Soil Potassium Deficiency Affects Soybean Phloem Nitrogen and Soybean Aphid Populations

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ABSTRACT The soybean aphid is an invasive pest in the midwest United States, with frequent population outbreaks. Previous work has shown that aphid population densities are higher on potassium-deficient soybean than on healthy soybean. The experiments reported here test the hypotheses that the potassium nutrition of the host plant affects the forms of phloem nitrogen available to soybean aphids, and subsequently, their abundance. In field surveys and an exclusion cage study when aphid populations were high, soybean plants with potassium deficiency symptoms had a higher density of soybean aphids than plants without deficiency symptoms. In clip cage experiments, this effect was caused by earlier aphid reproduction and higher numbers of aphid nymphs per mother on plants growing in lower-potassium soil. In phloem exudation samples, the percentage of asparagine, an important amino acid for aphid nutrition, increased with decreasing soil potassium, perhaps because of potassium's role in the nitrogen use of the plant. Taken together, these results show that soybean potassium deficiency can lead to higher populations of soybean aphid through a bottom-up effect. A possible mechanism for this relationship is that soybean potassium deficiency improves the nitrogen nutrition of these N-limited insects. By releasing these herbivores from N limitation, host plant potassium deficiency may allow soybean aphid populations to reach higher levels more rapidly in the field.

KEY WORDS Aphididae, Hemiptera, insect nutrition, nitrogen limitation, phloem nitrogen

The soybean aphid, *Aphis glycines* Matsumura, is an invasive species that was first discovered in the United States in 2000 (Venette and Ragsdale 2004). It has rapidly become the most important insect pest of soybeans, *Glycine max* L. Merr, in midwest North America, with natural populations of up to 30,000 aphids per plant during outbreaks (DiFonzo 2006).

During soybean aphid outbreaks in Michigan in 2000 and 2001, aphid population size and damage appeared greater on potassium-deficient plants than on plants lacking deficiency symptoms (DiFonzo and Hines 2002). Myers et al. (2005) showed that individual soybean aphids feeding on excised soybean leaves with potassium deficiency symptoms had greater fecundity than aphids feeding on leaves without symptoms. In a manipulative field experiment Myers and Gratton (2006) showed that aphids in clip cages had higher net reproduction and naturally occurring field populations had higher population growth rates and peaks in potassium deficient plots compared with plots with sufficient potassium fertilization. In addition, a broad field survey showed that soybean aphid population growth was negatively correlated with soil ma-

cronutrients, including potassium (Myers and Gratton 2006).

Potassium deficiency is reported to increase lifespan and rate of reproduction of the aphid *Rhopalosiphum padi* L. feeding on barley (Havlickova and Smetankova 1998). In addition, barley grown in potassium-rich soil had fewer *R. padi* than barley grown in potassium-deficient soil 7 days after infestation (Havlickova and Smetankova 1998). Potassium fertilization is linked to nitrogen levels in the plant. Van Emden (1966) found that an increase in potassium fertilization of Brussels sprouts leads to a decrease in soluble nitrogen, a correlate of phloem sap amino acid concentration.

The acquisition of nitrogenous compounds is the main nutritional challenge facing aphids (Terra 1988). Aphids are thought to obtain all of their dietary nitrogen from amino acids translocated in the phloem sap. Aphids are not known to use proteinases as part of their nutritional digestion, probably because high levels of proteinase inhibitors and extremely low protein concentrations in typical phloem sap make plant proteins a poor nitrogen source (Sandstrom and Moran 2001). The growth rates of many species, such as *R. padi*, are directly correlated to the concentration of amino acids in the phloem of their host plants (Weibull 1987). Potassium is an enzyme cofactor in

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the reaction that converts amino acids into proteins; thus, low potassium levels affect the type and quantity of amino acids translocated in the phloem (Mengel and Kirkby 2001). In soybeans, potassium deficiency leads to the accumulation of asparagine (Yamada et al. 2002).

Aphids may be limited not only by the total nitrogen in their diets but also by the form of nitrogen available, namely the amount of certain amino acids (Anderson et al. 2004). This limitation may be partially eliminated by conditions that cause the amino acid composition of phloem sap to change. Nitrogen fertilization of oats and barley changes both the total amount and composition of amino acids present in the phloem sap and increases relative growth rates of *R. padi* nymphs (Weibull 1987). In these plants, most amino acids remain at the same levels in the phloem sap after fertilization, but glutamic acid and aspartic acid levels increase relative to other amino acids (Weibull 1987). Peas that are resistant to *Acyrthosiphon pisum* (Harris) and wheat resistant to *Diuraphis noxia* (Mordvilko) have lower levels of free amino acids in their phloem compared with susceptible cultivars (Weibull 1987, Telang et al. 1999). Resistance to *R. padi* in barley is not related to a change in total concentration of amino acids in the phloem, but resistant barley has a reduced concentration of one particularly important amino acid, asparagine (Weibull 1988).

