Soybean Aphid Feeding Injury and Soybean Yield, Yield Components, and Seed Composition

Eric A. Beckendorf, Michael A. Catangui, and Walter E. Riedell*

ABSTRACT
Information that describes soybean aphid (Aphis glycines Matsumura) feeding injury effects on soybean [Glycine max (L.) Merr.] yield and seed composition is needed to develop better management practices for this invasive pest. This 2-yr controlled-infestation field study measured aphid populations and the effects of those populations on soybean seed yield, yield components (shoot biomass, pods plant−1, seeds pod−1, and 100 seed weight), and seed composition (oil and protein concentrations) when infested at the vegetative (V5) or reproductive (R2) development stages. In 2003, initial infestation rates of 10, 50, or 100 aphids plant−1 applied at V5 resulted in population peaks of 21,000, 18,000, and 12,000 aphids plant−1 and maximum cumulative aphid-days of 381,000, 327,000, and 242,000, respectively. In 2004, initial infestation rates of 1, 3, 10, 50, or 100 aphids plant−1 applied at V5 resulted in population peaks of 4,600, 9,400, 14,000, 22,000, and 21,000 aphids plant−1 and maximum cumulative aphid-days of 101,000, 229,000, 355,000, 514,000, and 537,000. In both years, the same infestation rates applied at R2 resulted in population peaks and cumulative aphid-day values that were about 42 to 88% lower than the V5 infestation dates. Seed yield, yield components, and seed oil concentration declined linearly as peak aphid numbers plant−1 and maximum cumulative aphid-days plant−1 increased. In contrast, seed protein concentration increased linearly with increasing peak aphid numbers plant−1. Relating these aphid population parameters at the plant growth stages studied enables producers to make informed decisions about the need for and timing of pest management treatments.

The soybean aphid, first reported to be in the United States in 2000, was detected in 21 U.S. states and 3 Canadian provinces by 2003 (Venette and Ragsdale, 2004). Because summer-reproducing female aphids reproduce asexually (parthenogenesis) and produce live young (viviparous), this invasive pest species has the ability to increase its population logarithmically in response to favorable environments (Takahashi et al., 1993). Winged female aphids are thought to be produced because of a combination of several stimuli including crowding (Lu and Chen, 1993), declining host plant quality (Hodgson et al., 2005), and changes in photoperiod and temperature (Ragsdale et al., 2004). Although winged aphids are not known to be strong flyers, individuals can travel great distances with the aid of jet-stream air currents (Dixon and Howard, 1986). Winged aphids may land on soybean, deposit a nymph, and move on in search of a new host, causing patchy infestations or "hot spots" within a field. In the fall of the year, males are produced and sexual reproduction takes place. Eggs produced from this sexual reproduction, which are deposited on buckthorn (Rhamnus cathartica L., or R. alnifolia L’Héritier), allow the insect to overwinter (Voegtlin et al., 2004).

Soybean aphids have piercing/sucking mouthparts that are used to remove phloem sap from stems, leaves, and pods. Even though initial feeding injury is not readily apparent, feeding by small populations can significantly affect photosynthesis (Macedo et al., 2003). As populations increase, feeding injury is manifest in small curled leaves, reduced number of branches, stuntimg, withered or shed flowers, and reduced pods (Wang et al., 1962; He et al., 1991). Large aphid populations produce copious amounts of honeydew, a sugary secretion that collects on leaves. Honeydew collects dust and dirt, and promotes a sooty mold growth, which in turn may also prevent light interception by leaves (He et al., 1991).

Accurate prediction of the level of yield loss caused by aphid feeding is considered to be the crux of integrated pest management for aphid pests (Kieckhefer et al., 1995). Despite the accumulating literature on the soybean aphid, there are currently few published data on the effects of soybean aphid populations on soybean yield and seed components for soybean grown in the United States. Thus, the objectives of this study were to quantify aphid populations and the injuries caused by those populations on plant biomass, seed yield, and components (total yield, pods plant−1, seeds pod−1, individual seed weight, oil concentration, protein concentration) in plants infested at the vegetative (V5) and reproductive (R2) development stage.


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Abbreviations: DOY, day of year; DM, dry matter; R stage, reproductive stage; V stage, vegetative stage.
MATERIALS AND METHODS

Soybean Field Conditions

Field studies were conducted in 2003 and 2004 on a Barnes clay loam soil (fine-loamy, mixed, superactive, frigid Calcid Hapludolls) at the Eastern South Dakota Soil and Water Research Farm near Brookings, SD. The plots used in this experiment were planted with winter wheat (Triticum aestivum L.) in the previous season. The 0.2 ha experimental site was tillaged using a chisel plow in the fall and a disk-harrow in the spring for seedbed preparation. Pioneer 91B91 Roundup Ready soybean seeds (Pioneer Hi-Bred International, Johnston, IA) were planted on 22 May 2003 and 27 May 2004 using a John Deere 7200 MaxEmerge 2 (Deere & Company, Moline, IL) planter. The seeds were inoculated with a N-fixing bacterium Bradyrhizobium japonicum (Kirchner) (Nitragin, Milwaukee, WI) before planting. Planting depth was 4 cm and the seedling rate was 558,000 seeds ha$^{-1}$. Rows were 72 cm apart and planted in an east–west direction. Fertilizer was applied in a band 5 cm deep and 5 cm to the side of seed furrow at a rate of 16.8 kg ha$^{-1}$ N, 43.8 kg ha$^{-1}$ P$_2$O$_5$, and 15.6 kg ha$^{-1}$ K$_2$O. Herbicides (alachlor and glyphosate) were applied at planting to manage weeds.

Soybean Aphid Field Cages

Experimental plots were enclosed in cages at the V2 (second node; Ritchie et al., 1999) developmental stage on 27 June 2003 and 28 June 2004. Each cage, which measured 1.5 by 1.5 m, was centered on two rows of soybean plants. Cages were used to keep other soybean pests (bean leaf beetles, grasshoppers, potato leafhoppers, and soybean leaf miners) from feeding on the soybean plants and potentially confounding the response variables being measured. The screening material was an amber-colored Lumite screen (18 by 14 mesh; BioQuip, Gardena, CA). Amber-colored Lumite screening is commonly used in field research and is known for good sunlight penetration and low air resistance (Bell and Baker, 2000; Lefko et al., 2000).

