# Nitrogen Fertilizer Management Effects on Soybean Nitrogen Components and Bean Leaf Beetle Populations

Walter E. Riedell,* Shannon L. Osborne, Jon G. Lundgren, and Joseph L. Pikul, Jr.

## ABSTRACT

**Bean leaf beetle [*Cerotoma trifurcata* (Förster)] (BLB) larvae consume soybean [*Glycine max* (L.) Merr.] root nodules. This study was conducted to determine if different rates of N contained in starter fertilizer affect soybean shoot N components and BLB populations. The effects of starter N fertilizer treatments, consisting of 112 kg ha$^{-1}$ of 24–16–11 (high-N treatment), 7–16–11 (intermediate-N), or 0–16–11 (no-N) percent elemental N–P–K, on soybean shoot $NO_3$–N and ureide-N and on BLB larval and adult abundance and body size were investigated on a Barnes clay loam (fine-loamy, mixed, superactive, frigid Calcic Hapludolls) near Brookings, SD. High-N fertilizer increased shoot $NO_3$–N from the beginning bloom to beginning pod stage and reduced ureide-N at the full bloom, beginning pod, and beginning seed stages when compared to the intermediate and no N treatments. Fertilizer N treatments had no effects on larval numbers or body size. High-N fertilizer resulted in greater adult emergence (1.73 insects m$^{-1}$) than no N (0.42 insects m$^{-1}$) in the hot and dry 2007 growing season, but not in the cooler and less-dry 2006 growing season. Across both years, adult hind tibia lengths, but not head capsule widths, were greater in insects that emerged from high-N (1.86 mm) and intermediate-N (1.85 mm) compared to no-N (1.79 mm) treatments. High-N starter fertilizer, which altered shoot N components and increased BLB adult numbers and size under hot and dry conditions, may increase BLB populations and intensify the damage caused by this insect pest.**

Over the past 30 yr, with the availability of improved and adapted soybean varieties, soybean cultivation has increased in the United States, especially in the Upper Midwest. In 2009, soybeans were planted on combined 6.2 million ha in Minnesota, North Dakota, and South Dakota. With an average yield of 2.5 Mg ha$^{-1}$, the value of soybeans harvested in these three northern states was $5.3 billion (National Agriculture Statistics Service, 2010). Soybean production and its overall contribution to farm profitability are of major importance to crop producers.

The BLB has long been recognized as a damaging native pest of legumes in the southeastern United States. (McConnell, 1915). With widespread cultivation of soybeans, coupled with milder winters, the BLB has increased in abundance across the Upper Midwest (Witkowski and Echtenkamp, 1996; Hammack et al., 2010). Adult BLB damage soybeans by feeding on shoots, leaves, and pods. The larval stage of the insect feeds on below-ground portions of the plant. Lundgren and Riedell (2008) found that root nodules were visibly damaged by larval feeding. External nodule surfaces were scarred, and frequently the nodule internal matrices were excavated by the larvae.

The application of N fertilizer is a cultural practice that affects soybean N components (Ray et al., 2006; Salvagiotti et al., 2008). In cooler northern U.S. climates, shoots of soybean grown with starter fertilizer containing up to 24 kg N ha$^{-1}$ had lower ureide levels than those grown with starter fertilizer that contained no N (Osborne and Riedell, 2006b). Ureides, which are the product of N fixation in soybean root nodules (Fujihara and Yamaguchi, 1978; Salvagiotti et al., 2009), are the principal form of fixed N that is translocated and accumulated in soybean shoot tissue (Thomas and Schrader, 1981; van Berkum et al., 1985). Ureide concentrations in soybean shoots are correlated with nodule dry weight and nodule N fixation (McClure and Israel, 1979; Herridge et al., 1990). Thus, the observed reductions in soybean shoot ureide levels (Osborne and Riedell, 2006b) suggest that starter N fertilizer may also have affected soybean root nodule dry weight as well as N fixation processes.

There is substantial interest to improve soybean cultural methods for increased yield and enhanced economic returns in the Upper Midwest region of the United States. Because BLB larvae consume soybean nodules, cultural practices that affect soybean nodule growth and function may also affect BLB populations in soybeans. Our hypothesis was that increasing N in starter fertilizer would suppress BLB larval and adult populations by reducing root nodule size and function. Our objective was to investigate the effects of starter fertilizer treatments containing different levels of N on soybean shoot N components, on BLB larval and adult population abundance, and on larval and adult body size.

## MATERIALS AND METHODS

### Long-Term Research Plots and Nitrogen Fertilization

Field experiments were conducted in 2006 and 2007 at the eastern South Dakota Soil and Water Research Farm near Brookings, SD (44°19’ N, 96°46’ W; 500 m elevation). Research plots were located on Barnes clay loam (with nearly-level topography; Maursetter et al., 1992). This soil series, which consists of very deep, well drained, moderately

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USDA-ARS, North Central Agricultural Research Lab., 2923 Medary Ave., Brookings, SD 57006. Received 8 Apr. 2011. *Corresponding author (walter.riedell@ars.usda.gov).

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Abbreviations: BLB, bean leaf beetle; DOY, day of year; GDD, growing degree day.

permeable soils that formed in loamy till (USDA NRCS Soil Series Search Query Facility, 2009), is characteristic of soils in eastern South Dakota and western Minnesota and is similar to soils common to the northern U.S. Corn Belt.

The long-term rotation study, consisting of soybean grown in annual rotation with maize (*Zea mays* L.) under three levels of N input and three replications, was established under dryland conditions in 1990. Crop rotation treatments were applied to six 90- by 30-m whole plots with three whole plots in soybean and three whole plots in maize. Whole plots were split into 30- by 30-m plots for application of the three N treatments. Plots and treatments were arranged in a randomized complete block design. Primary tillage for both crops was a fall chisel plow operation, and seed beds were prepared each spring with a tandem disk and field cultivator.

Starter fertilizer treatments for soybean and maize, applied in a band 5 cm to the side and 5 cm below the seed furrow, consisted of 112 kg ha$^{-1}$ of 24–16–11, 7–16–11, or 0–16–11 elemental N–P–K on the high N, intermediate N, and no N plots, respectively (Osborne and Riedell, 2006a). The soybean phase of the rotation received no additional N fertilizer. However, the maize phase of the rotation was fertilized with urea for a yield goal of 8.5 Mg ha$^{-1}$ (high N input plots), 5.3 Mg ha$^{-1}$ (intermediate N input plots), or maize not fertilized (no N input plots). All plots were treated with metolachlor and glyphosate herbicides immediately after planting. Additional information on these long-term research plots is in Pikul et al. (2001) and Riedell et al. (2009).

## Soybean Cultivation, Sampling, and Analysis

Experiments related to the influence of starter N fertilization on BLB were conducted on the soybean phase of the rotational study during the 2006 and 2007 growing seasons. Soil N levels (Gelderman et al., 1987) in the top 60 cm of the soil profile were determined in the fall (3 Nov. 2005 and 8 Nov. 2006) from plots after the maize phase of the rotation. For the high N input plots, soil N levels were 34.7 kg N ha$^{-1}$ in 2005 and 32.8 kg N ha$^{-1}$ in 2006. For the intermediate N input plots, soil N levels were 26.1 kg N ha$^{-1}$ in 2005 and 26.5 kg N ha$^{-1}$ in 2006, whereas that for the no N input plots was 20.2 kg N ha$^{-1}$ in 2005 and 25.8 kg N ha$^{-1}$ in 2006.