Some aphid species do not require all of what are normally considered to be the essential amino acids in their diet, and the required amino acids can vary between aphid species and populations (Srivastava 1987, Wilkinson and Douglas 2003). Aphids possess intracellular symbiotic bacteria, *Buchnera* sp., and secondary gut symbionts, which manufacture certain essential amino acids from nonessential amino acids in the aphid diet (Moran et al. 2003). *Buchnera* sp. may be able to synthesize essential amino acids at a higher rate than these substances can be ingested and absorbed across the gut wall (Douglas 1998).

Several nonessential amino acids have particular importance to aphids. In an artificial diet study on the aphid *Myzus persicae*, limited aphid reproduction occurred on artificial diets missing one of the normally nonessential amino acids glutamine, glutamic acid, asparagine, aspartic acid, alanine, or serine (Dadd and Krieger 1968). Glutamate and aspartate are the only amino acids transported across the mycetome membrane from aphid hemocoel to *Buchnera* cells (Moran et al. 2003). Increasing the amounts of these compounds in an aphid diet can increase adult weight of aphids (Dadd and Krieger 1968).

This paper tests the hypotheses that potassium-deficient soybeans offer soybean aphids a more favorable amino acid composition, leading to faster population increase.

Materials and Methods

Survey of Commercial Fields. Commercial soybean fields in southwest Michigan were surveyed on 13–14 August 2003 and 17–23 August 2004. The 2003 survey was conducted in eight fields in Van Buren, Calhoun, and Kalamazoo Counties; the 2004 survey was conducted in five fields in Van Buren and Calhoun Counties. In each year, fields with visual symptoms of potassium deficiency were chosen after discussion with local extension agents and agribusiness contacts.

In each field, paired sample sites were selected based on visual symptoms. One sample site in each pair was in the center of an area of severe potassium deficiency (stunted plants with chlorosis and necrosis around the outer leaf margin). The other sample site was a nearby topographically similar location lacking visual deficiency symptoms (green foliage, taller plants). At each site, soil cores, plant phloem, and aphid populations were sampled. There were three pairs of sample sites per field, although in each year of the survey, there was one field where only two paired samples were collected.

Soil cores (25 cm) were extracted from the center of each sample site (4 or 6 sites/field): one core per site in 2003 and three cores per site in 2004. The soil was submitted to the Michigan State University Soil and Plant Nutrient Laboratory for nutrient analysis. Three plants per sample site were chosen for phloem sampling, which took place as described below.

To sample aphid populations, three whole plants were randomly chosen and removed from each sample site. The plants were placed in coolers with ice packs that maintained the temperature at ≈4°C. Coolers were returned to the laboratory and stored in a cold room at 4°C overnight. In 2003, plants were individually placed in Whirl-pack bags (Nasco, Fort Atkinson, WI) filled with 70% ethanol and stored in the cold room until the number of aphids per plant could be counted under a dissecting microscope. In 2004, aphid numbers were much lower, so whole plant aphid counts were taken the next day. In addition to a whole plant count, the number of aphids per leaf was calculated by dividing the number of aphids per plant by the number of trifoliates per plant. In 2003, three fields with active entomopathogens were excluded from the analysis of aphid number and density because a majority of aphids appeared to be dead or dying from infection.

For each sample pair, the differences in average soil nutrient levels and average number of aphids per plant between the apparently deficient and nondeficient sample sites were calculated. Pairwise comparisons were made using the probT option of PROC MEANS in SAS version 8.2 (SAS Institute 1999). This option ran a *t*-test to determine the probability that the differences measured were significantly different from zero ($P < 0.05$).

Exclusion Cage Study. In 2004, an exclusion cage study was conducted to test the interaction between soil potassium levels and soybean aphid population size in a controlled manner. The study was conducted in a potassium-deficient commercial soybean field in Van Buren County, MI.

The field cages consisted of a 1-m^2 frame of 1.88 cm (3/4 in) diameter PVC tubing connected to four 1.5-m legs of the same material through PVC three-way

corner connectors (PlumbingStore.com). The legs of the cage were buried 0.5 m in the soil. The frame was covered with a cage sewn from no-see-um mesh (Venture Textiles, Braintree, MA). The edges of the cage were buried 0.25 m in the soil. A 30-cm^2 panel of bridal tulle fabric (mesh size, 1.8 mm; JoAnne Fabrics, Lansing, MI) was sewn into the top of each cage. This allowed alate aphids generated under crowded conditions in the cage to escape (A.J.W., unpublished data), preventing them from recolonizing the plants and causing the aphid population to reach artificially high levels. A vertical slit sealed with a strip of Velcro hook and loop fastener on one side of the cage was used as a door. Cages were sampled by opening the door and leaning into the cage. When aphid-free cages and cages containing aphids were sampled on the same day, the aphid-free cages were sampled first to prevent accidental infestation.