Each cage was equipped with a zippered opening that was located on the west end of the cage. The screening was fastened onto the frame using plastic ties, and the cage frame itself anchored to the soil using concrete reinforcing bars. Plant stands within each cage were thinned by hand to 30 soybean plants for each of the two rows enclosed in the cage (60 plants cage$^{-1}$). Color coded plastic ties loosely placed around the base of randomly chosen plants indicated when and which plants were to be removed for aphid counts or yield data. Thirty-two experimental plots were enclosed in cages in 2003 and 48 plots were enclosed in 2004.

Soybean Aphid Infestation

During the growing season, soybean plants were infested with known numbers of soybean aphids at the V5 (fifth node) and R2 (full bloom) soybean developmental stages (Ritchie et al., 1999). The V5 infestations were accomplished on 7 July 2003 and 12 July 2004; R2 infestations were accomplished on 23 July 2003 and 27 July 2004. The initial aphid treatments applied on V5 and R2 soybean plants during the 2003 growing seasons were 0, 10, 50, and 100 aphids plant$^{-1}$. In 2004, the initial treatments were 0, 1, 3, 10, 50, and 100 aphids plant$^{-1}$. The treatments were assigned to cages as a completely randomized experimental design. Each initial aphid population treatment per infestation period was replicated four times.

Infestation of the field plants was accomplished using aphids produced at the rearing facility at the North Central Agricultural Research Laboratory. The soybean aphids used in this research were clones of wild aphids collected from commercial soybean fields. The colony was maintained on Pioneer 91B01 soybean (Pioneer Hi-Bred International, Johnston, IA) inside environmental chambers (Conviron, CMP4030, Winnipeg, Canada) at 24°C and photoperiod of 16:8 (L:D). Leaves from infested laboratory-reared plants were collected; wingless soybean aphids (varying in age) were counted and leaves were cut into pieces that contained the desired soybean aphid numbers. The cut leaves containing the aphids were then placed in a 240 mL foam cup and covered with a lid. Cups filled with the desired treatment populations were taken to corresponding treatment cage on the field, and the leaf pieces containing known numbers of aphids were carefully placed on the uppermost growing point of each soybean plant. These known initial soybean aphid numbers were then allowed to establish on the plant and multiply freely. An attempt was made to keep the check (0 aphids plant$^{-1}$) cages aphid-free by applying insecticides ([0.02% pyrethrin plus 0.20% piperonyl butoxide (Schultz Plant Spray, Expert Gardener Houseplants and Gardens, Bridgeton, MO) in 2003; and 0.425% esfenvalerate (Ortho Bug B Gone Multi-purpose Insect Killer, The Scotts Miracle-Gro Co., Marysville, OH) in 2004]. In 2003, however, low levels of aphid infestations were detected in the check cages perhaps due to accidental introductions during initial cage set up. All of the check cages were aphid-free in 2004.

Soybean Aphid Population Measurement

Soybean plants that were to be sampled for aphid population measurements at specific dates or yields at harvest were randomly tagged using color-coded plastic ties at the time the cages were set on the field. Plants were sampled starting 3 d after initial aphid infestation at the V5 soybean development stage or 2 d after aphid infestation at the R2 soybean development stage, followed by biweekly sampling. Plant shoots were severed at ground level, placed in 49 L plastic bags, and stored in a freezer. Whole plant aphid populations were then counted using a magnifying lens and tally counter. Average aphid populations for two plants removed from each cage were used for statistical analyses. This sampling size appear to have been sufficient and very precise based on the resulting low variability within treatment means, standard errors, regression equations, and regression coefficients (Fig. 1 and 2; Tables 1, 2, 3, and 4).

Soybean aphid population peak for each treatment was determined by fitting a symmetrical bell-shaped curve to the data through nonlinear regression analysis (PROC NLIN; SAS Institute 1989). The mathematical equation fitted to the day of year (DOY) (X) and soybean aphid number plant$^{-1}$ (Y) data was:

\[ Y = [A][B(X - C)^2] \]
where \( A \) was the peak aphid number plant, \( B \) was a constant, and \( C \) was the DOY when the peak soybean aphid number plant\(^{-1} \) occurred.

Aphid-days and cumulative aphid-days were calculated according to Ruppel (1983). Maximum cumulative aphid-day for each treatment was determined by fitting a logistic curve to the data through nonlinear regression analysis (PROC NLIN; SAS Institute, 1989). The logistic equation fitted to the DOY (\( X \)) and cumulative aphid-day (\( Y \)) data was:

\[
Y = \frac{A}{1 + e^{B - CX}}
\]

where \( A \) was the maximum cumulative aphid-day, \( e \) was the base of natural logarithms, and \( B \) and \( C \) were constants.

### Soybean Yield, Seed Components, and Statistical Analysis

At the end of the growing season (29 Sept. 2003 and 6 Oct. 2004), plant biomass (g plant\(^{-1} \)) as well as yield component data (pods plant\(^{-1} \), seeds pod\(^{-1} \), and 100 seed weight) were taken from 10 randomly selected plants per cage at harvest. Seeds from these plants were pooled with harvested seeds from all remaining plants and were used for seed yield in each cage. Whole seed oil and protein concentrations (Osborne and Riedell, 2006) were measured using near infrared reflectance spectroscopy (Foss NIRSystems, Model 5000, Eden Prairie, MN). Peak aphid numbers and maximum cumulative aphid-days were then regressed against soybean yield components (pods plant\(^{-1} \), seeds pod\(^{-1} \), and 100 seed weight), plot yield, seed composition (seed oil and protein concentrations), and whole...
plant biomass using simple linear regression analyses (PROC REG; SAS Institute, 1989).

