Journal of Plant Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597277

Nitrogen Fixation, Ureide, and Nitrate Accumulation Responses to Soybean Aphid Injury in *Glycine max*

Walter E. Riedell [a]; Michael A. Catangui [b]; Eric A. Beckendorf [a]
[a] USDA-ARS, North Central Agricultural Research Lab, Brookings, South Dakota, USA [b] Plant Science Department, South Dakota State University, Brookings, South Dakota, USA

To cite this Article Riedell, Walter E., Catangui, Michael A. and Beckendorf, Eric A.'Nitrogen Fixation, Ureide, and Nitrate Accumulation Responses to Soybean Aphid Injury in *Glycine max*', Journal of Plant Nutrition, 32: 10, 1674 — 1686
To link to this Article: DOI: 10.1080/01904160903150925
URL: http://dx.doi.org/10.1080/01904160903150925

Journal of Plant Nutrition, 32: 1674–1686, 2009

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904160903150925

Taylor & Francis
Taylor & Francis Group

Nitrogen Fixation, Ureide, and Nitrate Accumulation Responses to Soybean Aphid Injury in *Glycine max*

Walter E. Riedell,[1] Michael A. Catangui,[2] and Eric A. Beckendorf[1]

[1]USDA-ARS, North Central Agricultural Research Lab, Brookings, South Dakota, USA
[2]Plant Science Department, South Dakota State University, Brookings, South Dakota, USA

ABSTRACT

There is little information available about soybean aphid (*Aphis glycines* Matsumura) effects on the physiology and mineral nutrition of soybean (*Glycine max* [L.] merr.). Controlled-environment studies were conducted to measure soybean aphid infestation effects on dry weight, nitrogen (N) fixation, ureide-N, and nitrate-N concentration and accumulation. Plants grown in perlite using –N nutrient solution culture were infested at the 3rd trifoliolate (V3) stage and measured for N fixation, nodule characteristics, and ureide-N concentration at the full pod (R4) stage. When compared to uninfested control plants, aphid infestation reduced total nodule volume per plant by 34%, nodule leghemoglobin per plant by 31%, plant N fixation rate by 80% and shoot ureide-N concentration by 20%. Soil-grown plants were infested at the first trifoliolate (V1) stage and shoots were measured for dry weight, nitrate-N, and ureide-N at the full bloom (R2) stage. Infestation reduced shoot dry weight by 63%, increased nitrate-N concentration by 75%, but did not significantly affect ureide-N concentration. Because nutrient concentration is a single-point measurement that results from the integration of two dynamic processes, nutrient accumulation and dry matter production, we conclude that aphid-induced reductions in N fixation, coupled with decreased dry weight accumulation, caused shoot ureide-N concentration to remain unchanged in aphid-injured plants when compared to uninfested plants. Because nitrate-N concentration was greater in aphid-damaged shoot tissue, we further conclude that nitrate-N accumulation was less sensitive to aphid injury than dry weight accumulation.

Received 5 March 2008; accepted 27 August 2008.

Address correspondence to Walter E. Riedell, USDA, Agricultural Research Service, North Central Agricultural Research Lab, 2923 Medary Avenue, Brookings SD, USA 57006. E-mail: walter.riedell@ars.usda.gov

Keywords: soybean, *Glycine max (*L.*)* Merrill, soybean aphid, *Aphis glycines* Matsumura, insect feeding injury, nitrogen, ureide, nitrate

INTRODUCTION

Soybean plants acquire nitrogen (N) from their environment by absorption of mineral N (e.g., nitrate-N) from the soil solution (Gan et al., 2004) as well as through the symbiotic fixation of atmospheric N_2 within their root nodules (McClure et al., 1980). Ureides, which originate predominately from N fixation (Fujihara and Yamaguchi, 1978), are the principle form of fixed N translocated and accumulated in soybean shoot tissue (van Berkum et al., 1985). Thus, soybeans obtain part of the N required for plant growth from a symbiotic microorganism. In return, the plant provides photoassimilates to support the growth and function of the nodule, the organ of the plant containing the N-fixing bacteria (Schubert, 1986). Nodule metabolism is dependent on photoassimilates to support respiration, nodule growth, and N fixation (Brown et al., 1995). Because photosynthate supply to nodules is an important limiting factor to N fixation, any stress that interferes with this supply will affect N fixation (Hardy and Havelka, 1976; Streeter et al., 1979; Layton and Boethel, 1989).