Soybean seeds (Pioneer '91B91') were planted (76 cm row spacing) 3.7 cm deep at a population of 489,000 live seeds ha$^{-1}$ on 23 May 2006 (day of year, DOY 143) and 2 May 2007 (DOY 122). Soybean plant development stages on 10 plants plot$^{-1}$ were measured using the method of Ritchie et al. (1997) starting on 16 June 2006 (DOY 167) and 12 June 2007 (DOY 163). Soybean shoot samples consisting of 4 m of row were removed from each soybean plot at regular intervals starting on 7 July 2006 (DOY 188) and 25 June 2007 (DOY 176) and ending on 29 Sept. 2006 (DOY 272) and 11 Sept. 2007 (DOY 254). Plant stems were severed at the soil level with pruning shears, and the plant shoots were placed into paper bags, 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). Ground plant material was analyzed for ureide-N (Patterson et al., 1982) and $NO_3$–N (Cataldo et al., 1975) using colorimetric techniques on hot water tissue extracts (Riedell et al., 2005; Osborne and Riedell, 2006a).

## Bean Leaf Beetle Larval and Adult Sampling

Sampling of BLB larvae began on 30 June 2006 (DOY 181) and 20 June 2007 (DOY 171) and ended on 20 Sept. 2006 (DOY 263) and 14 Sept. 2007 (DOY 257). Two soil core samples were taken from each soybean plot three times each week using a 10-cm diam. steel cylinder soil sampler (golf cup cutter). Plant shoots were first removed at the soil surface. The cylinder was centered directly over the crop row and pushed into the soil to a depth of about 12 cm (Anderson and Waldbauer, 1977). Each soil core contained portions of root systems from about three plants. There were 54 soil cores sampled per week (2 cores plot$^{-1}$ × 3 samples wk$^{-1}$ × 3 treatments × 3 replications).

Soil cores and associated root systems within plots were combined, gently broken apart by hand agitation, and layered on a steel screen (1.4 by 1.9 mm mesh size) suspended over a pan of water. These larval separation devices were then placed under incandescent lights for a period of at least 48 h. Larvae burrowed down to escape the heat/soil drying, and passed through the screen and into the water. Larvae were then removed from the water, counted, and stored in 70% ethanol. Larval head capsule widths, body lengths (posterior to anterior), and body diameter (at widest portion of the body) were measured with a dissecting microscope fitted with a micrometer ocular lens. Soil remaining on the screen after larval extraction was weighed.

Sampling for BLB adult emergence from the soil began on 6 July 2006 (DOY 187) and 20 June 2007 (DOY 171) and ended on 10 Oct. 2006 (DOY 283) and 19 Sept. 2007 (DOY 262). Emergence cages (0.89 m long, 0.46 m wide, 0.15 m tall; Fisher, 1980) were constructed of galvanized steel on the sides. Cages were open to the soil on the bottom and were covered with 18 by 14 mesh aluminum screening on the top. The screening was cut at one corner and a clear plexiglass tube (10 cm diam. by 15 cm tall) was inserted through the cut screen. The plexiglass tube was previously fitted with a 18 by 14 mesh plastic screen at the 7.5-cm mark that allowed insects to move upward, but not downward. Adult insects crawled up the cage sides, along the screen, and through the plexiglass tube where they were trapped in the top portion of the tube by the second screen.

In plot areas where soybean shoots had been harvested, cages were centered over the row with the long axis of the cage parallel to the crop row. The cages were pushed into the soil to a depth of about 5 cm with the corner containing the capped plexiglass tube at the highest portion of the cage. There were a total of 72 cages (8 cages plot$^{-1}$ × 3 plots treatment$^{-1}$ × 3 replications) for each year of the experiment. The eight cages were placed into a single row within each plot. Cages were left in place for a period of 2 wk, and then moved to other plant rows in the plot. Dispersion of cages within plots was such that cages within the row were separated by 1 m of undamaged plants and caged rows were separated from each other by a buffer (undamaged) row. Cages were checked weekly for adult beetles. Beetles were removed from cages, counted, and stored in 70% ethanol. Beetle head capsule width and hind tibia length were measured with a dissecting microscope fitted with a micrometer ocular lens.

**Table 1. Average monthly precipitation and air temperature for the 2006 and 2007 growing seasons near Brookings, SD.**

| Month | Average precipitation |  | Average air temperature |  |
|---|---|---|---|---|
|  | 2006 | 2007 | 2006 | 2007 |
|  | mm |  | °C |  |
| May | 51.3 (–23.6)† | 47.2 (–27.7) | 14.1 (+0.4) | 16.0 (+2.3) |
| June | 59.7 (–47.8) | 75.9 (–31.5) | 19.3 (+0.4) | 20.1 (+1.1) |
| July | 5.8 (–73.2) | 3.6 (–75.4) | 23.0 (+1.5) | 22.1 (+0.6) |
| August | 143.5 (+68.8) | 163.8 (+89.2) | 20.7 (+0.3) | 20.2 (–0.1) |
| September | 161.5 (+98.6) | 30.5 (–32.5) | 12.8 (–2.2) | 16.1 (+1.0) |

† Values in parentheses represent departure from normal (30-yr average).

### Environmental Monitoring

Soil matric potential (Watermark soil moisture sensor, Irrometer Company, Riverside, CA) and soil temperature (Hobo soil temperature sensor, Onset Inc., Bourne, MA) were measured in the plots at 15-cm depths over the course of the experiments. Hourly soil temperature readings were converted to soil growing degree days with a base temperature of 10°C (growing degree day, $GDD_{10}$) using software (SpecWare 8 basic, Spectrum Technologies Inc., Plainfield, IL). Monthly averages of precipitation and air temperature were obtained from a weather station located about 3 km from the experimental plots as published in annual climate data summaries available from the National Climatic Data Center (Ashville, NC).

### Statistical Analysis

Statistical differences were assessed using SAS version 9.2 with year and N input considered as fixed effects and replication considered as a random effect. Repeated measures procedures in PROC MIXED were applied for comparison of shoot nitrate N, ureide-N, and biomass data across sampling dates (Littell et al., 2006). Significant ($\alpha = 0.05$) main treatment effects on dependent variables were investigated by graphing means and standard errors. Nitrogen fertilizer treatment × sampling date interactions were further investigated by calculating mean separations (adjust = Tukey option in LSMEANS) appropriate for testing three N-treatment levels within sampling dates.

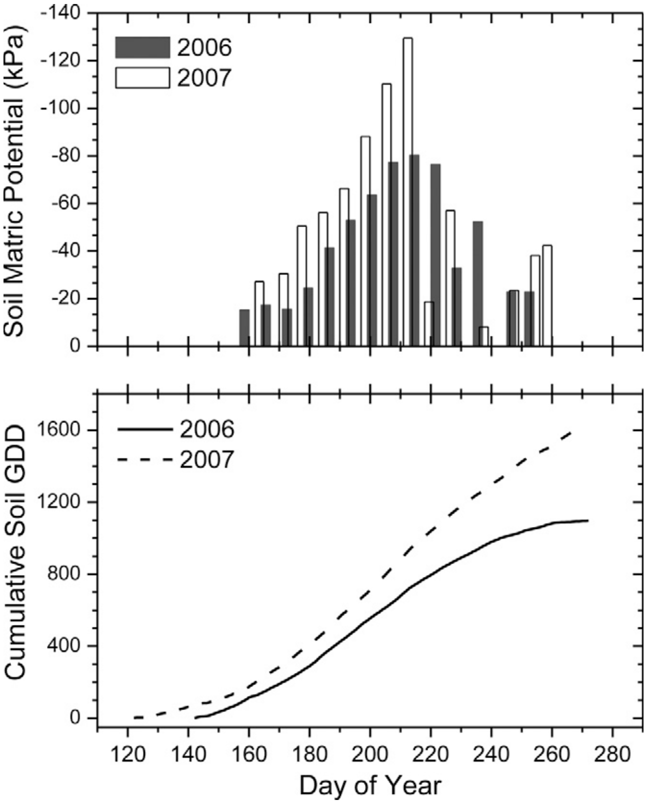

**Fig. 1. Soil matric potential and soil growing degree day (base 10) accumulation at 15-cm soil depth during the 2006 and 2007 growing season.**

Bean leaf beetle larval and adult populations were assessed by calculating and graphing the number of larvae or adults captured across all N fertilizer treatments within sampling dates during each year of the experiment. These graphs were assessed for the demonstration of two peaks which could be interpreted to represent two insect generational stages each year of the study.

