{g\,K\,kg^{-1}\,d^{-1}}$ for leaves and $-0.559\,\mathrm{g\,K\,kg^{-1}\,d^{-1}}$ for petioles. The leaf- and petiole-K concentrations at the R2 to R6 stages explained 48 to 80% and 41 to 85%, respectively, of the variation in relative yield (RY). Petiole-K concentration was a better predictor of RY than leaf-K at the R2 stage where the predicted critical range (CR) concentrations were 14.6 to 19.0 g leaf-K kg^{-1} and 30.1 to 38.3 g petiole-K kg^{-1}. The wider CR of petiole-K at the R2 stage followed by a greater linear decline rate across reproductive stages indicates that growth stage as well as deficiency and sufficiency thresholds for petiole-K could be more easily categorized than for leaves. Overall, the ability to interpret the K nutritional status in soybean tissues at numerous reproductive growth stages will improve K management.

Core Ideas

- Critical soybean tissue-K concentrations in the trifoliolate leaf and petiole can be developed for growth stages beyond the R2 stage by modeling the rate of tissue-K decline across time.
- Petiole-K concentrations are approximately twofold higher and decline at a greater rate than trifoliolate leaf-K concentrations and may be equally as good or a better tissue to sample for the diagnosis of K deficiency.
- The ability to interpret the K nutritional status in leaves, petioles, or both tissues at numerous reproductive growth stages allows plant K status to be monitored and possibly corrected during the growing season across a range of growth stages.

PLANT TISSUE ANALYSIS can be used to diagnose nutrient deficiencies before or after symptoms become visible provided a growth-stage-specific critical concentration has been previously determined. The critical nutrient concentration is a nutrient concentration range within a specific plant part above which near maximal yield is produced and below which yield loss is expected (Dow and Roberts, 1982; Mills and Jones, 1996). Critical concentrations are published for many crops and nutrients, but some critical concentrations have been developed by identifying the normal distribution of a population of plant samples (e.g., survey) rather than by correlating crop yield with tissue nutrient concentration from numerous site-years of fertilizer-response research. Although the survey-based nutrient concentration thresholds are of value, only research-based critical nutrient concentrations can be confidently used to determine how grain yield will be affected by tissue nutrient concentration.

Plant nutrient concentrations are known to vary among plant parts and plant development stages (Hanway and Weber, 1971; Sojka et al., 1985; Sadler et al., 1991). One of the most common problems encountered in diagnosing plant nutrient maladies from a tissue sample is that the growth stage at the time of tissue collection may not match that for which the diagnostic information was developed. For example, critical nutrient concentrations for soybean are published for the uppermost recently mature leaves at the R1–R2 stage (Fehr et al., 1971). Some of the proposed critical nutrient concentrations are from surveys (Mills and Jones, 1996) while others are research based (Grove et al., 1987; Slaton et al., 2010; Clover and Mallarino, 2013). The R1–R2 growth stage represents the beginning of plant reproductive growth when the proportion of season-total dry matter accumulation by soybean is generally <30% (Egli and Leggett, 1973; Bender et al., 2015). Despite widespread cultivation of soybean, critical nutrient concentrations have not been developed for growth stages other than the R1–R2 stage.

Potassium deficiency is among the most common nutrient maladies experienced by soybean, and yield increases from K fertilization are relatively common (Nelson et al., 1946; Jones et

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al., 1977; Camper and Lutz, 1977; Borges and Mallarino, 2000). The critical K concentration in the recently mature uppermost trifoliolate leaves has been the research focus by several scientists and is reported to be a good indicator of the RY potential of soybean. Grove et al. (1987), Slaton et al. (2010), and Clover and Mallarino (2013) reported leaf-K concentration at the R1–R2 stage was positively associated ($R^2 = 0.32–0.82$) with RY. However, Sartain et al. (1979) concluded that soybean seed yield was better correlated with the leaf-K concentration at the early pod stage than at early bloom. Miller et al. (1961) reported a strong relationship between soybean yield and leaf-K concentration at the R4 stage ($R^2 = 0.53–0.72$).

Potassium concentrations ≥19 g K kg^{-1} in the recently mature, uppermost leaves of soybean at the R1–R2 stage is considered sufficient and ≤15 g K kg^{-1} is considered deficient (Hanway and Johnson, 1985; Grove et al., 1987; Bell et al., 1995; Mills and Jones, 1996; Sabbe et al., 2000; Slaton et al., 2010). The concentration range of 15 to 19 g K kg^{-1} is considered critical. We are aware of no research that has quantified the critical K concentration in soybean leaves beyond the R2 growth stage. Research has demonstrated that the total leaf-K concentration of soybean peaks between the late vegetative and early reproductive stages and then declines gradually with time (Hanway and Weber, 1971; Sumner, 1977; Sojka et al., 1985). Drossopoulos et al. (1994) indicated that the K concentrations in the upper younger leaves of soybean peaked at the vegetative stage and plateaued up to the flowering (R1–R2) stage and then declined linearly until leaf senescence (R7).

Plant petioles can also be used to monitor the K nutritional status during the growing season (Miller et al., 1961; Bell et al., 1987; Cassman et al., 1989). Although we could not find conclusive information for soybean, the petiole-K concentration of the uppermost fully-expanded leaf is correlated with the yields of cotton (*Gossypium hirsutum* L., $R^2 = 0.53–0.61$; Cassman et al., 1989) and potato (*Solanum tuberosum* L.; $R^2 = 0.49–0.50$; Westermann et al., 1994). Petioles are used to monitor the N, P, and K nutritional status of several vegetable and fruit crops (Ludwick, 1990). Limited research has shown that the petiole-K concentration of soybean peaks near the same time as leaf-K concentration, but the petiole-K concentrations are almost double that of leaves and decline linearly with a wide range of concentration change across time (Hanway and Weber, 1971; Sojka et al., 1985).

The interpretation of the leaf-K concentration and perhaps the concentration of other nutrients at any stage beyond the R1–R2 may be possible if the rate of K concentration decline in plant tissues is relatively constant among years, cultivars, K fertility levels, and production systems. We could find no research that describes the dynamics of the uppermost mature, leaf-K or petiole-K concentrations across soybean developmental stages. Characterizing the change in soybean leaf- and petiole-K concentrations across time and soybean growth stages would be of value for diagnosing the K nutritional status of soybean at more than a single growth stage. Our goal was to propose critical K concentrations for soybean leaves and petioles across reproductive developmental stages with specific objectives of (i) characterizing the season-long dynamics of leaf- and petiole-K concentrations across a wide range of soil-K availabilities and cultivars of different MGs and growth habits (e.g., determinate or indeterminate)

and (ii) correlating soybean RY with the leaf- and petiole-K concentrations at each reproductive stage. Based on the cited literature we hypothesized that regardless of MG, growth habit, or K fertility level, both the leaf- and petiole-K concentrations would peak at blooming, increase as K fertility level increased, and then decline linearly across reproductive stages and would be good indicators of the K nutritional status of irrigated soybean.

MATERIALS AND METHODS
Experimental Sites and Treatments

Three experiments were conducted to evaluate the seasonal change in trifoliolate leaf-K concentration across a range of soybean MG at the Pine Tree Research Station (PTRS), Colt, AR, in 2012 (PTRS-12), 2013 (PTRS-13), and 2014 (PTRS-14a). Each experimental site, designed as randomized complete blocks, had been cropped to soybean in the previous year. Each of the PTRS-12, PTRS-13, and PTRS-14a research areas consisted of four adjacent tilled blocks that accommodated three, 7.6-m wide by 10-m long plots of each of three cultivars with each plot containing 20, 38-cm wide rows. Three glyphosate [*N*-(phosphonomethyl)glycine]-resistant soybean cultivars including a MG 3.9 (Armor 39-R16 for PTRS-12 and PTRS-13 and Asgrow 3934 for PTRS-14a), MG 4.7 (Armor 48-R40), and MG 5.3 (Armor 52-R15 for PTRS-12) or 5.5 (Armor 55-R22 for PTRS-13 and PTRS-14a) were selected and randomized within each block. The MG 3.9 and 4.7 cultivars exhibited an indeterminate growth habit and the MG 5.3 and 5.5 cultivars had a determinate growth habit.

Two additional experiments were conducted in 2014 to evaluate the seasonal change in trifoliolate leaf- and petiole-K concentrations across different K fertility levels and/or soybean MG at the Rice Research and Extension Center (RREC-14), Stuttgart, AR, and at the PTRS (PTRS-14b). Both of the experimental sites were cropped to rice (*Oryza sativa* L.) in 2013 and managed with no-tillage. The sites PTRS-14b, designed as strip-plot, and RREC-14, designed as randomized complete block, were long-term K fertilization trials that were established in 2000 and 2007, respectively, that offered a range of soil- and annual fertilizer-K rates for this experiment. Both sites consisted of five annual-K rates ranging from 0 to 150 kg K ha^{-1} yr^{-1} in 37.5 kg K ha^{-1} increments that were randomized within each block. The RREC-14 trial included a single indeterminate, MG 4.7 cultivar (Armor 47-R13) that was planted in each 4.6-m wide by 7.6-m long plot of six blocks and each plot contained twenty 18-cm wide rows. The PTRS-14b trial included one indeterminate, MG 4.8 cultivar (Armor 48-R66) and one determinate, MG 5.5 cultivar (Armor 55-R22). Both cultivars were strip-planted across five blocks where annual-K rate was the main-plot (4.0-m wide by 4.9-m long) and soybean cultivar was the subplot that contained ten 38-cm wide rows of each cultivar. Selected soil and agronomic information and the planting dates for each experimental site are listed in Table 1.

Soil Sampling and Analysis

A composite soil sample was collected from each block before applying any fertilizer and consisted of six, 2-cm o.d. soil cores from the 0- to 10-cm soil depth for the PTRS-12, PTRS-13, and PTRS-14a trials. A composite soil sample was collected from each main-plot (annual-K rate) of each block at RREC-14 and PTRS-14b. The composite soil samples were

oven-dried at 65°C, crushed to pass a 2-mm sieve, and analyzed for soil organic matter by combustion (Schulte and Hopkins, 1996) and soil pH in a 1:2 v/v soil/water mixture (Sikora and Kissel, 2014). A subsample of 2.00 ± 0.05 g soil was extracted with Mehlich-3 solution (Helmke and Sparks, 1996) for determining nutrient concentrations by inductively coupled plasma atomic emission spectroscopy (ICP–AES, Arcos-160 SOP, Spectro, NJ). The mean values of selected soil physicochemical properties for each experimental site are listed in Table 1.

Crop Management

The seeding rate of each cultivar at each experimental site ranged from 65 to 75 kg ha^{-1} for a common target seed population of 417,600 seeds ha^{-1}. To ensure that plant nutrition was not limiting to plant growth and yield, the research area at each site was fertilized as outlined in Table 1. All research areas were flood-irrigated as needed, and irrigation and pest management followed University of Arkansas Cooperative Extension Service recommendations (University of Arkansas, 2000).

