INITIAL STUDIES ON SOIL NITROGEN MANAGEMENT, SOYBEAN NITROGEN RELATIONS,AND BEAN LEAF BEETLE BIOLOGY

W.E. Riedell, J.G. Lundgren, S.L. Osborne, and J.L. Pikul, Jr.
USDA, ARS, North Central Agricultural Research Laboratory, Brookings, SD 57006.
wriedell@ngirl.ars.usda.gov 605-693-5207

ABSTRACT

Bean leaf beetles (*Cerotoma trifurcata*) are serious insect pests of soybeans (*Glycine max*). This study was conducted to determine if soil nitrogen (N) input treatments would impact the biology of this emerging pest species. The experiment was conducted in the soybean phase of a long-term corn/soybean rotation study near Brookings SD. Soil N input treatments were: corn fertilized for a yield goal of 8.5 Mg ha^{-1} (high N input), 5.3 Mg ha^{-1} (medium N input), or corn not fertilized (no N input). Corn and soybean plots also were treated with banded starter fertilizer (112 kg ha^{-1}) as 14-16-11, 7-16-11, or 0-16-11 elemental N-P-K on the high N, medium N, and no N input treatments, respectively. Soybeans grown on high N input plots showed similar shoot ureide-N concentrations at all development stages studied. Bean leaf beetle larvae were present in the soil from just prior to the beginning flowering stage until the beginning maturity stage (about 65 days). There were no consistent N input treatment effects observed in the cumulative number of larvae found in the plots. Our findings suggest that the soil N management treatments imposed during the 2005 growing season had little effect upon ureide-N concentration, which in turn suggests that there were few differences in soybean root nodulation and atmospheric N fixation across treatments. Thus it was not surprising to find that there were no major effects of soil N management treatments on bean leaf beetle larval populations

INTRODUCTION

The bean leaf beetle is a soybean insect pest of increasing in economic importance in the north central U.S. (Witkowski and Echtenkamp, 1996). Lifecycle studies (Higley and Boethel, 1994) indicate that it overwinters as an adult beetle in undisturbed areas (shelterbelts and grass borders) around soybean fields. Beetles leave overwintering sites in early April, mate, move to spring legumes (alfalfa and sweet clover) and then to emerging soybean. Beetles feed upon soybean shoot tissue and begin laying eggs. The eggs are laid within about 8 cm of the plant stem in the upper 5 cm of soil profile (Kogan et al., 1980). A female normally lives about 40 days and lays 125 to 250 eggs. Overwintered adults begin to die as egg laying is completed.

Eggs hatch in about 10 days (depending on soil temperature) and the resulting larvae, which have three distinct developmental stages, live in the soil where they feed upon soybean roots and root nodules for 3 - 4 wks (Herzog et al., 1974). Larvae then pupate and adults emerge from the soil about a week later. Total developmental time from egg to adult normally ranges from 25 to 40 days. There are two generations per year in Nebraska (Hunt et al., 1994) and east-central South Dakota (Hammack and Pikul, 2003).

Economic thresholds for bean leaf beetle on soybean have been established on the basis of damage to above-ground parts of soybean plants (Hunt et al. 1994). The economic importance of larval feeding on roots and nodules is not fully understood, although this kind of injury is likely to have a serious impact on yields (Kogan et al. 1980). The literature contains only a few reports on the effects of bean leaf beetle larval feeding on below-ground organs of soybeans. Newsom et al. (1978) reported that nodule destruction by larvae reduced N fixation two fold. Larval feeding damage to soybean nodules by a related species, *Cerotoma arcuata,* was more deleterious to plant growth and yield than was defoliation by adults (Teixeira et al. 1996).

In the northern Great Plains, N starter fertilizer applied to soybeans planted in late May reduced ureide levels (ureides are indicators of N fixation; van Berkum et al., 1985) through mid July (Osborne and Riedell, 2004). Because soybean root nodules are an important food source for bean leaf beetle larvae (Marrone and Stinner, 1983) and because first generation adult beetles begin to emerge from soybean fields in mid-July, it is possible that soil management tactics that affect soybean nodulation, such as starter N application, could have an effect on bean leaf beetle biology which in turn may impact yield production. Our objective was to investigate the potential relationships between soil N management, soybean N relations, and bean leaf beetle biology.