The soybean aphid, a major soybean insect pest in the north central U.S. (Myers et al., 2005), uses its syringe-like mouth parts to remove sap from the phloem of shoot organs. Feeding injury caused by soybean aphids includes leaf yellowing, leaf growth abnormalities, premature leaf senescence, and reduced canopy leaf area (Beckendorf et al., 2008). Macedo et al. (2003) also demonstrated that large reductions in photosynthesis occurred when soybeans were injured by low numbers of soybean aphids. Photosynthetic rate reductions of up to 50% were measured on infested leaflets with no apparent symptoms of aphid injury (Macedo et al., 2003). Because nodules and their subtending root system receive 15 to 30% of the net photosynthetic energy derived by the plant (Rawsthorne et al., 1980), we postulate that N fixation will be reduced in plants infested with soybean aphids. We further hypothesize that shoot ureide-N concentration, which is dependent upon N fixation in soybean (McClure and Israel, 1979), will be reduced in soybean aphid-injured plants. Because there is a higher energy requirement for the development, maintenance, and functioning of the symbiotic N fixation system in comparison to nitrate-N absorption and accumulation (George and Singleton, 1992) and because nitrate-N is a storage form of N in plants that does not need to be assimilated by roots (Marschner, 2002), we also postulate that shoot nitrate-N concentration would respond differently to aphid injury than ureide-N concentration.

We felt that a controlled environment study of soybean aphid effects on N fixation as well as on ureide-N and nitrate-N relations would be a step toward understanding the mechanism of how soybean plants are injured by this insect

pest. Thus, the objectives of this study were to measure the effects of soybean aphid infestation on soybean root nodule characteristics and N fixation as well as on plant dry weight and the shoot concentration and accumulation of ureide-N and nitrate-N. Because of the increasing importance of the soybean aphid, examination of soybean N responses to injury caused by this insect pest is of considerable theoretical and practical importance.

MATERIALS AND METHODS

Aphid Colony and Soybean Growth Conditions

Aphids used in the study came from colonies maintained on soybeans (Asgrow '0801' or '0803') in a growth chamber. Colony aphids were collected from natural populations in a soybean field near Aurora SD during the 2005 growing season. Colonies originated from newly-deposited nymphs, and thus were not viroliferous.

Growth chamber and greenhouse experiments were conducted at the North Central Agricultural Research Laboratory in Brookings, SD, USA. Soybean seeds (Pioneer '91B01''), treated with *Bradyrhizobium japonicum* ('S' culture; Nitragin Inc., Milwaukee, WI, USA), were used in both experiments. For the N fixation and the ureide-N concentration growth chamber experiment, soybean plants were grown using –N nutrient solutions (Coolong and Randle, 2003) in a dual cabinet controlled environment chamber (Model GC8-2; Environmental Growth Chambers, Chagrin Falls, OH, USA) set at a constant 65% relative humidity (RH) and 16°C with a daily 12h:12h light:dark photoperiod with 530 μMol s^{-1} m^{-2} PAR at canopy height provided by cool white fluorescent lamps.

For the ureide-N and nitrate-N concentration and accumulation greenhouse experiment, plants were grown in soil under natural light supplemented with a daily 12h:12h light:dark photoperiod from metal halide and high pressure sodium lamps providing 250 μMol s^{-1} m^{-2} PAR at canopy height. Supplemental lighting was provided during the first and last 3-h of the daily photoperiod. Greenhouse temperatures were maintained at 25°C (days) and 14°C (nights) with 20 to 50% RH. The Barnes sandy clay loam soil used in these experiments was obtained from continuous corn fields (Pikul et al., 2005) near Brookings, SD.