The study site had an initial soil potassium level of 67 ppm as measured by a soil test just after planting. The field had a pH of 6.3, a phosphorus level of 14 ppm, calcium level of 1173 ppm, magnesium level of 161 ppm, and a cation exchange capacity of 8.6 me/100 g. Five blocked replicates of untreated and potassium-amended strips, 6.1 by 36.6 m, were established in the field. Fertilization was done on 13 May by broadcasting potash fertilizer (0–0-62; Mason Elevator, Mason, MI) as typically done by producers in no-till fields in the area. The fertilizer rate of 256.8 kg/ha potash was determined based on the initial soil test value and the Tri-State Fertilizer Recommendations (Vitosh et al. 1995). Four cages were established in each strip on 13 May. On 28 May, the soybeans in each cage were thinned to 10 plants per cage. One cage per strip was infested with one soybean aphid per plant. The remaining cages were maintained aphid-free and used for phloem sampling. The aphid source was a laboratory colony established from field-collected soybean aphids in 2003, and maintained at Michigan State University (27°C, 24-h photoperiod). Three soil cores (25 cm) per cage were extracted on 4 June and analyzed as described for the commercial field sampling.

The number of soybean aphids per plant was counted two or three times per week from 28 May until 15 July. From 28 May until 30 June, all plants in each infested cage were counted. As the aphid population increased in each cage, the number of plants counted was reduced. Thus, five plants per cage were sampled on 2 July and three plants per cage were sampled between 6 and 15 July. Aphid counting ceased on 15 July because of the very high aphid populations (>22,000 aphids/plant).

Uninfested plants in two of the cages per strip were phloem sampled on 18 June, 36 d after fertilization when plants were in the V3–V5 growth stage (three to five leaves). One cage per strip used for the 18 June phloem sampling had been infested with aphids on 28 May. However, three plants per cage were manually maintained aphid free until phloem sampling took place. The second cage per strip was an uninfested control. Plants in the two remaining aphid-free cages per strip were phloem-sampled on 15 July, 63 d after

fertilization when plants were in the R2 (full flower) growth stage.

The aphid population data were analyzed by PROC MIXED in SAS version 8.2 (SAS Institute 1999), with potassium treatment, date, and the potassium–date interaction as fixed factors and replicate as a random factor.

Clip Cage Studies. Clip cage experiments were conducted in 2004 to determine the effect of soil potassium fertilization on individual aphids. Clip cages consisted of a chamber made of 1-cm-diameter PVC pipe open on one side, with the opposite side covered by no-see-um mesh. The open side of the cage was pressed against a leaf surface, and a plastic cover slip was placed on the back side of the leaf. A hair clip was used to hold the cage in place.

Clip cage experiments took place in the field in the same untreated and amended strips used for the 2004 exclusion cage study. The experiment was conducted twice with five replications. In each case, four clip cages per strip (4 cages per strip by 2 strips per replicate by 5 replicates = 40 cages per experiment) were used.

Because maternal effects can affect aphid reproduction (Dixon 1998), the following procedure was used to control for possible birth order and nutrition effects. Adult aphids were removed from the colony maintained at Michigan State University and placed on excised soybean leaves in petri dishes. Over the course of the day, the adult aphids were checked for reproduction. Groups of ≈10 nymphs were removed and placed on excised soybean leaves with petioles inserted into 1-ml Eppendorf tubes filled with water and sealed with Parafilm (American National Can, Menasha, WI). Individual leaves were kept in petri dishes and maintained in a growth chamber at 27°C with a 24-h photoperiod. After 3–5 d, the nymphs were removed from these leaves and placed onto fresh excised soybean leaves in groups of one to three per leaf. The aphids were checked daily for reproduction. When the first nymph of each mother was deposited, the mothers were removed, and the petri dishes were sealed with laboratory tape. These petri dishes, containing first-born nymphs <24 h old, were placed into coolers at ≈4°C and transported to the field. In the field, individual nymphs were removed from the excised leaves using a fine camel hair paintbrush and placed on the underside of the second fully expanded leaf from the top of the plant. Each aphid was enclosed in a clip cage. The clip cages were monitored two to three times per week until live aphids were no longer present.

The first set of 40 clip cages was each infested with an individual soybean aphid nymph on 10 June (28 d after K fertilization). In these cages, the nymph matured to adulthood and remained in the cage for the duration of its lifetime. Cages were checked two to three times per week for survival of the mother and for reproduction. On each sample date, nymphs were removed from the clip cage and killed. The age of the aphid at first reproduction, total number of nymphs

produced, and the lifespan of the aphid were measured.

