EVALUATION OF SOYBEAN HIGH-INPUT MANAGEMENT SYSTEMS AND THEIR EFFECT ON YIELD, ISOFLAVONES, OIL CONTENT AND FATTY ACIDS IN SOYBEAN SEED

By

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ABSTRACT

EVALUATION OF SOYBEAN HIGH-INPUT MANAGEMENT SYSTEMS AND THEIR EFFECT ON YIELD, ISOFLAVONES, OIL CONTENT AND FATTY ACID SIN SOYBEAN SEED

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Many agronomic products are sold to soybean growers that are used to help protect or increase soybean yield. The purpose of this study was to investigate combinations of products to test for synergy in comparison to individual inputs. Field research was conducted at two locations in Michigan during 2012-2014, with various agronomic inputs, and combinations of inputs applied to soybean. Results showed no increased yield for many of the individual products, but higher yields were found with the high input combination of products. When analyzing all Michigan locations together, the Combination treatment increased yield by 10.4%. When analyzing each site/year individually, 3 of the 5 site years showed response to at least five treatments.

Paired comparisons were made between treatments receiving a designated management input and those without the input. Year and location had a significant effect on isoflavone concentrations. The research confirms an interaction between the field environment and management inputs on soybean isoflavone concentrations.

Total oil content was not greatly affected although there was a slight negative correlation of total oil with soybean yield over all locations. There were significant differences in all five fatty acids in relation to agronomic practices in individual site years but the effects were not consistent between sites. All five fatty acids were significantly correlated to yield.

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KEY TO ABBREVIATIONS

ANOVA	Analysis Of Variance
GC-MS	Gas Chromatography Mass Spectrometry
HPLC	High Performance Liquid chromatography
LCO	Lipo-chitooligosaccharide Growth Promoter
NOAA	National Oceanic and Atmospheric Administration
RT	Retention Time
ST	Seed Treatment
UPLC	Ultra Performance Liquid Chromatography
UTC	Untreated Control

CHAPTER 1

EVALUATION OF SOYBEAN SEED YIELD IN RESPONSE TO AGRONOMIC INPUTS

ABSTRACT

Many agronomic products are sold to soybean growers that are used to help protect or increase soybean yield. There has been little research on grain yield with combinations of products in comparison to individual products. The purpose of this study was to investigate combinations of products to test for synergy in comparison to individual inputs. Field research was conducted at 2 locations in Michigan during 2012-2014 with various agronomic inputs, and combinations of inputs applied to soybean. Results showed no increased yield for many of the individual products, but higher yields were found with the high input combination of products. When analyzing all Michigan locations together, the Combination treatment increased yield by 10.4%. Some individual treatments and the combination treatments increased yield, but was not significantly different from the Untreated Control (UTC). When analyzing each site/year individually, 3 of the 5 site years showed response to at least five treatments. The Combination treatment and the Combination without foliar fungicide and insecticide both had the highest response, showing increases at 4 of the 5 site years each.

INTRODUCTION

The role of soybeans has changed from the time soybeans were introduced in the US in the mid-19th century, from a forage crop, to a rotation crop for corn, and to the major crop that it is today. Large-scale production of soybeans began in the 20th century in the United States, and the land area planted to soybeans expanded rapidly. Soybeans now are the second most planted field crop in the United States after corn with 89.5 million acres planted in 2017. (USDA 2017).

Many growers are attempting to maximize yield by increasing the number of inputs used in their production system. Agrochemical companies have developed many novel products that claim to have yield and profitability benefits. Some of these products show positive results under certain circumstances but are marketed to be used with broad application. Other products, despite aggressive marketing campaigns, fail to increase yields when evaluated under replicated, scientifically-based research methods.

Growers are interested in which inputs will increase yields when grain prices are elevated. During the month of August 2012, growers in the US received $16.20 per bushel (USDA NASS 2017). During the month of February 2016, growers in the US received $8.57 per bushel (USDA NASS 2017). When grain markets are low, growers are also interested in knowing which inputs can be eliminated without reducing yields or profitability.