To determine BLB larval stages, data on head capsule widths, combined across the 2 yr of the study, were graphed as histograms showing frequency of individuals observed within successively larger 0.05-mm-wide size increments (Hammack et al., 2003). These histograms demonstrated three peaks that were interpreted to represent peak frequency of the head capsule widths of three larval stages. Assuming a normal distribution of head capsule widths, multiple Gaussian curves were fit to the histograms using a nonlinear least squares regression method available in Origin 6.1 software (Hammack et al., 2003; OriginLab Corp., 1999).

Data on larval and adult numbers, head capsule widths, larval body size, and adult hind tibia length were analyzed using PROC MIXED procedures appropriate for analysis for a randomized complete block experiment conducted over 2 yr (Littell et al., 2006). With the occurrence of a significant test for main effects, means were separated using the adjust = Tukey option in LSMEANS.

## RESULTS AND DISCUSSION

### Growing Season Environments

Growing season precipitation was considerably below normal for May through July in 2006 and 2007 (Table 1). Soil matric potential at 15-cm depth steadily became more negative from about 19 June (DOY 170) to 29 July (DOY 210) during both years, with readings in 2006 being less negative than those in 2007 (Fig. 1). August precipitation was well above normal in both years (Table 1). Precipitation in September 2006 was considerably above normal, and that in 2007 was below normal.

The intensity of rainfall events was different across the 2-yr experiment. An 80 mm rainfall occurred on 13 Aug. 2006 (DOY 225) and a single event dropped 112 mm rainfall on 1–2 Sept. 2006 (DOY 244–245). In 2007, 60, and 71 mm rainfall events were recorded on 4 August (DOY 216) and 18–20 August (DOY 230–232). These differences in the rain intensity across years caused the soil matric potential in 2006 to become gradually less negative from about 8 August (DOY 220) to 9 September (DOY 252). In 2007, soil matric potential indicated periods of dryer soil conditions interspaced between wetter conditions from 29 July (DOY 210) to 15 September (DOY 258; Fig. 1).

Average air temperatures were above normal for the months of May through July during both 2006 and 2007 (Table 1). August air temperature was above normal in 2006 and slightly below normal in 2007. Air temperature in September 2006 was well below normal and above normal in 2007. In 2006, approximately a 20 d period of time, from planting on 23 May (DOY 142) to

**Table 2. Relationship of soybean shoot sample date with day of year (DOY), soil growing degree day accumulation ($GDD_{10}$), and soybean shoot reproductive stage.**

| | Sample date | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| | | | | | | 2006 | | | | | |
| DOY | 189 | 196 | 203 | 211 | 217 | 224 | 231 | 238 | 243 | 256 | 272 |
| Soil $GDD_{10}$† | 424 | 503 | 590 | 694 | 765 | 838 | 900 | 962 | 1000 | 1061 | 1097 |
| R stage‡ | 1.1 | 1.7 | 2.1 | 2.8 | 4.2 | 5.2 | 5.8 | 6.0 | 6.0 | 6.2 | 8.0 |
| | | | | | | 2007 | | | | | |
| DOY | 171 | 185 | 193 | 200 | 207 | 214 | 221 | 228 | 236 | 243 | 254 |
| Soil $GDD_{10}$ | 295 | 480 | 607 | 713 | 833 | 954 | 1053 | 1153 | 1251 | 1332 | 1464 |
| R stage | 1.0 | 2.2 | 3.0 | 3.7 | 4.6 | 5.3 | 5.9 | 6.0 | 6.0 | 6.7 | 7.8 |

† Values represent soil growing degree day accumulation at a base of 10°C. Soil temperature was taken at 15- cm depth with probes placed within the soybean row.

‡ Soybean reproductive developmental stages were R1, beginning bloom; R2, full bloom; R3, beginning pod; R4, beginning pod; R5, beginning seed; R6, full seed; R7, beginning maturity; and R8, full maturity (Ritchie et al., 1997).

about 14 June (DOY 165), was needed before GDD accumulation reached a linear rate (Fig. 1). This linear phase lasted until about 18 August (DOY 230) after which the rate of soil GDD accumulation became less than linear. In 2007, a 38-d period of time from 3 May (DOY 122) to about 9 June (DOY 160) was needed before GDD accumulation reached a linear rate. This linear rate remained fairly constant for the remainder of the growing season. The slope of soil GDD linear accumulation rate appeared to be lower in 2006 than in 2007 (Fig. 1). Taken together, the more negative soil matric potential as well as the greater soil GDD accumulation rate recorded in 2007 suggest that this growing season was hotter and dryer than that in 2006.

## Soybean Plant Growth and Nitrogen Compounds

Analysis of plant samples taken from the plots at roughly 7 d intervals (Table 2) during both years of the study revealed a significant ($P = 0.0001$) sampling date effect on soybean shoot dry weight. Shoot dry weight gradually increased from 59 g $m^{-1}$ of row at the R1 stage to about 483 g $m^{-1}$ at the R6 (full seed) and R7 (beginning maturity) stages and dropped to 315 g $m^{-1}$ at the R8 (full maturity) stage. This slight decrease at the R8 likely resulted because of leaf drop in mature plants. There were no significant effects of year or N fertilizer treatment and no significant two- or three-way interactions between year, starter N fertilizer treatment, or sample date for shoot dry weight. This lack of starter N fertilizer effects on shoot dry weight agrees with previous observations (Riedell et al., 2005). Ray et al. (2006) suggested that, in most soils, soybeans do not respond to low rates (25–35 kg N $ha^{-1}$) of preplant N fertilization. In contrast, linear increases in R1 and R3 shoot biomass were observed in response to increasing rates (from 0–24 kg N $ha^{-1}$) of N in starter fertilizer when early-planted soybeans encountered cool spring temperatures for several weeks after planting (Osborne and Riedell, 2006a).

Statistical analysis of shoot $NO_3$–N, ureide-N, and total N data revealed significant sample date effects for these variables. Shoot $NO_3$–N concentrations remained nearly level at about 1 g $kg^{-1}$ dry weight at the R1 and R2 development stages, with concentrations dropping off during the R3 (beginning pod) through R8 development stage (Fig. 2). In contrast, soybean shoot ureide-N concentrations gradually increased from about 0.4 g $kg^{-1}$ dry weight at the R1 development stage to about 3 g $kg^{-1}$ dry weight at R5 development stage, after which the shoot concentrations decreased (Fig. 2). Total N concentrations dropped from about 37 g $kg^{-1}$ dry weight at the R1 stage to about 30 g $kg^{-1}$ at the R3 stage after which concentrations remained steady until increasing to about 32 g $kg^{-1}$ at the R7 and R8 stages (Fig. 2).

Soybeans accumulate N from two different sources: soil N and atmospheric N. Shoot $NO_3$–N is first absorbed from the soil and then translocated to shoots (McClure and Israel, 1979). Ureides are synthesized from products of N fixation in root nodules (Thomas and Schrader, 1981) after which they are translocated and accumulated in shoots (Herridge and Peoples, 1990). Ureide concentrations in soybean shoots increase from the beginning flowering (R1) through the full pod (R4) and beginning flowering (R5) stages (Riedell et al., 2005) after which concentrations rapidly decrease as ureides are assimilated into other N compounds and translocated to developing fruits (Thomas and Schrader, 1981).

