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Potassium Fertilization Effects on Isoflavone Concentrations in Soybean [*Glycine max* (L.) Merr.]

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Soybean isoflavone concentrations vary widely, but the contribution of soil fertility and nutrient management to this variability is unknown. Field experiments from 1998 to 2000 on soils with low to high exchangeable potassium (K) concentrations evaluated K application and placement effects on isoflavone concentrations and composition of soybean in various tillage and row-width systems. Soybean seed yield and concentrations of daidzein, genistein, glycitein, leaf K, and seed K were measured. Significant increases in daidzein, genistein, and total isoflavone were observed with direct deep-banded K or residual surface-applied K on low-K soils. Positive effects of K fertilization on isoflavones were less frequent on medium- to high-testing K soils. Both individual and total isoflavones were often positively correlated with seed yield, leaf K, and seed K on low-K soils. Appropriate K management could be an effective approach to increase isoflavone concentrations for soybeans produced on low- to medium-K soils.

KEYWORDS: Potassium; soybean; *Glycine max* (L.) Merr.; isoflavone; daidzein; genistein; glycitein; yield; seed K; leaf K; correlation

INTRODUCTION

Isoflavones are a group of phytochemicals in some legumes that are thought to contribute to the healthful effects of soybean in human and animal diets. Researchers have found that the potential role of soybean-based foods in prevention of chronic diseases including cancer, heart disease, osteoporosis, and menopausal symptoms (*1*−*3*) is promising because isoflavones in soybean seeds possess functions of antiestrogens (*4*, *5*), antioxidants (*6*), and tyrosine protein kinase inhibitors (*7*).

Significant genetic and environmental impacts on isoflavone concentrations in soybean seeds have been reported (*8*−*12*). Wang et al. (*12*) observed that total isoflavone concentrations ranged from 1161 to 2743 μg g^{-1} in 210 soybean cultivars grown in South Dakota. Hoeck et al. (*8*) showed that the genotype, genotype × year, genotype × location, and genotype × year × location interactions were all significant for both total and individual isoflavone concentrations. Eldridge and Kwolek (*9*) reported that total isoflavone concentrations in soybean seeds varied from 1160 to 3090 μg g^{-1} among four soybean cultivars grown in the same environment and from 460 to 1950 μg g^{-1} among four locations with the same variety. Wang and Murphy observed in 1994 (*11*) that total isoflavone concentrations of Vinton 81 soybean ranged from 1176 to 3309 μg g^{-1} among years at the same location and from 1176 to 1749 μg g^{-1} among locations within the same year; thus, year seemed to influence isoflavone concentrations more than location. Kitamura et al. (*13*) and Tsukamoto et al. (*10*) showed that isoflavone concentrations were significantly lower in soybean seeds that developed in high temperatures during seed filling than those in seeds exposed to low temperatures during the filling period.

Previous research in soil fertility and nutrient management has focused mainly on the optimum levels of nutrients for high or economically optimum crop yield. The impacts of soil fertility levels and management practices on crop quality frequently are not evaluated. Further understanding of the relationships between isoflavone concentrations and soil and crop management factors is essential to soybean growers who may be given financial incentives to produce high-isoflavone soybean, and to soybean breeders for the selection of soybean varieties that have high genetic potential for individual and total isoflavones. In addition, nutritional scientists have interest in discovering the impact of agricultural management practices oriented toward increasing yield on the contents of phytochemicals in foods.

None of the previous investigations have evaluated nutrient management effects on individual and total isoflavone responses,

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or the relationships of isoflavones with other seed attributes such as yield or potassium (K) concentrations. Aside from nitrogen, K is the second most abundant nutrient in soybean seeds. Typical K concentrations in soybean trifoliolate leaves and seeds are on the order of 10 to 30 g kg^{-1} (*14*−*17*). Potassium may be essential in isoflavone synthesis because K is an essential activator for more than 60 enzymes that catalyze a variety of metabolic activities (*18*). The objectives of this study were to (1) evaluate K fertilization effects on isoflavone concentrations and composition of soybean in various tillage and row-width systems, and (2) determine the relationships of isoflavone concentrations with seed yield and leaf K and seed K concentrations.