A second set of 40 clip cages was started on 14 July (62 d after fertilization) with methods modified to further control for maternal effect. In these cages, the nymph remained until it matured and deposited five nymphs. The mother aphid was removed, and her progeny were evaluated for the remainder of study for survivorship, the date of first reproduction, and total nymph production. As in the first trial, on each sample date, the nymphs produced were removed and killed.

Clip cage data for each experiment were analyzed separately using the PROC MIXED procedure of SAS version 8.2 (SAS Institute 1999) using replicate as a random factor. Mean comparisons were conducted using Fisher protected least significant difference (LSD; $P < 0.05$).

Analysis of Phloem Samples. Phloem samples were taken using a modification of the phloem exudation method of King and Zeevart (1974). A 10 mM solution of ethylenediaminetetraacetate (EDTA) was prepared and adjusted to pH 7.0 with 1 N NaOH. Aliquots (5 ml) of the EDTA solution were placed in two dram vials and sealed with Parafilm. In the field, a slit was cut in the Parafilm covering of each vial. A soybean leaf (the second fully unfurled trifoliate leaf counting down from the growth point) was cut off at the petiole while submerged in a dish of the EDTA solution. The petiole was immediately inserted through the slit in the Parafilm into an EDTA vial. Vials were placed in a cooler at $\approx 4°C$, returned to the laboratory, and placed in a cold room at $4°C$. The leaves were removed from the vials after 24 h, and the vials were sealed with a lid. The exudate samples were stored at $-80°C$ until free amino acid analysis could be performed.

Free amino acid analyses for 18 physiological amino acids in the phloem samples was performed using reverse-phase high-pressure liquid chromatography (HPLC) with a Waters system (Waters 2690 Separations Module, Waters 474 Scanning Fluorescence Detector, Waters Temperature Control Module, Millennium Software Package, and the Acc-Q tag reagent kit; Waters, Milford, MA).

Because the exact amount of phloem that bled into the exudate samples was unknown (King and Zeevart 1974), it was not possible to directly compare the quantities of amino acids contained in the phloem exudate. Instead, qualitative differences in the profile of amino acids, as defined by the proportion of each amino acid present in the total free amino acids of the exudate, were evaluated. The phloem amino acid data from the three studies was analyzed as a single regression. A small constant (0.04), chosen by visual inspection for uniformity of the transformed data, was added to the proportion of each amino acid to allow transformation, and the proportions were log-transformed to satisfy assumptions of linearity and homoscedasticity. A line was fit to the data, and the significance of soil potassium level, variables indicating each experiment, and the timing of sampling (for the cage experiment) in the regression model were tested. To assess the 18 regressions of individual amino

acid levels with a true α value of 0.05, a Bonferroni-adjusted P value of 0.0028 was used to assess the fit of each regression (Weisberg 2005). Because aphid counts and phloem samples were not taken from the same plants, a direct comparison of phloem samples and aphid numbers was not conducted. All regressions were done using the Arc version 1.06 software package (www.stat.umn.edu/arc/).

Results

Commercial Fields. In 2003, an outbreak year for soybean aphid, potassium deficiency symptoms were present in many fields in southwest Michigan, and field sampling was relatively easy. In 2004, aphid numbers were low across the Midwest, and it took much longer to locate fields exhibiting potassium deficiency symptoms. However, in both years, paired sample sites within a field that differed in soil potassium level were successfully chosen. In 2003, sample sites with potassium deficiency symptoms (hereafter referred to as deficient areas) had an average soil potassium level of 29 ppm with a range of 15–65 ppm compared with an average of 42 ppm and a range of 22–83 ppm for samples sites lacking deficiency symptoms (hereafter referred to as symptomless areas; difference = 11.47 ppm, $t = 3.4$, $P = 0.0004$). In 2004, the deficient areas had an average soil potassium level of 34 ppm with a range of 28–83 ppm; symptomless areas had an average of 53 ppm with a range of 38–83 ppm (difference = 19.8 ppm, $t = 5.48$, $P = 0.0001$). It should be noted that all of the fields included in this study had soil potassium levels below the 150 ppm recommended for Michigan soybean production (Vitosh et al. 1995). In 2004, soil magnesium was also significantly different within pairs (difference = 41.1 ppm, $t = 2.81$, $P = 0.0149$). Both potassium and magnesium deficiency often occur in sandy soils (Foth and Ellis 1997).

In 2003, differences in aphid density mirrored soil potassium levels. While there was no difference in total aphid number per plant within pairs (deficient areas = 1,760 aphids/plant, symptomless areas = 1,560 aphids/plant, $n = 10$, $t = 0.66$, $P = 0.54$), plants from symptomless areas had more leaves per plant than plants from deficient areas (deficient areas = 10.1 leaves, symptomless areas = 15.1 leaves, $n = 19$, $t = 3.34$, $P = 0.003$). When expressed as the number of soybean aphids per leaf, aphid density was 50% greater in deficient compared with symptomless areas (deficient areas = 174 aphids/leaf, symptomless areas = 103 aphids/leaf, $n = 10$, $t = 2.57$, $P = 0.03$). Leaves from deficient plants were also smaller. In 2004, under low aphid conditions, there was no difference in soybean aphid numbers (deficient areas = 18.8 aphids/plant, symptomless areas = 42.3 aphids/plant, $n = 14$, $t = 1.57$, $P = 0.1414$) or density (difference = 2.3 aphids per leaf, $t = 1.81$, $P = 0.0935$) between deficient and symptomless areas.