Plant Sampling and Analysis

For PTRS-12, PTRS-13, and PTRS-14a trials, a fully-expanded, trifoliolate leaf (without petiole) from one of the top three nodes of 15 plants plot^{-1} was collected 7 to 12 times at 6 to 15 d intervals, beginning at the V5 stage until the R7 stage (leaf senescence). For RREC-14 and PTRS-14b, a trifoliolate leaf and petiole from the third node from the top of 15 plants plot^{-1} was collected 10 times, every 6 to 10 d from the V5 to R6 stages. The average plant development stage, as described by Fehr et al. (1971), was recorded at each sampling time. The date, days after emergence (DAE), and growth stage that plant samples were

collected, in each trial, are outlined in Table 2. In both sampling methods (one of the top three nodes vs. third node from the top), the collected tissue samples were similar regarding nodal position. However, we collected tissue samples from the specific position (third node) of the plant at RREC-14 and PTRS-14b to avoid positional variation in the tissue-K concentrations (Hanway and Weber, 1971; Sojka et al., 1985; Sadler et al., 1991). The collected plant samples were dried at 60°C in a forced-draft oven for 7 d. For samples collected at RREC-14 and PTRS-14b, the trifoliolate leaves were separated from the petioles after drying. Leaf and petiole samples were ground in a Wiley mill to <1 mm diam. particle size. A 0.250±0.005-g subsample was weighed, digested with concentrated HNO$_3$ and 30% H$_2$O$_2$ (Jones and Case, 1990), and the digests were analyzed by ICP–AES for K concentration.

At maturity, a 10 to 15 m^2 area within each block of each cultivar, excluding the border rows, was harvested with a small-plot combine for seed yield determination. The seed weight was adjusted to a uniform seed moisture content of 130 g H$_2$O kg^{-1}. The RY was calculated for each cultivar at PTRS-14b and RREC-14 by dividing the actual mean yield of soybean receiving each annual-K fertilization rate by the highest actual mean yield of soybean fertilized with K and multiplying by 100.

Statistical Analysis

Soybean seed yield data from each site-year were analyzed using the MIXED procedure of SAS (v9.4, SAS Inst., Cary, NC). Data from PTRS-12, PTRS-13, and PTRS-14a were analyzed separately using a model that included the fixed effect of three soybean MG cultivars and the random effect of block. The ANOVA for RREC-14 included annual-K fertilization

Table 1. Selected soil and agronomic information, soil physicochemical property means, and nutrient management for research trials conducted at the Pine Tree Research Station (PTRS) in 2012 (PTRS-12), 2013 (PTRS-13), and 2014 (PTRS-14a) and for long-term K fertilization trials conducted with five annual fertilizer-K rates at the Rice Research and Extension Center (RREC-14) and Pine Tree Research Station (PTRS-14b) in 2014.

Site-year	Annual-K rate	Soil classification		Date planted	Organic matter	Soil pH	Mehlich-3 extractable soil nutrients					Nutrient supplied‡§		
		Series	Group†				P	K	Ca	Mg	S	P	K	B
	kg K ha^{-1} yr^{-1}			day/month	g kg^{-1}		———————— mg kg^{-1} ————————					——— kg ha^{-1} ———		
PTRS-12	–	Calhoun	TG	22 May	22	7.1	15	64	1643	302	8	25	73¶	0.6
PTRS-13	–	Calloway	AF	26 June	23	7.3	58	96	1762	287	11	0	70	0.6
PTRS-14a	–	Calhoun	TG	22 May	26	7.1	13	68	1628	263	7	35	100	0.6
RREC-14	0	Dewitt	TA	24 Apr.	23	5.5	32	99	883	101	6	26	0	0.0
	38	Dewitt	TA	24 Apr.	–	5.6	31	124	912	105	7	26	38	0.0
	75	Dewitt	TA	24 Apr.	–	5.5	30	139	816	96	7	26	75	0.0
	113	Dewitt	TA	24 Apr.	–	5.5	32	152	834	95	6	26	113	0.0
	150	Dewitt	TA	24 Apr.	–	5.5	33	177	805	94	7	26	150	0.0
PTRS-14b	0	Calhoun	TG	22 May	30	7.9	30	76	2755	409	24	26	0	1.1
	38	Calhoun	TG	22 May	–	7.9	26	88	2788	395	26	26	38	1.1
	75	Calhoun	TG	22 May	–	7.8	28	93	2685	385	28	26	75	1.1
	113	Calhoun	TG	22 May	–	7.8	30	107	2671	394	27	26	113	1.1
	150	Calhoun	TG	22 May	–	7.8	29	128	2635	387	26	26	150	1.1

† AF, Aquic Fraglossudalfs; TA, Typic Albaqualfs; TG, Typic Glossaqualfs.

‡ Phosphorus, K, and B were applied as triple superphosphate (200 g P kg^{-1}), muriate of potash (500 g K kg^{-1}), and Na$_2$B$_8$O$_{13}$·4H$_2$O (205 g B kg^{-1}), respectively. All fertilizers at each site were broadcast to the soil surface before planting except for B at PTRS-12, PTRS-13, and PTRS-14, which was sprayed after soybean emergence.

§ For PTRS-12, 22 kg S and 11 kg Mg ha^{-1} were applied as K$_2$SO$_4$·2MgSO$_4$ (180 g K, 215 g S, and 105 g Mg kg^{-1}) and at RREC-14, 435 kg ha^{-1} pelleted lime was applied 2 wk before planting.

¶ A total of 73 kg K ha^{-1} was applied at 55 kg K ha^{-1} as muriate of potash (500 g K kg^{-1}) and at 18 kg K ha^{-1} as K$_2$SO$_4$·2MgSO$_4$ (180 g K, 215 g S, and 105 g Mg kg^{-1}).

rate as a fixed effect and block as a random effect. The statistical model for PTRS-14b was a strip-plot treatment structure that included the fixed effects of annual-K fertilization rate as the main-plot factor and soybean cultivar as the subplot factor and the random effect of block. The means were separated by Fisher's protected LSD with differences interpreted as significant at the 0.05 probability level.

The replicate data of soybean leaf- and petiole-K (if sampled) concentrations of each cultivar and/or annual fertilizer-K rate of each site-year were regressed independently against DAE to predict the peak K concentration with the DAE that corresponded to the specific growth stage. The regression analyses were performed with a linear-slope model (model that has two

Table 2. The date, days after emergence (DAE), and growth stage that plant samples were collected for three cultivars belonging to different maturity groups (MG) for research conducted at the Pine Tree Research Station (PTRS) in 2012 (PTRS-12), 2013 (PTRS-13), and 2014 (PTRS-14a) and for one or two cultivars in long-term K fertilization trials conducted at the Rice Research and Extension Center (RREC-14) and Pine Tree Research Station (PTRS-14b) in 2014.

Sample time	Sample date	DAE	Growth stage†‡		
			MG 3.9	MG 4.7/4.8§	MG 5.3/5.5¶
			PTRS-12		
1	26 June	28	R1	R0	V5
2	11 July	43	R2	R2	R0
3	24 July	56	R3	R2	R2
4	7 Aug.	70	R5	R4	R3
5	22 Aug.	85	R5.5	R5	R5
6	5 Sept.	99	R6	R5.5	R5.5
7	12 Sept.	106	R7	–	–
	19 Sept.	113	–	R6.5	–
	3 Oct.	127	–	–	R7
			PTRS-13		
1	25 July	22	R1	V6	V6
2	6 Aug.	34	R3	R2	V9
3	15 Aug.	43	R4	R3	R2
4	27 Aug.	55	R5	R5	R4
5	7 Sept.	66	R5.5	R5.5	R5
6	18 Sept.	77	R6	R5.5	R5.5
7	28 Sept.	87	R7	R6.5	R5.5
8	8 Oct.	97	–	–	R6.5
			PTRS-14a		
1	25 June	27	V6	V6	V6
2	3 July	35	R2	R1	V8
3	9 July	41	R3	R2	R0
4	16 July	48	R3.5	R3	R1
5	24 July	56	R4	R3	R2
6	30 July	62	R5	R4	R3
7	6 Aug.	69	R5.5	R5	R4
8	14 Aug.	77	R5.5	R5.5	R4
9	21 Aug.	84	R6	R5.5	R5
10	31 Aug.	94	R7	R6.5	R5.5
11	10 Sept.	104	–	R7	R6
12	20 Sept.	114	–	–	R6.5
			RREC-14		
1	18 June	48	–	R1	–
2	26 June	56	–	R2	–
3	2 July	62	–	R3	–
4	10 July	70	–	R4	–
5	16 July	76	–	R4.5	–
6	23 July	83	–	R5	–
7	30 July	91	–	R5.5	–
8	6 Aug.	97	–	R5.5	–
9	13 Aug.	104	–	R6	–
10	21 Aug.	112	–	R6	–

(continued)

linear segments connected with each other; Schabenberger and Pierce, 2002) using the NLIN procedure of SAS.

To predict the decline rate of the leaf-K concentration across soybean cultivars after K concentration peaked, the replicate leaf-K concentration data from the R2 (where K concentration peaked) to R7 stages of PTRS-12, PTRS-13, and PTRS-14a were regressed together against DAE with a linear model using the MIXED procedure of SAS. The linear model included soybean cultivar ($n = 3$) as the fixed effect and site-year ($n = 3$) and block ($n = 4$) as random effects.

To predict the diminishing trend of leaf- and petiole-K concentrations across annual-K fertilization rates after K concentration peaked, the replicate data from the growth stage where K concentrations peaked to the R6 stage were regressed against DAE by trial (RREC-14 and PTRS-14b) using the MIXED procedure. The petiole- and leaf-K concentrations peaked at the R1 and R2 stages, respectively, for the MG 4.7 cultivar at RREC-14 and the MG 5.5 cultivar at PTRS-14b and at the R2 and R3 stages for the MG 4.8 cultivar at PTRS-14b. The linear regression models included the fixed effect of annual-K fertilization rates ($n = 5$) for RREC-14 and the fixed effects of annual-K fertilization rates ($n = 5$) and soybean cultivar ($n = 2$) and their interaction for PTRS-14b. Both models also included five (PTRS-14b) or six (RREC-14) blocks as a random effect.

A linear model with the GLM procedure and a linear-plateau (LP) model with the NLIN procedure were used to correlate soybean RY with the leaf- and petiole-K concentrations at the R1 to R6 stages. The studentized residuals distribution for all variables was tested to identify outliers (studentized residual > ±2.5) and the models were refit by omitting the outliers when appropriate.

The CR of nutrient concentrations is defined multiple ways in the literature with definitions encompassing the concentrations at which 80 to 100% of the maximum growth or yield is produced (Ulrich and Hills, 1967, 1973; Dow and Roberts, 1982; Lanyon and Smith, 1985). The CR of K concentrations for leaves and petioles was calculated at 90 (lower) to 95% (upper) of the maximum (100%) predicted RY for the linear model and, for the LP model, the 90% confidence limits (CL)

of the join point defined the lower and upper boundaries of the CR. Leaf- and petiole-K concentrations above the CR were considered as sufficient, below the CR as deficient, and within the CR as critical.