Statistical analysis also revealed significant effects of N fertilizer treatment on shoot $NO_3$–N (0.53 g $kg^{-1}$ dry weight for high N fertilizer treatment, 0.47 for intermediate, and 0.41 for no N; $P = 0.006$), shoot ureide-N (1.36 g $kg^{-1}$ dry weight for high fertilizer treatment, 1.52 for intermediate, and 1.68 for no N; $P = 0.006$), and total shoot N (31.3 g $kg^{-1}$ dry weight for high fertilizer treatment, 31.8 for intermediate, and 32.1 for no N; $P = 0.005$). Significant sample date by N fertilizer

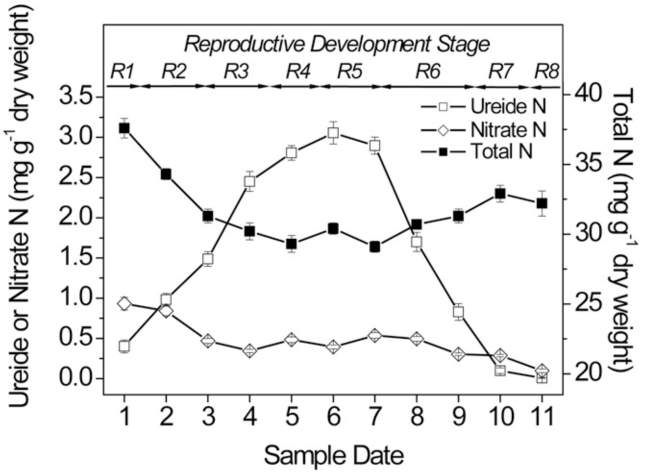

**Fig. 2. Shoot concentrations of nitrate N, ureide-N, and total N in relation to soybean reproductive developmental stage and sample date. See Table 2 for additional information on sample dates. Symbols represent means ± standard errors for plants sampled across all N input treatments across both years of the 2-yr experiment.**

**Table 3. Soybean shoot nitrate N and ureide N concentration within N fertilizer input level treatments for data combined across years.**

| N input | Sample date† | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| | g $kg^{-1}$ dry weight | | | | | | | | | | |
| | Shoot nitrate N concentration | | | | | | | | | | |
| No N | 0.82 b‡ | 0.73 b | 0.32 b | 0.26 a | 0.36 a | 0.33 a | 0.51 a | 0.46 a | 0.29 a | 0.28 a | 0.08 a |
| Intermediate | 0.88 b | 0.78 b | 0.50 ab | 0.35 a | 0.53 a | 0.39 a | 0.55 a | 0.51 a | 0.30 a | 0.30 a | 0.11 a |
| High N | 1.10 a | 1.01 a | 0.58 a | 0.42 a | 0.56 a | 0.45 a | 0.55 a | 0.51 a | 0.32 a | 0.28 a | 0.10 a |
| | Shoot ureide N concentration | | | | | | | | | | |
| No N | 0.58 a | 1.12 a | 1.74 a | 2.98 a | 2.92 a | 3.37 a | 2.95 ab | 1.83 a | 0.85 a | 0.10 a | 0.02 a |
| Intermediate | 0.35 a | 0.91 a | 1.47 ab | 2.35 b | 2.87 a | 2.96 ab | 3.19 a | 1.70 a | 0.85 a | 0.08 a | 0.01 a |
| High N | 0.26 a | 0.91 a | 1.24 b | 2.03 b | 2.63 a | 2.84 b | 2.54 b | 1.56 a | 0.79 a | 0.12 a | 0.01 a |

† See Table 2 for additional information on sample dates.

‡ Values marked with the same letter within columns and within nitrate or ureide N components are not significantly different (Tukey Test, $\alpha = 0.05$).

treatment interactions for shoot $NO_3$–N ($P = 0.0001$) and ureide-N ($P = 0.0001$) suggest that shoot $NO_3$–N and shoot ureide-N each responded differently to N fertilizer treatments across sample dates. Shoot $NO_3$–N concentrations in the high N fertilizer treatment were significantly greater than those in the no N input treatment for the first three sample dates (from R1 until the early R3 development stages) as well as those in the intermediate N fertilizer treatment for the first two sample dates (R1 and R2 development stages; Table 3). There were no significant N fertilizer treatment effects on shoot $NO_3$–N concentration beyond the early R3 development stage (Table 3). Because soil-derived N is translocated from roots to shoots in the $NO_3$–N form (McClure and Israel, 1979; Osborne and Riedell, 2006b), it was not unexpected to observe transient increases in shoot $NO_3$–N in plants grown in soil that was treated with higher levels of starter fertilizer N.

In contrast, soybean shoot ureide-N concentrations were significantly less in the high N starter fertilizer treatments than the no N starter fertilizer treatments for sample dates 3, 4, and 6 (from the late R2, R3, and R5 soybean development stages; Table 3). The high N starter fertilizer treatment also had lower ureide-N concentration than the intermediate N treatment at sample date 7 (late R5 soybean development stage; Table 3). There were no significant effects of N fertilizer treatments on shoot ureide-N concentration for the remaining sample dates. These results support and extend the findings of Diatloff et al. (1991) and Yinbo et al. (1997) who reported that application of fertilizer N depressed ureide levels when sampling occurred during early flowering but not when sampling occurred during late flowering.

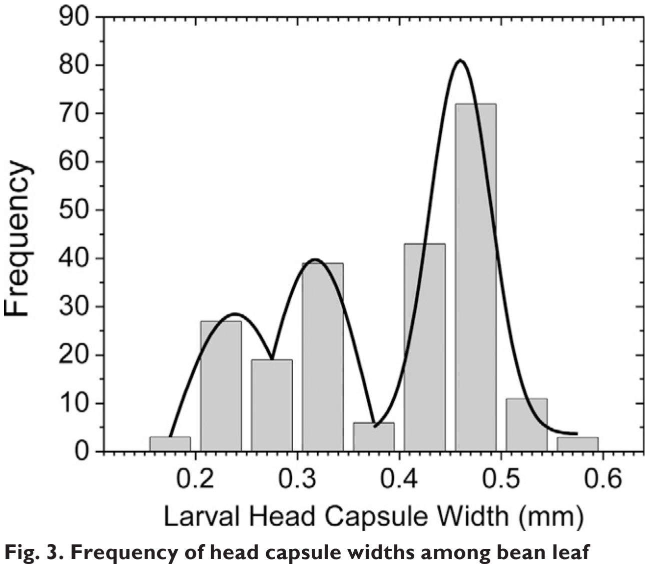

**Fig. 3. Frequency of head capsule widths among bean leaf beetle larvae recovered from soil cores taken across N fertilizer treatments during the 2006 and 2007 growing seasons. Multiple Gaussian curves were fit to the histograms using a nonlinear least squares regression method.**

## Bean Leaf Beetle Larval Morphology and Phenology

Overwintering adult BLB become active in early spring, mid- to late May in southern Minnesota and eastern South Dakota (Loughran and Ragsdale, 1986; Hammack et al., 2010), mate, move first to spring forages (alfalfa [*Medicago sativa* L.] and sweet clover [*Melilotus* spp.]) and later to emerging soybeans where they feed on soybean shoot tissue and begin laying eggs (Higley and Boethel, 1994). Eggs are laid within about 8 cm of the plant stem in the upper 5 cm of soil profile (Kogan et al., 1980). After hatching, insects, as larvae, feed on soybean roots and nodules, pupate, and emerge from the soil as adults.