One input which has gained popularity in recent years is the use of seed treatments. There are more seed treatments coming onto the market each year, each promising to protect the investment of seeds and higher yields. The most common seed treatments are fungicides and insecticides. Research results have been mixed as to yield increases from the use of seed treatments, and also if they can increase or protect yields enough to pay for their cost.

Schultz and Thelen (2008) found that fungicidal seed treatment improved yield on 3 of 16 site-years. Gaspar et all (2104) also discovered that fungicide only seed treatments did not show consistent yield increases. Their data showed that fungicide and insecticide seed treatments increased plant stand but yield increases were variable. Cox and Cherney (2011) had similar results showing that seed-applied insecticide/fungicides mostly increased early soybean plant establishment and had only a small increase in yield. Seed treatments had an interaction with cultivar, and could increase yields enough to make them cost effective in a study by Esker and Conley (2011).

Soybeans are a legume and are able to fix nitrogen by utilizing a soil bacteria *Bradyrhizobia japonicum*. Fields that have a history of soybean generally have enough natural *Bradyrhizobia japonicum* in the soil and inoculation is not required. Growers and seed companies, however, are often choosing to add this bacteria to the seed as an inoculant. A study by De Bruin et al. (2009) evaluated 51 inoculant products. Inoculant products had similar effect and did not increase yields at 63 environments. A study by Schultz and Thelen (2008) found that yields increased in 6 of the 14 site years and an average increase of 85.6 kg ha−1 using inoculation on fields where soybeans have been grown before. Inoculum is relatively inexpensive and can be thought of as insurance in case there is not enough natural bacteria in the soil.

Since soybean is able to fix nitrogen, nitrogen fertilizer is generally not used when growing soybean. Yet the question remains, can added nitrogen increase soybean yield. Wesley et al.

(1997) applied various sources of nitrogen fertilizer at the R3 growth stage and found soybean yield increased significantly by late-season N application at six of eight sites; the average increase was 464 kg ha-1 or 11.8 %.

Other nutrients can also be applied as a foliar fertilizer. Studies have shown that a foliar fertilizer can increase yield when soil is low in certain nutrients (Nelson et all, 2005, Ross et all, 2006). Other studies had shown that there is little or no increase in yield when there is no deficiency in soil nutrients. (Haq and Mallarino, 2000). In 16 cultivar-location-year trials conducted by Poole et al, 1982, soybean seed yield increased significantly by the foliar fertilizer treatments over the control only once.

Foliar fungicides are intended to protect plants from fungus. The prophylactic use of fungicides is being advertised by chemical companies as a way to increase soybean grain yield. Results of fungicide studies have been mixed. Some studies support this claim and have shown that fungicides can increase yield when disease levels are low (Henry et al, 2011). Other studies showed no differences in yield when there was no disease present (Hanna et al, 2008). Swoboda and Pedersen (2008) found that pyraclostrobin applied at R3 increased plant biomass by 10% by increasing stem biomass but fungicides applied in the absence of foliar disease did not produce nonfungicidal physiological effect or associated yield improvement.

Foliar insecticides can work effectively when there is insect pressure. Some studies have shown that yield can be increased by spraying insecticides in the absence of insect pressure. A study by Henry et al, (2011) reported an increase of 150 kg ha−1 by an R4 application of lambda-cyhalothrin.

Some techniques have been attempted to modify plant growth to increase yield. One is the use of lactofen to increase branching. If the growing point on the main stem (apical meristem) on a soybean plant is removed or killed, the plant will branch and make new growing points on the braches. Experiments have been conducted with a chemical that burns the plant and kills the apical meristem, such as lactofen, to test if increased branching and increased yields can be achieved. Results on the yield response to defoliant applications have been inconsisitent (Orlowski, 2016).