RESULTS AND DISCUSSION
Soybean Seed Yield

Soybean seed yield was not significantly affected by soybean cultivar and averaged 3953 kg ha^{-1} at PTRS-12 ($P = 0.079$) and 2968 kg ha^{-1} at PTRS-13 ($P = 0.473$). Soybean yields were different among cultivars at PTRS-14a ($P = 0.001$) with the MG 4.7 cultivar (4418 kg ha^{-1}) producing 13 and 74% greater yields (LSD 0.05 = 445 kg ha^{-1}) than the MG 5.5 (3898 kg ha^{-1}) and 3.9 (2533 kg ha^{-1}) cultivars, respectively. Soybean yield was significantly affected by annual-K fertilization rate at RREC-14 and by the main effects of annual-K fertilization rate and soybean cultivar at PTRS-14b (Table 3). For RREC-14 and PTRS-14b, soybean receiving 38 to 150 kg K ha^{-1} yr^{-1} produced similar yields that were 9 to 15% and 12 to 19% greater, respectively, than the yield of soybean receiving no fertilizer-K. Averaged across annual-K

Table 3. Soybean seed yield as affected by annual fertilizer-K rate for long-term K fertilization trials conducted at the Rice Research and Extension Center (RREC-14) and Pine Tree Research Station (PTRS-14b) in 2014.

Annual fertilizer-K rate or source of variation	Seed yield	
	RREC-14	PTRS-14b
	———— kg ha^{-1} ————	
0	4465	3271
38	4905	3665
75	4885	3771
113	5120	3877
150	5114	3901
LSD(0.05)	283	378
	———— P values ————	
Annual fertilizer-K rate (K)	0.001	0.027
Cultivar (C)	–	0.002
K × C	–	0.565

Table 2 (continued).

Sample time	Sample date	DAE	Growth stage†‡		
			MG 3.9	MG 4.7/4.8§	MG 5.3/5.5¶
		PTRS-14b			
1	3 July	35	–	R1	V8
2	9 July	41	–	R2	V9
3	16 July	48	–	R2	R1
4	24 July	56	–	R3	R2
5	30 July	62	–	R3.5	R3
6	6 Aug.	69	–	R4.5	R3.5
7	14 Aug.	77	–	R5	R4
8	21 Aug.	84	–	R5.5	R5
9	31 Aug.	94	–	R5.5	R5.5
10	10 Sept.	104	–	R6	R5.5

† Growth stage key: Fehr et al. (1971).

‡ Additional growth stages not defined by Fehr et al. (1971): R0, Plants had flower clusters, but the flowers were not open; R3.5, R4.5, and R6.5, one-half of the plants were in the growth stages immediately above and below the listed value; and R5.5, areas of pod cavities at one of the top four nodes were 50% filled by developing seeds.

§ A MG 4.7 cultivar was used for PTRS-12, PTRS-13, PTRS-14a, and RREC-14 and a MG 4.8 cultivar was used for PTRS-14b.

¶ A MG 5.3 cultivar was used for PTRS-12 and a MG 5.5 cultivar was used for PTRS-13, PTRS-14a, and PTRS-14b.

Table 4. Intercept and slope coefficients predicting the number of days after emergence (DAE) that corresponded to a specific growth stage where trifoliolate leaf- and/or petiole-K concentrations (KC) peaked for research trials conducted at the Pine Tree Research Station (PTRS) in 2012 (PTRS-12), 2013 (PTRS-13), and 2014 (PTRS-14a) with three soybean cultivars belonging to different maturity groups (MG) and for long-term K fertilization trials conducted at the Rice Research and Extension Center (RREC-14) and Pine Tree Research Station (PTRS-14b) in 2014 with five annual fertilizer-K rates and one or two cultivars.

Site-year	MG	Annual fertilizer-K rate	Linear-slope model†‡ coefficients				Peak or join point		
			Intercept	First slope	Second slope	R^2	KC	DAE	Growth stage§
		kg K ha^{-1} yr^{-1}	g K kg^{-1}	—— g K kg^{-1} d^{-1} ——			g K kg^{-1}	days	
			Trifoliolate leaf						
PTRS-12	3.9	–	8.9	0.159¶	–0.173	0.80	16.1	45	R2
	4.7	–	8.9	0.168	–0.172	0.89	18.1	55	R2
	5.3	–	5.5	0.215	–0.170	0.83	16.3	50	R1.5
PTRS-13	3.9	–	19.4	0.063¶	–0.139	0.89	20.5	18¶	R1
	4.7	–	20.5	–0.008¶	–0.076	0.48	20.2	33	R2
	5.5	–	16.4	0.105¶	–0.236	0.96	20.4	38	R1.5
PTRS-14a	3.9	–	17.5	0.077¶	–0.255	0.90	20.8	43	R3
	4.7	–	14.2	0.124	–0.295	0.94	20.6	52	R3
	5.5	–	15.6	0.131¶	–0.267	0.89	21.8	47¶	R1
RREC-14	4.7	0	15.8¶	0.034¶	–0.202	0.86	17.6	53¶	R1.5
		38	14.4¶	0.092¶	–0.228	0.89	19.6	56¶	R2
		75	6.6¶	0.258¶	–0.206	0.83	21.0	56¶	R2
		113	14.9¶	0.114¶	–0.199	0.85	21.3	56¶	R2
		150	13.9¶	0.150¶	–0.193	0.79	22.3	56¶	R2
PTRS-14b	4.8	0	1.3¶	0.225	–0.188	0.57	14.4	58	R3
		38	1.7¶	0.264	–0.225	0.85	17.0	58	R3
		75	3.4	0.277	–0.223	0.87	19.5	58	R3
		113	1.8¶	0.329	–0.200	0.77	20.9	58	R3
		150	0.7¶	0.350	–0.168	0.83	20.3	56	R3
	5.5	0	3.0¶	0.179	–0.136	0.77	12.3	52	R1.5
		38	–1.0¶	0.325	–0.192	0.81	15.9	52	R1.5
		75	–7.1	0.519	–0.212	0.91	19.4	51	R1.5
		113	1.8¶	0.299	–0.213	0.80	19.4	59	R2
		150	2.1¶	0.295	–0.194	0.80	19.5	59	R2
			Petiole						
RREC-14	4.7	0	32.2	0.025¶	–0.505	0.91	33.4	48¶	R1
		38	38.6	0.053¶	–0.597	0.93	41.1	48¶	R1
		75	42.4	0.043¶	–0.551	0.91	44.5	48¶	R1
		113	46.1	0.043¶	–0.572	0.91	48.2	48¶	R1
		150	46.9	0.043¶	–0.516	0.90	49.0	48¶	R1
PTRS-14b	4.8	0	–21.2¶	1.019	–0.511	0.67	28.7	49	R2
		38	–75.2	2.653	–0.674	0.92	41.5	44	R2
		75	–17.3	1.320	–0.734	0.91	47.4	49	R2
		113	–12.4¶	1.284	–0.740	0.91	51.8	50	R2
		150	–7.7¶	1.228	–0.697	0.90	53.7	50	R2
	5.5	0	–13.4¶	0.755	–0.354	0.76	22.8	48	R1
		38	–53.9	1.964	–0.539	0.86	34.5	45	R1
		75	–68.5	2.453	–0.650	0.89	44.3	46	R1
		113	–24.7	1.375	–0.596	0.86	44.1	50	R1
		150	–29.2	1.521	–0.534	0.88	45.3	49	R1

† Data were analyzed for each site-year, cultivar (MG), and/or annual fertilizer-K rate. Each model was significant at the 0.0001 probability level.

‡ The linear-slope model [KC = {intercept + (first slope × DAE)} + {intercept + (second slope × DAE)}] is a model that has two linear segments connected with each other (Schabenberger and Pierce, 2002).

§ Growth stages R1 and R2 as defined by Fehr et al. (1971). Additional growth stage R1.5 is defined as one-half of plants were at the R1 stage and one-half of plants were at the R2 stage.

¶ Coefficients and join points are not significantly different from zero at the 0.05 probability level.

fertilization rates at PTRS-14b, the determinate MG 5.5 cultivar (4092 kg ha^{-1}) produced 24% greater seed yield than the indeterminate MG 4.8 cultivar (3302 kg ha^{-1}). The seed yield increases attributed to K fertilization in our studies (RREC-14 and PTRS-14b) were within the typical range (5–25%) of soybean yield responses to fertilizer-K reported in the literature (Coale and Grove, 1990; Mallarino et al., 1991; Slaton et al., 2010, 2013; Clover and Mallarino, 2013; Parvej et al., 2015).