MATERIALS AND METHODS

Plant Material. Soybean cultivars OAC Bayfield, First Line (FL) 2801R, NK S08-80 and NK S19-90 were used in this study. All cultivars were conventional soybean except FL 2801R, which is a Roundup Ready cultivar. The cultivars were selected to represent a range of maturity groups (MG) of soybean commonly grown in southwestern Ontario. The maturity groups of these cultivars are listed as follows: OAC Bayfield, MG 0.4; FL 2801R, MG 0.8; NK S08-80, MG 0.8; and NK S19-90, MG 1.9. In previous studies conducted for the soybean seed industry in Ontario, these authors have found the cultivar OAC Bayfield to be among those having relatively low total isoflavone concentrations, whereas FL 2801R was found to be among a group of cultivars with medium to high total isoflavone contents (unpublished data). No previous information was available to the authors on seed isoflavone concentrations for NK S08-80 and NK S19-90.

Field Experiments. Soybean seed samples used in this study were collected from field experiments conducted in Ontario, Canada, with various statistical designs, but which all involved the determination of soybean responses to K fertilizer application and placement. There were five experimental sites in total, which will be referred to as Paris direct, Strathroy direct, Kirkton direct, Paris residual, and Kirkton residual, respectively. In these site names, "direct" infers that K fertilization treatments were imposed directly to the soybean crop; while "residual" implies that K fertilizers were applied to the corn preceding the soybean crop, and no K fertilizer was applied after corn or during the soybean season. Soybean seed samples were collected from 1998 to 2000 in the three direct K experiments, but only from 1998 to 1999 for the residual K investigations. At each location, experiments were conducted at adjacent sites in the same field or in adjacent fields for successive seasons. The daily air temperatures and rainfall were recorded on-site or collected from the closest weather station during the entire growing season for each experiment. Primary physical and chemical properties of the soils at all locations are presented in **Table 1**. The K fertilization and other management treatments for each experiment are described below.

Paris Direct. Field experiments were conducted on a private farm near Paris, Brant County, Ontario, from 1998 through 2000. A randomized complete-block split-plot design with four replicates was used in this study. Potassium placement methods were randomly assigned to the whole plots, and soybean row-widths were assigned to the subplots. Four K placement methods were spring-applied as follows. (1) Surface broadcast: fertilizer was evenly broadcasted on the soil surface. (2) 76-cm Band: potassium was placed 10-cm deep in bands spaced 76 cm apart. (3) 38-cm Band: potassium was placed 10-cm deep in bands at 38-cm centers. (4) Zero K: no K fertilizer was applied.

When K was applied, the rate was 100 kg K ha^{-1} as muriate of potash (0-0-50). Soybean row widths included 76, 38, and 19 cm, such that row spacings corresponded to fertilizer bands as well as the narrow-row production practices typical for soybean in the region. Soybean was planted in 4, 8, and 16 rows, 21 m long, with 76-, 38-, and 19-cm row widths, respectively, in each plot. Soybean cultivar OAC Bayfield was planted no-till in each season. Further details on site characteristics, soil K stratification, and soybean responses to treatments are available in Yin and Vyn (*16*).