MATERIALS AND METHODS

Experimental plots used in this study were established in 1990 on Barnes clay loam soil at the Eastern South Dakota Soil and Water Research Farm near Brookings SD. Soybean was grown in rotation with corn (*Zea mays*), and both crops were present each year. Starting in 1995, a chisel plow operation in the fall was the primary tillage. Seed beds were prepared each spring using a tandem disk and field cultivator. Nitrogen treatments were: corn phase of the rotation fertilized for a yield goal of 8.5 Mg ha^{-1} (high N input), 5.3 Mg ha^{-1} (medium N input), or corn not fertilized (no N input). In addition, corn and soybean crops were treated with starter fertilizer (2 x 2 band; 112 kg ha^{-1}) as 14-16-11, 7-16-11, or 0-16-11 elemental N-P-K on the high N, medium N, and no N plots, respectively. The long-term rotation study consisted of two crop types, three levels of N input, and three replications for a total of 18 plots. Each plot was 30 x 30 m. Additional information on experimental plots can be obtained from Pikul et al. (2001).

Spring tillage operations were conducted on soybean plots on early May. Plots were treated with herbicide (recommended rates of Dual II Magnum and Round Up Ultra Max) on 19 May 2005 (DOY 139). 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 27 May 2005 (DOY 149). Sensors to record soil moisture and temperature (15 cm depth) were installed in the plots on 9 June 2005 (DOY 160).

Soybean shoot samples (2 m of row from each plot) were taken on 11 July (DOY 192), 28 July (DOY 209), 8 August (DOY 220), 24 August (DOY 236), and 14 September (DOY 257). Shoot tissue was dried to a constant weight at 60 C, ground to pass a 2-mm screen in a Wiley mill, weighed, and analyzed for total N (Pikul et al., 2001), ureide-N (Patterson et al., 1981), and NO_3-N (Catalado et al., 1975). Resulting data were averaged within treatments by sample dates, the standard error of the mean was calculated, and the data plotted on graphs.

After field cultivation (8 July 2005; DOY 189), bean leaf beetle adult emergence cages (0.89 m long, 0.46 m wide, 0.15 m tall) were placed into plots (4 per plot) and checked every week for the presence of adult beetles. Beetles were removed from cages, counted, and stored in 70 % ethanol. Cages were moved to new locations in each field every two weeks. The cumulative numbers of beetles collected over the growing season within soil N management treatments were plotted.

To measure bean leaf beetle larval numbers, soil samples were taken from plots using a 10 cm diam. steel cylinder (golf cup cutter) pushed into the soil to a depth of 12.5 cm. The cup cutter was centered directly over the row, and portions of root system were also removed with the soil. Two soil cores were taken from each plot every 2 d. Soil from these samples was placed on a steel screen (1.4 x 1.9 mm mesh size) suspended over a pan of water. These larval separation devices were then placed under incandescent lights (12 bulbs, 75 watts each) for a period of at least 48 hr. Larvae were then removed from the water, counted, and stored in ethanol. Dried soil remaining on the screen was weighed and placed into plastic bags for later chemical analysis. The cumulative numbers of larvae collected over the growing season within soil N management treatments were plotted.

Sampling for adults began on 11 July (DOY 192) and ended on 3 October 2005 (DOY 276). Larval sampling began on 13 July (DOY 194) and ended on 23 September 2005 (DOY 266). Plots were harvested with a Massey Ferguson MF8-XP plot combine equipped with an electronic weigh bucket on 30 September 2005 (DOY 273). Seed moisture and test weight were measured using a Dickey-John GAC 2000 grain analysis system. Seed oil and protein were measured with a Foss NIR. Resulting data were subjected to analysis of variance procedures in SAS.

RESULTS

Climate conditions during May 2005 (14 out of 31 d receiving measurable precipitation; air temperatures 1.1 C below 30-yr average) were not conducive to field work and crop planting. Thus, planting of soybean in 2005 was delayed about 3 wk past the average sowing date (first week of May; Pikul et al., 2001). Once planted, however, soybean experienced excellent growing conditions (near normal precipitation; air temperatures of 3.5 and 2.5 C above average in June and July, respectively). Over the course of the experiment, soybean plots were relatively free of weeds, had only incidental infestations of soybean aphids, and had a fair amount of adult bean leaf beetle feeding damage. There were no major differences in shoot dry mass accumulation noted between N fertilization treatments through the R5 (beginning seed) development stage. Plants grown on high N input plots showed greater shoot dry mass than the other treatments at the R6 (full seed) and R7 (beginning maturity) stages (Fig. 1, top).