A frequency graph (Hammack et al., 2003) of head capsule widths for larvae obtained from soil samples across all N input treatments and years of the study ($n = 226$) revealed three separate size categories that were assumed to differentiate three larval stages (Fig. 3). Based on frequency distribution, we considered head capsule widths from first, second, and third stage larvae to range from 0.170 mm to 0.290, 0.291 to 0.409, and 0.410 to 0.580 mm, respectively. There are no published data on BLB head capsule widths available. However, these head capsule size ranges are similar to those reported for larvae of a closely related Coleopteran species, the western corn rootworm (*Diabrotica virgifera vigifera*; Hammack et al., 2003).

When larval stage (Fig. 3) was considered an independent factor, statistical analysis revealed a significant ($P = 0.0008$) effect of larval stage on the number of larvae extracted from the soil. A mean ± SEM value of 0.15 ± 0.04 larvae $kg^{-1}$ of soil occurred for first stage larvae whereas that for second stage was 0.13 ± 0.02 larvae $kg^{-1}$ of soil and for third stage was 0.41 ± 0.06 larvae $kg^{-1}$ of soil. These results support and extend those of Anderson and Waldbauer (1977) who, using a brine floatation technique to separate BLB larvae from soil, found 1.7-fold greater numbers of third stage larvae compared to second and first stage larvae in soil samples (5.1 by 5.1 cm to a depth of 7.6 cm) which were centered directly over the plant row.

Larval head capsule width, as well as body length, and body diameter were also significantly different across larval stages.

Head capsule width (mean ± SEM) for first stage larvae was 0.232 ± 0.003 mm, second stage was 0.338 ± 0.004 mm, and third stage was 0.470 ± 0.004 mm. These values are similar to those of western corn rootworm larvae (Hammack et al., 2003). Body length for first stage BLB larvae was 2.25 ± 0.23 mm, 4.14 ± 0.15 mm for second stage, and 8.14 ± 0.22 mm for third stage. First stage BLB larvae had a body diameter of 0.34 ± 0.03 mm, while second stage body diameter was 0.58 ± 0.02 mm, and third stage body diameter was 1.13 ± 0.03 mm.

A significant year × stage interaction ($P = 0.03$) for larval body diameter suggests that larval stages responded differently across the 2 yr of the study. Body diameters of first and second stage larvae were not significantly different across years, but third stage larvae were significantly smaller in 2006 than in 2007 (Fig. 4). A trend ($P = 0.09$) for the year × stage interaction for larval body length also suggested that first and second stage larvae were similar in size between years but that third stage larvae were shorter in 2006 (7.91 ± 0.25 mm) than in 2007 (8.95 ± 0.41 mm). Thus, larval body diameter and length data reported in the current study suggest that third stage larvae had better growth in the 2007 growing season which was hotter and dryer than the 2006 growing season.

In 2006, larvae found in the soil over the course of the growing season had two distinct population peaks; the first during the R1 soybean development stage (about DOY 184–195) and the second during the R4 through R7 stages from about DOY 219 to 263 (Fig. 5). A more uniform population distribution over time was observed in 2007 (Fig. 5). Larval data, which show a bimodal frequency in 2006, would seem to support the contention that, in the northern U.S. soybean growing regions, BLB have two generations per year (Hammack et al., 2010) while the more uniform larval frequency data from 2007 do not.

### Nitrogen Fertilizer and Bean Leaf Beetle Larvae

Bean leaf beetle larvae were first observed in soil samples at the R1 soybean development stage (DOY 184, 3 July 2006 and DOY 176, 25 June 2007; Fig. 5). Larvae continued to be present in soil samples taken beyond the R6 soybean development stage (DOY 263, 20 Sept. 2006 and DOY 257, 14 Sept. 2007; Fig. 5). Thus, larvae were present in the soil for a total time period of about 79 d in 2006 and 81 d in 2007. These data confirm and extend the data of Riedell et al. (2005) who observed larvae in soil samples for a period of 68 d from the R1 through R7 soybean development stage. There were no significant effects of N fertilizer treatment, year, N fertilizer treatment × year interactions, or N fertilizer treatment by larval stage interactions on the total number of larvae.

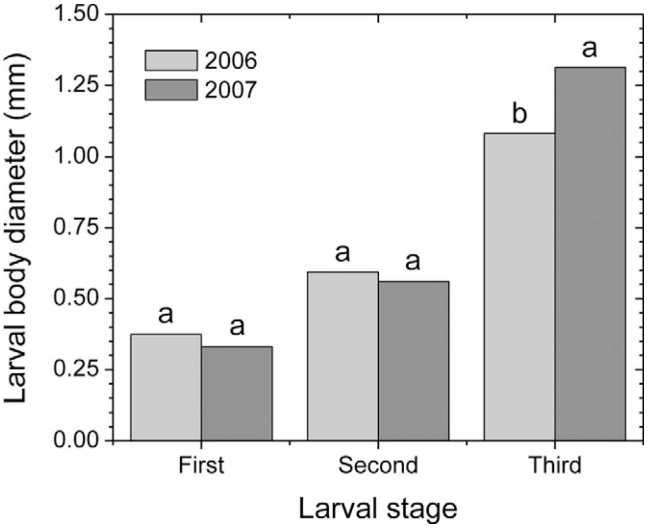

**Fig. 4. Larval body diameter across all N treatments within each larval stage and year of the 2 yr study. Bars marked with the same letter are not significantly different within larval stage.**

Larval head capsule width, larval body length, and body diameter were not significantly affected by N fertilizer treatment, year, or two-way interactions of N fertilizer treatment by year or N fertilizer treatment by larval stage. In western corn rootworm, larval head capsule width is thought to indicate larval development stage, but larval body size is thought to represent the growth of these larval stages (Chege et al., 2005). Thus, larval head capsule width and body size data reported in the current study suggest that fertilizer N input treatments had no effect on the larval development or the growth of the larvae. In contrast, Riedell et al. (2005) observed that high starter N fertilizer treatments (16 kg N ha$^{-1}$) were detrimental to larval growth when compared with the intermediate (8 kg N ha$^{-1}$) and no N fertilizer treatments. These data were obtained from a single growing season that was characterized by unusually warm temperatures, late soybean planting (27 May 2005), higher BLB recoveries, and no significant N fertilizer treatment effects on shoot ureide-N concentrations (Riedell et al., 2005).

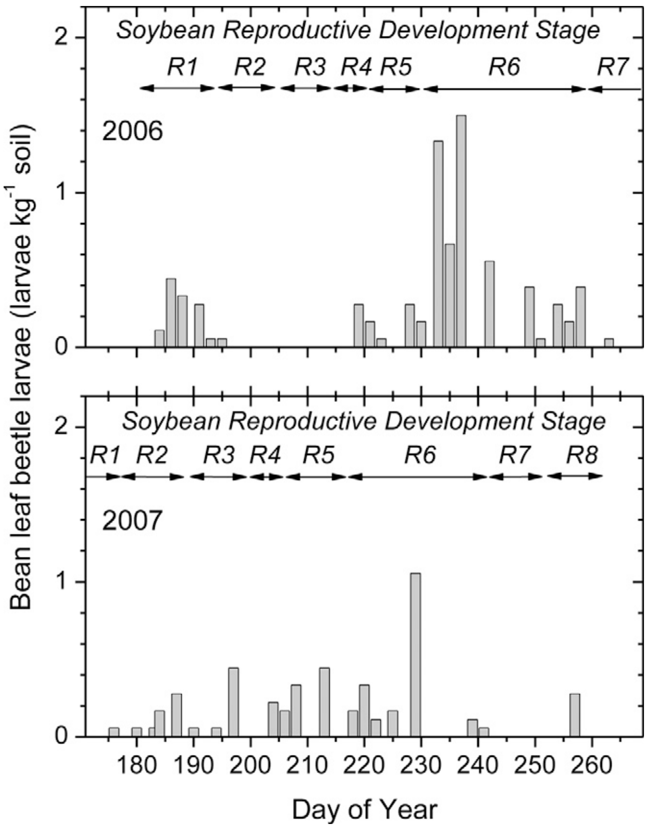

**Fig. 5. Bean leaf beetle larvae obtained from plots across all N input levels within each year of the experiment. Bars represent the total number of larvae kg$^{-1}$ of soil. Larval sampling was initiated in late June (DOY 181 in 2006 and DOY 171 in 2007) and ended in mid-September (DOY 263 in 2006 and DOY 257 in 2007).**