Strathroy Direct and Kirkton Direct. Field investigations were conducted near Strathroy and Kirkton, Ontario, from 1998 to 2000. A randomized complete-block experimental design with four replicates was used in each season at both locations. There were 13 treatments in total including K application timing and methods, conservation tillage systems, and soybean row-widths. The rate of K applied was 100 kg K ha^{-1} at both locations. Soybean cultivar NK S19-90 was used in 1998 and NK S08-80 was grown in 1999 and 2000 at Strathroy. Soybean cultivar OAC Bayfield was planted in 1998, and FL 2801R was grown in 1999 and 2000 at Kirkton. Soybean was no-till planted in 4 rows spaced 76 cm apart or 8 rows spaced 38 cm apart, in 21 m length, for each plot at both locations. Detailed information about soil characteristics and soybean responses to K treatments was described in Yin and Vyn (*17*). Because of resource limitations, only 38-cm no-till soybean in the four K treatments of fall band, spring band, fall broadcast, and zero K was evaluated in this study.

Table 1. Initial Soil Physical and Chemical Properties of Soil Ap Horizon (0–15 cm) and Soybean Planting Dates for the Experimental Sites (1998–2000)

location and property (unit)	1998	1999	2000
Paris direct			
texture	silt loam	loam	sandy loam
pH	6.6	6.4	6.0
soil-test K (mg L^{-1})[a]	35	36	54
planting date (month/day)	05/19	05/14	05/26
Strathroy direct			
texture	clay	clay	loam
pH	7.4	6.8	7.3
soil-test K (mg L^{-1})[a]	155	134	96
planting date (month/day)	05/23	06/05	06/01
Kirkton direct			
texture	silt loam	silt loam	silt loam
pH	7.0	6.8	7.7
soil-test K (mg L^{-1})[a]	92	73	90
planting date (month/day)	05/26	05/19	05/30
Paris residual			
texture	silt loam	silt loam	
pH	5.8	6.4	
soil-test K (mg L^{-1})[a]	60	47	
planting date (month/day)	05/19	05/17	
Kirkton residual			
texture	silt loam	silt loam	
pH	7.2	6.4	
soil-test K (mg L^{-1})[a]	85	90	
planting date (month/day)	05/21	05/20	

[a] Ammonium acetate extractable K (mg K per L of soil) (*19*).

Paris Residual. Field experiments involving a corn−soybean rotation were conducted near Paris, Brant County, Ontario, from 1997 through 1999. For the corn season, the experiments were conducted using a randomized complete-block design with four replicates in both 1997 and 1998. Spring K placement methods included deep band, surface broadcast, and zero K. Where K was applied, it was at a rate of 100 kg K ha^{-1} as muriate of potash (0-0-50). Corn was planted in 76-cm rows.

The same plots were used for subsequent no-till soybean in 1998 and 1999. Soybean cultivar FL 2801R was planted in 19-cm rows in both seasons. Each plot consisted of 16 rows of 21-m length. The soybean crop received no K treatments other than the residual fertilization from the previous year's no-till corn.

Kirkton Residual. Field investigations were conducted on a private farm from 1997 through 1999 near Kirkton, Perth County, Ontario. For the corn season, a randomized complete-block split split-plot design with four replications was used. Tillage systems including no-till, fall zone-till, and fall moldboard plow were randomly assigned to the whole plots; fall K application rates of 0, 42, and 84 kg K ha^{-1} were assigned to the subplots, and spring K rates of 0 and 42 kg K ha^{-1} were assigned to the sub subplots. Additional information about treatments and crop management practices associated with the previous corn was communicated previously (*20*).

The identical experimental design and plot arrangement as the previous corn year were used for the subsequent no-till soybean in each season. No K fertilizer was applied after corn or during the soybean season. Soybean were planted no-till in the same direction as the

previous corn rows. Soybean cultivar FL 2801R was used in both 1998 and 1999. Soybean was planted in 8 rows, 21-m long, spaced 38 cm apart for each plot in both seasons. Further details about treatments and soybean management practices were presented in Yin and Vyn (*21*). Only the plots receiving either zero K, or 84 kg K ha^{-1} of fall K plus 42 kg K ha^{-1} of spring K, in each tillage system were evaluated for isoflavone responses.