RESULTS AND DISCUSSION

Soybean Aphid Populations

Initial infestation rates of 10, 50, and 100 soybean aphids plant\(^{-1}\) at V5 (7 July 2003) resulted in peaks of 21,209, 17,694, and 11,828 soybean aphids plant\(^{-1}\) on 19 Aug., 15 Aug., and 17 Aug. 2003, respectively (Table 1). The same initial infestation levels introduced 16 d later on R2 soybean (23 July 2003) resulted in lower peaks of 3161, 6844, and 7082 soybean aphids plant\(^{-1}\) on 21 August, 4 September, and 24 August 2003. Unplanned introduction of low levels of soybean aphids in the check plots peaked at 705 soybean aphids plant\(^{-1}\) on 8 August and 192 soybean aphids plant\(^{-1}\) on 13 August for the V5 and R2 initial infestations. These unplanned infestations added serendipitous data to our experiment. In 2003, the lowest planned initial infestation level of 10 soybean aphids plant\(^{-1}\) still gave rise to very high aphid numbers and affected soybean yield components and quality in similar magnitude as the 50 and 100 soybean aphids plant\(^{-1}\) initial treatments (Table 1).

In 2004, initial infestations with 1, 3, 10, 50, and 100 soybean aphids plant\(^{-1}\) at V5 (12 July 2004) resulted in peaks of 4,627; 9,381; 14,402; 21,717; and 20,711 soybean aphids plant\(^{-1}\) on 13 Sept., 4 Sept., 25 Aug., 23 Aug., and 19 Aug. 2004, respectively (Table 3). The same initial infestation levels introduced 15 d later on R2 soybean (27 July 2004) resulted in lower peaks of 2050, 2756, 2994, 6050, 6487 soybean aphids plant\(^{-1}\) on 8 September, 9 September, 31 August, and 29 August. Check treatments in both V5 and R2 infestations remained at zero aphids plant\(^{-1}\) throughout the growing season (Table 3). Cumulative growing degree days (GDD, 10°C baseline) from initial infestation with aphids at V5 through R5 were 766 GDD in 2003 and 653 GDD in 2004 (Beckendorf, 2005). For the later initial infestation date, the cumulative GDD were 517 GDD in 2003 and 407 GDD in 2004 from R2 through R5. Peak aphid numbers, when averaged across treatments, were similar in both years perhaps because of the similarity in the cumulative GDD in 2003 and 2004.

Accurate measurement of yield effects caused by aphid feeding involves assessment of the aphid population density on plants plus the duration of their feeding (Kieckhefer et al., 1995). Aphid-day unitage (e.g., one aphid feeding on one plant for a 24 h period) measures the combined intensity and duration of the soybean aphids on the soybean plants (Kieckhefer et al., 1995; Ruppel, 1983). Aphid-day values are obtained by multiplying aphid population numbers by the number of days that the aphids were on the host plant. Maximum cumulative aphid-days represented the highest possible intensity and duration of soybean aphid exposure that the soybean plants were exposed to during the growing seasons (Tables 1, 2, 3, and 4).
Table 1. Effects of soybean aphid infestations starting at the V5 and R2 stages on soybean yield components during the 2003 season.

<table>
<thead>
<tr>
<th>Soybean aphids plant$^{-1}$</th>
<th>Max. cumulative aphid-days (X)</th>
<th>Pods plant$^{-1}$ (Y$_1$)</th>
<th>Seeds pod$^{-1}$ (Y$_2$)</th>
<th>100 seeds wt. (g) (Y$_3$)</th>
<th>Yield (kg ha$^{-1}$) (Y$_4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial†</td>
<td>Peak</td>
<td>Aphid infestation starting at V5 stage (7 July; DOY = 188)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (2.50 ± 0.64)$^‡$‡</td>
<td>704.74 ± 324.36§</td>
<td>7926.40 ± 2625.63</td>
<td>32.30 ± 2.59</td>
<td>2.32 ± 0.05</td>
<td>13.15 ± 0.39</td>
</tr>
<tr>
<td>10 (12.50 ± 3.97)</td>
<td>21,209.40 ± 6,418.48</td>
<td>380,632.75 ± 102,561.87</td>
<td>19.22 ± 3.43</td>
<td>2.12 ± 0.10</td>
<td>11.67 ± 0.26</td>
</tr>
<tr>
<td>50 (30.25 ± 8.81)</td>
<td>17,693.78 ± 3366.63</td>
<td>326,719.25 ± 49,162.48</td>
<td>18.12 ± 2.25</td>
<td>2.12 ± 0.10</td>
<td>12.53 ± 0.44</td>
</tr>
<tr>
<td>100 (148.00 ± 36.26)</td>
<td>11,827.95 ± 2,652.41</td>
<td>241,938.50 ± 56,648.81</td>
<td>18.75 ± 3.62</td>
<td>2.11 ± 0.13</td>
<td>12.53 ± 0.57</td>
</tr>
</tbody>
</table>

Regression equations:

\[
Y_1 = -0.00004X + 31.31 \\
R^2 = 0.86
\]

\[
Y_2 = -0.0000006X + 2.30 \\
R^2 = 0.85
\]

\[
Y_3 = -0.000003X + 13.25 \\
R^2 = 0.78
\]

\[
Y_4 = -0.0029X + 2,090.00 \\
R^2 = 0.93
\]

| Initial†                   | Peak                           | Aphid infestation starting at R2 stage (23 July; DOY = 204) |
| 0 (48.00 ± 27.16)         | 191.54 ± 56.73§                | 4,407.43 ± 1,432.19      | 26.75 ± 1.89             | 2.34 ± 0.06               | 14.77 ± 0.43              | 2,761.65 ± 68.33          |
| 10 (133.50 ± 75.45)       | 3,160.90 ± 1,127.71            | 75,219.23 ± 32,311.27    | 27.75 ± 1.49             | 2.24 ± 0.06               | 12.98 ± 0.62              | 2,078.85 ± 165.12         |
| 50 (55.75 ± 21.38)        | 6,844.05 ± 4,714.21            | 81,336.20 ± 33,461.21    | 19.25 ± 1.38             | 2.24 ± 0.04               | 13.58 ± 0.29              | 2,280.13 ± 129.97         |
| 100 (59.75 ± 9.45)        | 7,082.10 ± 2,224.81            | 130,544.03 ± 42,803.72   | 20.25 ± 2.50             | 2.16 ± 0.04               | 13.31 ± 0.22              | 2,020.53 ± 147.71         |

Regression equations:

\[
Y_5 = -0.000003X + 20.26 \\
R^2 = 0.41
\]

\[
Y_6 = -0.000001X + 2.35 \\
R^2 = 1.00
\]

\[
Y_7 = -0.00001X + 14.55 \\
R^2 = 0.66
\]

\[
Y_8 = -0.0060X + 2,721.40 \\
R^2 = 0.85
\]

† Soybean aphids may have been accidentally introduced during initial cage set up.
‡ Values represent treatments while values in parentheses (mean ± standard error) represent aphid counts 3 d after infestation.
§ Values represent mean ± standard error.