Aphid-Day Measurement

Accurate measurement of aphid feeding effects on plants involves assessment of the aphid population density on plants plus the duration of their feeding. Aphid-day (aphid-d) unitage (e.g., one aphid feeding on one plant for a 24 h

period equals one aphid-d) measures the combined intensity and duration of the aphids on plants (Kieckhefer et al., 1995). Aphid-d values were obtained by multiplying aphid population numbers by the number of days that the aphids were on the host plant. Cumulative aphid-d values (Ruppel, 1983) represent the intensity and duration of soybean aphid feeding injury to which the soybean plants were exposed during the experiments.

Soybean N Fixation and Ureide-N Growth Chamber Experiment

Polyvinyl chloride pots (15 cm tall and 7.5 cm diam.) were filled with coarse perlite to within 3.5 cm of the pot top. Three *Bradyrhizobium*-treated seeds were placed on the perlite and covered with 2.5 cm of fine vermiculite. All pots were watered with N-free nutrient solution (Coolong and Randle, 2003). Plants were thinned to 1 uniform seedling pot^{-1} after 14 d of growth. After 38 d of growth under these conditions, when the plants were in the 3rd trifoliolate (V3; Ritchie et al., 1997) stage, 300 soybean aphids plant^{-1} were applied (Table 1) to plants in each of 10 pots while plants in an additional 10 pots were uninfested. To prohibit aphid contamination of control plants, infested and uninfested plants were placed into different cabinets of the dual growth chamber. Aphid numbers per plant were estimated every 6 days. By 16 d after infestation, infested plants received a cumulative infestation total of about 28 000 aphid-d (Table 1, Figure 1).

Sixteen days after infestation, when the 54-d-old plants were in the R4 (full pod; Ritchie et al., 1997) stage, root system nitrogenase activity (Curtis, 2004) was estimated by measuring H_2 evolution using an open-flow gas exchange system (Qubit Systems Inc., Kingston, Ontario, Canada). Nitrogenase activity was measured under normal atmospheric gasses [apparent nitrogenase activity (ANA)] as well as under an artificial gas mixture of an 80:20 ratio of argon

Table 1
Summary of experimental aphid treatments imposed for the growth chamber and greenhouse experiments

Experiment	Aphids applied	Shoot harvest	Plant stage at infestation†	Plant stage at harvest†	Initial aphid infestation rate	Cumulative aphid infestation
	Days after planting				aphids plant^{-1}	aphid-d
Growth chamber	38	54	V3	R4	300	28 000
Greenhouse	19	49	V1	R2	350	114 000

†V1, first trifoliolate leaf expanded; V3, third trifoliolate leaf expanded, R2, full bloom; R4, full pod (after Ritchie et al., 1997).

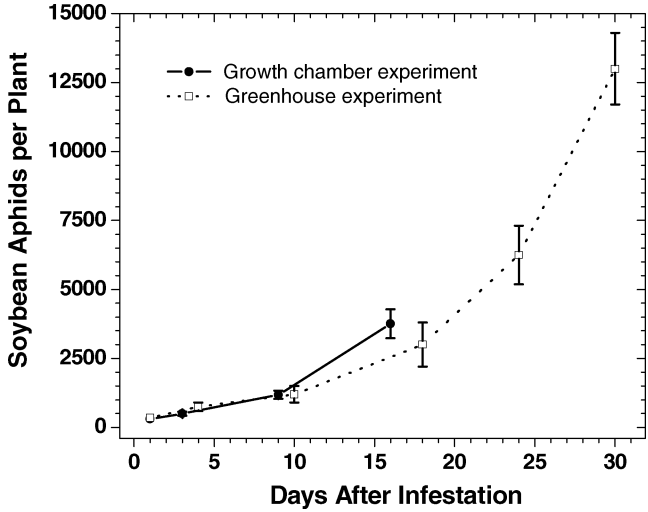

Figure 1. The number of soybean aphids present on shoots during the course of the N fixation growth chamber experiment and the ureide-N and nitrate-N accumulation greenhouse experiment. Aphids were applied on day 1 of the experiment. Symbols represent the average ± standard deviation for 10 replicate samples per treatment for the growth chamber experiment and 3 replicate samples per treatment for the greenhouse experiment.