Seasonal Dynamics of Trifoliolate Leaf Potassium Concentration

The linear-slope model showed that regardless of soybean cultivar, annual-K rate, or site-year, the K concentration in the uppermost, recently-mature trifoliolate leaves increased linearly or plateaued from the mid-vegetative (V5–V7) to the early reproductive stages (R1–R3) and then declined linearly throughout reproductive growth (Table 4; Fig. 1a–1l). The initial linear increase and plateau of leaf-K concentration was not evident for the MG 3.9 cultivar at PTRS-13 (Fig. 1d) because
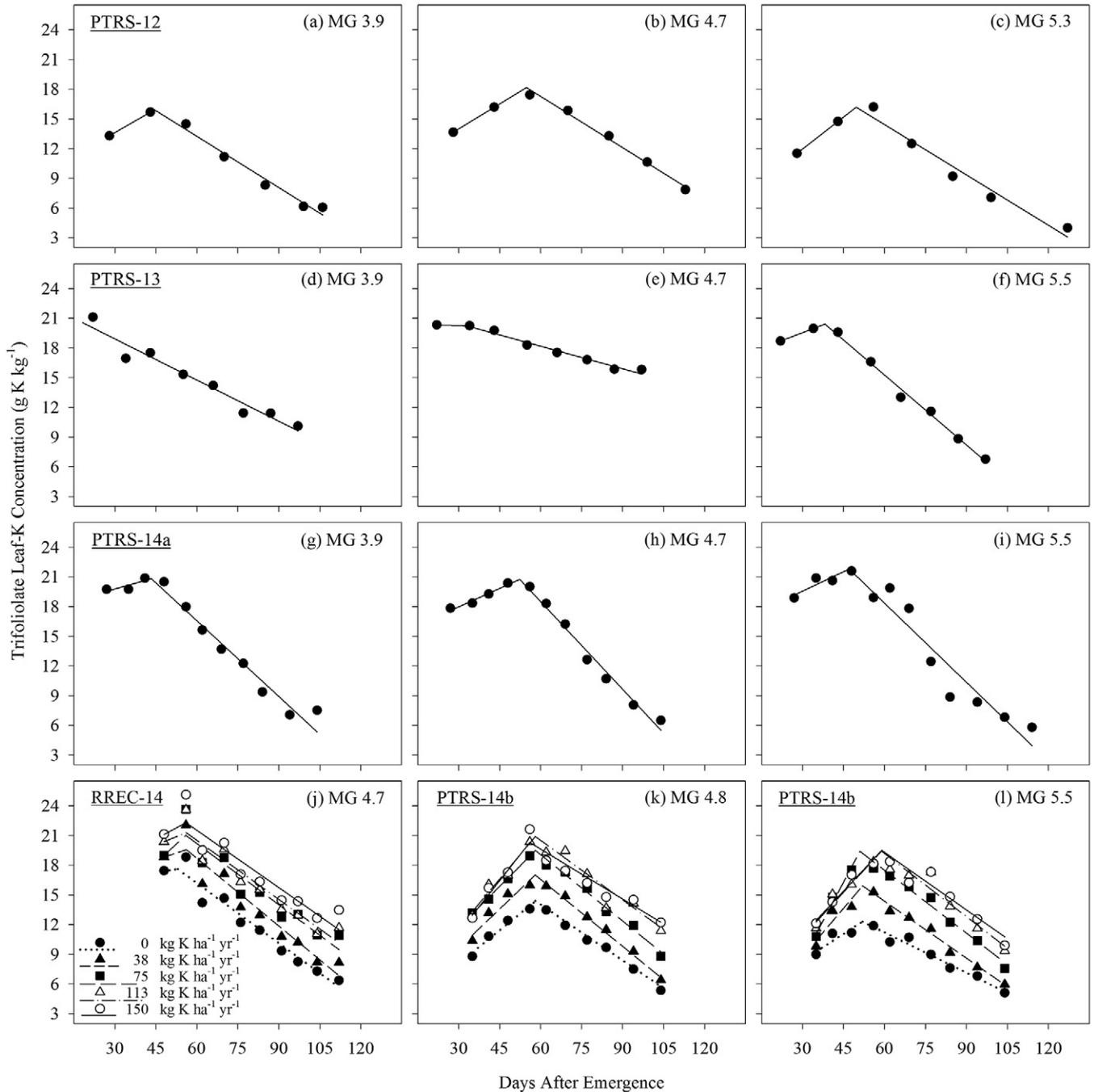
Fig. 1. Soybean trifoliolate leaf-K concentration change as a function of days after emergence as predicted with a linear-slope model for research trials conducted at the Pine Tree Research Station (PTRS) in 2012 (a–c; PTRS-12), 2013 (d–f; PTRS-13), and 2014 (g–i; PTRS-14a) with soybean cultivars representing three different maturity groups (MG) and for long-term K fertilization trials conducted at the Rice Research and Extension Center (j; RREC-14) and Pine Tree Research Station (k–l; PTRS-14b) in 2014 with five annual fertilizer-K rates and one or two soybean cultivars, respectively. Data for each trial were analyzed by cultivar and annual fertilizer-K rate. Model coefficients and time and growth stage where K concentration peaked are listed in Table 4.

the first leaf samples were collected at the onset of reproductive growth (Table 2). The leaf-K concentration decline as soybean progressed toward maturity in our five trials agrees with previous research and is attributed to increased dry matter production (i.e., dilution) and translocation of K to the developing seeds (Hanway and Weber, 1971; Sale and Campbell, 1980; Drossopoulos et al., 1994).

Three soybean cultivars belonging to different MGs and planted at different times each year were used in PTRS-12, PTRS-13, and PTRS-14a trials. The leaf-K concentration of each cultivar within each trial peaked at different DAE that coincided, on average, with the R2 stage (Table 4; Fig. 1a–1i). The leaf-K concentration of soybean fertilized with five annual fertilizer-K rates peaked at the R2 stage for the MG 4.7 cultivar at RREC-14 (Fig. 1j) and the MG 5.5 cultivar at PTRS-14b (Fig. 1l) and at the R3 stage for the MG 4.7 cultivar at PTRS-14b (Fig. 1k; Table 4). Among site-years, soybean cultivars, and annual fertilizer-K rates, the peak leaf-K concentrations ranged from 12.3 to 21.8 g K kg^{-1}, which represents K concentrations that would be considered deficient to optimal at the R1–R2 stage (Hanway and Johnson, 1985; Slaton et al., 2010; Clover and Mallarino, 2013).

Leaf-K concentration at the 5 site-years of research declined from the R2–R3 growth stage to the R6–R7 stage, so we examined the results to determine if the rate of decline was uniform among site-years, cultivars, and K availability levels. The linear model showed that regardless of soybean cultivar or annual-K fertilization rate, the leaf-K concentration declined linearly with plant development. Within each trial a common negative slope was derived from the sources of variation (e.g., cultivars, site-years, or fertilizer-K rates) with average slope values of −0.196 g K kg^{-1} d^{-1} for PTRS-12, PTRS-13, and PTRS-14a (Fig. 2a); −0.203 g K kg^{-1} d^{-1} for RREC-14 (Fig. 2b); and −0.194 g K kg^{-1} d^{-1} (Fig. 2c, 2d) for PTRS-14b (Table 5). The intercept was different among treatments within each trial indicating that the rate of decline was independent of cultivar or K availability. Averaged across PTRS-12, PTRS-13, and PTRS-14a, the leaf-K concentration among cultivars followed the order of cultivar MG 4.7 > MG 5.3/5.5 > MG 3.9 (Table 5; Fig. 2a). The leaf-K concentration increased either numerically or statistically with each increase in annual fertilizer-K rate for RREC-14 (Fig. 2b) and PTRS-14b (Fig. 2c, 2d). Research suggests that K fertilization increases leaf-K concentration when soybean yields respond positively to K fertilization, however, luxury consumption of K
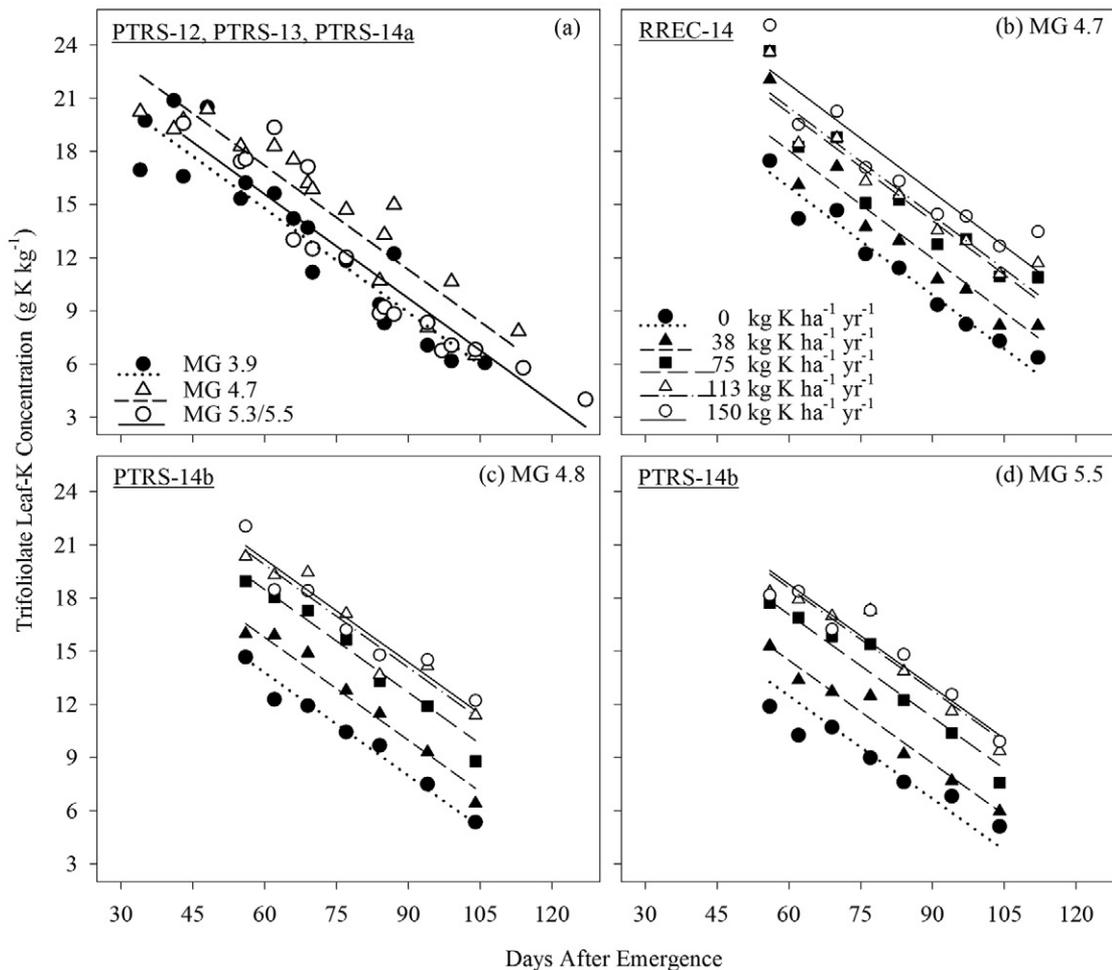
Fig. 2. Soybean trifoliolate leaf-K concentration change as a function of days after emergence from the growth stage where K concentrations peaked to the R6 or R7 stages as predicted with a linear model for research trials conducted at the Pine Tree Research Station (a; PTRS) in 2012 (PTRS-12), 2013 (PTRS-13), and 2014 (PTRS-14a) with soybean cultivars representing three different maturity groups (MG) and for long-term K fertilization trials conducted at the Rice Research and Extension Center (b; RREC-14) and Pine Tree Research Station (c–d; PTRS-14b) in 2014 with five annual fertilizer-K rates and one or two soybean MG cultivars, respectively. Model coefficients are listed in Table 5. The growth stage of each cultivar of each trial that corresponded to a specific day after emergence is listed in Table 2.

can increase leaf-K concentrations without a corresponding yield increase (Randall et al., 1997; Yin and Vyn, 2003; Clover and Mallarino, 2013).

When the leaf-K concentrations from all five trials between the R2 and R7 growth stages were regressed, the average slope was -0.198 g K kg^{-1} d^{-1} (Table 5). The similarity of the linear slope values among the five trials encompassing cultivars having a MG range of 3.9 to 5.5, different seeding dates, and multiple K availability levels suggests that predicting critical leaf-K concentrations beyond the R2 stage is possible. When we regressed leaf-K concentrations of the top three nodes from Sadler et al. (1991) or K concentrations in the upper younger leaves from Drossopoulos et al. (1994) against DAE, leaf-K concentrations decreased linearly from the R2 to R6 stages at rates of -0.252 and -0.257 g K kg^{-1} d^{-1}, respectively. The reason for relatively greater decline rates in their studies, compared to our study, is unknown, but may be due to inclusion of younger leaves from the topmost node in their study, which contain less K than leaves from nodes below the topmost node (Sadler et al., 1991) or may be due to differences in soybean genotype (Keogh et al., 1977), environment (Keogh et al., 1977; Grove et al., 1987; Nelson et al., 2005), or K availability (Clover and Mallarino, 2013).