Exclusion Cage Study. Plants were infested with aphids on 28 May 2004. When the cages were checked on 1 June, aphids were found in each cage, indicating that the infestation was successful. Between 1 June
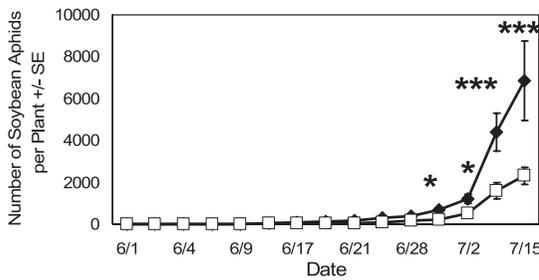
Fig. 1. Number of soybean aphids per plant in exclusion cages in a potassium-deficient commercial soybean field, Hartford, MI, 2004. Cages were placed over soybean growing in unfertilized or potassium-fertilized strips. *Sample dates where the treatments were different at $P < 0.05$. ***Sample dates where the treatments were different at $P < 0.0001$. ◆, no potassium fertilization; □, potassium fertilization.

and 28 June, aphid numbers slowly increased to an average of 288 per plant and did not significantly differ by treatment. Two days later on 30 June, the average number of aphids per plant in cages in the unfertilized treatment (703) was significantly greater than the average number of aphids per plant in cages in the potassium-fertilized treatment (233; df = 1162, t = 2.41, $P = 0.0160$). Cages in unfertilized strips continued to have more aphids per plant than cages in fertilized areas over the next two sample dates (2 July, df = 1162, t = 2.24, $P = 0.0252$; 8 July, df = 1162, t = 7.82, $P < 0.0001$; Fig. 1). By 15 July, 48 d after fertilization, the number of aphids in the unfertilized treatment averaged 6,858 per plant compared with 2,315 per plant in the fertilized treatment (df = 1162, t = 10.52, $P < 0.0001$). The aphid populations in the unfertilized cages were as high as 22,000 aphids per plant.

The experiment was terminated on 15 July because counting aphids in the cages was no longer feasible.

Clip Cages. In the first clip cage experiment (10 June, 28 d after fertilization), 29 aphids that survived over 2 d in clip cages were followed. The lifespan of the aphid ($n = 29$, $F = 0.07$, $P = 0.80$), age of the aphid at first reproduction ($n = 29$, $F = 0.93$, $P = 0.35$), and total number of nymphs produced ($n = 29$, $F = 0.76$, $P = 0.39$) were not significantly different by treatment. On average, aphids lived 13.7 d in the cage, first reproduced on day 10 of their life, and produced 6.7 nymphs each.

In the later experiment (14 July, 62 d after fertilization), there were significant differences in aphid reproductive performance on unfertilized and fertilized plants. The aphids produced nymphs at an earlier age (unfertilized = 8.8 d, fertilized = 11 d, $n = 39$, $F =$ 6.82, $P = 0.0282$), and the total number of nymphs produced per cage was higher (unfertilized = 88.2, fertilized = 71.2, $n = 39$, $F = 5.89$, $P = 0.0305$) for aphids in clip cages placed on unfertilized plants.

Amino Acid Analysis. The full regression model, including study, timing and soil potassium, was significant for 14 of the 18 amino acids extracted from the leaves (Table 1). However, soil potassium level contributed toward the significance of the model for only one amino acid, asparagine (Fig. 2). In the cases of aspartic acid, glycine, tyrosine, and valine, proportions in both years of the survey differed from the exclusion cage study. In the 2003 survey, proportions of glutamine, leucine, lysine, phenylalanine, and serine significantly differed from proportions in the exclusion cage study. In the 2004 survey, asparagine, glutamine, isoleucine, and proline significantly differed from the proportions found in the exclusion cage study. Asparagine, leucine, lysine, and tyrosine significantly dif-

Table 1. Coefficients for the effect of soil potassium, F value for the entire model, and t values for model components for each amino acid in the phloem exudate of soybeans in the regression model