Few studies have tested multiple inputs in a high input system against individual inputs. (Boring kitchen sink, Orlowski, Marburger). A study by Bluck found a very small potential for high-input production systems to enhance crop yield without the presence of diseases, insects, or nutrient deficiencies (Bluck, 2015). A multi-state study conducted by Orlowski et al, 2016 found that several treatments in the north region increased soybean yield, and the greatest yield increases were found in treatments that contained several inputs. Even with higher yields, the high input system indicated low probability of recouping product application expenses. A Similar study by Marburger et al (2016) tested 6 cultivars suitable for each location and found the two high input systems yielded 5.5% and 3.5% higher than the standard practice, but only found interaction with management system and cultivar at 3 of 53 site years.

The objective of this study was to determine the yield response of soybean to a number of agronomic inputs and management practices in Michigan.

MATERIALS AND METHODS

Site Description

Field experiments were established in 2012, 2013, and 2014 at Breckenridge, MI (N 43° 29' 27.6'', W 84° 24' 30.24'') and at East Lansing, MI (N 42° 42' 35.64'', W 84° 28' 14.16'). The East Lansing location was established at the Michigan State University Agronomy Research Farm in Ingham County on a Capac Loam soil (fine-loamy, mixed, mesic, Aeric Ochraqualfs). The Breckenridge location was in Midland County, MI and has a Parkhill Loam soil (fine-loamy, mixed, semiactive, nonacid mesic Mollic Epiaquepts). Fields were chosen to be average or high yielding fields to represent modern production agriculture in the Great Lakes Region. Standard fertility practices were used.

Crops were rotated and fields were rotated so the experiment was not always in the same exact field and location. Each soybean trial followed corn, with the exception of the Breckenridge location in 2014, which followed soybeans but had corn in the 2012 year. Tillage on each plot was done by field cultivating in the spring to prepare a seed bed and most locations were chisel plowed the previous fall.

Plot Size and Shape

All studies were planted at 432,250 seeds ha-1 (175,000 seeds per acre), in 6 rows 38 cm apart using a 6 row planter with an Almaco 36 cell flood cone over each John Deere 7000 row unit. A 76 cm “guess row” spacing was left between plots to allow space to walk while spraying treatments and taking notes. Planted plot size was 3 by 12 m and plots were trimmed prior to harvest to 10.4 m in length, and the center 4 rows were harvested. The Breckenridge site was planted on May 21, 2012; May 9, 2013, and May 25, 2014. Planting dates at East Lansing were May 9, 2013 and May 22, 2014.

Tillage and pest management operations were performed according to university best management practices. Weeds were controlled by an application of glyphosate when weeds were less than 5 cm in height and soybeans were in the V3 growth stage. The application was repeated later in the season if necessary. Plots were machine harvested and soybean grain yield was measured with calibrated weight scales and adjusted to 13% moisture. Soybean seeds were collected at harvest from the experimental field locations for quality analysis.

Plot Maintenance and Notes

Stand counts were done at V2-V3 stage, and again just prior to harvest. This was done by counting the plants in one meter of row on each of 3 rows per plot. The exact location in the plot was marked with garden stakes so the same area could be counted both times. Throughout the season, notes were taken on stage of crop, insect and disease pressure, and other opportunity notes.

Since insecticidal seed treatments and foliar insecticides were used as treatments in the study, a blanket insecticide was not used across the entire location. Japanese beetles (*Popillia japonica*), bean leaf beetles (*Cerotoma trifurcate*), aphids (*Aphis glycines*), and other insects were found in these plots, but they were below university thresholds and were not expected to have a great effect on yield.

