Effect of Soybean Cyst Nematode on Fatty Acid Levels of Soybean Seed

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Abstract
Growing soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) resistant varieties in fields infested with SCN can improve yield, but the impact of SCN on seed quality of lines with modified seed composition is unknown. The objective of this study was to determine if SCN resistant or susceptible rootstock influenced the composition of seed of soybean lines developed for desirable elevated levels of unsaturated fatty acids (FA). Five soybean lines with modified or normal levels of FA were Y-grafted to generate a 5×5 combination of plants with a SCN susceptible (SCNS) or resistant (SCNR) rootstock having a self-grafted branch, a scion (alien) grafted branch, or non-grafted branch; control plants were not grafted. In 2004 and 2005 plants were transplanted into blocks of Asgrow 3302 (SCNR) or Asgrow 3701 (SCNS) verified to be infested with SCN. Seeds were harvested at physiologic maturity (R8) in 2004 and 2005 and analyzed for five unsaturated FA. Differences in FA levels between 2004 and 2005 were significant (*P* < 0.05), but there were no significant (*P* = 0.05) block × year effects. Significant differences were observed between the FA levels of seed harvested from non-grafted control plants and self-grafted or non-grafted branches of grafted plants within lines. Seed oleic acid of the mid-oleic line S03-1379-2 was significantly greater when grafted onto an SCNR line than when grafted onto SCNS lines. The low linolenate trait appeared to be insensitive to SCN presence as seed linolenic acid of the low linolenic line IA-3017 was not different when grafted onto SCNR or SCNS lines. SCN can negatively impact the expression of seed quality of soybean if grown where SCN infestations are evident.

Introduction
New soybean genotypes with modified fatty acid (FA) composition are being developed and will be important in meeting industry demands for food, fuel, and other uses (23). It is important to determine best growing conditions for different genotypes with modified oil to insure that FA levels desired by the industry are consistently met. Evaluation of the oil profile of genotypes with modified FA across environments is necessary to determine their utility in plant breeding programs and commerce (15).

The influence of environmental stresses on the FA profile of soybean oil has been addressed in many studies, but generally limited to stresses associated with temperature and moisture (6,18,24). Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is the most widespread pest of soybean in the USA and worldwide impeding soybean growth and development by interfering with root function (22). High population levels of SCN have been reported to have a considerable effect on soybean yields of susceptible cultivars (23) and constitute an environmental stress. Resistance or susceptibility to SCN is an expressed interaction between a soybean genotype and nematode: a genotype × environment interaction that can have a considerable influence on plant growth and development. Leaf chlorosis and plant stunting may occur if nematode population densities are high or infection is associated with one or more secondary problems such as nutrient deficiency or root rot (11), but the primary measurable effect of infection by SCN is yield reduction in the absence of symptoms (22).
FA modifications in soybean cultivars are a relatively recent option for producers and SCN may be encountered during commercial production. The first low-saturate cultivar that was grown commercially in 1996 was developed jointly by Iowa State University and Pioneer Hi-Bred International, Inc. Several large seed companies are marketing soybean cultivars with < 3% linolenic and some cultivars produce a seed linolenic content as low as 1% of total oil. Mid-oleic (> 56%) germplasm (4) and experimental lines have been developed and some cultivars are scheduled for commercial availability in 2009. Seed buyers can pay producers of soybeans with modified FA a premium over the price of conventional soybeans.

This research investigated the effects of SCN on soybean seed composition. The objectives of this study were to assess the stability of soybean FA composition of genotypes with varied FA levels grafted onto SCN resistant (SCNR) or susceptible (SCNS) rootstocks when grown in an SCN infested site.

**Soybean Lines, Grafting, and Field Study**

Five soybean lines were pruned above the cotyledons during early seedling growth to promote development of two axillary branches. Plants were Y-grafted as described by Pantalone et al. (14) to generate a 5 × 5 combination of plants with a SCN susceptible or resistant rootstock having a self-grafted branch, and a scion (alien) grafted branch or non-grafted branch. Control plants were not grafted. The soybean lines used were: (i) SN97-6946 (SCNR, resistance derived from PI-88788) with conventional FA ratio; (ii) Magellan (SCNS) with conventional FA ratio; (iii) S02-1379-2 with higher than typical oleic (SCNS); (iv) IA 3017 with lower than typical linolenic (SCNS); and (v) Williams 82 with conventional FA ratio (SCNS).