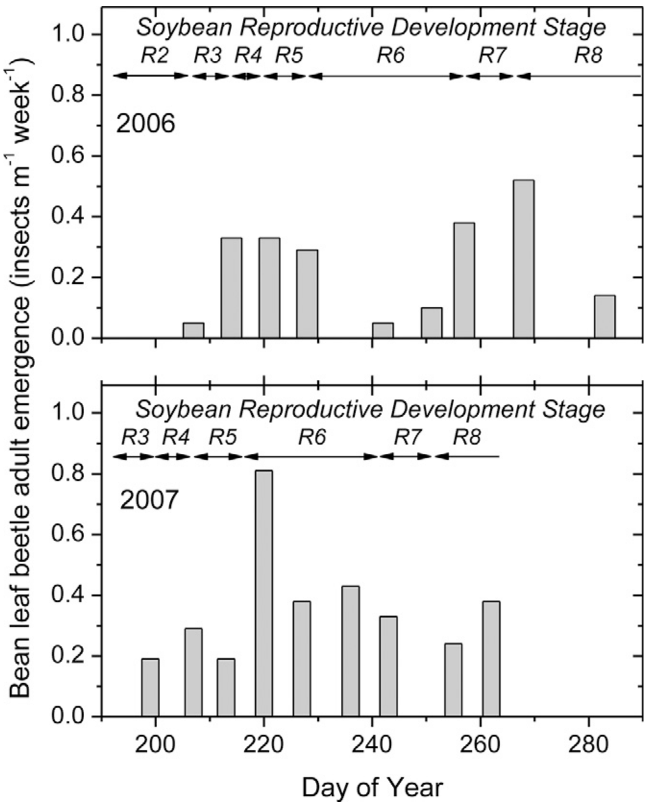

**Fig. 6. Bean leaf beetle adults that emerged from plots across all N input levels within each year of the experiment. Bars represent the total number of adults m$^{-1}$ of row wk$^{-1}$. Adult sampling was initiated on 6 July 2006 (DOY 187) and 20 June 2007 (DOY 171) and ended on 10 Oct. 2006 (DOY 283) and 19 Sept. 2007 (DOY 262).**

## Bean Leaf Beetle Adult Phenology and Morphology

Bean leaf beetle adults first emerged from the soil at the R2 to R3 soybean development stage (DOY 207, 26 July 2006 and DOY 199, 18 July 2007) and continued to emerge from the soil until the R8 soybean development stage (DOY 283, 10 Oct. 2006 and DOY 262, 19 Sept. 2007; Fig. 6). Over the course of the growing seasons, the total number of adults collected from all cages was 47 in 2006 and 69 in 2007. These results confirm the observations of Loughran and Ragsdale (1986) and Hammack et al. (2010) who, using sweep-net sampling procedures in southern Minnesota and eastern South Dakota, reported that newly emerged adults were first discovered in soybean plots during July and were present through harvest.

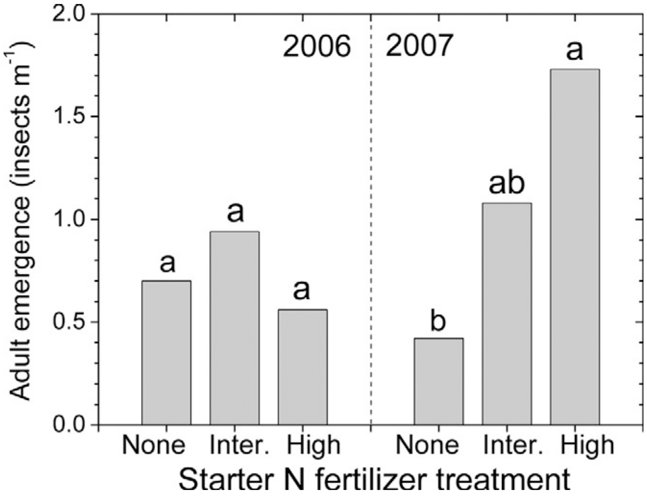

**Fig. 7. Bean leaf beetle adults obtained from emergence cages in plots within N starter fertilizer treatments and within each year of the 2-yr experiment.**

The number of adults m$^{-1}$ of row that emerged over the course of the two growing seasons reveals two distinct adult emergence peaks in 2006 and a more uniform emergence distribution over time in 2007 (Fig. 6). In 2006, adults comprising the first generation peak were emerging from the soil during the R2 to R5 soybean development stages (about DOY 207–230) while the second generation was present during the R6 through R8 stages from about DOY 242 to 283. These results are similar to those of Waldbauer and Kogan (1976) and Kogan et al. (1974) who reported, using sweep-net sampling, two distinct population peaks of adult BLB; first generation adults were present from July (DOY 180–196) to mid-August (DOY 220–230) and second generation during September (DOY 244) to harvest.

In the present study, about 38 d separated the initiation of first generation emergence from that of the second generation in 2006 (Fig. 6). This number approximates first to second generation times of 46 d in eastern South Dakota (Hammack et al., 2010) and 40 d in eastern Nebraska (Witkowski and Echtenkamp, 1996). Because the rate of soil GDD accumulation was greater in 2007 than 2006 (Fig. 1) and insect development proceeds faster at higher temperatures, it can be postulated that first to second adult generation time would be less in 2007 than 2006. In 2007, initial emergence of first generation adults occurred on DOY 199 (Fig. 6). Given a generation time of 38 d in 2006, second generation adults should have begun to emerge sooner than DOY 237 in 2007. Thus, we speculate that the break between first and second generations occurred between DOY 227 and 236 in 2007 (Fig. 6).

When data from both adult generations across both years were analyzed, year had no significant main effects on adult head capsule width. A significant year × generation interaction ($P = 0.001$) suggested that head capsule width in the two adult generations responded differently to year. In first generation adults, but not in the second, head capsule widths were smaller in 2006 (1.27 ± 0.02 mm) than in 2007 (1.34 ± 0.01 mm). Because increased beetle head capsule width is related to increased fitness in western corn rootworm adults (Oyediran et al., 2004), these data suggest that first generation BLB that emerged from soil in 2006 had less fitness than those that emerged in 2007. This suggestion is supported by a year × generation interaction trend ($P = 0.09$) whereby another morphological measure positively related to adult fitness, hind tibia length (Li et al., 2009), was lower in first generation adults in 2006 (1.78 ± 0.03 mm) than in 2007 (1.85 ± 0.02 mm).

The potential reasons for this increased first generation adult vigor under drought conditions are not readily apparent. Soil conditions when larvae that gave rise to first generation adults were feeding on soybean roots and nodules (DOY 184–195 in 2006; DOY 176–197 in 2007; Fig. 3) were characterized by intensifying soil drying and increasing soil temperature for both years but with 2006 being less dry and slightly cooler (slower rate of GDD accumulation) than 2007 (Fig. 1). Thus, it may be that BLB first generation larvae, when they experience drought conditions of severe soil drying and warm soil temperatures, may have a faster growth rate that in turn may give rise to more vigorous first generation adults than those larvae who experience less intense soil drying and cooler soil temperatures. Additionally, the physiological processes associated with N fixation are generally decreased in response to water deficit (Purcell and King, 1996). Nitrogen fixing activities are more likely to continue under water-deficit conditions in larger 4-mm-diam. root nodules than with smaller 2-mm-diam. nodules (King and Purcell, 2001). Approximately 25% of the volume of a 2-mm-diam. nodule is comprised of tissue infected with N-fixing bacteria whereas a 4-mm-diam. nodule has about 60% infected tissue (Weisz and Sinclair, 1988). Because larvae will feed on the nodule internal matrices (Lundgren and Riedell, 2008) and because larvae prefer to feed within nodules under drought conditions (Marrone and Stinner, 1984), we speculate that larger diameter nodules under drought conditions were more nutritious to larvae that developed into first generation adults. This speculation is supported by our observation of increased larval body size (Fig. 4) in the hotter and dryer 2007 growing season than in 2006.