Description of Treatments

Inputs used were commercially available products marketed to increase or protect potential grain yield. Three separate seed treatment combinations were used in this study. The first seed treatment was a fungicide, pyraclostrobin, applied at 0.031 mg ai per seed, metalaxyl applied at 0.049 mg ai per seed, and fluxapuroxad applied at 0.0161 mg ai per seed marketed as Acceleron. (Table 1.1). The second seed treatment was pyraclostrobin with the addition of an insecticide imidacloprid (N-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl}nitramide) at 0.2336 mg a.i. per seed, clothianidin [1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine] at 0.13 mg a.i. per seed, and Bacillus firmus at 0.026 mg a.i. per seed. The third seed treatment was Max seed treatment and contained the same products as the fungicide and insecticide system, with the addition of a nematacide and biologicals (*Bradyrhizobium japonicum*) and lipo-chitooligosaccharide (LCO) at an application rate of 1.83 mL per kg seed and included a foliar applied LCO at a rate of 292 mL ha^{-1}. The nitrogen treatment consisted of 84 kg ha^{-1} of urea plus an urease inhibitor (Agrotain, Koch Agronomic Services, Wichita, KS) at 82 ml Mg-1, and 84 kg ha^{-1} of polymer coated urea (44-0-0) at soybean growth stage V4. A defoliant, lactofen, was applied at 240 g a.i. ha^{-1} with 1% crop oil concentrate at growth stage V4. Foliar fertilizer, (11-8-5-0.1-0.05-0.04-0.02-0.00025-0.00025% N-P_2O_5-K_2O-Fe-Mn-Zn-B-Co-Mo) was applied at 4676 ml ha^{-1} at growth stage R1. An antioxidant, N,N'-diformyl urea, was applied at 1169 mL ha^{-1} at growth stage R3. A foliar fungicide pyraclostrobin was applied in 2012 at 108 g a.i. ha^{-1} at growth stage R3. In the years 2013 and 2014 the foliar fungicide was a combination product containing pyraclostrobin applied at 194 g a.i. ha^{-1} and fluxapyroxad at 97 g a.i. ha^{-1}. Foliar insecticide lambda cyhalothrin was used in 2012 at 35 g a.i. ha^{-1} while in the years 2013 and 2014 the foliar insecticide was a combination product containing lambda cyhalothrin applied at 31 g a.i. ha^{-1} and thiamethoxam at 41 g a.i. ha^{-1} at growth stage R3. All field treatments with rates and timing are provided in Table 1.2.

Table 1.1. Description of agronomic management inputs

		F	Max.	N	Defoliant	Fertilizer	Antioxidant	F	I
1	UTC	-	-	-	-	-	-	-	-
2	Antioxidant	-	-	-	-	-	+	-	-
3	Seed Fungicide	+	-	-	-	-	-	-	-
4	Seed F+I+B	+	-	-	-	-	-	-	-
5	Seed Max ST	+	+	-	-	-	-	-	-
6	Foliar fertilizer	-	-	-	-	+	-	-	-
7	Foliar Defoliant	-	-	-	+	-	-	-	-
8	Foliar Fungicide	-	-	-	-	-	-	+	-
9	Foliar Insecticide	-	-	-	-	-	-	-	+
10	Foliar I + F	-	-	-	-	-	-	+	+
11	Nitrogen	-	-	+	-	-	-	-	-
12	Combination	+	+	+	-	+	+	+	+
13	Combination + Defoliant	+	+	+	+	+	+	+	+
14	Combination - Nitrogen	+	+	-	-	+	+	+	+
15	Combination - Foliar F	+	+	+	-	+	+	-	+
16	Combination - Foliar F+I	+	+	+	-	+	+	-	-

B: Biologicals
F: Fungicides
I: Insecticides
Max: Maximum seed treatment
N: Nitrogen treatment
ST: Seed treatment
UTC: Untreated control