Leaf K Measurement. Leaf samples consisting of 20 most-recently developed and fully expanded trifoliolate leaves (petiole included) from 20 soybean plants were collected at initial flowering stage (R1) in mid-to-late July from each plot in all experiments. Leaf tissues were dried in a forced-air oven at 65 °C for at least 3 d, and then ground in a Wiley mill (Arthur K. Thomas Co., Philadelphia, PA) to pass through a 1-mm screen. Leaf samples were analyzed for K concentrations using a dry ash method (*23*).

Seed Evaluation. After soybean reached maturity, seed yield was determined by using a plot combine to harvest a central strip of soybean 1.0-m wide for the entire plot length from each plot, and adjusting the yield to a moisture content of 130 g kg^{-1}. Seed samples were taken at harvest for the determination of isoflavone and K concentrations. All these samples, which were stored under identical conditions and contained 80−85 g kg^{-1} moisture, were ground for isoflavone analysis. Seed samples were dried in a forced air oven at 65 °C for at least 3 d, and ground in a mill to pass through a 1-mm screen for seed K determination.

Concentrations of daidzein, genistein, and glycitein were determined using a high-performance liquid chromatography (HPLC) method modified from Franke et al. (*22*) after acid hydrolysis of the endogenous 12 isoflavones to their aglycon forms (daidzein, genistein, and glycitein), which were summed to obtain total isoflavone concentrations. The aglycon weight corresponded to approximately 55% of the weight in the naturally occurring glycosylated forms.

All the chemicals and solvents used in this measurement (e.g., ethanol, methanol, and hydrochloric acid) were analytical grade or HPLC grade. "Nano Pure" or equivalent HPLC-grade water was used. The three standard aglycons of isoflavone (daidzein, genistein, and glycitein) were obtained from Indofine Chemical Co (Sommerville, NJ).

Finely ground soybean seed was weighed in duplicate samples of 0.5000 g each and dispersed in 10 mL of ethanol plus 2 mL of concentrated HCl. The resulting solutions were hydrolyzed by heating to 125 °C for 2 h in a sand bath. After the samples were cooled, they swere centrifuged at 3000 rpm for 10 min. The clear aliquot was filtered through a 0.45-μm PTFE filter. Individual hydrolyzed daidzein, genistein, and glycitein were separated on a HPLC equipped with a photodiode array (PDA) detector (200−300 nm) using the following instrumental conditions: HPLC, Waters 600E multi-solvent delivery system with a 717 plus auto-sampler and a PDA detector set to collect spectra from 200 to 300 nm; HPLC column, Waters Nove Pak C_{18} column (3.9 × 150 mm, 5-μm particle size) with C_{18} guard column; HPLC mobile phases, solvent A was 4% aqueous acetic acid and solvent B was 100% HPLC grade methanol; flow rate, 1.5 mL min^{-1}; and injection volume, 5 μL. HPLC mobile phases were solvent A (4% aq. acetic acid) and solvent B (100% methanol), and the solvent system was as follows (% solvent A/% solvent B): 0 min (70/30), 12.5 min (65/35), 13 min (50/50), 15 min (30/70), 22.5 min (25/75), and 23 min (70/30). Recovery was monitored by the addition of a recovery standard, flavone, to the sample prior to hydrolysis.

Using this program, daidzein (R_T = 13.09 min) and glycitein (R_T = 16.16 min) were well separated near the beginning of the run, while genistein (R_T = 19.62 min) eluted later; the recovery standard (flavone) was eluted (R_T = 22.89 min) within a 25-min run time.

The Millennium software (version 3.2) associated with the Waters HPLC instrument was used to generate linear calibration curves based on peak areas for the 3 isoflavone standards run with each sample batch. The software also computed the μg of isoflavone per g of sample for each of the calibrated isoflavones when sample weight and dilution volumes were entered. Peak areas were generally used to quantitatively analyze the isoflavone concentrations. In addition, seed samples were analyzed for K concentrations using a dry ash method (*23*).