Relative Soybean Yield and Trifoliolate Leaf Potassium Concentration

The linear and LP models showed significant relationships between soybean RY and trifoliolate leaf-K concentration at the R2 (Fig. 3a; LP only), R3 (Fig. 3b), R4 (Fig. 3c), R5 (Fig. 3d), R5.5 (Fig. 3e), and R6 (Fig. 3f) stages using data from RREC-14 and PTRS-14b (Table 6). The LP models for the R3 to R6 stages are not shown because the plateau segment of the LP models was either not evident (for the R5.5 and R6 stages) or very narrow (for the R3, R4, and R5 stages). The relationships at the R1 stage for both the linear and LP models and at the R2 stage for the linear model were not significant at the 0.05 probability level (Table 6). The leaf-K across reproductive developmental stages explained 48 to 80% of the variation in RY. The leaf-K concentration from the R3 to R5.5 stages accounted for 8 to 18% more RY variation than the K concentration at the R2 stage. The relationships reported by Miller et al. (1961) and Sartain et al. (1979) suggest that tissue-K concentration at the R3 to R4 stages may be better correlated with RY than tissue-K concentration at the R1–R2 stage (Grove et al., 1987; Slaton et al., 2010; Clover and Mallarino, 2013). The potential reasons for the stronger relationships for the R3 to R5.5 stages compared to the R2 stage (Table 6) may be because pod and seed numbers are better predictors of yield than flower number and flowers tend to abort more frequently than pods (Wiebold et al., 1981). The relationship between RY and leaf-K concentration at the R6 stage compared to the R3 to R5.5 stages was weaker and may be due to fewer data points. However, monitoring the K nutritional status of soybean plants as a guide for timely fertilization requires that the relationship be predicted using the earliest possible growth stage to avoid potential yield losses. Research-based information describing soybean yield response to K fertilization timing is scant and warrants further research. Nelson et al. (2005) showed that foliar-applied K at the V4, R1–R2, and R3–R4 stages increased soybean yield, but the yield increase was greater from K applied at the V4 or R1–R2 than at the R3–R4 stages.

Critical Trifoliolate Leaf Potassium Concentration

The CR of K concentrations in the trifoliolate leaves at the R2 stage, represented by the 90% CL of the LP model join point, was 14.6 to 19.0 g K kg^{-1} (Table 6; Fig. 3a). Leaf-K concentrations ≤14.6 g K kg^{-1} were considered deficient and ≥19.0 g K kg^{-1} were considered sufficient. Leaf-K concentrations ≤15.0 g K kg^{-1} at the R2 stage have been reported as deficient and research clearly shows significant yield loss occurs below this threshold (Hanway and Johnson, 1985; Bell et al., 1995; Mills and Jones, 1996; Sabbe et al., 2000; Slaton et al., 2010). Our sufficient R2 leaf-K concentration of 19.0 g K kg^{-1} was within the 18.9 to 25.0 g K kg^{-1} range reported in the literature (Hanway and Johnson, 1985; Grove et al., 1987; Bell et al., 1995; Mills and Jones, 1996; Sabbe et al., 2000; Slaton et al., 2010).

The deficient and sufficient leaf-K concentrations that define the CR were based on the 90% CL of the LP model join point

Table 5. Intercept and linear slope coefficients predicting the decline rate of trifoliolate leaf-K concentration (LKC) as a function of time (d after emergence, DAE) for three soybean cultivars belonging to three maturity groups (MG) for research trials conducted at the Pine Tree Research Station (PTRS) in 2012 (PTRS-12), 2013 (PTRS-13), and 2014 (PTRS-14a) and for long-term K fertilization trials conducted at the Rice Research and Extension Center (RREC-14) and Pine Tree Research Station (PTRS-14b) in 2014 that included five annual fertilizer-K rates and one or two cultivars.

Cultivar MG	Annual-K rate	Linear model[†] coefficients		
		Intercept	Slope	R^2
	kg K ha^{-1} yr^{-1}	g K kg^{-1}	g K kg^{-1} d^{-1}	
Averaged across PTRS-12, PTRS-13, and PTRS-14a				
MG 3.9	–	26.5c[‡]	–0.196	0.82
MG 4.7	–	28.9a	–0.196	
MG 5.3/5.5[§]	–	27.3b	–0.196	
SE		0.6	0.0065	
RREC-14				
MG 4.7	0	28.2d	–0.203	0.87
	38	30.2c	–0.203	
	75	32.3b	–0.203	
	113	32.6b	–0.203	
	150	33.9a	–0.203	
SE		0.5	0.0055	
PTRS-14b				
MG 4.8	0	25.4e	–0.194	0.86
	38	27.4d	–0.194	
	75	30.1b	–0.194	
	113	31.5a	–0.194	
	150	31.8a	–0.194	
MG 5.5	0	24.1f	–0.194	
	38	26.1e	–0.194	
	75	28.7c	–0.194	
	113	30.2b	–0.194	
	150	30.4b	–0.194	
SE		0.5	0.0053	
Average slope (all site-years)			–0.198	
Average SE (all site-years)			0.0058	

† LKC = intercept + (slope × DAE).

‡ Within each site-year, values in the same column followed by different letters are significantly different at the 0.05 probability level.

§ A MG 5.3 cultivar was used for PTRS-12 and a MG 5.5 cultivar was used for PTRS-13, PTRS-14a, and PTRS-14b.

for the R2 stage plus the calculated 90 (lower) and 95% (upper) RY predicted from the linear model for the R3 to R6 stages, respectively (Table 6). The R3, R4, R5, R5.5, and R6 growth stages corresponded to 10, 20, 30, 40, and 50 d after full-bloom (R2) based on our field observations (Table 2) and tend to follow the observations reported by Zhang et al. (2004). Linear regression of these lower and upper boundary CR concentrations showed K concentrations declined linearly from the R2 to R6 stages with slope values of -0.204 and -0.203 g K kg^{-1} d^{-1}, respectively (Fig. 4a; $P = 0.004$ and $R^2 = 0.91$ for the lower boundary; $P = 0.001$ and $R^2 = 0.97$ for the upper boundary),

which were very close to the overall average rate of decline of -0.198 g K kg^{-1} d^{-1} (Table 5). Albeit, slightly more complex, a quadratic model was also fit to the predicted CR points from Table 6 (Fig. 4b) and improved the R^2 of the lower ($P < 0.001$; $R^2 = 0.99$) and upper ($P = 0.002$; $R^2 = 0.99$) boundaries in defining the CR across growth stages. The linear and quadratic predictions are compared to the model based on the overall linear leaf-K decline rate (-0.198 g K kg^{-1} d^{-1}) and the CR of leaf-K at the R2 stage (14.6–19.0 g K kg^{-1}) to define the CR boundaries between the R2 and R6 stages (Fig. 4c). Each of the models assume that across time, leaf-K concentrations equal or
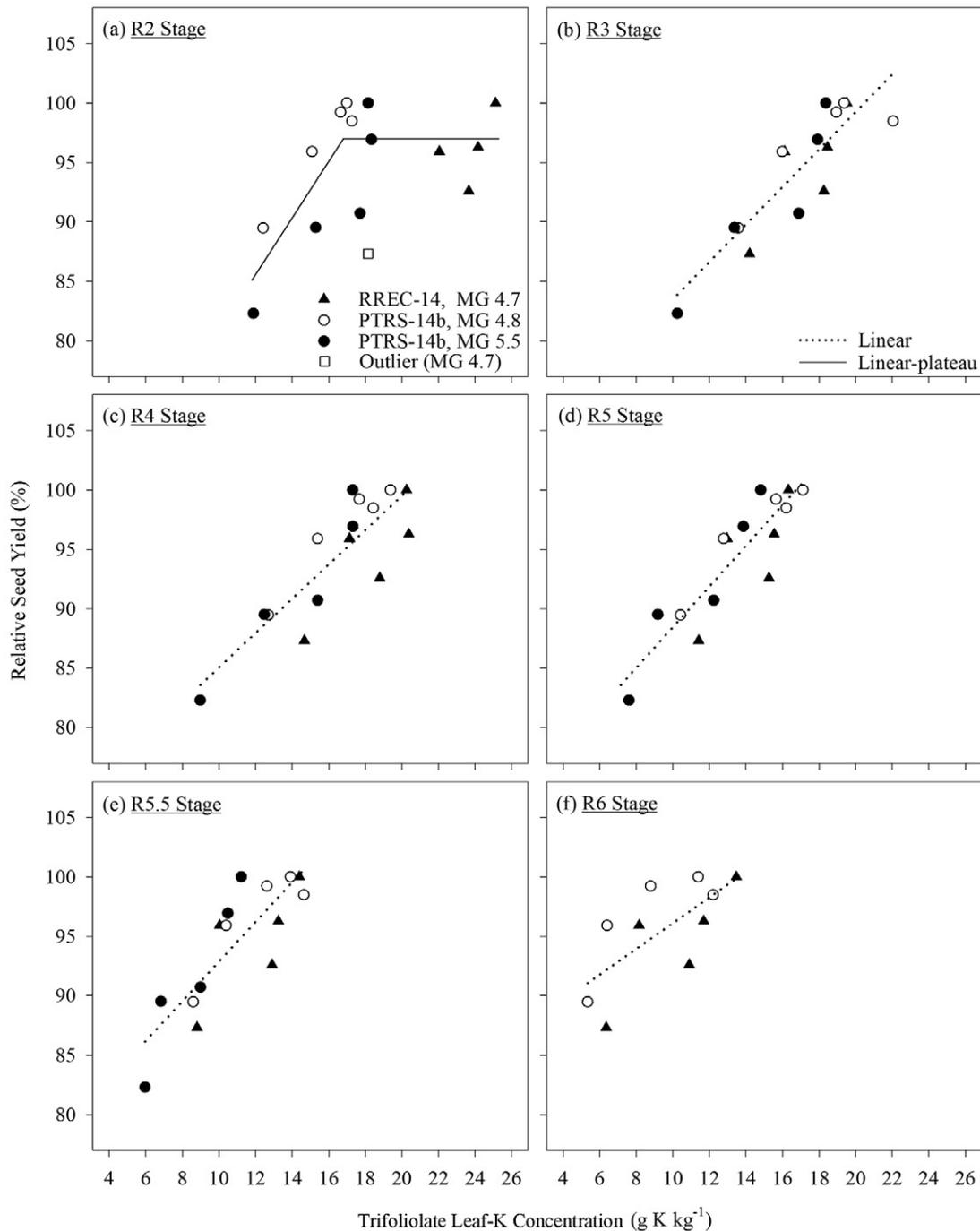
Fig. 3. Relationships between relative soybean seed yield and trifoliolate leaf-K concentration at the (a) R2, (b) R3, (c) R4, (d) R5, (e) R5.5, and (f) R6 stages for long-term K fertilization trials conducted at the Rice Research and Extension Center (RREC-14) with a maturity group (MG) 4.7 cultivar and at the Pine Tree Research Station (PTRS-14b) with MG 4.8 and 5.5 cultivars in 2014. Mean data of each annual fertilizer-K rate for each cultivar and trial were used to model these relationships. Model coefficients are listed in Table 6.