### Nitrogen Fertilizer and Bean Leaf Beetle Adults

The total number of adults that emerged from the soil was significantly ($P = 0.05$) affected by N fertilizer treatments. Soil under the high N fertilizer treatment produced a mean ± SEM of 1.15 ± 0.41 insects $m^{-1}$ of row, whereas intermediate N produced 1.01 ± 0.31 and no N treatment produced 0.56 ± 0.16. However, a significant N fertilizer × year interaction ($P = 0.02$) suggested that adult emergence responded differently to N fertilizer treatments across the 2 yr of the study. In 2006, N starter fertilizer had no significant effects on adult emergence (Fig. 7). In 2007, significantly greater numbers of adults emerged from plots given the high N fertilizer treatments than from the no N fertilizer plots whereas adult emergence for the intermediate N fertilizer treatment was intermediate between the other two fertilizer treatments (Fig. 7). These results suggest that during a hot and very dry growing season, but not during slightly cooler and less dry growing season, increasing amounts of N fertilizer applied as starter increased the number of adult BLB that emerged from soybean plots.

When data for all adults from both years were analyzed, there were no significant fertilizer N treatment effects and no significant two-way fertilizer by generation or fertilizer × year interactions on adult head capsule width. In contrast, adult hind tibia length was significantly ($P = 0.02$) larger in the high and intermediate N fertilizer treatments than the no N treatment (1.86 ± 0.01 mm for high N fertilizer, 1.85 ± 0.02 mm for intermediate N, and 1.79 ± 0.03 mm for no N). There were no significant two-way fertilizer by generation or fertilizer × year interactions on adult hind tibia length. In most insects, increased hind tibia lengths are positively correlated with increased lifetime fecundity in young female adults as well as with increased life span in males (Honěk, 1993; Li et al., 2009). Extrapolating these findings to BLB adults suggests that high and intermediate N fertilizer treatment may have resulted in increased fecundity and lifespan in adult BLB.

## CONCLUSIONS

Shoots of plants fertilized with high-N fertilizer contained greater concentrations of soil-derived $NO_3$–N during the R1 through R3 plant development stages compared with the no-N treatment whereas N-fixation-derived ureide-N concentrations were generally less in the high-N fertilizer N treatment than with the no-N treatment during R2 through R5 stages in plant development. Larvae that gave rise to BLB adults were feeding on plant root systems during the R1 through R7 soybean developmental stages across both years of the experiment Because of the demonstrated relationships between ureide concentrations, nodule morphology, and nodule N fixation capacity (McClure and Israel, 1979; Herridge et al., 1990), high-N fertilizer treatment used in the present study may have resulted in reduced nodule N dry weight and reduced N fixation during the time larvae were feeding on root systems while no-N fertilizer treatment may have had opposite effects. Because BLB larvae feed on soybean root nodules (Lundgren and Riedell, 2008), our hypothesis was that larval numbers and body size would have been reduced under high-N treatments and enhanced under no N. Thus, the finding that starter N fertilizer had no effect on larval numbers or body size was unexpected. We speculate that other factors were important in determining larval responses to N fertilizer treatments. For example, increases in N fixation lead to increased carbohydrate concentrations that are several fold greater in nodules than other plant organs (Streeter, 1980). For western corn rootworm, Pleau et al. (2002) found that larval mass declined precipitously as carbohydrate concentration in artificial diets was increased above an optimum level. In the current study, perhaps increased nodule carbohydrate levels nullified potential positive effects of enhanced nodule size and quality in the no-N treatment on larval numbers and fitness. Additional research is needed to confirm these speculations.

In contrast to the results observed for larvae, high N starter fertilizer increased BLB adult emergence and adult fitness (increased hind tibia length) during the hot and very dry 2007 growing season. We speculate that increasing starter fertilizer N under drought conditions changed soybean physiology and morphology which, in turn, positively impacted BLB adults. Additional research is needed to further elucidate the exact physiological mechanisms involved in these responses. Regardless of the mechanisms involved, however, our data suggest that increasing N levels in starter fertilizer may interact with growing season environments to increase adult emergence and adult fitness, which in turn may increase populations and intensify the damage caused by this insect pest in soybeans during hot and dry growing seasons.

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## REFERENCES

Anderson, T.E., and G.P. Waldbauer. 1977. Development and field testing of a quantitative technique for extracting bean leaf beetle larvae and pupae from soil. Environ. Entomol. 6:633–636.

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. Commun. Soil Sci. Plant Anal. 6:71–80. doi:10.1080/00103627509366547

Chege, P.G., T.L. Clark, and B.E. Hibbard. 2005. Alternate host phenology affects survivorship, growth, and development of western corn rootworm (Coleoptera: Chrysomelidae) larvae. Environ. Entomol. 34:1441–1447. doi:10.1603/0046-225X-34.6.1441

Diatloff, A., R.J. Redden, and D.F. Herridge. 1991. Correlation between xylem ureide levels and nodulation in field-grown *Phaseolus vulgaris*. Aust. J. Exp. Agric. 31:679–682. doi:10.1071/EA9910679

Fisher, J.R. 1980. A modified emergence trap for quantitative adult corn rootworm studies (Coleoptera: Chrysomelidae). J. Kans. Entomol. Soc. 53:363–366.

Fujihara, S., and M. Yamaguchi. 1978. Probable site of allantoin formation in nodulating soybean plants. Phytochemistry 17:1239–1243. doi:10.1016/S0031-9422(00)94564-3

Gelderman, R., R. Neal, S. Swartos, and L. Anderson. 1987. Soil testing procedures in use at the South Dakota State Soil Testing Laboratory. Pamphlet 101. Plant Sci. Dep., South Dakota State Univ., Brookings.

Hammack, L., M.M. Ellsbury, R.L. Roehrdanz, and J.L. Pikul, Jr. 2003. Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 96:1153–1159. doi:10.1603/0022-0493-96.4.1153

Hammack, L., J.L. Pikul, Jr., and M.S. West. 2010. Phenology and abundance of bean leaf beetle (Coleoptera: Chrysomelidae) in eastern South Dakota on alfalfa and soybean relative to tillage, fertilization and yield. Environ. Entomol. 39:727–737. doi:10.1603/EN09153

Herridge, D.F., F.J. Bergersen, and M.B. Peoples. 1990. Measurement of nitrogen fixation by soybean in the field using the ureide and natural $^{15}$N abundance methods. Plant Physiol. 93:708–716. doi:10.1104/pp.93.2.708

Herridge, D.F., and M.B. Peoples. 1990. Ureide assay for measuring nitrogen fixation by nodulated soybean calibrated by $^{15}$N methods. Plant Physiol. 93:495–503. doi:10.1104/pp.93.2.495

Higley, L.G., and D.J. Boethel. 1994. Handbook of soybean insect pests. Entomological Soc. of Am. Annapolis, MD.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: A general relationship. Oikos 66:483–492. doi:10.2307/3544943

King, C.A., and L.C. Purcell. 2001. Soybean nodule size and relationship to nitrogen fixation response to water deficit. Crop Sci. 41:1099–1107. doi:10.2135/cropsci2001.4141099x

Kogan, M., W.G. Ruesink, and K. McDowell. 1974. Spatial and temporal distribution patterns of the bean leaf beetle, *Cerotoma trifurcata* (Forster), on soybeans in Illinois. Environ. Entomol. 3:607–617.