Statistical Analysis. Data were analyzed using an analysis of variance appropriate for a randomized complete-block split-plot design for Paris direct and Kirkton residual, and a randomized complete block-design for Strathroy direct, Kirkton direct, and Paris residual. For the Kirkton residual experiments, the three tillage systems were treated as whole plots, while the zero K and the combined fall K (84 kg ha^{-1}) plus spring K (42 kg ha^{-1}) treatments were regarded as subplots. Mean separations were accomplished using Fisher's protected LSD test. Probability levels lower than 0.05 were categorized as significant. Pearson product−moment correlation coefficients were calculated based on the data from all plots in each experiment to describe the relationships of daidzein, genistein, glycitein, and total isoflavone with leaf K, seed K, and seed yield.

RESULTS AND DISCUSSION

According to current Ontario soil-test K interpretations, soil K is considered low at <61 mg L^{-1}, medium at 61−120 mg L^{-1}, high at 121−150 mg L^{-1}, very high at 151−250 mg L^{-1}, and excessive at >250 mg L^{-1} for soybean (*24*). Soybean yield responses to K fertilization are expected on soils with low to medium K levels. On the basis of the above standards, initial soil-test K concentrations were low for both direct and residual K experiments at Paris (**Table 1**). Initial soil K concentrations at Strathroy direct were very high in 1998, high in 1999, and medium in 2000. Soil K levels at Kirkton direct and Kirkton residual were in the medium range for all seasons.

A test of error homogeneity was conducted across crop years for each measurement at all locations; the homogeneous errors indicated that the data of each measurement could be combined over years at each location. In addition, there was no significant year × treatment interaction on each measurement at all locations. Therefore, the discussion of isoflavone responses to K fertilization, tillage, and row-width treatments will be limited to the average results across years.

Paris Direct. Both deep-banded K treatments resulted in significant gains in daidzein, genistein, and total isoflavone concentrations compared with those of zero K and surface-broadcast K (**Table 2**). However, the differences in glycitein were significant only between 38-cm banded K and zero K or surface broadcast K. Surface broadcasting did not result in significant increases in either individual or total isoflavone contents compared with the zero K treatment. There were no significant differences between the 38- and 76-cm banded K treatments in individual or total isoflavone concentrations. Individual or total isoflavone contents were not affected significantly by row width (data not presented).

Response patterns of individual and total isoflavone concentrations to K application and placement were very similar to those of seed yield (**Table 2**). Isoflavones and yield responded significantly and positively to K applications only when fertilizer was placed in deep bands on these low-K soils. Moreover, positive correlations of daidzein, genistein, and total isoflavone contents with seed yield were observed in all three seasons at this location (**Table 3**).

Overall, the positive responses of isoflavone concentrations to band placement of K fertilizer coincided with the improvements in leaf K and seed K concentrations (**Table 2**). In addition, the correlation between total isoflavone contents and leaf K or seed K concentrations was significant in both 1998 and 1999, but not significant in 2000 (**Table 3**, **Figure 1**). Daidzein and genistein concentrations showed trends similar to that of total isoflavone. Glycitein concentrations were positively correlated with leaf K and seed K in 1998 and 2000.

Strathroy Direct. Spring band and fall broadcast K treatments significantly increased daidzein, genistein, and total isoflavone concentrations compared with those of zero K (**Table 2**). Spring band was superior to fall band for daidzein and total