less than the lower boundary would be deficient, concentrations equal or greater than the upper boundary would be sufficient, and concentrations between these two boundaries would be considered to be in the CR(Fig. 4c). For the linear model in Fig. 4c, the calculated lower and upper leaf-K concentration boundaries were 12.6 and 17.0 g K kg^{-1} at 10 d after full bloom (R3), respectively, 10.6 and 15.0 g K kg^{-1} at 20 d (R4), 8.7 and 13.1 g K kg^{-1} at 30 d (R5), 6.7 and 11.1 g K kg^{-1} at 40 d (R5.5), and 4.7 and 9.1 g K kg^{-1} at 50 d (R6). Compared to the linear model in Fig. 4c, the calculated lower threshold of the linear (Fig. 4a) and quadratic (Fig. 4b) models indicating K deficiency from the R2 to R5 stages were 1.1 to 2.5 g K kg^{-1} greater. Regardless

Table 6. Relationships between soybean relative yield (RY) and trifoliolate leaf-and petiole-K concentrations (KC) at the R2 to R6 growth stage as predicted with linear (L) and linear-plateau (LP) models for long-term K fertilization trials conducted at the Rice Research and Extension Center (RREC-14) and Pine Tree Research Station in 2014 (PTRS-14b).

Growth stage	Model†	Coefficients				Critical-K concentration‡	
		Intercept	Slope	R^2	$P > F$	90% RY	95% RY
		g K kg^{-1}	g K kg^{-1} d^{-1}			——— g K kg^{-1} ———	
			Trifoliolate leaf				
R1	L	94.2	0.060	0.01	0.862	–	–
	SE	5.5	0.340	–	–	–	–
	LP	70.5	2.272	0.12	0.497	–	–
	SE	28.0	2.827	–	–	–	–
R2§	L	83.1	0.642	0.26	0.063	–	–
	SE	5.8	0.313	–	–	–	–
	LP	57.0	2.380	0.62	0.005	14.6	19.0
	SE	12.5	0.870	–	–	–	–
R3	L	67.7	1.577	0.77	<0.001	14.1	17.3
	SE	4.0	0.236	–	–	–	–
R4¶	L	70.6	1.446	0.73	<0.001	13.4	16.9
	SE	4.1	0.246	–	–	–	–
R5	L	71.3	1.714	0.80	<0.001	10.9	13.8
	SE	3.3	0.238	–	–	–	–
R5.5#	L	76.2	1.667	0.70	<0.001	8.3	11.3
	SE	3.4	0.302	–	–	–	–
R6§	L	85.3	1.083	0.48	0.026	4.3	9.0
	SE	3.9	0.396	–	–	–	–
			Petiole				
R1	L	93.7	0.039	0.01	0.735	–	–
	SE	4.4	0.114	–	–	–	–
	LP	90.5	0.107	0.05	0.434	–	–
	SE	5.0	0.132	–	–	–	–
R2§	L	71.8	0.605	0.85	<0.001	30.1	38.3
	SE	2.8	0.072	–	–	–	–
R3	L	78.7	0.427	0.81	<0.001	26.5	38.2
	SE	2.2	0.060	–	–	–	–
R4¶	L	77.8	0.608	0.81	<0.001	20.1	28.3
	SE	2.3	0.081	–	–	–	–
R5	L	81.7	0.559	0.75	<0.001	14.8	23.8
	SE	2.2	0.090	–	–	–	–
R5.5#	L	85.2	0.603	0.65	0.001	8.0	16.3
	SE	2.0	0.122	–	–	–	–
R6§	L	90.6	0.552	0.41	0.045	−1.1	8.0
	SE	2.4	0.232	–	–	–	–

† RY = intercept + (slope × KC). The L and LP models for leaf and petiole at the R1 stage and L model for the leaf at the R2 stage were not significant at the 0.05 probability level.

‡ Critical range (CR) K concentrations for leaf and petiole were calculated at 90 and 95% RY from the L model. The negative (−1.1 g K kg^{-1}) predicted petiole-K concentration for the lower CR threshold at the R6 is not possible and is an artifact of the limited data available at the R6 growth stage (Fig. 7f). For the leaf at the R2 stage, the 90% confidence limits (14.6–19.0 g K kg^{-1}) of the join point (16.8 g K kg^{-1} at 97.0% RY) of the LP model were considered as CR K concentrations. Tissue-K concentrations above and below the CR were considered as sufficient and deficient, respectively.

§ Tissue samples were collected twice during the R2 growth stage for the MG 4.8 cultivar at PTRS-14b and during the R6 growth stage for the MG 4.7 cultivar at RREC-14 (Table 2). Tissue-K concentrations from the second sampling of both growth stages were used in regression analyses.

¶ Tissue samples were not collected at the R4 growth stage for the MG 4.8 cultivar at PTRS-14b (Table 2). The mean of K concentrations for the R3.5 and R4.5 stages were used for regression analyses.

Tissue samples were collected twice during R5.5 growth stage for both RREC-14 and PTRS-14b (Table 2). The mean K concentrations of the two sample times was used in regression analyses.

of the model, the actual CR will vary with the duration of each reproductive growth stage and require knowledge of the actual growth stage or the date of the R2 stage (i.e., to calculate the days after R2 stage). Additional research is needed to validate whether these preliminary leaf-K concentration models can accurately predict K deficiency beyond the R2 stage.

Seasonal Dynamics of Petiole Potassium Concentration

The K concentration of petioles from the third node from the top of the plant also increased linearly during late vegetative growth, peaked during early reproductive growth (R1–R2), and declined linearly as soybean progressed into reproductive development (Table 4; Fig. 5b, 5c). The absence of an initial linear increase of the petiole-K concentration from the vegetative to the early reproductive stage for RREC-14 (Fig. 5a) was because the first sample coincided with the onset of reproductive growth (Table 2). The linear-slope model showed that the peak petiole-K concentration (22.8–53.7 g K kg^{-1}), across annual-K fertilizer rates and soybean cultivars, occurred 44 to 50 DAE, at the R1 stage for the MG 4.7 cultivar at RREC-14 and the MG 5.5 cultivar at PTRS-14b and at the R2 stage for the MG 4.8 cultivar at PTRS-14b (Table 4; Fig. 5). Hanway and Weber (1971) and Sojka et al. (1985) also showed petiole-K concentrations peak near blooming and decline linearly with time. The peak petiole-K concentrations (22.8–53.7 g K kg^{-1}) in our study were about double that of the peak leaf-K

concentrations (12.3–22.3 g K kg^{-1}; Table 4), which agrees with the findings of Hanway and Weber (1971) and Sojka et al. (1985).

We also evaluated the linear decline of the petiole-K concentration after K concentration peaked at the R1–R2 stage across five annual-K fertilization rates with one (RREC-14) or two (PTRS-14b) soybean cultivars. The petiole-K concentration decreased linearly with a common slope coefficient of −0.554 g K kg^{-1} d^{-1} regardless of annual-fertilizer-K rate for RREC-14 (Table 7; Fig. 6a). For PTRS-14b, the slope values were different among annual fertilizer-K rates ranging from −0.400 to −0.608 g K kg^{-1} d^{-1} due to the significant interaction between annual fertilizer-K rate and DAE (Fig. 6b, 6c). However, the slope values of −0.520 to −0.608 g K kg^{-1} d^{-1} for soybean fertilized with 38 to 150 kg K ha^{-1} yr^{-1} were statistically similar and higher than the slope value of −0.400 g K kg^{-1} d^{-1} for soybean receiving 0 kg K ha^{-1} yr^{-1}. The average decline rate of petiole-K concentration for the K-fertilized soybean grown at PTRS-14b was −0.564 g K kg^{-1} d^{-1} (Table 7). The lower decline rate for soybean receiving no fertilizer-K at PTRS-14b was probably due to the already very low petiole-K concentrations (e.g., intercepts) present in both cultivars at the R2 stage caused by low soil-K availability. The slope values for soybean grown at RREC-14 and for soybean grown at PTRS-14b were not statistically compared, but were numerically similar, averaging −0.559 g K kg^{-1} d^{-1}.

The linear model also indicated that the petiole-K concentration for a particular time or reproductive stage increased with each increase in annual-K fertilization rate for RREC-14
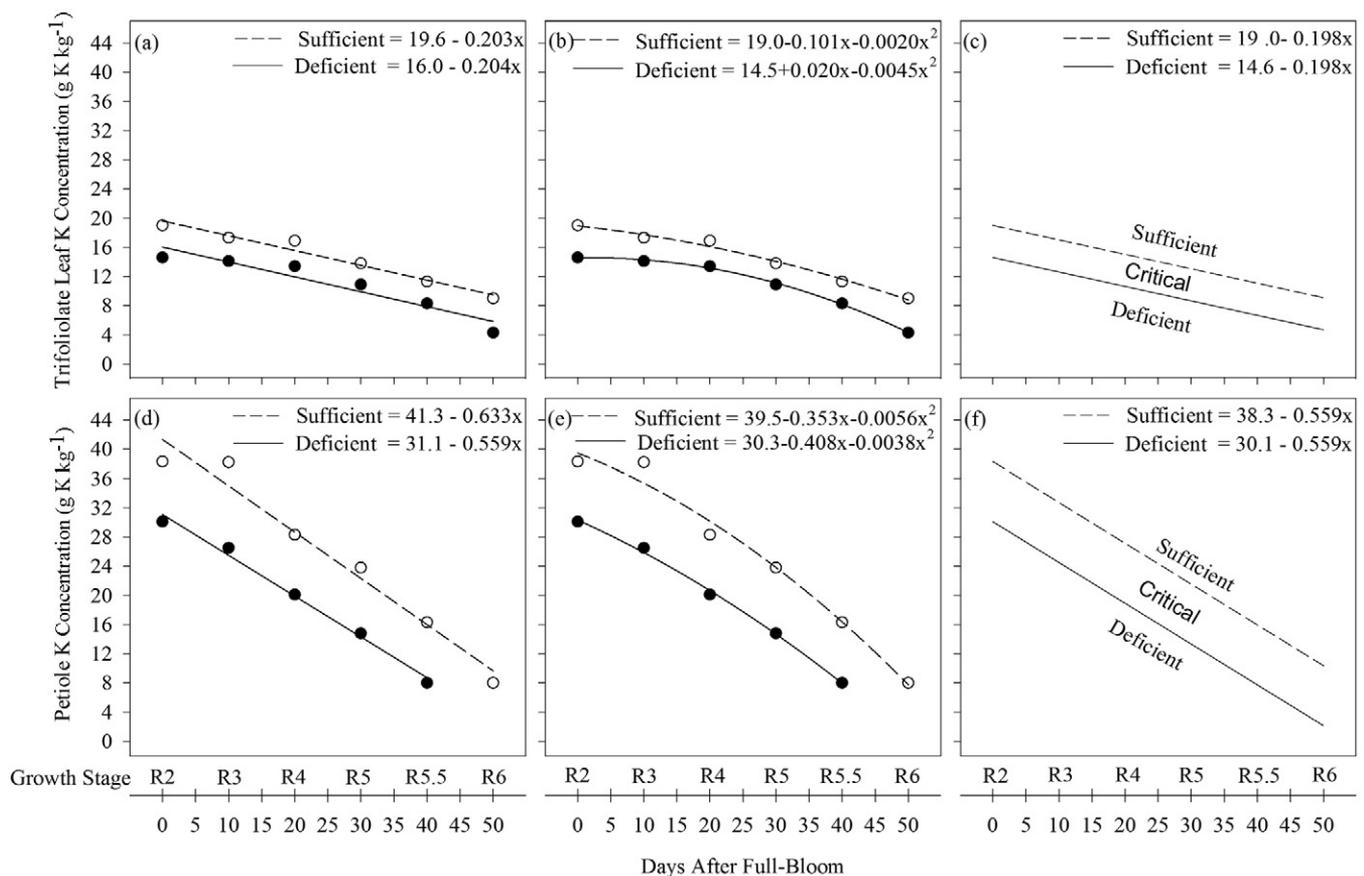
Fig. 4. Predicted soybean critical tissue-K concentrations across time in the (a–c) trifoliolate leaves and (d–f) petioles from the R2 (full-bloom) to R6 (full-seed) stages. The prediction of the deficient petiole-K threshold was extended only to R5.5 stage due to the negative predicted petiole-K concentration, listed in Table 6, for the lower critical range threshold at the R6 stage. The duration of each reproductive growth stage may vary with the planting date and soybean maturity group.