Table 2. Effects of soybean aphid infestations starting at the V5 and R2 stages on soybean seed composition and plant biomass during the 2003 season.

<table>
<thead>
<tr>
<th>Soybean aphids plant$^{-1}$</th>
<th>Max. cumulative aphid-days (X)</th>
<th>Seed oil conc. (%) (Y$_5$)</th>
<th>Seed protein conc. (%) (Y$_6$)</th>
<th>Biomass (g DM) (Y$_7$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial†</td>
<td>Peak</td>
<td>Aphid infestation starting at V5 stage (7 July; DOY = 188)</td>
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<td></td>
</tr>
<tr>
<td>0 (2.50 ± 0.64)$^‡$‡</td>
<td>704.74 ± 324.36§</td>
<td>7926.40 ± 2625.63</td>
<td>20.18 ± 0.28</td>
<td>39.07 ± 0.44</td>
</tr>
<tr>
<td>10 (12.50 ± 3.97)</td>
<td>21,209.40 ± 6,418.48</td>
<td>380,632.75 ± 102,561.87</td>
<td>18.70 ± 0.36</td>
<td>41.72 ± 0.76</td>
</tr>
<tr>
<td>50 (30.25 ± 8.81)</td>
<td>17,693.78 ± 3366.63</td>
<td>326,719.25 ± 49,162.48</td>
<td>19.62 ± 0.34</td>
<td>40.04 ± 0.28</td>
</tr>
<tr>
<td>100 (148.00 ± 36.26)</td>
<td>11,827.95 ± 2,652.41</td>
<td>241,938.50 ± 56,648.81</td>
<td>19.48 ± 0.30</td>
<td>40.63 ± 0.58</td>
</tr>
</tbody>
</table>

Regression equations:

\[
Y_5 = -0.000003X + 20.26 \\
R^2 = 0.75
\]

\[
Y_6 = 0.000006X + 39.00 \\
R^2 = 0.72
\]

\[
Y_7 = -0.00002X + 16.40 \\
R^2 = 0.86
\]

| Initial†                   | Peak                           | Aphid infestation starting at R2 stage (23 July; DOY = 204) |
| 0 (48.00 ± 27.16)         | 191.54 ± 56.73§                | 4,407.43 ± 1,432.19      | 20.09 ± 0.26             | 38.20 ± 0.09           | 16.38 ± 1.08              |
| 10 (133.50 ± 75.45)       | 3,160.90 ± 1,127.71            | 75,219.23 ± 32,311.27    | 19.88 ± 0.08             | 39.04 ± 0.55           | 15.31 ± 1.74              |
| 50 (55.75 ± 21.38)        | 6,844.05 ± 4,714.21            | 81,336.20 ± 33,461.21    | 19.88 ± 0.23             | 39.27 ± 0.36           | 10.68 ± 0.97              |
| 100 (59.75 ± 9.45)        | 7,082.10 ± 2,224.81            | 130,544.03 ± 42,803.72   | 19.40 ± 0.22             | 39.31 ± 0.14           | 11.73 ± 1.70              |

Regression equations:

\[
Y_5 = -0.000005X + 20.19 \\
R^2 = 0.85
\]

\[
Y_6 = 0.000009X + 38.28 \\
R^2 = 0.87
\]

\[
Y_7 = -0.00006X + 2721.40 \\
R^2 = 0.85
\]

† Soybean aphids may have been accidentally introduced during initial cage set up.
‡ Values represent treatments while values in parentheses (mean ± standard error) represent aphid counts 3 d after infestation.
§ Values represent mean ± standard error.
Table 3. Effects of soybean aphid infestations starting at the V5 and R2 stages on soybean yield components during the 2004 season.

<table>
<thead>
<tr>
<th>Soybean aphids plant−1</th>
<th>100 seeds wt. (g)</th>
<th>YLD (kg ha−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphid infestation starting at V5 stage (12 July; DOY = 194)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.00 ± 0.00)‡</td>
<td>0.00 ± 0.00†</td>
<td>27.00 ± 0.63</td>
</tr>
<tr>
<td>1 (2.00 ± 0.41)</td>
<td>26.75 ± 1.19</td>
<td>25.50 ± 0.63</td>
</tr>
<tr>
<td>3 (2.00 ± 0.77)</td>
<td>16.23 ± 1.19</td>
<td>15.25 ± 0.77</td>
</tr>
<tr>
<td>50 (417.75 ± 6.34)</td>
<td>2.00 ± 0.06</td>
<td>1.85 ± 0.06</td>
</tr>
<tr>
<td>Regression equations:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Y_1 = 17.48 ± 0.40$</td>
<td>$Y_2 = 16.95 ± 0.40$</td>
<td>$Y_3 = 16.19 ± 0.40$</td>
</tr>
<tr>
<td>$R^2 = 0.80$</td>
<td>$R^2 = 0.85$</td>
<td>$R^2 = 0.30$</td>
</tr>
</tbody>
</table>

| Aphid infestation starting at R2 stage (27 July; DOY = 209) |                  |               |
| 0 (0.00 ± 0.00)§  | 0.00 ± 0.00  | 29.25 ± 0.63 |
| 1 (5.75 ± 3.09)  | 27.50 ± 1.97  | 26.25 ± 1.59 |
| 3 (5.25 ± 1.03)  | 16.23 ± 1.03  | 15.15 ± 0.77 |
| 50 (417.75 ± 6.34) | 2.00 ± 0.06  | 1.85 ± 0.06  |
| Regression equations: |                  |               |
| $Y_1 = 17.90 ± 0.38$ | $Y_2 = 17.75 ± 0.39$ | $Y_3 = 17.50 ± 0.38$ | $Y_4 = 17.25 ± 0.38$ |
| $R^2 = 0.71$ | $R^2 = 0.85$ | $R^2 = 0.85$ | $R^2 = 0.85$ |

Initial infestations with 10, 50, and 100 soybean aphids plant−1 at V5 (7 July 2003) resulted in maximum cumulative aphid-days plant−1 values of 380,633; 326,719; and 241,939 (Table 1). The same initial infestation levels introduced on R2 soybean (23 July 2003) resulted in lower maximum cumulative aphid-days plant−1 values of 75,219; 81,336; and 130,544. Cumulative aphid-days values, determined for the unplanned introduction of soybean aphid in the check plots, totaled 7926 and 4407 cumulative aphid-days plant−1 for the V5 and R2 initial infestations (Table 1).