(Ar): oxygen (O_2) [total nitrogenase activity (TNA)]. Nitrogen fixation rate was then calculated using the following formula (Curtis, 2004):

$$N_2\,\text{Fixation Rate} = (\text{TNA} - \text{ANA})/3$$

Plants were then removed from pots, and shoots and root systems gently washed free of aphids, honeydew, and perlite using a stream of tap water followed by a distilled water rinse. Root nodules were removed by hand from each root system, photographed with a digital camera, and then placed into the freezer. Quantitative nodule characteristics were measured using digital image (ImageJ; http://rsb.info.nih.gov/ij/) analysis software. Colorimetric methods were used to estimate nodule leghemoblobin (Wilson and Reisenauer, 1963). Shoots were severed from the roots and the resulting organs were dried to constant weight at 60°C in a forced-air oven, weighed, and ground to pass a 2 mm screen in a Wiley mill (Arthur Thomas Co., Philadelphia, PA, USA). Colorimetric methods were used to estimate ureide-N (Patterson et al., 1981).

Ureide-N and Nitrate-N Greenhouse Experiment

Soil was mixed in a 1:1 (v:v) ratio with coarse vermiculite and added to plastic containers (34 cm wide, 54 cm long, and 40 cm deep). The containers had drainage holes in the bottom that were covered by a 10 cm deep layer of 1 cm

diam. rock. Containers were watered to field capacity and allowed to drain for 24 h prior to planting. Five seeds per container were planted 4 cm deep with 4 cm spacing between seeds in the center of each container. After seeding emergence and early growth, 1 uniform seedling per container was selected and the others were removed. Over the course of the experiment, soil matric potential was monitored in each container at a 30 cm depth using tensiometers (Irrometer Co., Riverside, CA, USA). Containers were watered to field capacity when matric potential readings of 50 kPa were observed.

Soybean plants with fully-expanded first trifoliolate leaves (V1; Ritchie et al., 1997; 19 days after planting) were infested with about 350 aphids per plant (Table 1). Uninfested plants in separate containers served as the control. There were three replicate containers per treatment, and containers were arranged in a completely random design on the greenhouse bench. Aphid numbers per plant were estimated every 4 to 10 days. When plants reached the full bloom stage (R2; Ritchie et al., 1997), shoots were harvested (49 DAP; 114 000 aphid-d; Table 1; Figure 1). Plant shoots were then washed under a gentle stream of distilled water to remove aphids and their excretions. Shoots were dried to constant weight at 60 C in a forced air oven, weighed, and ground to pass a 2 mm screen in a Wiley mill (Arthur Thomas Co., Philadelphia, PA, USA). Colorimetric methods were used to estimate ureide-N (Patterson et al., 1981) and nitrate-N (Cataldo et al., 1975).

Data Analysis

Data from both experiments were analyzed separately using procedures for the comparison of two means (PROC TTEST) in SAS. For the greenhouse experiment, qualitative relationships between shoot dry weight, N component accumulation, and N component concentration were also examined for ureide-N and nitrate-N using the method of Jarrell and Beverly (1981). A sequence of symbols representing statistically ($P = 0.1$) significant differences ($\uparrow$ = increased, $\downarrow$ = decreased, 0 = no change) in these parameters between soybeans infested with soybean aphid and uninfested control plants was generated using the results of a t test appropriate for the comparison of the two means. The response patterns of the symbol sequences were used to determine potential response characteristics for these qualitative relationships.