Table 2. Potassium Fertilization Effects on Leaf K, Yield, Seed K, and Individual and Total Isoflavones at Paris Direct, Strathroy Direct, and Kirkton Direct Averaged over 1998 to 2000

location	treatment	leaf K g kg^{-1}	yield Mg ha^{-1}	seed K g kg^{-1}	isoflavone μg g^{-1} daidzein	genistein	glycitein	total
Paris direct	F test	***[a]	*	***	***	***	*	***
	surface broadcast	20.2c[b]	2.43b	16.1c	531b	683b	108b	1323b
	76-cm band	21.5b	2.59a	16.5b	570a	733a	110ab	1414a
	38-cm band	22.3a	2.63a	17.0a	573a	759a	119a	1451a
	zero K	17.1d	2.39b	14.9d	504b	641b	104b	1248b
Strathroy direct	F test	**	ns	ns	*	*	ns	*
	fall band	23.6a	3.31	17.6	1201b	1071ab	174	2446bc
	spring band	24.4a	3.48	17.9	1361a	1117a	172	2650a
	fall broadcast	23.9a	3.21	17.7	1322a	1101a	163	2586ab
	zero K	21.2b	3.46	17.6	1204b	1021b	155	2380c
Kirkton direct	F test	**	ns	ns	ns	ns	ns	ns
	fall band	25.7a	3.17	17.8	702	897	132	1732
	spring band	25.2a	3.12	17.9	700	896	144	1739
	fall broadcast	25.8a	2.99	17.9	664	860	139	1663
	zero K	22.5b	3.11	17.6	692	862	138	1692

[a] *, **, and *** indicate the treatment effect is statistically significant at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively. Nonsignificant effect is denoted by ns. [b] Means in a column within each location followed by the same letter are not significant at $P = 0.05$ according to Fisher's protected LSD.

Table 3. Correlation Coefficients of Isoflavone Concentrations with Leaf K, Seed K, and Seed Yield at Paris Direct (1998–2000)[a]

correlation variable pair		1998		1999		2000	
isoflavone	other attribute	R[b]	sig[c]	R	sig	R	sig
daidzein	leaf K	0.24	ns	0.80	***[d]	0.14	ns
	seed K	0.38	**	0.71	***	0.23	ns
	yield	0.52	***	0.68	***	0.35	*
genistein	leaf K	0.38	**	0.82	***	–0.04	ns
	seed K	0.56	***	0.78	***	0.16	ns
	yield	0.55	***	0.70	***	0.37	*
glycitein	leaf K	0.38	**	0.17	ns	0.49	***
	seed K	0.60	***	0.09	ns	0.53	***
	yield	0.52	***	0.08	ns	0.10	ns
total	leaf K	0.38	**	0.83	***	0.11	ns
	seed K	0.57	***	0.76	***	0.28	ns
	yield	0.60	***	0.70	***	0.39	**

[a] Values are averaged over the K placement and row width treatments for each year. [b] R, correlation coefficient. [c] Sig, significance of R. [d] *, **, and *** indicate the treatment effect is statistically significant at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively. Nonsignificant effect is denoted by ns.

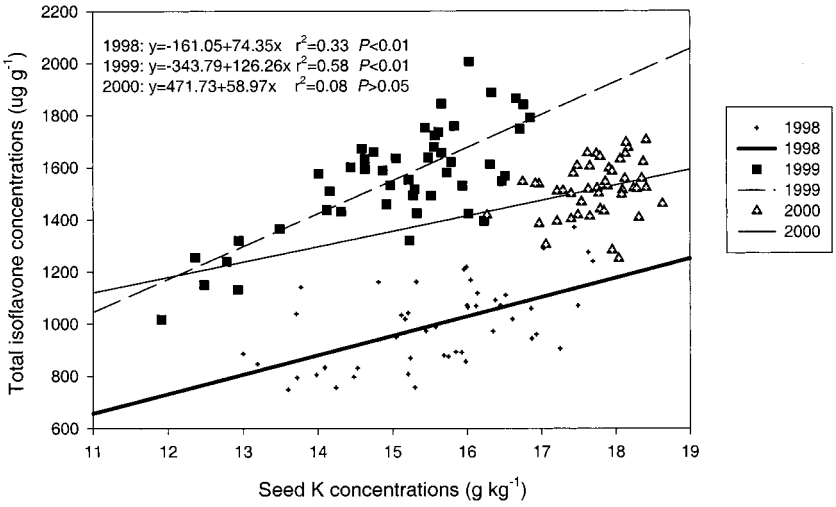

Figure 1. Regression of total isoflavone concentrations with seed K concentrations at Paris direct (1998–2000).