Kogan, M., G.P. Waldbauer, G. Boiteau, and C.E. Eastman. 1980. Sampling bean leaf beetles on soybean. p. 201–236. *In* M. Kogan and D.C. Herzog (ed.) Sampling methods in soybean entomology. Springer, New York.

Li, H., S. Toepfer, and U. Kuhlmann. 2009. Relationship between phenotypic traits and selected fitness components of *Diabrotica virgifera virgifera*. Entomol. Exp. Appl. 131:254–263. doi:10.1111/j.1570-7458.2009.00856.x

Littell, R.C., G.A. Milliken, W.W. Stroup, R.D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models, 2nd ed. SAS Inst., Cary, NC.

Loughran, J.C., and D.W. Ragsdale. 1986. Life cycle of the bean leaf beetle, *Cerotoma trifurcaae* (Coleoptera: Chrysomelidae), in southern Minnesota. Ann. Entomol. Soc. Am. 79:34–38.

Lundgren, J.G., and W.E. Riedell. 2008. Soybean nitrogen relations and root characteristics after *Cerotoma trifurcata* (Coleoptera:Chrysomelidae) larval feeding injury. J. Entomol. Sci. 43:107–116.

Marrone, P.G., and R.E. Stinner. 1984. Influence of soil physical factors on survival and development of the larvae and pupae of the bean leaf beetle, *Certoma trifurcata* (Coleoptera: Chrysomelidae). Can. Entomol. 116:1015–1023. doi:10.4039/Ent1161015-7

Maursetter, J.M., T.E. Schumacher, G.D. Lemme, and M.J. Lindstrom. 1992. Final report on the initial soil properties of the Eastern South Dakota Soil and Water Research Farm. Plant Sci. Dep., South Dakota State Univ., Brookings.

McClure, P.R., and D.W. Israel. 1979. Transport of nitrogen in the xylem of soybean plants. Plant Physiol. 64:411–416. doi:10.1104/pp.64.3.411

McConnell, W.R. 1915. A unique type of insect injury. J. Econ. Entomol. 8:261–266.

National Agriculture Statistics Service. 2010. Data and statistics. Available at http://www.nass.usda.gov/Data_and_Statistics/ (accessed 1 Oct. 2010; verified 25 June). USDA-NASS, Washington, DC.

OriginLab Corp. 1999. Origin user's manual, version 6. OriginLab Corp., Northampton, MA.

Osborne, S.L., and W.E. Riedell. 2006a. Starter nitrogen fertilizer impact on soybean yield and quality in the northern Great Plains. Agron. J. 98:1569–1574. doi:10.2134/agronj2006.0089

Osborne, S.L., and W.E. Riedell. 2006b. Soybean growth response to low rates of nitrogen applied at planting in the northern Great Plains. J. Plant Nutr. 29:985–1002. doi:10.1080/01904160600686007

Oyediran, I.O., B.E. Hibbard, and T.L. Clark. 2004. Prairie grasses as hosts of western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 33:740–747. doi:10.1603/0046-225X-33.3.740

Patterson, T.G., R. Glenister, and T.A. LaRue. 1982. Simple estimate of ureides in soybean tissue. Anal. Biochem. 119:90–95. doi:10.1016/0003-2697(82)90670-4

Pikul, J.L., Jr., L. Carpenter-Boggs, M. Vigil, T.E. Schumacher, M.J. Lindstrom, and W.E. Riedell. 2001. Crop yield and soil condition under ridge and chisel-plow tillage in the northern Corn Belt, USA. Soil Tillage Res. 60:21–33. doi:10.1016/S0167-1987(01)00174-X

Pleau, M.J., J.E. Huesing, G.P. Head, and D.J. Feir. 2002. Development of an artificial diet for the western corn rootworm. Entomol. Exp. Appl. 105:1–11. doi:10.1046/j.1570-7458.2002.01027.x

Purcell, L.C., and C.A. King. 1996. Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. J. Plant Nutr. 19:969–993. doi:10.1080/01904169609365173

Ray, J.D., L.G. Heatherly, and F.B. Fritschi. 2006. Influence of large amounts of nitrogen on nonirrigated and irrigated soybean. Crop Sci. 46:52–60. doi:10.2135/cropsci2005.0043

Riedell, W.E., J.G. Lundgren, S.L. Osborne, and J.L. Pikul, Jr. 2005. Effects of soil nitrogen management on soybean nitrogen relations and bean leaf beetle (Coleoptera: Chrysomelidae) biology. J. Agric. Urban Entomol. 22:181–190.

Riedell, W.E., J.L. Pikul, Jr., A.A. Jaradat, and T.E. Schumacher. 2009. Crop rotation and nitrogen input effects on soil fertility, maize mineral nutrition, yield, and seed composition. Agron. J. 101:870–879. doi:10.2134/agronj2008.0186x

Ritchie, S.W., J.J. Hanway, H.E. Thompson, and G.O. Benson. 1997. How a soybean plant develops. Spec. Rep. 53. Iowa State Univ. Coop. Ext. Serv., Ames.

Salvagiotti, F., K.G. Cassman, J.E. Specht, D.T. Walters, A. Weiss, and A. Dobermann. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Field Crops Res. 108:1–13. doi:10.1016/j.fcr.2008.03.001

Salvagiotti, F., J.E. Specht, K.G. Cassman, D.T. Walters, A. Weiss, and A. Dobermann. 2009. Growth and nitrogen fixation in high-yielding soybean: Impact of nitrogen fertilization. Agron. J. 101:958–970. doi:10.2134/agronj2008.0173x

Streeter, J.G. 1980. Carbohydrates in soybean nodules: II. Distribution of compounds in seedlings during the onset of nitrogen fixation. Plant Physiol. 66:471–476. doi:10.1104/pp.66.3.471

Thomas, R.J., and L.E. Schrader. 1981. Ureide metabolism in higher plants. Phytochemistry 20:361–371. doi:10.1016/S0031-9422(00)84147-3

USDA NRCS Soil Series Search Query Facility. 2009. Barnes series. Available at http://soilseries.sc.egov.usda.gov/OSD_Docs/B/BARNES.html (accessed 1 Oct. 2010; verified 1 July 2011). NRCS Soil Survey Div., Washington, DC.

van Berkum, P., C. Sloger, D.F. Wever, P.B. Cregan, and H.H. Keyser. 1985. Relationship between ureide N and $N_2$ fixation, aboveground N accumulation, acetylene reduction, and nodule mass in greenhouse and field studies with *Glycine max* L. (Merr). Plant Physiol. 77:53–58. doi:10.1104/pp.77.1.53

Waldbauer, G.P., and M. Kogan. 1976. Bean leaf beetle: Phenological relationship with soybean in Illinois. Environ. Entomol. 5:35–44.

Weisz, P.R., and T.R. Sinclair. 1988. A rapid non-destructive assay to quantify soybean nodule gas permeability. Plant Soil 105:69–78. doi:10.1007/BF02371144

Witkowski, J.F., and G.W. Echtenkamp. 1996. Influence of planting date and insecticide on the bean leaf beetle (Coleoptera: Chrysomelidae) abundance and damage in Nebraska soybean. J. Econ. Entomol. 89:189–196.

Yinbo, G., M.B. Peoples, and B. Rerkasem. 1997. The effect of N fertilizer strategy on $N_2$ fixation, growth and yield of vegetable soybean. Field Crops Res. 51:221–229. doi:10.1016/S0378-4290(96)03464-8