Time-course measurements of total N revealed concentrations of 3.6 % (medium N input treatment) to 3.8 % (high and no N input treatments) when measured just prior to the R1 (beginning bloom) development stage (Fig. 1, bottom). Total N concentrations then decreased linearly until the R6 stage followed by a slight increase after the R7 development stage (Fig. 1, bottom). There were no consistent N input treatment effects on total N beyond those noted at the R1 development stage.

Shoot NO_3-N concentrations remained nearly level from prior to the R1 until the R3 (beginning pod) development stage, with concentrations dropping off during the R5 through R7 development stages (Fig. 2, top). Of interest is the relatively higher NO_3-N concentrations recorded in the high and medium N input treatments as compared to the no N input treatment. In contrast to this, ureide-N concentrations gradually increased from just prior to R1 until the R5 development stages, after which the concentrations decreased (Fig 2, bottom). N input treatments had only minor effect upon ureide-N concentration, with the no N treatment showing only slightly higher concentrations during the first three sample dates.

Bean leaf beetle larvae were first found in the soybean plot cages just prior to the R1 stage (DOY 194; 13 July 2005; Fig. 3, top). Larvae were present in plots until the R7 stage, for a total time period of about 65 d. There were no biologically-meaningful N input treatment effects observed in the cumulative number of larvae found in the plots (Fig. 3, top). The first emergence of bean leaf beetle adults was observed when the soybean plants reached the R3 development

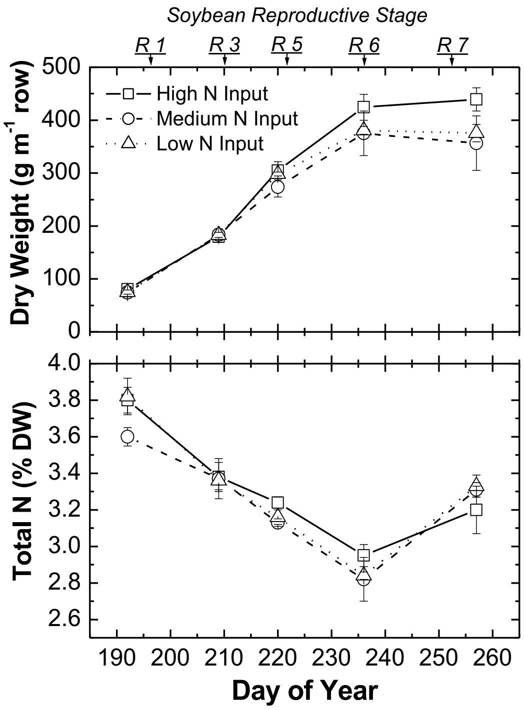

Figure 1. Dry weight (top) and total N (bottom) of shoot tissue samples taken from plots treated with different input N levels. Samples were taken on 11 July (DOY 192), 28 July (DOY 209), 8 August (DOY 220), 24 August (DOY 236), and 14 September (DOY 257). Symbols represent data means ± standard errors for three replicate samples per treatment.

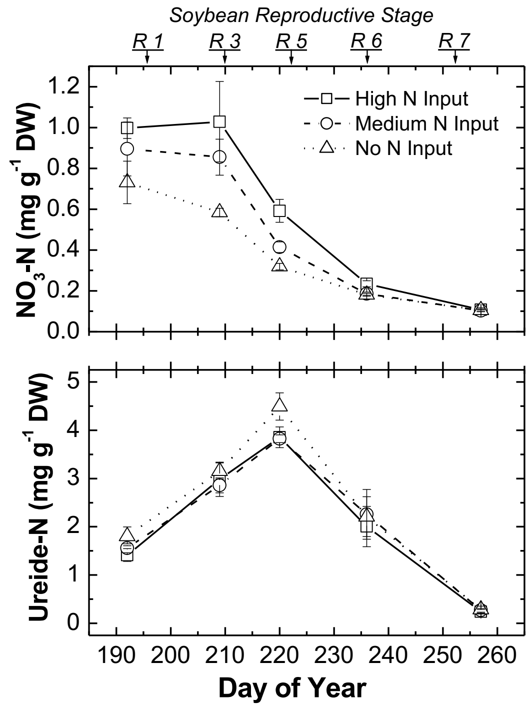

Figure 2. Concentrations of NO_3-N (top) and ureide-N (bottom) of soybean shoot tissue samples taken during the 2005 growing season from soybean plots treated with different input N levels. Symbols represent data means ± standard errors for three replicate samples per treatment.