RESULTS AND DISCUSSION

Aphid Effects on Nodules, N Fixation, and Shoot Ureide-N Concentration

Aphid populations (aphids per plant) on infested plants grown in perlite with –N nutrient solutions under growth chamber conditions remained relatively low

for about 9 d after initial infestation (Figure 1). This lag phase in population growth was followed by a logarithmic increase in aphid numbers starting about 9 days after infestation and continuing until harvest. Over the course of this experiment, cumulative soybean aphid infestation was about 28,000 aphid-d (Table 1). This level of infestation applied at the V3 crop development stage (38 days after planting) significantly reduced shoot and root dry weights when plants were harvested 54 days after planting at the full pod (R4) development stage (Table 2). Aphid injury reduced total nodule volume per plant but had no effect on the number of root nodules per plant (Table 2). Thus, nodules on infested plants were of smaller average volume than those from control plants. Aphid infestation also reduced nodule leghemoglobin per plant and plant N fixation rate when compared to control (Table 2). Leghemoglobin is a hemoprotein that provides precise control of the O_2 levels within the nodule (Sato et al., 2001). Nodule leghemoglobin levels are positively related to N fixation rate (González et al., 2001).

Taken together, our data indicate that reduced N fixation rates in soybean plants injured by aphids were accompanied by reductions in average nodule volume and nodule leghemoglobin accumulation as well as shoot and root dry weight. These observations support and extend those of Layton and Boethel (1989) and Russin et al. (1990) who found that soybean leaf defoliation caused by soybean looper (*Pseudoplusia includens* Walker) larvae resulted in reduced N fixation rate, reduced root system dry weight, reduced nodule dry weight, but had no effect on the number of nodules per plant.

Decreased N fixation under drought stress conditions in soybean has been shown to be related to decreased nodule size (Purcell et al., 1997). Under conditions of drought stress, large nodules provide a greater sink for phloem-derived delivery of sugars and water than small nodules (King and Purcell, 2001). We speculate that, in our experiment, a reduction in photosynthesis caused by aphid infestation (Macedo et al., 2003) reduced the level of photoassimilate available for nodule growth and N fixation. We further speculate that the reduction in average nodule size decreased nodule sink strength and subsequent photoassimilate delivery to the nodules. The fact that aphid infestation caused about a 34% reduction in nodule volume per plant and an 80% reduction in N fixation rate supports this speculation. However, additional data on the effect of aphid infestation on photosynthesis and the delivery of photoassimilates to the nodules would be needed to substantiate these speculations.

Shoot ureide-N concentration in infested plants was about 20% lower than control plants (Table 2). Because ureides are N fixation products that are stored in soybean shoots (Fujihara and Yamaguchi, 1978; van Berkum et al., 1985), shoot ureide-N concentration data summarize the impact of aphid feeding on N fixation over the 16 days that elapsed between initial infestation and shoot harvest. While the N fixation rate data (Table 2) provide an indication of nodule activity at a single time point at the end of the experiment, the ureide-N concentration data (Table 2) may be a more reliable measure (Streeter, 2003)

Table 2

The effect of soybean aphid infestation on soybean dry weight, nodule characteristics, nodule leghemoglobin accumulation, N fixation rate, and shoot ureide-N concentration Plants grown in perlite in a growth chamber using –N nutrient solution culture were infested 38 d after planting (at the V3 development stage) and were harvested 54 d after planting (at the R4 development stage). Infested plants received a cumulative aphid infestation of about 28000 aphid-d

Treatment	Shoot dry wt. (g plant^{-1})	Root dry wt (g plant^{-1})	Nodule number (no plant^{-1})	Nodule volume (ml plant^{-1})	Leghemoglobin (mg plant^{-1})	N fixation rate (μmol hr^{-1})	Ureide-N (g kg^{-1})
Control	1.8	0.4	60.8	0.59	7.4	5.25	5.4
Infested	1.1	0.3	59.5	0.39	5.1	1.03	4.3
Pr > t	0.0003[†]	0.001	0.82	0.0004	0.0004	0.0001	0.005

[†]Probability (t statistic) of a significant aphid treatment effect on dependent variables.

of the impact of aphid feeding on nodule activity over the 16 d time period between initial infestation and shoot harvest.