(intercept, Table 7; Fig. 6a). Regardless of soybean cultivar at PRTS-14b, the petiole-K concentration during the reproductive stages (R2–R6) increased statistically or numerically as annual fertilizer-K rate increased (Fig. 6b, 6c). We could find no information in the literature describing the seasonal dynamics of petiole-K concentration as affected by K availability. Miller et al. (1961) showed that petiole-K concentrations on both the lower- and upper-half of soybean plants at the R4 stage increased as fertilizer-K rate increased.
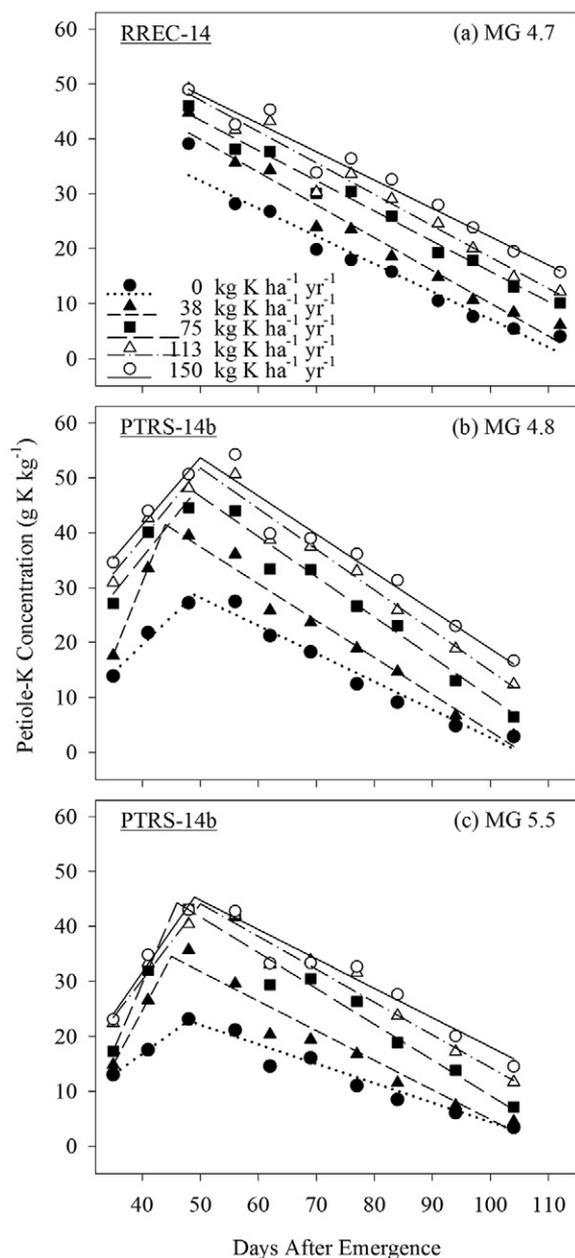
Fig. 5. Soybean petiole-K concentration change as a function of days after emergence as predicted with a linear-slope model for long-term K fertilization trials conducted at the Rice Research and Extension Center (a; RREC-14) and Pine Tree Research Station (b–c; PTRS-14b) in 2014 with five annual fertilizer-K rates and one or two soybean maturity groups (MG) cultivars, respectively. Data for each trial were analyzed by cultivar and annual fertilizer-K rate. Model coefficients and time and growth stage where K concentration peaked are listed in Table 4.

Relative Soybean Yield and Petiole Potassium Concentration

The relationships between RY and petiole-K concentrations at the R1 to R6 stages were developed to predict critical petiole-K concentrations for specific growth stages using a process similar to that described for trifoliolate leaves. The linear and LP models showed significant relationships between RY and petiole-K concentrations at the R2, R3, R4, R5, R5.5, and R6 stages (Table 6; Fig. 7a–7f). The LP models are not shown because the plateau portion of the LP models was very narrow for the R2 to R5 stages and absent for the R5.5 and R6 stages. The relationship at the R1 stage was not significant for either the linear or LP models (Table 6). The petiole-K concentrations from the R2 to R6 stages accounted for 41 to 85% of the variability in RY. The linear model showed that the petiole-K concentration at the R2 stage had the highest numerical R^2 value and it declined as plant development advanced to the R3 to R6 stages (Table 6). We could find no research that evaluated the relationship between relative soybean yield and petiole-K concentration of the uppermost recently mature leaves at any growth stage. However, when we regressed soybean yield from

Table 7. Intercept and linear slope coefficients predicting the decline rate of petiole-K concentration (PKC) as a function of time (d after emergence, DAE) as affected by five annual fertilizer-K rates and one or two soybean cultivars belonging to different maturity groups (MGs) for long-term K fertilization trials conducted at the Rice Research and Extension Center (RREC-14) and Pine Tree Research Station (PTRS-14b) in 2014.

Cultivar MG	Annual-K rate	Linear model† coefficients		
		Intercept	Slope	R^2
	kg K ha^{-1} yr^{-1}	g K kg^{-1}	g K kg^{-1} d^{-1}	
		RREC-14		
MG 4.7	0	62.0e‡	–0.554	0.92
	38	66.3d	–0.554	
	75	71.0c	–0.554	
	113	74.1b	–0.554	
	150	77.0a	–0.554	
SE		1.0	0.0105	
		PTRS-14b		
MG 4.8	0	45.0c	–0.400b	0.88
	38	62.0b	–0.561a	
	75	72.3a	–0.608a	
	113	73.9a	–0.565a	
	150	72.9a	–0.520a	
MG 5.5	0	42.4c	–0.400b	
	38	59.4b	–0.561a	
	75	69.7a	–0.608a	
	113	71.3a	–0.565a	
	150	70.3a	–0.520a	
SE		2.1	0.0272	
Average slope (PTRS-14b)			–0.564§	
Average slope (all site-years)			–0.559¶	
Average SE (all site-years)			0.0189	

† PKC = intercept + (slope × DAE).

‡ Different letters next to mean values in the same column within each site-year represent significant difference at the 0.05 probability level.

§ Average slope for the annual fertilizer-K rates of 38 to 150 kg K ha^{-1} yr^{-1} at PTRS-14b.

¶ Average slope for the annual fertilizer-K rates 0 to 150 kg K ha^{-1} yr^{-1} at RREC-14 and 38 to 150 kg K ha^{-1} yr^{-1} at PTRS-14b.

Miller et al. (1961) against the petiole-K concentrations of the upper-half of the plant at the R4 stage, we found a LP relationship that explained 75% of the variation in actual soybean yield [kg grain ha^{-1} = 78.1 + 142.9x; x0 (join point) = 13.46 g petiole-K kg^{-1}; $P < 0.001$]. Cassman et al. (1989) showed that the petiole-K concentration of the uppermost fully-expanded leaf of cotton at the full-bloom stage accounted for 53 to 61% variation in relative cotton yield.
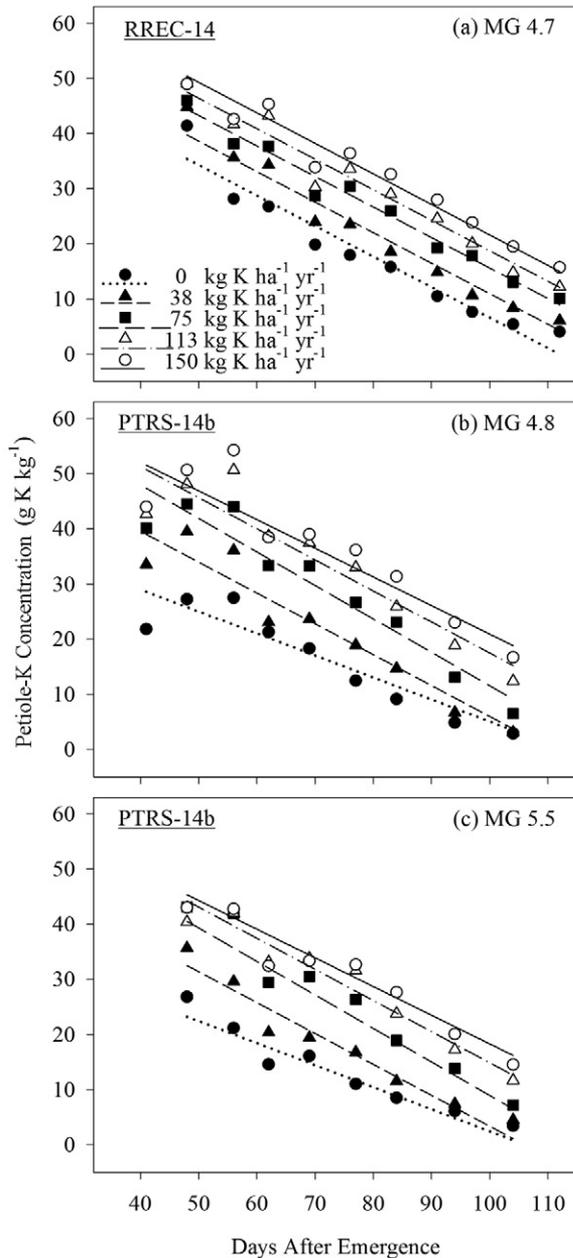
Fig. 6. Soybean petiole-K concentration change as a function of days after emergence from the growth stage where K concentrations peaked to the R6 stages as predicted with a linear model for long-term K fertilization trials conducted at the Rice Research and Extension Center (a; RREC-14) and Pine Tree Research Station (b–c; PTRS-14b) in 2014 with five annual fertilizer-K rates and one or two soybean maturity groups (MG) cultivars, respectively. Model coefficients are listed in Table 7. The growth stage of each cultivar of each trial that corresponded to a specific day after emergence is listed in Table 2.