stage (DOY 209; 28 July 2005; Fig. 3, bottom). Adults continued to emerge until after the plants reached the R8 (full maturity) development stage (DOY 276; 3 October 2005), for a total time period of about 70 days. The rate at which adults emerged from the high N input treatments appeared to be greater than the rates seen in the other treatments (Fig. 3, bottom).

There were no significant effects of N input treatment on soybean seed yield or seed quality (Table 1). Previous experiments conducted on these same long-term experimental plots documented significant effects of input N treatments on yield in 8 of the 11 years reported (Pikul et al., 2001). Average (over 11 yr) yields were: 2300, 1984, and 1797 kg ha^{-1} for the high H, medium N, and no N input treatments, respectively. Thus, the yield data recorded in 2005 represent an increase of 27, 48, and 58 % over the respective 11-yr averages.

DISCUSSION

Soybean plants accumulate N from two different sources: soil N and atmospheric N. Even with the ability to fix atmospheric N, however, soybean crops remove 150 to 200 kg N ha^{-1} from the soil (Varvel and Peterson, 1992). Atmospheric N_2 fixation, nodulation, and ureide-N concentrations decrease proportionally as NO_3 concentrations supplied to the plants are increased (van Berkum et al., 1985). In our experiment, due to the late planting of soybean and the above average air temperatures recorded during the first two months after planting, it appears that the

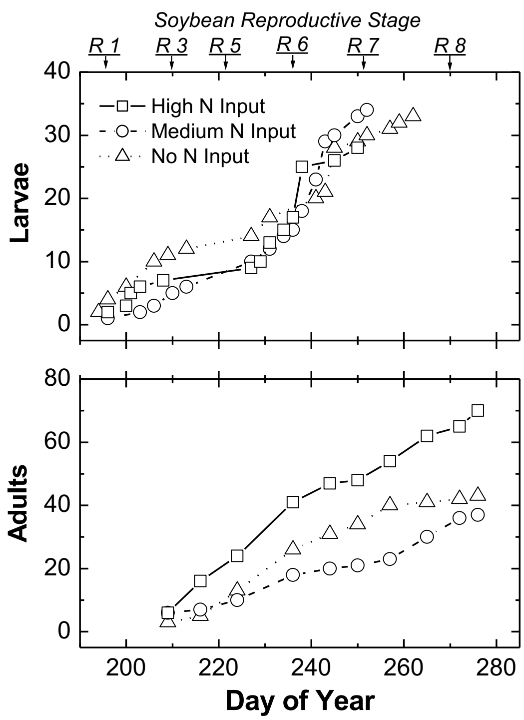

Figure 3. Cumulative numbers of bean leaf beetle larvae (left, top; larval number per 0.2 m^3 of soil volume) in soil samples or cumulative numbers of bean leaf beetle adults (left, bottom; adult number per 5 m^2 of soil surface area) that emerged during the 2005 growing season from soybean plots treated with different input N levels.

Table 1. Grain yield and seed quality of soybeans grown in annual rotation with corn on Barnes clay loam soil near Brookings SD in 2005. Prob. > *F* values represent the probability associated with the *F* statistic (ANOVA).

Treatment	Yield	Oil	Protein
	(kg ha^{-1})	*(percent)*	
High N	2926	19.5	37.3
Medium N	2933	19.4	37.5
No N	2845	19.5	37.3
Prob. > *F*	0.31	0.79	0.82

soybeans under the no N input treatment fixed atmospheric N at rates equal to the other treatments. Such a contention is supported by our ureide-N concentration data (Fig. 2, bottom) and our yield and seed quality data (Table 1) which showed no major differences across N input treatments. Osborne and Riedell (2004) also found that R1 ureide concentrations of late-planted soybeans (with 16 C soil temperature) were less than when soybeans were planted earlier (with 8-12 C soil temperature). Taken together, these data suggest that a critical soil NO_3 concentration was available to the plants in all of the N input treatments in 2005. This, in turn, caused the plants to decrease atmospheric N fixation and reduce accumulation of ureide-N in an equal manner across all input N treatments (see van Berkum et al., 1985). Additional data on soil NO_3 levels in this experiment are needed to confirm these speculations.