The study was conducted in 2004 and 2005 in a field near Columbia, MO, verified to be infested with SCN Race 3 in 2003. The field was divided into four blocks with two blocks each randomly chosen and no-till planted with glyphosate-resistant Asgrow lines 3302 (SCNS) or 3701 (SCNR, resistance derived from PI-88788) on 28 June 2003, 3 June 2004, and 7 June 2005. The planting placement of the Asgrow lines was the same in 2003, 2004, and 2005. The field was sprayed with glyphosate at the rate of 1.5 lb ai/acre after seedling emergence. Grafted plants and controls were randomly transplanted among the Asgrow lines on 15 June 2004 and 20 June 2005 and grown to physiologic maturity (R8). Ultra Blazer (acifluorfen) was applied at the rate of 1 pt/acre tank mixed with Poast (sethoxydim) at 1.5 pt/acre on 16 July 2004 and 22 July 2005 to control broadleaved and grass weeds. Irrigation was applied as needed.

Greenhouse bioassays were performed for each soybean line and Asgrow cultivar as described by Rao-Arelli et al. (17) using eggs collected from Asgrow 3302 and 3701 plants grown at the field site during 2003. Seeds of the five soybean lines and Asgrow 3302 and 3701 were germinated, planted and inoculated with SCN eggs. Ten seedlings were grown for 4 to 5 days prior to their inoculation with 1200 ± 25 eggs in 5 mL of suspension (distilled water) with an automatic pipetter (16). Approximately 30 days after inoculation, plant roots were individually washed with a strong jet of water to dislodge SCN white females and cysts. These were counted under a stereomicroscope, and female index (FI) was calculated for the number of females developing on each line in each replication (9). Each seedling represented a single replication within a genotype, and the test was completely randomized. Data were combined for ANOVA (SAS Institute Inc., Cary, NC) of female indices and means were separated with Fisher’s LSD based on a significant F test. Ratings of resistant/susceptible reactions of the soybean lines were based on Schmitt and Shannon (20). The bioassay demonstrated that line SN97-6946 and Asgrow 3701 exhibited resistance to Race 3 (mean cyst number of 6.3 per plant) and Asgrow 3302 and the four other soybean lines were susceptible (mean cyst number of 60.6 per plant).

Nematode egg densities were determined at soybean harvest 2003, 2004, and 2005. Plots within each block were sampled for SCN cysts and eggs in October. Ten 2.5-cm-diameter × 20-cm-deep soil cores were collected from random points within the rows of soybean plants and mixed. The soil samples...
were stored at 4°C before being processed. Cysts were extracted from a 100 cm³ subsample of soil on a 150-µm-pore sieve and mechanically ruptured to release eggs (13). The nematode population density was expressed as number of eggs per 100 cm³ of soil. Numbers of SCN eggs were significantly higher in soil derived from the plots of Asgrow 3302 in 2003, 2004, and 2005 with an average of 5341, 10030, and 14612 eggs per 100 cm³ compared to 1635, 2967, and 1563 for Asgrow 3307.

**Investigated Effects of SCN on Soybean Seed Composition**

Concentrations of palmitic, stearic, oleic, linoleic, and linolenic acids as a percentage of the total fatty acids in the seed oil were evaluated for each plant by randomly selecting pods that were later threshed by hand. From each sample seeds were randomly selected for FA analysis. Seeds were manually chipped to obtain a tissue sample fatty acid extraction. Tissue was extracted in 5 mL chloroform:hexane:methanol (8:5:2, v/v/v) overnight. Derivitization was done by transferring 100 µL of extract to a vial and adding 75 µL of methylating reagent (0.25 M methanolic sodium methoxide:petroleum ether:ethyl ether, 1:5:2 v/v/v). Hexane was added to dilute samples to approximately 1 mL. An Agilent (Palo Alto, CA) series 6890 capillary gas chromatograph fitted with a flame ionization detector (275°C) was used with an AT-Silar capillary column (Alltech Associates, Deerfield, IL). Standard fatty acid mixtures (1) were used as calibration reference standards.

Composition comparisons were made between seed produced from lines grafted on SCNR or SCNS rootstock and their respective non-grafted controls. Data was analyzed as a randomized complete block (RCB) using PROC GLM (SAS Institute Inc.) for FA content. Years, blocks (Asgrow lines), and entries were considered fixed effects while replications were considered random. Oleic acid content showed heterogeneity of variance within each year. Variance increased with increasing levels of oleic and a square root transformation was used to normalize the data and yield a homogeneous variance. Within entry comparisons were made between non-grafted control plants and self-grafted or non-grafted branches of grafted plants to determine if there was an effect due to grafting. Mean comparisons were made using Fisher’s LSD at $P = 0.05$ significance level.