Critical Petiole Potassium Concentration

The critical petiole-K concentrations at the R2 stage that represent 90 and 95% RY were 30.1 and 38.3 g K kg^{-1} (Table 6; Fig. 7a), which were more than double the concentrations that defined the critical leaf-K concentration at the R2 stage (14.6 and 19.0 g K kg^{-1}). The petiole-K concentrations of ≤30.1 and ≥38.3 g K kg^{-1} at the R2 stage were, therefore, considered deficient and sufficient, respectively, petiole-K concentration thresholds.

The predicted deficient and sufficient petiole-K concentrations as defined by 90 and 95% RY, respectively, decreased from 30.1 and 38.3 g K kg^{-1} at the R2 stage to –1.1 and 8.0 g K kg^{-1} at the R6 stage (Table 6). Note that the predicted petiole-K concentration for the lower CR threshold at the R6 was negative, which is not possible and is an artifact of the limited data available at the R6 growth stage (Fig. 7f). For this reason, the prediction of the deficient petiole-K threshold was extended only to R5.5. The predicted deficient and sufficient petiole-K concentrations also declined linearly from the R2 to R5.5 or R6 stages (0–40 or 50 d after R2) with slope values of –0.559 and –0.633 g K kg^{-1} d^{-1}, respectively (Fig. 4d; $P = 0.001$ and $R^2 = 0.99$ for deficient concentrations; $P = 0.001$ and $R^2 = 0.97$ for sufficient concentrations), which were close to our average slope of –0.559 g K kg^{-1} d^{-1} (Table 7). A quadratic model of the deficient and sufficient petiole-K concentrations from the R2 to R5.5 or R6 stages (Table 6) was also significant (Fig. 4e; $P = 0.003$ and $R^2 = 0.99$ for deficient concentration; $P = 0.003$ and $R^2 = 0.98$ for sufficient concentration). As was done for the leaf tissue, the critical petiole-K concentration thresholds at the R2 stage (30.1–38.3 g K kg^{-1}) and the average rate of petiole-K decline (–0.559 g K kg^{-1} d^{-1}) from the R2 stage were used to define the critical petiole-K concentrations across reproductive developmental stages (Fig. 4f). This preliminary model shows that the deficient petiole-K concentrations at 10 (R3), 20 (R4), 30 (R5), 40 (R5.5), and 50 (R6) d after full bloom were 24.5, 18.9, 13.3, 7.7, and 2.2 g K kg^{-1}, respectively. Each of the three models predict slightly different values defining K deficiency at each growth stage that differ by 0 to 2.0 g petiole-K kg^{-1} between the R2 and R5 stages. Additional research is needed to determine which of the models is most accurate and to validate the proposed critical petiole-K concentrations across soybean developmental stages.

We did not compare K concentrations between leaves and petioles, but our research showed that the petiole-K concentrations across annual fertilizer-K rates had a wider range of change from the R2 to R6 stages (30.3–49.3 g K kg^{-1} at the R2 stage to 3.3–19.3 g K kg^{-1} at the R6 stage; Fig. 6) than the leaf-K concentrations (15.2–21.3 g K kg^{-1} to 5.7–12.1 g K kg^{-1}; Fig. 2). The intercept and linear slope values were approximately two and three times, respectively, greater for the petiole-K (Table 7) than leaf-K (Table 5) concentrations. The wider range of critical petiole-K concentrations from one growth stage to the other suggests that growth stage as well as deficiency and sufficiency thresholds for petiole-K concentrations could be more easily categorized. For example, according to our models the predicted CR petiole-K concentrations were 24.5 to 32.7 g K kg^{-1} at R2 stage and 7.7 to 15.9 g K kg^{-1} at R5.5 stage (Fig. 4f), which are numerically higher than the values and ranges of the critical leaf-K concentrations of 12.6 to 17.0 g K kg^{-1} at R2 stage and 6.7 to 11.1 g K kg^{-1} at R5.5 stage (Fig. 4c). Our results suggest that soybean petioles may

be an equally good or perhaps better tissue than trifoliolate leaves for monitoring the K nutritional status of soybean during reproductive growth. Mills and Jones (1996) summarized that petioles are a better tissue than the uppermost fully-expanded leaves for monitoring the nutritional status in cotton (*Gossypium hirsutum* L.), potato (*Solanum tuberosum* L.), sugar beet (*Beta vulgaris* L.), and some vegetables.

CONCLUSIONS

The trifoliolate leaf- and petiole-K concentrations both peaked around blooming (R2 stage) and then declined linearly at a constant rate of -0.198 g K kg^{-1} d^{-1} for the leaves and -0.559 g K kg^{-1} d^{-1} for the petioles, regardless of soybean cultivar or annual fertilizer-K rate. The uniform rate of decline in K concentrations for each of these tissues may extend our ability to interpret tissue-K concentrations at growth stages beyond R2. The petiole- and leaf-K concentrations from the
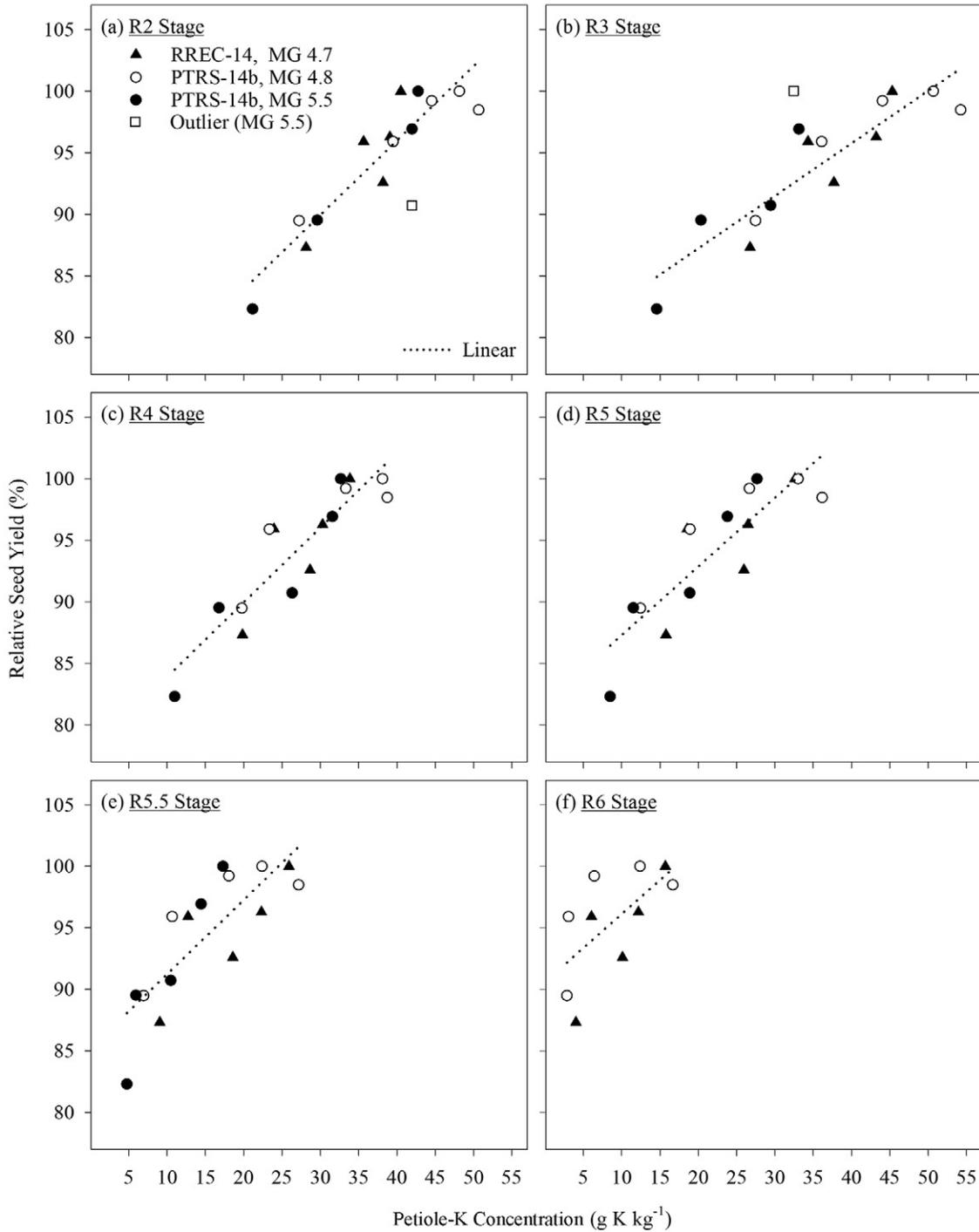
Fig. 7. Relationships between relative soybean seed yield and petiole-K concentration at the (a) R2, (b) R3, (c) R4, (d) R5, (e) R5.5, and (f) R6 stages for long-term K fertilization trials conducted at the Rice Research and Extension Center (RREC-14) with a maturity group (MG) 4.7 cultivar and at the Pine Tree Research Station (PTRS-14b) with MG 4.8 and 5.5 cultivars in 2014. Mean data of each annual fertilizer-K rate for each cultivar and trial were used to model these relationships. Model coefficients are listed in Table 6.

R2 to R5.5 stages were highly correlated with RY. Petiole-K concentrations at the R2 stage explained more of the RY variability than leaf-K concentrations, but the strength of the relationships at the R3 to R5.5 stages was comparable. The twofold higher value and range of critical K concentrations at the R2 stage followed by a nearly threefold greater linear decline rate across the reproductive stages for the petioles (30.1–38.3 g K kg^{-1} with −0.559 g K kg^{-1} d^{-1}) compared to the leaves (14.6–19.0 g K kg^{-1} with −0.198 g K kg^{-1} d^{-1}) may allow for more accurate separation of the deficient- and sufficient-K concentrations within a growth stage as well as between the growth stages. Our models represent the first proposed critical tissue-K concentrations for soybean across multiple growth stages. Overall, the ability to interpret the K nutritional status in leaves, petioles, or both tissues at numerous reproductive growth stages will improve K management and help agricultural practitioners to monitor and possibly correct K nutritional problems across a range of growth stages.

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