Our findings suggest that the soil N management treatments imposed during the 2005 growing season had little effect upon ureide-N concentration, which in turn suggests that there were few differences in soybean root nodulation and atmospheric N fixation across treatments. Thus it was not surprising to find that there were no major effects of soil N management treatments on bean leaf beetle larval populations (Fig. 3, top). In contrast, we did observe an increased rate of bean leaf beetle adult emergence from the high N treatment as compared to the other treatments (Fig. 3, bottom). These findings on adult beetles are in contrast to those of

Burleigh et al. (1980) and Hammack and Pikul (2003) who reported a significant trend of lower bean leaf beetle adult densities with increasing amounts of N fertilizer applied to soybeans. It is difficult to speculate as to the reasons why we obtained these results for adult emergence. Additional data from a more-normal soybean growing season are needed.

ACKNOWLEDGEMENTS

The authors thank J. Brandt, L. Draper, D. Hartman, D. Harris, M. Pravecek and K Dagel for excellent technical assistance and Drs. K. Dashiell, L. Hesler, and K. Rosentrater for critical review of an earlier version of this manuscript. The USDA, ARS offers its programs to all eligible persons regardless of race, color, age, sex, or national origin. Mention of a commercial or proprietary product does not constitute endorsement by the USDA.

REFERENCES

Burleigh, J.G., R.W. Katayama, and L. Hubbard. 1980. Effect of nitrogen fertilization on abundance of the bean leaf beetle in soybean. Arkansas Farm Res. 29:5.

Catalado, D.A., M. Haroon, L.E. Schrader, and V.L. Youngs. 1975. Rapid coloimetric determination of nitrate in plant tissue by nitration of salicylic acid. Comm. Soil Sci. Plant Anal. 6:71-80.

Hammack, L., and J.L. Pikul, Jr. 2003. Bean leaf beetle phenology and abundance in South Dakota soybeans. 2003 Annual Meeting Entomol. Soc. Am. Abstract D0359.

Herzog, D.C., C.E. Eastman, and D.L. Newsom. 1974. Laboratory rearing of the bean leaf beetle. J. Econ. Entomol. 67:794-795.

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

Hunt, T., J.F. Witkowski, R. Wright, K. Jarvi. 1994. The bean leaf beetle in soybeans. NebGuide #G90-974. Cooperative Extension, Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln. http://ianrpubs.unl.edu/insects/g974.htm

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 (eds.) Sampling methods in soybean entomology. Springer, NY.

Marrone, P. G., and R. E. Stinner. 1983. Bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae): physical factors affecting larval movement in soil. Environmental Entomology 12: 1283-1285.

Newsom, L.D., E.P. Dunigan, C.E. Eastman, R.L. Hutchinson, and R.M. McPherson. 1978. Insect injury reduces nitrogen fixation in soybeans. Louisiana Agriculture 21:15-16

Osborne, S.L., and W.E. Riedell. 2004. Effect of starter nitrogen fertilizer on nitrogen fixation of soybeans in the northern Great Plains. P. 145-149. In: A.J. Schlegel (ed.) Proc. Great Plains Soil Fert. Conf. 2-3 March 2004. Denver CO.

Patterson, T.G., G. Glenister, and T.A. LaRue. 1981. Simple estimate of ureides in soybean tissue. Anal. Biochem 119:90-95.

Pikul Jr., J.L., L. Carpenter-Boggs, M. Vigil, T.E. Schumacher, M.J. Lindstrom, 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.

Teixeira, M.L.F., H.L.C. Coutinho, and A.A. Franco. 1996. Effects of *Cerotoma arcuata* (Coleoptera: Chrysomelidae) on predation of nodules and on N fixation of *Phaseolus vulgaris*. J. Econ. Entomol. 89:165-169.

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*. Plant Physiol 77:53-58.

Varvel, G.E., and T.A. Peterson. 1992. Nitrogen fertilizer recovery by soybean in monoculture and rotation systems. Agron. J. 84:215-218.

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