isoflavone contents. However, daidzein, genistein, or total isoflavone concentrations were not affected by fall-banded K. Potassium applications did not significantly increase glycitein relative to zero K no matter how the K fertilizer was placed (**Table 2**). Increases in either yield or seed K concentrations with K applications − relative to those of zero K − were not significant, which suggests that the increases in daidzein, genistein, and total isoflavone concentrations could occur independently of yield and seed K increases. However, leaf K concentrations at the initial flowering stage showed a similar response to K applications as did daidzein, genistein, and total isoflavone (**Table 2**). Although isoflavone synthesis may be related to leaf K status at this location, significant correlations of individual or total isoflavone contents with leaf K concentra-

Table 4. Potassium Fertilization Effects on Leaf K, Yield, Seed K, Individual and Total Isoflavone Concentrations at Paris Residual and Kirkton Residual Averaged over 1998 and 1999

location	treatment	leaf K g kg^{-1}	yield Mg ha^{-1}	seed K g kg^{-1}	isoflavone μg g^{-1} daidzein	genistein	glycitein	total
Paris residual	F test	**[a]	ns	***	**	**	ns	*
	deep band	16.6a[b]	2.48	16.7a	723b	675b	108	1505b
	surface broadcast	17.6a	2.60	16.8a	909a	823a	107	1838a
	zero K	12.3b	2.47	15.3b	751b	683b	92	1527b
Kirkton residual	F test	***	*	***	**	ns	ns	*
	124 kg K ha^{-1}	22.0a	3.72a	17.3a	1112a	903	109	2124a
	zero K	18.2b	3.47b	16.5b	1047b	852	114	2013b

[a] *, **, and *** indicate the treatment effect is statistically significant at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively. Nonsignificant effect is denoted by ns. [b] Means in a column within each location followed by the same letter are not significant at $P = 0.05$ according to Fisher's protected LSD.

tions, seed yield, and seed K contents were rarely observed in each season (data not presented).

Kirkton Direct. Individual and total isoflavone contents were not affected by K applications even though leaf K concentrations at the initial flowering stage were significantly increased by applied K (**Table 2**). Band placement had no benefits relative to surface broadcast for increasing either individual or total isoflavone concentrations on these soils with medium K levels. Responses of seed yield, seed K, and isoflavone concentrations to K applications were not significant (**Table 2**). Correlations of individual or total isoflavone contents with leaf K concentrations, seed yield, and seed K contents were also seldom significant in each season (data not presented).

Paris Residual. Surface broadcasting of K fertilizer to corn prior to soybean significantly increased daidzein, genistein, and total isoflavone concentrations compared with those of zero K and deep-banded K (**Table 4**). Daidzein, genistein, and total isoflavone concentrations were not increased by deep-banded K relative to those of zero K. Potassium applications did not result in significant increases in glycitein relative to zero K, regardless of how the K fertilizer was placed (**Table 4**). Unlike the responses of total isoflavone, significant yield responses to K fertilization were not observed at this location (**Table 4**). Both deep placement and surface application of K fertilizer significantly increased leaf K and seed K concentrations compared with those of zero K. The results of this experiment suggest that isoflavone increases can occur independently of yield increases, but may be associated with leaf K and seed K concentrations. Both individual and total isoflavone concentrations were positively correlated with seed yield, leaf K concentrations, and seed K contents in 1998 but not in 1999 (data not presented).