In 2004, initial infestation rates of 1, 3, 10, 50, and 100 soybean aphids plant−1 at V5 (12 July 2004) resulted in maximum cumulative aphid-days plant−1 of 101,076; 229,437; 355,045; 514,571; and 537,135 (Table 3). The same initial infestation levels introduced on R2 soybean (27 July 2004) resulted in lower maximum cumulative aphid-days plant−1 of 34,323; 57,701; 43,926; 125,143; and 148,818.

The soybean aphid had high reproductive potential (Tables 1 through 4). A single aphid plant−1 introduced at V5 in 2004, for example, multiplied to a peak of 4627 aphids plant−1 and resulted in 101,076 maximum cumulative aphid-days plant−1. However, a single aphid plant−1 introduced 15 d later at R2 resulted only in a peak of about 2050 aphids plant−1, and 34,323 maximum cumulative aphid-days plant−1. The resulting peak aphid number from an initial infestation of one soybean aphid plant−1 was 56% lower when introduced at R2 than at V5. The resulting maximum cumulative aphid-days plant−1 was 66% lower at R2 than at V5. Similar trends were also observed for the other initial soybean aphid infestation levels (Tables 1 through 4). In general, the R2 infestations were 56% to 79% lower in resulting peak aphid numbers and 66 to 88% lower in resulting maximum cumulative aphid-days plant−1 than the V5 infestations.

The lower reproductive potential of the soybean aphid when introduced at R2 may have been a result of the fewer number of days that the insects were allowed to reproduce on soybean. In general, we observed that soybean aphid numbers peaked when the plants were at or near the R5 stage and drastically declined thereafter (Beckendorf, 2005). Changes in plant host physiology during the growing season have been observed to affect soybean aphid populations (Ragsdale et al., 2004; van den Berg et al., 1997).

Specifically, aphid reproduction has been shown to be positively correlated with the N content of the host plant in soybean (Hu et al., 1992; Myers and Gratton, 2006) and other plants (Nevo and Coll, 2001; Pettit et al., 1994). In soybean, the accumulation of N fixation products (e.g., ureides) by soybean shoots increases rapidly starting at R1, peaks at about R5, and dramatically drops to near 0 by R7 (Riedell et al., 2005; Osborne and Riedell, 2006). Thus, the decline in aphid numbers after R5 may have been due to a decline in phloem sap N concentrations in leaves and stems which in turn reduced the level of N available for soybean aphid nutrition and reproduction.

It is commonly assumed that the decline in aphid numbers in late summer is mainly due to changes in photoperiod and temperature that signal aphids to start producing winged forms and migrate to buckthorns for overwintering.
ous flower bugs (e.g., multicolored Asian lady beetle (Harmonia axyridis Pallas) larvae and adults) were immediately removed if they were observed to be in the caged plants. Although the mesh size of the cage screening used was large enough to allow entry of parasitic wasps, aphid microbial pathogens, and insidious flower bugs [Orius insidiosus (Say)], large numbers of these natural enemies were not observed in the cages.

Because experimental plants infested with aphids were enclosed in screened cages, the population dynamics in this study likely represented the total reproductive potential of soybean aphids in the absence of regulation from natural predators and competition from other soybean pests. Large-bodied natural predators [e.g., multicolored Asian lady beetle (Harmonia axyridis Pallas) larvae and adults] were immediately removed if they were observed to be in the caged plants. Although the mesh size of the cage screening used was large enough to allow entry of parasitic wasps, aphid microbial pathogens, and insidious flower bugs [Orius insidiosus (Say)], large numbers of these natural enemies were not observed in the cages.

Many of the other published soybean aphid studies have used small cages clipped onto soybean leaves (Rutledge and O’Neil, 2006; Myers and Gratton, 2006) or field cages of various dimensions (Fox et al., 2004; Li et al., 2004; Liu et al., 2004). In these studies as well as the current study, the use of field cages may have influenced the microclimate inside the cage which in turn may have influenced soybean aphid mortality and soybean growth and development (Hand and Keaster, 1967). In contrast, Fox et al. (2004) reported that cages had minimal effects on temperature, relative humidity, or soybean growth. The use of field cages in quantifying injuries caused by various insect pests of soybean and alfalfa has an excellent track record of precision and fidelity to actual field conditions (Hutchins et al., 1989; Hutchins and Pedigo, 1989; Lefko et al., 2000; Pedigo, 1989; Peterson et al., 1998; Smelser and Pedigo, 1992). Smelser and Pedigo (1992) is the basis of the current economic threshold of the bean leaf beetle [Ceratoma trifurcata (Forster)] in the United States. Despite using small cages (2 by 1 m) and relatively few plants (five to eight plants per plot) to measure quality and yield, data from this study has stood the test of time. Economic thresholds based on the results from this study have been used throughout the Midwest in managing the bean leaf beetle. This study proves that small-plot research can be very precise and is applicable not just to the state where it was performed (Ames, IA, in this study) but also the whole U.S. Midwestern soybean growing area.

### Impact of Soybean Aphids on Soybean Yield Components

Peak aphid numbers and maximum cumulative aphid-days had direct impacts on soybean yield components both in 2003 and 2004 (Fig. 1). Strong negative linear relationships were detected between aphid numbers and the soybean yield and components. Seed yield, pods plant$^{-1}$, seeds pod$^{-1}$, and individual seed weight declined linearly as peak aphid numbers plant$^{-1}$ and maximum cumulative aphid-days plant$^{-1}$ increased. Soybean aphids seemed to have reduced yield components more severely when introduced at V5 than at R2 (Fig. 1). For example, the number of pods plant$^{-1}$ were reduced from an average of about 32 pods plant$^{-1}$ to 19 pods plant$^{-1}$ (41% loss) in 2003 and 27 pods plant$^{-1}$ to nine pods plant$^{-1}$ (67% loss) in 2004 when the soybean aphids were introduced at V5 (Tables 1 and 3). When the soybean aphids were introduced at R2, the reductions in pod numbers were from 27 to 20 pods plant$^{-1}$ (26% loss) in 2003 and from 28 to 24 pods plant$^{-1}$ (14% loss) in 2004. However, these observed differences between the impacts of V5 and R2-introduced aphids on

### Table 4. Effects of soybean aphid infestations starting at the V5 and R2 stages on soybean seed composition and plant biomass during the 2004 season.