Amino acid	Soil K coefficient	Whole model F	t values			
			K	2003 survey	2004 survey	Timing in cages
Alanine	4.2E−04	2.41	0.28	−2.23	−2.24	−2.77
Arginine	2.9E−03	2.59	1.84	−0.85	1.11	−0.60
Asparagine	−5.8E−03	30.01[a]	−3.14[a]	0.08	7.16[a]	3.02[a]
Aspartic acid	4.6E−03	16.08[a]	2.02	−6.37[a]	−4.25[a]	−0.47
Glutamine	−1.6E−03	40.72[a]	−0.94	−7.25[a]	2.52	−1.92
Glutamic acid	6.4E−04	23.31[a]	0.32	2.77	−4.72[a]	−0.05
Glycine	8.1E−04	11.79[a]	0.44	4.48[a]	3.03[a]	−1.82
Histidine	−2.1E−04	1.18	−0.51	−2.02	−1.23	−1.67
Isoleucine	6.9E−04	4.96[a]	0.86	−2.87	−3.94[a]	−2.81
Leucine	1.3E−03	26.24[a]	1.11	−8.72[a]	−2.68	−5.24[a]
Lysine	−2.1E−03	18.00[a]	−2.34	−6.94[a]	−1.60	−4.10[a]
Methionine	−5.4E−04	4.56[a]	−1.61	0.47	2.75	−0.38
Phenylalanine	1.6E−04	8.31[a]	0.37	3.96[a]	−0.03	0.05
Proline	1.9E−03	8.59[a]	1.58	−1.98	−4.84[a]	−2.41
Serine	7.7E−04	20.89[a]	0.49	−5.45[a]	1.01	0.77
Threonine	−2.4E−04	1.39	−0.28	−0.92	−1.90	−1.87
Tyrosine	−8.0E−06	8.88[a]	−0.02	−5.47[a]	−5.03[a]	−3.96[a]
Valine	−3.7E−04	4.60[a]	−0.29	−3.77[a]	−3.96[a]	−2.05

[a] $P < 0.0028$, corresponding to a true P value of 0.05 for all comparisons. t values for the 2003 and 2004 survey compare the amino acid levels found in the surveys to those found in the exclusion cage study. t values for timing in cages compare 15 July phloem collection to the 18 June collection in the controlled cage study.
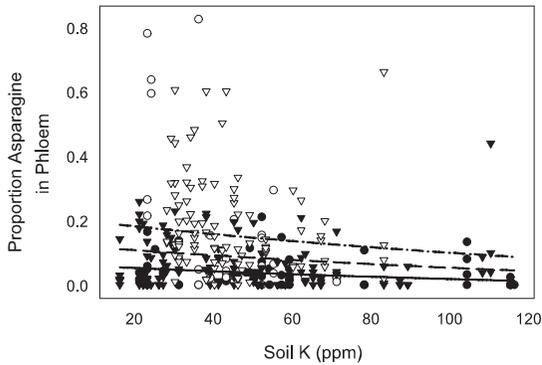
Fig. 2. Proportion of asparagine in the amino acid profile of soybean phloem sap from plants growing in soils with varying levels of soil potassium, southwestern Michigan, 2003–2004. Data come from two surveys of commercial fields and an artificially fertilized cage experiment. The regressions for each study are parallel and significant at Bonferroni-adjusted $P < 0.05$. ● and solid line, early sampling of cage experiment; ○ and dashed line, late sampling of cage experiment; ▼, 2003 survey (regression line not distinguishable from early sampling of cage experiment); ∇ and dashed-dot line, 2004 survey.

fered between the early and later phloem collections in the exclusion cage study.

Discussion

The reproductive rate of aphids in clip cages was higher on unfertilized plants compared with fertilized plants. Unfertilized plants harbored more soybean aphids than plants growing in higher potassium soils at the same location in the exclusion cage experiment in 2004. When commercial fields with high aphid populations were surveyed in 2003, deficient areas had denser populations of soybean aphids than symptomless areas. In the 2004 field survey, soybean aphid populations were very low and did not differ on plants growing in soils with different levels of potassium.

These results contrast with the plot studies of Myers et al. (2005) and Myers and Gratton (2006), where differences in aphid populations were not detectable with high aphid populations but were detectable in a year when aphid populations were lower. Myers et al. (2005) concluded that differences under high aphid populations were masked by aphid immigration between plots. In our commercial field survey in 2003 and field cage study, we detected differences in soybean aphid populations at economically damaging levels. Our 2004 field survey, which did not detect aphid population differences at very low aphid levels, represents a snapshot, whereas Myers and Gratton (2006) detected differences in low aphid populations by repeated sampling. Thus, Myers and Gratton were able to capture peak abundance in their study, whereas the 2004 field survey did not necessarily capture peak abundance. In addition, the aphid populations encountered in the 2004 survey were extremely low,

often <30 aphids per plant, so that differences may not have been detectable. However, in the 2004 cage study, which had higher aphid populations and was repeatedly sampled, the effect of potassium deficiency on aphids was again apparent.