Aphid Effects On Shoot Nitrate-N And Ureide-N Concentration And Accumulation

Soybean plants grown under greenhouse conditions in soil and infested with soybean aphids at the first trifoliolate (V1; Ritchie et al., 1997) stage had unifoliolate leaves and older trifoliolate leaves with symptoms of interveinal chlorosis and necrosis at harvest. Infested plants were also shorter than control plants. These symptoms were consistent with the soybean aphid plant injury symptoms reported by others (Macedo et al., 2003; Myers et al., 2005). In our aphid infestation treatments, initial aphid populations of 350 aphids plant^{-1} built up to an average of about 13 000 aphids plant^{-1} over the 30 day period between the initial V1 infestation and the R2 crop development stage when shoots were harvested (Figure 1). The average cumulative aphid-d for the infestations in this experiment was 114 000 (Table 1). This level of aphid infestation has been shown to consistently reduce shoot dry weight accumulation when plants were infested with aphids at the V5 development stage and grown under field conditions (Beckendorf et al., 2008).

Shoot dry weight was about 63% less in infested plants than in uninfested controls (Table 3). The soybean aphid treatment significantly reduced nitrate-N and ureide-N accumulation (on a mg plant^{-1} basis) when plants were infested at V1 (Table 3). When shoot concentrations (on a g kg^{-1} basis) are considered, plants infested with aphids had shoot ureide-N concentrations that were not significantly different across the two treatments (Table 3). In contrast, nitrate-N concentrations were significantly greater (by about 75%) in infested plants than control plants (Table 3). These contrasting results suggest that shoot ureide-N and nitrate-N concentrations were responding to aphid infestation in a different manner.

Table 3

The effect of soybean aphid infestation on the accumulation of soybean shoot dry weight and the concentration and accumulation of nitrate-N and ureide-N. Plants grown in the greenhouse in soil were infested 19 d after planting (V1 crop development stage) and shoots were harvested 49 d after planting (R2 crop development stage)

Treatment	Dry weight (g plant^{-1})	Nitrate-N (g kg^{-1})	Ureide-N (g kg^{-1})	Nitrate-N (mg plant^{-1})	Ureide-N (mg plant^{-1})
Control	6.41	0.66	0.87	4.27	5.66
Infested	2.36	1.15	0.88	2.73	2.16
Pr > t	0.0006†	0.0588†	0.9611	0.0943	0.0686

†Probability (t statistic) of a significant aphid treatment effect on dependent variables within the V1 crop development stage infestation treatment.

Table 4
Qualitative representation of differences in N component accumulation, dry weight accumulation, and N component concentration between uninfested soybean plants and plants infested with soybean aphids at the V1 developmental stage and harvested at the R2 developmental stage

N component	Δ Accumulation	Δ Dry weight	Δ Concentration	Response[†]
Nitrate-N	↓[‡]	↓	↑	Concentration effect
Ureide-N	↓	↓	0	No concentration change

[†]Response determined by sequence of the three qualitative symbols for each element (after Jarrell and Beverly, 1981).

[‡]Symbols represent more (↑), less (↓), or no change (0) in infested plants relative to uninfested control plants (*t* statistic, $P = 0.1$).

Shoot nutrient concentration is a single-point measurement that results from the integration of two dynamic processes, nutrient accumulation and dry matter production. Thus, consideration of a crop's nutrient status should be based upon nutrient concentration as well as dry weight and nutrient accumulation responses (Jarrell and Beverly, 1981). In soybean plants infested with aphids compared with uninfested plants, shoot ureide-N concentration remained the same while shoot dry weight and ureide-N accumulation were reduced (Table 4). In contrast to ureide-N, nitrate-N shoot concentration was greater in infested plants than control while dry weight and nitrate-N accumulation were reduced (Table 4). Thus, aphid infestation had no effect upon shoot ureide-N concentration but did have a "concentration" effect upon shoot nitrate-N concentration.

Because ureide-N originates predominately from the energy-demanding process of N fixation (Fujihara and Yamaguchi, 1978; van Berkum et al., 1985), it is likely that aphid-induced reductions in N fixation (Table 2), coupled with decreased dry weight accumulation, were both instrumental in allowing shoot ureide-N concentration to remain unchanged in aphid-injured plants. Nitrate-N, which is readily absorbed directly from the soil, is a storage form of N in plants that does not need to be assimilated by roots (Engels and Marschner, 1995; Marschner, 2002). Because nitrate-N concentration was greater in aphid-injured shoot tissue, we conclude that nitrate-N accumulation was less sensitive to aphid injury than dry weight accumulation.