<table>
<thead>
<tr>
<th>Soybean aphids plant$^{-1}$</th>
<th>Max. cumulative aphid-days (X)</th>
<th>Seed oil conc. (%) (Y$_1$)</th>
<th>Seed protein conc. (%) (Y$_2$)</th>
<th>Biomass (g DM) (Y$_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Peak</td>
<td>Aphid infestation starting at V5 stage (12 July; DOY = 194)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.00 ± 0.00)†</td>
<td>0.00 ± 0.00‡</td>
<td>19.08 ± 1.72</td>
<td>40.74 ± 0.41</td>
<td>19.08 ± 0.00</td>
</tr>
<tr>
<td>1 (2.00 ± 0.41)</td>
<td>4,626.90 ± 1,125.36</td>
<td>101,075.80 ± 7,868.09</td>
<td>18.86 ± 0.25</td>
<td>40.50 ± 0.18</td>
</tr>
<tr>
<td>3 (3.25 ± 0.75)</td>
<td>9,380.95 ± 20.72.56</td>
<td>229,437.00 ± 5,964.00</td>
<td>18.80 ± 0.17</td>
<td>40.94 ± 0.24</td>
</tr>
<tr>
<td>10 (8.25 ± 1.03)</td>
<td>14,401.53 ± 2.697.61</td>
<td>355,045.00 ± 35,841.20</td>
<td>18.26 ± 0.25</td>
<td>42.67 ± 0.38</td>
</tr>
<tr>
<td>50 (49.75 ± 6.34)</td>
<td>21,717.45 ± 4,620.91</td>
<td>514,571.25 ± 79,812.48</td>
<td>17.46 ± 0.18</td>
<td>43.66 ± 0.21</td>
</tr>
<tr>
<td>100 (71.75 ± 11.38)</td>
<td>20,710.53 ± 1,202.14</td>
<td>537,135.25 ± 45,596.40</td>
<td>17.61 ± 0.42</td>
<td>43.42 ± 0.80</td>
</tr>
</tbody>
</table>

Regression equations:

Aphid infestation starting at R2 stage (27 July; DOY = 209)

| Initial                     | Peak                           | Regression equations: |                                 |                      |
| 0 (0.00 ± 0.00)§            | 0.00 ± 0.00                    | $Y_5 = -0.000003X + 19.08$ | $R^2 = 0.90$                  | $Y_6 = 0.000006X + 40.42$ | $R^2 = 0.91$ | $Y_7 = -0.00002X + 18.24$ | $R^2 = 0.98$ |
| 1 (5.75 ± 3.09)             | 2,049.83 ± 536.94              | $Y_5 = -0.000003X + 19.08$ | $R^2 = 0.67$                  | $Y_6 = 0.000009X + 40.03$ | $R^2 = 0.69$ |
| 3 (4.75 ± 1.25)             | 2,993.78 ± 1,431.57            | $Y_5 = -0.000003X + 19.08$ | $R^2 = 0.67$                  | $Y_6 = 0.000009X + 40.03$ | $R^2 = 0.69$ |
| 10 (4.25 ± 1.65)            | 6,049.70 ± 579.84              | $Y_5 = -0.000003X + 19.08$ | $R^2 = 0.67$                  | $Y_6 = 0.000009X + 40.03$ | $R^2 = 0.69$ |
| 50 (38.00 ± 9.60)           | 148,817.05 ± 15,542.38         | $Y_5 = -0.000003X + 19.08$ | $R^2 = 0.67$                  | $Y_6 = 0.000009X + 40.03$ | $R^2 = 0.69$ |
| 100 (106.75 ± 11.94)        | 6,486.63 ± 408.10              | $Y_5 = -0.000003X + 19.08$ | $R^2 = 0.67$                  | $Y_6 = 0.000009X + 40.03$ | $R^2 = 0.69$ |

† Values represent treatments while values in parentheses (mean ± standard error) represent aphid counts 2 d after infestation.

‡ Values represent treatments while values in parentheses (mean ± standard error) represent aphid counts 3 d after infestation.

§ Values represent treatments while values in parentheses (mean ± standard error) represent aphid counts 2 d after infestation.
pods plant$^{-1}$ and the other yield components may have been mainly due to soybean aphid reaching much higher peaks and maximum cumulative aphid-days when allowed to multiply on soybean plants starting earlier at V5 than later at R2 (Fig. 1; Tables 1 and 3). That is, V5-introduced aphids had more time to multiply, reach higher peaks, and inflict more injury to the host plant than the R2-introduced aphids.

The unit impact on yield components per unit increase in peak aphid numbers and maximum cumulative aphid-days may be better determined by looking at the slopes of the regression curves. In 2003 (Fig. 1A), with increasing peak aphid numbers, it was estimated that 0.7 pod plant$^{-1}$ was lost for every 1000 unit increase in peak soybean aphid numbers plant$^{-1}$ when initially introduced at V5 ($R^2 = 0.80$). When introduced at R2, the estimated loss was 1.2 pods plant$^{-1}$ for every 1000 unit increase in soybean aphid peak numbers plant$^{-1}$ ($R^2 = 0.78$). The slopes of the regression curves indicated that the impact of soybean aphids on soybean yield components may have in fact been more severe when the soybean aphids were introduced at R2 than when introduced at V5. It is generally accepted that R stages soybean are more sensitive to environmental stress than V stages soybean (Kalton et al., 1949; Ritchie et al., 1999; Stone and Pedigo, 1972).