As all entries were transplanted into populations of the two Asgrow lines (blocks), a comparison was made between blocks for the five FA (Table 1). Mean palmitic and stearic acid levels were not different among the transplants interplanted into either Asgrow population, but oleic acid was significantly lower and linoleic and linolenic acids were significantly higher for transplants grown within Asgrow 3302 (SCNS) compared to those interplanted into Asgrow 3703 (SCNR). Differences in FA levels between 2004 and 2005 were significant. There were no significant ($P = 0.05$) block × year effects for FA.
Table 1. Mean comparisons for block and year effects on levels of fatty acids in two Asgrow varieties with conventional levels of fatty acids. There were no significant block × year effects for fatty acids.

<table>
<thead>
<tr>
<th></th>
<th>Palmitic</th>
<th>Stearic</th>
<th>Oleic</th>
<th>Linoleic</th>
<th>Linolenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asgrow 3302 (SCNS)</td>
<td>11.06</td>
<td>3.91</td>
<td>29.14</td>
<td>49.98</td>
<td>5.92</td>
</tr>
<tr>
<td>Asgrow 3701 (SCNR)</td>
<td>11.00</td>
<td>3.96</td>
<td>30.56</td>
<td>48.88</td>
<td>5.60</td>
</tr>
<tr>
<td>Mean</td>
<td>11.0</td>
<td>3.9</td>
<td>29.9</td>
<td>49.4</td>
<td>5.8</td>
</tr>
<tr>
<td>LSD05</td>
<td>NSx</td>
<td>NS</td>
<td>0.8</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>2004</td>
<td>11.09</td>
<td>3.88</td>
<td>27.27</td>
<td>51.64</td>
<td>6.12</td>
</tr>
<tr>
<td>2005</td>
<td>10.98</td>
<td>3.98</td>
<td>31.86</td>
<td>47.72</td>
<td>5.47</td>
</tr>
<tr>
<td>Mean</td>
<td>11.0</td>
<td>3.9</td>
<td>29.6</td>
<td>49.7</td>
<td>5.8</td>
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<tr>
<td>LSD05</td>
<td>0.1</td>
<td>0.1</td>
<td>0.8</td>
<td>0.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

x NS = not significant at $P \leq 0.05$ according to Fisher’s LSD.
Y SCNS = soybean cyst nematode susceptible;
SCNR = soybean cyst nematode resistant.

Significant differences were observed between the FA levels of seed harvested from non-grafted control plants and self-grafted or non-grafted branches of grafted plants within lines (Table 2). Oleic acid was significantly lower for the non-grafted control of SO2-1379-2 and SN07-6942 over their respective self-grafted or non-grafted branches, but significantly greater for Magellan and Williams 82. Oleic acid differences for IA3017 among the non-grafted control and self-grafted or non-grafted branches were not significant. Linoleic levels within lines inversely mimicked oleic acid levels. Among lines there was no consistent trend of effects in FA levels and grafting.
Table 2. Mean comparisons of fatty acid levels in seed produced on branches of non-grafted control plants versus grafted plants with self-grafted and non-grafted branches.\textsuperscript{x} Soybean lines with modified or unmodified fatty acids in seeds were grafted onto soybean cyst nematode susceptible (SCNS) or resistant (SCNR) rootstock and field grown in 2004 and 2005. The line SO2-1379-2 produces more oleic acid and IA-3017 produces less linolenic acid than the other three lines with conventional levels of fatty acids.

\textsuperscript{x} Soybean lines with modified or unmodified FA were grafted onto soybean cyst nematode susceptible (SCNS) or resistant (SCNR) rootstock and field grown in 2004 and 2005. The line SO2-1379-2 produces more oleic acid and IA-3017 produces less linolenic acid than the other three lines with conventional levels of fatty acids.

\textsuperscript{y} NS = not significant at $P \leq 0.05$ according to Fisher’s LSD.

Self-grafts of soybean lines with modified or unmodified FA were compared to their grafts onto SCNR or SCNS rootstocks (Table 3). Oleic acid of SO2-1379-2 seeds was significantly higher on the SCNR rootstock of SN97-6946. There was no significant effect observed in the linolenic acid of IA-3017 seeds produced on either SCNR or SCNS rootstocks, but oleic acid was significantly lower and linoleic acid was significantly higher when IA-3017 was grafted onto Williams 82 (SCNS). Palmitic and linolenic acid levels of Williams 82 were significantly greater when grafted on SCNR rootstock while oleic acid was significantly lower, but overall the FA levels changed little. The FA levels of self-grafted Magellan were not different than Magellan grafts onto SCNR or SCNS rootstocks (data not shown).
Table 3. Mean comparisons of self-grafted (in italics) soybean lines with modified fatty acid or unmodified fatty acid profiles in seeds versus when grafted onto soybean cyst nematode susceptible (SCNS) or resistant (SCNR) rootstock.