CONCLUSIONS

The capacity for N uptake and shoot biomass accumulation is dependent upon environmental conditions (Lawlor, 2002). Results reported in this work illustrate that soybean N relations are affected by soybean aphid injury. Taken

together, measurements of N fixation, dry weight accumulation, and ureide-N and nitrate-N concentration and accumulation provide insights on the impact of the soybean aphid on soybean physiology. Determination of the physiological basis of soybean aphid injury may be valuable for focusing additional research efforts aimed at developing aphid tolerant soybean varieties or crop management options that ameliorate yield loss to aphid feeding injury in soybeans.

ACKNOWLEDGMENTS

Research supported in part by a grant from the South Dakota Soybean Research and Promotion Council. The authors thank A. Rohweder, K. Anderson, and J. Zebill, and L. Draper for technical assistance. Mention of trade names or commercial products in this publication is solely for the purpose of providing information to the reader and does not imply recommendation or endorsement by the USDA. USDA offers its programs to all eligible persons regardless of race, color, age, sex, or national origin.

REFERENCES

Beckendorf, E. A., M. A. Catangui, and W. E. Riedell. 2008. Soybean aphid feeding injury and soybean yield, yield components, and seed composition. *Agronomy Journal* 100: 237–246.

Brown, S. M., K. J. Oparka, J. I. Sprent, and K. B. Walsh. 1995. Symplastic transport in soybean root nodules. *Soil Biology and Biochemistry* 27: 387–399.

Cataldo, D. A., M. Haroon, L. E. Schrader, and V. L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis* 6: 71–80.

Coolong, T. W., and W. M. Randle. 2003. Sulfur and nitrogen availability interact to affect the flavor biosynthetic pathway in onion. *Journal of the American Society for Horticultural Science* 128: 776–783.

Curtis, J. H. 2004. Nitrogen Fixation Laboratory Package. Kingston, Canada: Qubit Systems Inc.

Engels, C., and H. Marschner. 1995. Plant uptake and utilization of nitrogen. In: *Nitrogen Fertilization in the Environment*, ed. P. E. Bacon, pp. 41–81. New York: Marcel Dekker.

Fujihara, S., and M. Yamaguchi. 1978. Probable site of allantoin formation in nodulating soybean plants. *Phytochemistry* 17: 1239–1243.

Gan, Y., I. Stulen, H. van Keulen, and P. J. C. Kuiper. 2004. Low concentration of nitrate and ammonium stimulate nodulation and N_2 fixation while inhibiting specific nodulation (nodule DW g^{-1} root dry weight) and specific

N_2 fixation (N_2 fixed g^{-1} root dry weight) in soybean. *Plant and Soil* 258: 281–292.

George, T., and P. W. Singleton. 1992. Nitrogen assimilation traits and dinitrogen fixation in soybean and common bean. *Agronomy Journal* 84: 1020–1028.

González, E. M., L. Gálvez, and C. Arrese-Igor. 2001. Abscisic acid induces a decline in nitrogen fixation that involves leghaemoglobin, but is independent of sucrose synthase activity. *Journal of Experimental Botany* 52: 285–293.

Hardy, R. W. F., and U. D. Havelka. 1976. Photosynthesis as a major factor limiting nitrogen fixation by field-grown legumes with emphasis on soybeans. In: *Symbiotic Nitrogen Fixation in Plants*, ed. P. S. Nutman, pp. 421–439. Cambridge, UK: Cambridge University.

Jarrell, W. M., and R. B. Beverly. 1981. The dilution effect in plant nutrition studies. *Advances in Agronomy* 34: 197–224.

Kieckhefer, R. W., J. L. Gellner, and W. E. Riedell. 1995. Evaluation of the aphid-day standard as a predictor of yield loss caused by cereal aphids. *Agronomy Journal* 87: 785–788.

King, C. A., and L. C. Purcell. 2001. Soybean nodule size and relationship to nitrogen fixation response to water deficit. *Crop Science* 41: 1099–1107.