Only one amino acid, asparagine, was significantly correlated with soil potassium level. The proportion of asparagine in phloem amino acids decreased as soil potassium level increased. In the exclusion cage study, between the early phloem sampling (which took place before aphid populations differed by treatment) and the late phloem sampling (after differences in the number of aphids per plant became apparent), the proportion of asparagine increased, whereas the proportions of three other amino acids, including the essential amino acids leucine and lysine, decreased. The 2004 survey, which had no detectable differences in aphid population, had higher proportions of asparagine and lower proportions of thee other amino acids including the essential amino acid isoleucine than the exclusion cage study. These results suggest that higher asparagine levels contribute to higher aphid growth under potassium deficiency. The results of the 2004 survey indicate that isoleucine concentrations may also play a role. It is important to note that the phloem sampling methods show only proportions of amino acids and not the total amino acid concentration in phloem (Douglas 2003). If nitrogen translocation in general increased as a result of potassium deficiency, the total availability of any amino acid, including those with lesser proportions, may have increased. Such an increase in amino acid availability could have influenced the aphids and would not be shown by these methods.

Despite the limitations of phloem exudate sampling, several important patterns emerge. First, asparagine was the only phloem amino acid correlated with soil potassium level in our study. Asparagine is extremely important in the nitrogen nutrition of many aphids (Dadd and Krieger 1968, Weibull 1987), and increased levels of this nutrient may help to relieve nitrogen limitation. This pattern occurred regardless of whether the aphid population was high, low, or absent. Our clip cage studies showed that soybean aphid reproduction is greater on unfertilized plants even if large soybean aphid populations are not present. Thus, aphids are reacting to the nutritional status of the plant and not inducing a nutritional change.

Also, by comparing the asparagine levels between our three studies, we observed a pattern seen in plant resistance studies. Experiments that took place when natural infestations of soybean aphids were low had a higher proportion of asparagine than experiments with high numbers of soybean aphids. In other systems, a reduction in the proportion of nutritionally important amino acids including asparagine is a component of plant resistance to aphids (Auclair et al. 1957, Weibull 1988, Ponder et al. 2000).

The proportion of asparagine in soybean phloem may decrease with increasing soil potassium level because of its role as a nitrogen storage molecule in the soybean plant. Because potassium is involved in the

formation of proteins from amino acids (Mengel and Kirkby 2001, Marschner 2002), amino acids are likely to build up in the shoot when the plant is deficient in potassium, causing increased levels of asparagine to be translocated in the phloem. Under potassium deficiency, soybean plants are known to accumulate asparagine (Yamada et al. 2002).

Through this mechanism, soil potassium deficiency is likely to lead to increased levels of phloem asparagine when nitrogen fixation is occurring in soybean. Aphids are severely nitrogen limited (Terra 1988), and asparagine is one of the most important nitrogen sources in the aphid diet (Dadd and Krieger 1968). Because the negative correlation between soil potassium level and the proportion of phloem asparagine mirrors the negative correlation between soybean aphid density and soil potassium, the percentage of asparagine in the phloem amino acid profile may impact the size of soybean aphid populations under outbreak conditions by alleviating part of the aphids' dietary nitrogen limitation.

The plant stress hypothesis supports the idea that soybean aphid fecundity should be higher on potassium-deficient plants (Waring and Cobb 1989). This hypothesis states that herbivorous insects perform better on host plants that are under environmental stress due to increased translocation of nutrients. Although experimental evidence for this hypothesis is mixed, it has been noted that phloem feeders such as aphids often exhibit a negative response to potassium fertilization (Waring and Cobb 1989).

The enhanced nitrogen nutrition available to soybean aphids feeding on potassium-deficient soybean may increase the tendency of the aphid population to reach outbreak levels. From a practical standpoint, this means that soybean growers should manage soil potassium levels in their fields as part of their integrated pest management (IPM) plan for the soybean aphid. This also shows a bottom-up effect of plant nutrition on a plant–herbivore interaction. If low levels of essential amino acids in plant phloem evolved to resist phloem-feeding herbivores, this represents a trade-off in which plant nutrition affects resistance to insects.

References Cited

Anderson, T. R., M. Boersma, and D. Raubenheimer. 2004. Stoichiometry: linking elements to biochemicals. Ecology 85: 1193–1202.

Auclair, J., J. B. Maltais, and J. J. Cartier. 1957. Factors in resistance of peas to the pea aphid, *Acrythosiphon pisum* (Harr.) (Homoptera: Aphididae). II. Amino acids. Can. Entomol. 89: 457–464.

Dadd, R., and D. Krieger. 1968. Dietary amino acid requirements of the aphid, *Myzus persicae.* J. Insect Physiol. 14: 741–764.

DiFonzo, C. D. 2006. Multi-state soybean aphid RAMP project: soybean aphid IPM on a landscape scale (SAILS) (http://www.soybeans.umn.edu/pdfs/2006aphid/RAMPNewsJune06.pdf).