<table>
<thead>
<tr>
<th>Scion</th>
<th>Rootstock</th>
<th>Palmitic (% )</th>
<th>Stearic (%)</th>
<th>Oleic (%)</th>
<th>Linoleic (%)</th>
<th>Linolenic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO2-1379-2</td>
<td>SO2-1379-2</td>
<td>10.4</td>
<td>4.1</td>
<td>44.1</td>
<td>38.6</td>
<td>3.2</td>
</tr>
<tr>
<td>(SCNS)</td>
<td>SN97-6946</td>
<td>10.1</td>
<td>4.0</td>
<td>46.0*</td>
<td>36.9</td>
<td>2.9*</td>
</tr>
<tr>
<td>Magellan (SCNS)</td>
<td>10.2</td>
<td>4.1</td>
<td>42.4</td>
<td>39.1</td>
<td>2.9*</td>
<td></td>
</tr>
<tr>
<td>Williams 82 (SCNS)</td>
<td>10.1</td>
<td>3.9</td>
<td>42.7</td>
<td>40.5*</td>
<td>2.8*</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.2</td>
<td>4.0</td>
<td>43.8</td>
<td>38.8</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>IA3017 (low linolenic line)</td>
<td>IA3017 (SCNS)</td>
<td>10.7</td>
<td>4.0</td>
<td>37.4</td>
<td>46.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Magellan (SCNS)</td>
<td>11.5</td>
<td>3.7</td>
<td>29.6</td>
<td>53.2</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Williams 82 (SCNS)</td>
<td>11.2</td>
<td>4.7*</td>
<td>27.3*</td>
<td>55.4*</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>11.2</td>
<td>4.1</td>
<td>31.0</td>
<td>52.2</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Williams 82 (conventional fatty acid line)</td>
<td>IA3017 (SCNS)</td>
<td>11.2</td>
<td>4.2</td>
<td>25.0</td>
<td>52.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Magellan (SCNS)</td>
<td>11.2</td>
<td>4.2</td>
<td>25.2</td>
<td>52.3</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Williams 82 (SCNS)</td>
<td>11.3</td>
<td>4.1</td>
<td>24.5</td>
<td>53.0</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>11.3</td>
<td>4.1</td>
<td>24.5</td>
<td>53.0</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different than the self-graft at $P \leq 0.05$ according to Fisher’s LSD; NS = not significant.

### Summary and Considerations

Stresses during soybean seed development can reduce size, quality, and yield of soybean seeds (2,5,7,8,21). SCN feeding on soybean roots interferes with root function, impedes soybean growth and development, and can result in significant yield loss (22). SCN has been reported to alter seed yield, but not effect total oil concentrations. The seed oil concentration in many soybean cultivars has been reported to be positively correlated to stress conditions where the crop is grown (10). Sucrose is the major carbohydrate translocated in the phloem from source (photosynthetic tissues) to sink tissues, like developing seed. Stress induced by SCN could inhibit photosynthesis and the production of sucrose, as-well-as extract sucrose from the phloem. The extraction of sucrose from the phloem by the nematode *H. schachtii* has been documented in infested *Arabidopsis thaliana* (3). Direct sucrose extraction by SCN from the phloem may have visible impacts on soybean growth, development, and yield or subtle effects like altered seed composition.

This study demonstrated that seed quality can be affected by SCN when soybean lines are grown in soils where SCN are present and that grafting of SCN susceptible lines onto SCN resistant rootstocks can have a positive effect on levels of desirable fatty acids. Although grafting is an impractical solution to SCN presence this research demonstrates that SCN may influence seed quality through its effects on fatty acid levels. Cultivars with modified seed composition may need SCN resistance to retain the expected trait expression levels to meet production incentives, especially if yield is reduced where SCN is present.

Recent reports (12) cite the loss of resistance to SCN in prominent soybean production areas, because the source of resistance traces to a single genetic parent, PI 88788 (19). Soybean breeders at Iowa State University have anticipated this SCN resistance problem and have recently released several soybean lines with SCN resistance derived from uncommon sources. Since new
races of SCN pose a threat to sustained use of single-gene resistance in varieties and the expression of the low linolenic trait, soybean breeders may need to include new sources of SCN resistance in all new releases to allow gene expression in environments where SCN is present.

Disclaimers and Acknowledgment

Mention of a trademark, vendor, or proprietary product does not constitute a guarantee or warranty of the product by the USDA or the Univ. of Missouri and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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