Lawlor, D. W. 2002. Carbon and nitrogen assimilation in relation to yield: Mechanisms are the key to understanding production systems. *Journal of Experimental Botany* 53: 773–787.

Layton, M. B., and D. J. Boethel. 1989. Response of soybean growth and N_2-fixing ability to progressive insect-induced defoliation. *Journal of Economic Entomology* 82: 273–280.

Macedo, T. B., C. S. Bastos, L. G. Higley, K. R. Ostlie, and S. Madhavan. 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *Journal of Economic Entomology* 96: 188–193.

Marschner, H. 2002. *Mineral Nutrition of Higher Plants*. London: Academic Press.

McClure, P. R., and D. W. Israel. 1979. Transport of nitrogen in the xylem of soybean plants. *Plant Physiology* 64: 411–416.

McClure, P. R., D. W. Israel, and R. J. Volk. 1980. Evaluation of the relative ureide content of xylem sap as an indicator of N_2 fixation in soybeans. *Plant Physiology* 66: 720–725.

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. *Journal of Economic Entomology* 98: 113–120.

Patterson, T. G., R. Glenister, and T. A. LaRue. 1981. Simple estimate of ureides in soybean tissue. *Analytical Biochemistry* 119: 90–95.

Pikul, J. L., L. Hammack, and W. E. Riedell. 2005. Corn yield, nitrogen use and corn rootworm infestation of rotations in the northern corn belt. *Agronomy Journal* 97: 854–863.

Purcell, L. C., M. deSilva, C. A. King, and W. H. Kim. 1997. Biomass accumulation and allocation in soybean associated with genotypic differences in tolerance of nitrogen fixation to water deficits. *Plant and Soil* 196: 101–113.

Rawsthorne, S., F. R. Minchin, R. J. Summerfield, C. Cookson, and J. Coombs. 1980. Carbon and nitrogen metabolism in legume root nodules. *Phytochemistry* 19: 341–355.

Ritchie, S. W., J. J. Hanway, H. E. Thompson, and G. O. Benson. 1997. How a soybean plant develops. Special Report 53. Ames IA: Iowa State University Cooperative Extension Service.

Ruppel, R. F. 1983. Cumulative insect-days as an index of crop protection. *Journal of Economic Entomology* 76: 375–377.

Russin, J. S., M. B. Layton, D. J. Boethel, E. C. McGawley, J. P. Snow, and G. T. Berggren. 1990. Growth, nodule development, and N_2-fixing ability in soybean damaged by an insect-fungus-nematode pest complex. *Journal of Economic Entomology* 83: 247–254.

Sato, T., N. Onoma, H. Fujikake, N. Ohtake, K. Sueyoshi, and T. Ohyama. 2001. Changes in four leghemoglobin components in nodules of hypernodulating soybean (*Glycine max* [L.] Merr.) mutant and its parent in the early nodule development stage. *Plant and Soil* 237: 129–135.

Schubert, K. R. 1986. Products of biological nitrogen fixation in higher plants: Synthesis, transport, and metabolism. *Annual Review of Plant Physiology* 37: 539–574.

Streeter, J. G. 2003. Effects of drought on nitrogen fixation in soybean root nodules. *Plant, Cell and Environment* 26: 1199–1204.

Streeter, J. G., H. J. Medersik, and R. A. Ahmad. 1979. Coupling between photosynthesis and nitrogen fixation. In: *World Soybean Research Conference II: Proceedings*, ed. F. T. Corbin, pp. 129–137. Boulder, CO: Westview.

van Berkum, P., C. Sloger, D. F. Wever, P. B. Cregan, and H. H. Keyser. 1985. Relationship between ureide N and N2 fixation, aboveground N accumulation, acetylene reduction, and nodule mass in greenhouse and field studies with *Glycine max* L. (Merr.). *Plant Physiology* 77: 53–58.

Wilson, D. O., and H. M. Reisenauer. 1963. Determination of leghemoglobin in legume nodules. *Analytical Chemistry* 6: 27–30.