DiFonzo, C. D., and R. Hines. 2002. Soybean aphid in Michigan: update from the 2001 season. Michigan State University Extension Publication E-2748, East Lansing, MI.

Dixon, A. 1998. Aphid ecology, 2nd ed. Chapman & Hall, London, UK.

Douglas, A. 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera.* Annu. Rev. Entomol. 43: 17–37.

Douglas, A. 2003. The nutritional physiology of aphids. Adv. Insect Physiol. 31: 73–140.

Foth, H. D., and B. G. Ellis. 1997. Soil fertility, 2nd ed. Lewis Publishers, Boca Raton, FL.

Havlickova, H., and M. Smetankova. 1998. Effect of potassium and magnesium fertilization on barley preference by the bird cherry-oat aphid *Rhopalosiphum padi.* Rostlinna Vyroba. 44: 379–383.

King, R. W., and J.A.D. Zeevart. 1974. Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol. 53: 96–103.

Marschner, H. 2002. Mineral nutrition of higher plants. Academic, New York.

Mengel, K., and E. A. Kirkby. 2001. Principles of plant nutrition, 5th ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Moran, N. A., G. R. Plague, J. P. Sandstrom, and J. L. Wilcox. 2003. A genomic perspective on nutrient provisioning by bacterial symbionts of insects. Proc. Natl. Acad. Sci. U.S.A. 100: 14543–14548.

Myers, S. W., and C. Gratton. 2006. Influence of potassium fertility on soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), population dynamics at a field and regional scale. Environ. Entomol. 35: 219–227.

Myers, S. W., C. Gratton, R. P. Wolkowski, D. B. Hogg, and J. L. Wedberg. 2005. Effect of soil potassium availability on soybean aphid (Hemiptera: Aphididae) population dynamics and soybean yield. J. Econ. Entomol. 98: 113–120.

Ponder, K., J. Pritchard, R. Harrington, and J. Bale. 2000. Difficulties in location and acceptance of phloem sap combined with reduced concentration of phloem amino acids explain lowered performance of the aphid *Rhopalosiphum padi* of nitrogen deficient barley (*Hordeum vulgare*) seedlings. Entomol. Exp. Appl. 97: 203–210.

Sandstrom, J. P., and N. A. Moran. 2001. Amino acid budgets in three aphid species using the same host plant. Physiol. Entomol. 26: 202–211.

SAS Institute. 1999. SAS online doc. SAS Institute, Cary, NC.

Srivastava, P. 1987. Nutritional physiology, pp. 99–121. *In* A. Minks and P. Harrewijn (eds.), Aphids: their biology, natural enemies and control. Elsevier, Amsterdam, The Netherlands.

Telang, A., J. Sandstrom, E. Dyreson, and N. Moran. 1999. Feeding damage by *Diuraphis noxia* results in a nutritionally enhanced phloem diet. Entomol. Exp. Appl. 91: 403– 412.

Terra, W. 1988. Physiology and biochemistry of insect digestion: an evolutionary perspective. Brazil. J. Med. Biol. Res. 21: 675–734.

Van Emden, H. 1966. Studies on the relations of insect and host plant III. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* (Hemiptera: Aphididae) on Brussels sprout plants supplied with different rates of nitrogen and potassium. Entomol. Exp. Appl. 9: 444–460.

Venette, R., and D. W. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? Ann. Entomol. Soc. Am. 97: 219–226.

Vitosh, M. L., J. W. Johnson, and D. B. Mengel. 1995. Michigan State University extension bulletin E-2567: tri-state fertilizer recommendations for corn, soybeans, wheat, and alfalfa. Michigan State University, East Lansing, MI.

Waring, G. L., and N. S. Cobb. 1989. The impact of plant stress on herbivore population dynamics. pp. 167–226. *In* E. A. Bernays (ed.), Insect-plant interactions. CRC, Boca Raton, FL.

Weibull, J. 1987. Seasonal changes in the free amino acids of oat and barley phloem sap in relation to plant growth stage and growth of *Rhopalosiphum padi.* Ann. Appl. Biol. 111: 729–737.

Weibull, J. 1988. Free amino acids in the phloem sap from oats and barley resistant to *Rhopalosiphum padi.* Phytochemistry 27: 2069–2072.

Weisberg, S. 2005. Applied linear regression, 3rd ed. Wiley-Interscience, Hoboken, NJ.

Wilkinson, T. L., and A. E. Douglas. 2003. Phloem amino acids and the host plant range of the polyphagous aphid, *Aphis fabae.* Entomol. Exp. Appl. 106: 103–113.

Yamada, S., M. Osaki, T. Shinano, M. Yamada, I. Miyoko, and A. T. Permana. 2002. Effect of potassium nutrition on current photosynthesized carbon distribution to carbon and nitrogen compounds among rice, soybean, and sunflower. J. Plant Nutr. 25: 1957–1973.