During the 2004 season (Fig. 1B), the reduction in pod number was similar in the V5- and R2-infested soybean at 0.9 and 0.7 pod plant$^{-1}$ for every 1000 unit increase in peak soybean aphid numbers ($R^2 = 0.87–0.96$). The pod number plant$^{-1}$ may be the most important yield component that determines eventual soybean yield at harvest (Herbert and Litchfield, 1982; Mathew et al., 2000).

The number of seeds pod$^{-1}$ and 100 seed weight were also negatively affected by the soybean aphids (Fig. 1C–D). Number of seeds pod$^{-1}$ declined linearly with increasing peak aphid numbers. Predicted number of seeds pod$^{-1}$ in aphid free plants in 2004 was 2.3 seeds pod$^{-1}$. Soybean aphids introduced at V5 and R2 reduced number of seeds pod$^{-1}$ by 0.03 and 0.04 seeds pod$^{-1}$ for every 1000 unit increase in peak soybean aphid numbers ($R^2 = 0.75–0.94$; Fig. 1D). The 100 seed weight also declined linearly with increasing aphid numbers in 2003 and 2004 (Fig. 1E–F) which suggests that soybean plants infested with high numbers of aphids also produced smaller seeds. In both years, the R2-introduced aphids appeared to have caused a more drastic reduction in seed size than the V5-introduced ones ($R^2 = 0.42–0.85$; Fig. 1E–F).

Linear reductions in seed yields were observed in 2003 and 2004 (Fig. 1G–H). In 2004, plot yields declined at the rate of 134 kg ha$^{-1}$ for every 1000 increase in peak numbers by soybean aphids introduced at V5 ($R^2 = 0.95$). Soybean aphids introduced at R2 reduced yields by 192 kg ha$^{-1}$ per 1000 aphids plant$^{-1}$ increase in peak numbers ($R^2 = 0.89$). In terms of maximum cumulative aphid-days, there was a decline in yield of 470 kg ha$^{-1}$ for every 100,000 unit increase in maximum cumulative aphid-days plant$^{-1}$ was observed ($R^2 = 0.90$).

Yield reductions during the 2003 season were less than the 2004 season. In 2003, the yield reduction caused by soybean aphids introduced at V5 was 50.9 kg ha$^{-1}$ per 1000 unit increase in peak aphid numbers plant$^{-1}$ ($R^2 = 0.88$; Fig. 1G) or 290 kg ha$^{-1}$ per 100,000 unit increase in maximum cumulative aphid-days plant$^{-1}$ ($R^2 = 0.93$; Table 1). When introduced at R2, the yield reduction caused by the aphids was 76.3 kg ha$^{-1}$ per 1000 unit increase in peak aphid numbers plant$^{-1}$ (Fig. 1G) or 600 kg ha$^{-1}$ per 100,000 unit increase in maximum cumulative aphid-days plant$^{-1}$ (Table 1).

Although the unit impact on yield per unit increase in peak aphid numbers appear to indicate that the soybean aphids were more injurious to soybean when infestations started at R2 than at V5, it must be noted that the maximum aphid numbers realized or attained on the R2-infested soybean were 56 to 79% lower in resulting peak aphid numbers and 66 to 88% lower in resulting maximum cumulative aphid-days plant$^{-1}$ than the V5 infestations (Fig. 1G–H; Tables 1 and 3). This had the overall effect of the total realized or maximum possible yield loss being actually lower in the R2-infested than in the V5-infested plants.

The maximum possible yield loss caused by aphids in 2003 was 1044 kg ha$^{-1}$ (52% yield loss) in the V5-infested soybean and 526 kg ha$^{-1}$ (20% yield loss) for the R2-infested soybean (Fig. 1G–H). In 2004, the maximum possible yield loss was 2914 kg ha$^{-1}$ (88% yield loss) for V5-infested soybean and 1245 kg ha$^{-1}$ (39% yield loss) for R2-infested soybean. It appears, therefore, that the maximum possible yield loss due to aphids was governed by the maximum aphid numbers attained on the host plants, which in turn was influenced by duration that the aphids were present and the physiological state (e.g., N content of the phloem sap) of the host plants. In general, V5-introduced soybean aphids reproduced to much higher peak numbers and inflicted more damage than the R2-introduced aphids (Fig. 1G–H).

**Soybean Aphid Effects on Biomass, Seed Oil, and Protein Concentrations**

Linear reductions in seed oil concentration were associated with increasing peak aphid numbers plant$^{-1}$ and maximum cumulative aphid-days plant$^{-1}$ (Fig. 2A–F; Tables 2 and 4). In 2003, soybean aphids introduced at V5 reduced seed oil concentration by 0.06% point per 1000 unit increase in peak aphid numbers plant$^{-1}$, or 0.03% point per 10,000 unit increase in maximum cumulative aphid-days plant$^{-1}$ (Fig. 2A; Table 2). Soybean aphids introduced at R2 reduced seed oil concentration by 0.07% point per 1000 unit increase in peak aphid numbers plant$^{-1}$ or 0.05% point per 10,000 unit increase in maximum cumulative aphid-days plant$^{-1}$. During the 2004 season, the seed oil concentration reductions due to aphids introduced at V5 were 0.08% point per 1000 unit increase in peak aphid numbers plant$^{-1}$ or 0.03% point per 10,000 unit increase in maximum cumulative aphid-days plant$^{-1}$ (Table 4). When introduced at R2, the soybean aphids reduced seed oil concentration by 0.1% point per 1000 unit increase in peak aphid numbers plant$^{-1}$ or 0.05% point per 10,000 unit increase in maximum cumulative aphid-days plant$^{-1}$.

Seed oil concentration at or >19% is considered desirable for marketability; soybean aphids can potentially reduce seed oil concentration below this level. In 2004, for example, our study indicated that peak soybean aphid numbers >2875 soybean aphids plant$^{-1}$ for infestations starting at V5 or 4300 soybean aphids plant$^{-1}$ for infestations starting at R2 would
have reduced seed oil concentration below 19% (Table 4). For the 2003 season, the number of aphids that would have reduced seed oil concentration below 19% were much higher at >21,000 soybean aphids plant\(^{-1}\) for infestations starting at V5 and >15,714 soybean aphids plant\(^{-1}\) for infestations starting at R2 (Table 2). Across the 2 yr of the experiment, soybean seed yields were about 39% higher in 2004 than 2003 (Tables 1 and 3) indicating that the negative impact of soybean aphids on seed oil concentration may be more substantial during years conducive to higher soybean yields.