Differences in daidzein, genistein, and total isoflavone contents between deep-band and surface-broadcast treatments to the previous corn crop may be due to the overall lower availability of residual banded K fertilizer in the soybean rooting profile. According to another investigation (*25*), the residual effects of deep-banded K on subsequent no-till soybean varied with soybean row widths. As the subsequent no-till soybean in this study was planted in narrow rows (19 cm), 75% of soybean rows were not positioned in the residual K fertilizer bands. Such a planting system would substantially lower the overall availability of residual banded K to subsequent no-till soybean. Therefore, deep banding of K fertilizer to previous corn would be less desirable for subsequent no-till narrow-row soybean relative to broadcast-applied K on low-testing soils if K fertilizer was applied before corn in a 2-yr rotation of corn−soybean.

Kirkton Residual. Responses of daidzein, genistein, glycitein, and total isoflavone to residual K applications were similar among the three tillage systems used for the previous corn crop (data not presented). Averaged over three tillage treatments, K applications to preceding corn significantly increased daidzein and total isoflavone concentrations compared with zero K, but genistein or glycitein was not enhanced by K applications (**Table 4**). In addition, tillage systems did not affect either individual or total isoflavone concentrations (data not shown). Therefore, the adoption of conventional tillage practices would not seem to be beneficial to increasing isoflavone contents. Responses of leaf K concentrations, seed yield, and seed K contents to K applications showed tendencies similar to those of daidzein, genistein, and total isoflavone (**Table 4**), which agreed with the results from the Paris direct experiments in that increases in isoflavones were accompanied by increases in leaf K concentrations, seed yield, and seed K contents. The correlations of daidzein, genistein, or total isoflavone with seed yield were consistently positive, whereas correlations of individual or total isoflavone contents with leaf K or seed K concentrations were seldom significant at this location (data not shown).

In summary, significant and positive responses of daidzein, genistein, and total isoflavone to direct deep-banded K or residual surface-applied K were observed on low-K soils. Potassium application and placement effects on isoflavones were sometimes significant on medium- to high-testing soils. Glycitein concentrations rarely responded to K applications. In addition, appropriate K fertilization practices were more beneficial to isoflavone concentrations than changes in other management factors such as tillage systems and row widths.

In a recent genetic mapping study, Meksem et al. (*26*) observed that one quantitative trait locus (QTL) for genistein concentrations was closely linked to a seed yield QTL in the mapping population of Essex × Forrest. Although the genetic material used in our study is of different origin and maturity group from that used by Meksem et al. (*26*), the effects of the two linked QTLs for genistein concentrations and yield may be a more universal genetic phenomenon in soybean. Furthermore, the positive correlation of total isoflavone with seed yield suggests that there was no tradeoff of seed yield for isoflavones in soybean; rather, isoflavone concentrations significantly increased as seed yield went up. This positive relationship between total isoflavone and seed yield is very encouraging, as it suggests that high soybean yield could be compatible with high quality from an isoflavone-based functional-food perspective.

Significant correlations between isoflavone contents and leaf K or seed K concentrations mainly existed on low-K soils in this study (such as Paris direct). Nevertheless, it is interesting that isoflavone concentrations responded positively to K fertilizer applications even when seed yield or (and) seed K concentrations themselves weren't increased on some of these medium-

and high-K soils. However, significant isoflavone increases were always accompanied by significant leaf K increases. Whether leaf K or seed K is directly involved in isoflavone synthesis is unknown, although K is well-known as an essential activator for many enzymes in various metabolic pathways in plants. It is possible that the observed K fertilization effects on isoflavones were due to the stimulation of some specific enzymes involved in isoflavone synthesis, as was suggested for lycopene synthesis in tomato (*27*). Overall, the generally positive correlations between isoflavones and seed K concentrations, as well as between isoflavones and leaf K concentrations, suggest that K concentrations in soybean leaves and seeds may be among the important factors controlling isoflavone concentrations on low-K soils. Adoption of optimum K fertilization practices in terms of appropriate rates and placements (the latter especially important for no-till soybean) to increase seed yield when soil K is limiting may simultaneously increase daidzein, genistein, and total isoflavone contents of soybean seeds.

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