Seed protein concentration increased with increasing peak aphid numbers and maximum cumulative aphid-days (Tables 2 and 4). This makes seed protein concentration the only plant variable that was positively correlated with soybean aphids. Soybean aphids introduced at V5 in 2003 increased seed protein concentration by 0.1% point for every 1000 unit increase in peak soybean aphid number plant\(^{-1}\), or 0.6% point per 10,000 unit increase in maximum cumulative aphid-days plant\(^{-1}\) (Table 2). The increase in seed protein was similar in soybean plants that were infested with aphids starting at R2. In 2004, V5-introduced aphids increased seed protein by 0.2% point per 1000 unit increase in peak aphid number plant\(^{-1}\) or 0.6% point per 10,000 unit increase in maximum cumulative aphid-days plant\(^{-1}\) (Table 4).

Our data do not allow determination of the plant physiological mechanism that increased soybean seed protein concentration with increasing aphid numbers. However, the timing of stress occurrence may dictate whether protein or oil concentration depositions in the seeds are increased or decreased (Rose, 1988; Yazdi-Samadi et al., 1977). Higher seed protein and lower seed oil concentrations are associated with moisture stress occurring late in the soybean podfill stages, while lower seed protein and higher oil concentrations are associated with moisture stress occurring early in the podfill stage. In the present study, an increase in seed protein concentration with a concomitant decrease in seed oil concentration was recorded as the peak soybean aphid numbers increased (Tables 2 and 4). According to Yazdi-Samadi et al. (1977), protein deposition in the developing seeds begins at 10 to 12 d after flowering; oil deposition begins at 15 to 20 d after flowering depending on soybean variety. We recorded peak soybean aphid numbers occurring at R5 (beginning seed stage) in late July through early August whether the aphid infestation started at V5 or R2. The soybean R5 stage (and peak aphid populations) occurred at about 28 d after full bloom (R2) in 2003 and 2004. This may mean that protein deposition was already progressing rapidly while the oil deposition just starting when peak aphid injuries occurred. Thus, the stress or injury caused by soybean aphids to the soybean plant mainly occurred later in the podfill stage and thus affected oil deposition more than protein deposition in the soybean seeds.

Whole aboveground biomass plant\(^{-1}\) at harvest (stems, pods, and seeds), expressed as g dry matter plant\(^{-1}\), declined with increasing peak aphid numbers plant\(^{-1}\) and maximum cumulative aphid-days plant\(^{-1}\) (Tables 2 and 4). In 2003, when the soybean aphids were introduced at V5, the reduction in biomass was 0.4 g plant\(^{-1}\) for every 1000 unit increase in peak aphid numbers plant\(^{-1}\) or 0.2 g plant\(^{-1}\) for every 10,000 unit increase in maximum cumulative aphid-days; for soybean plants infested starting at R2, the reduction in biomass was 0.8 g plant\(^{-1}\) per 1000 unit increase in peak aphid numbers plant\(^{-1}\) or 0.4 g plant\(^{-1}\) per 10,000 unit increase in maximum cumulative aphid-days plant\(^{-1}\) (Table 2). The decline in biomass was more pronounced in 2004 where it declined by up to 1.0 g plant\(^{-1}\) per 1000 unit increase in peak aphid number plant\(^{-1}\) in plants initially infested with soybean aphids at R2 (Table 4). These observed declines in biomass both summarize and support the associated declines in yield components (pods plant\(^{-1}\), seeds pod\(^{-1}\), weight of 100 seeds, plot yield) and seed oil concentration in 2003 and 2004 (Tables 1 through 4).

CONCLUSIONS

The soybean aphid, *Aphis glycines* Matsumura, has a very high reproductive rate and is highly injurious to soybean. In 2004, for example, one soybean aphid plant\(^{-1}\) introduced at V5, reached a peak of 4627 aphids plant\(^{-1}\) and reduced seed yield by 38%. This reduction in yield was likely due to reductions in the number of pods plant\(^{-1}\), seeds pod\(^{-1}\), and seed size. Our findings appear to support the conclusion reached by Macedo et al. (2003) that low populations of soybean aphids can cause physiological injury to soybean even in the absence of outward plant injury symptoms.

The unit impact on yield per unit increase in peak aphid numbers indicated that the soybean aphids were generally more injurious to soybean when infestations started at R2 than at V5. However, the maximum aphid numbers realized or attained on the R2-infested soybean were 56 to 79% lower in resulting peak aphid numbers and 66 to 88% lower in resulting maximum cumulative aphid-days plant\(^{-1}\) than the V5 infestations (Fig. 1G–H; Tables 1 and 3). The overall effect of this was that the total realized or maximum possible yield loss was actually lower in the R2-infested than in the V5-infested plants.

The maximum possible yield loss due to the aphids was governed by the maximum aphid numbers attained on the host plants, which in turn were influenced by duration that the aphids were present on, and the physiological state (e.g., N content of the phloem sap) of the host plants. In general, V5-introduced soybean aphids reproduced to much higher peak numbers and inflicted more damage than the R2-introduced aphids (Fig. 1G–H).

The soybean host plant itself, perhaps through expected changes in physiological and agronomic characteristics as it matures, appeared to also influence the maximum possible aphid population that can be attained on the host; this can eventually alter the severity of injuries inflicted by the pest to the host. Taken together, these observations and speculations suggest that we should be managing aphid populations differently based on soybean plant developmental stage and dates of initial aphid infestation. That is, plant stage-specific economic injury levels can and must be developed for the soybean aphid in the future.

Lastly, soybean aphids do not just affect soybean yield and yield components; they also affect seed oil and protein concentrations. And these effects on yield and composition will need to be combined to arrive at a more complete soybean aphid management recommendation. Such an endeavor should be a high priority given the economic importance of the soybean to U.S. agricultural economy and the current paucity of accurate
decision-making tools that reflect the interrelationships among aphid injury to soybean, soybean market value, aphid control cost, and soybean yield potential.

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REFERENCES


He, F., Y. Fanyue, X. Wanmin, L. Xiaoping, and W. Yanquin. 1991. Optimal race, color, age, sex, or national origin.