Table 1.2. Product rates and timing

Product†	Product Use	Active Ingredient	Product Rate	Timing
			mL kg seed^{-1}	
Acceleron F	fungicide	pyraclostrobin + metalaxyl + fluxapyroxad	1.04	seed
Acceleron I	insecticide	imidacloprid	2.6	seed
Poncho/Votivo	insecticide and nematicide	clothianidin + Bacillus firmus	1.83	seed
Optiomize	LCO	Bradyrhizobium japonicum + LCO	1.83	seed
			kg ha^{-1}	
Urea	nitrogen fertilizer	46–0–0%N–P2O5–K2O	84	V4
ESN	nitrogen fertilizer	44–0–0%N–P2O5–K2O	84	V4
			mL ha^{-1}	
Cobra	defoliant	lactofen	877	V4
Ratchet	LCO	LCO	292	V4-V6
Task Force II	foliar fertilizer	11-8-5-0.1-0.05-0.04-0.04-0.02-0.00025-0.00025% N-P2O5-K2O-Fe-Mn-Zn-B-Co-Mo	4676	R1
Bio-Forge	antioxidant	N,N’-diformyl urea	1169	R3
Headline (2012)	fungicide	pyraclostrobin	438	R3
Priaxor (2013-2014)	fungicide	fluxapyroxad + pyraclostrobin	585	R3
Warrior II (2012)	insecticide	lambda-cyhalothrin	140	R3
Endigo	insecticide	lambda-cyhalothrin + thiamethoxam	292	R3

†Acceleron Fungicide and Acceleron Insecticide (Monsanto Co., St. Louis, MO); Optimize (Novozymes, Brookfield, WI);
ESN [environmentally smart nitrogen (polymer-coated urea)] (Agrium, Calgary, Alberta, Canada);
Ratchet (Novozymes, Brookfield, WI); Task Force 2 (Loveland Products, Inc., Greeley, CO);
Bio-Forge (Stoller USA, Inc., Houston, TX); Headline (BASF Corp., Florham Park, NJ) used in 2012;
Priaxor (BASF Corp., Florham Park, NJ) used in 2013–2014;
Warrior II used in 2012;
Endigo used in 2013–2014 (Syngenta Crop Protection, LLC, Greensboro, NC).
Table modified from Orlowski et al., Crop Science 2016.

Each study consisted of 4 replications of each treatment in a randomized complete block design. The cultivar used at Breckenridge in 2012 was Asgrow AG2731 and Asgrow AG2431 for the years 2013 and 2014 and at Michigan State University was Asgrow AG2731 for both years.

Harvest

Plots were machine harvested using an Almaco SP20 plot harvester. Only the center four rows of the plot was harvested for yield. Grain was harvested into mesh bags and brought into the Michigan State University agronomy farm building where they were weighed and moisture was taken. A subsample was kept for grain analysis at MSU, and another was sent to Minnesota State University for analysis done by NIR.

Isoflavone data for this trial can be found in an article published in 2017 titled "Determination of Isoflavone (Genistein and Daidzein) Concentration of Soybean Seed as Affected by Environment and Management Inputs" (Laurenz 2017). Fatty Acid data is also available in an unpublished report titled "Determination of the Fatty Acid Profile of Soybean as Affected by Environment and Management Inputs."

Statistical Analysis

Data were analyzed using PROC GLIMMIX in SAS 9.4. (SAS Institute, Cary NC). Analysis was run on combined locations and each location individually. Contrast statements were run to compare all treatments containing a certain agronomic input against treatments that did not contain the respective input.

RESULTS AND DISCUSSION

Climatological Summary

Mean monthly air temperatures were generally higher in 2012 and 2013 compared to the 30-year average for both locations (Table 1.3). Temperatures for 2014 were lower than the 2012 and 2013 years and slightly lower than the 30-year average. Despite drought conditions throughout much of Michigan in 2012, the Breckenridge location received some timely rains and ended the season with 4.4 cm over the 30-year average. The Breckenridge location received almost the same rainfall in 2014 as 2012, but was 9.8 cm lower than the 30-year average in 2013. Rainfall at the East Lansing location in the years 2013 and 2014 was very near the 30-year average.

Rainfall was considerably below the 30-year average in 2013 for both locations during the critical grain fill month of September. The Breckenridge and East Lansing locations received only 40% and 22% of the 30-year average rainfall in the month of September respectively.

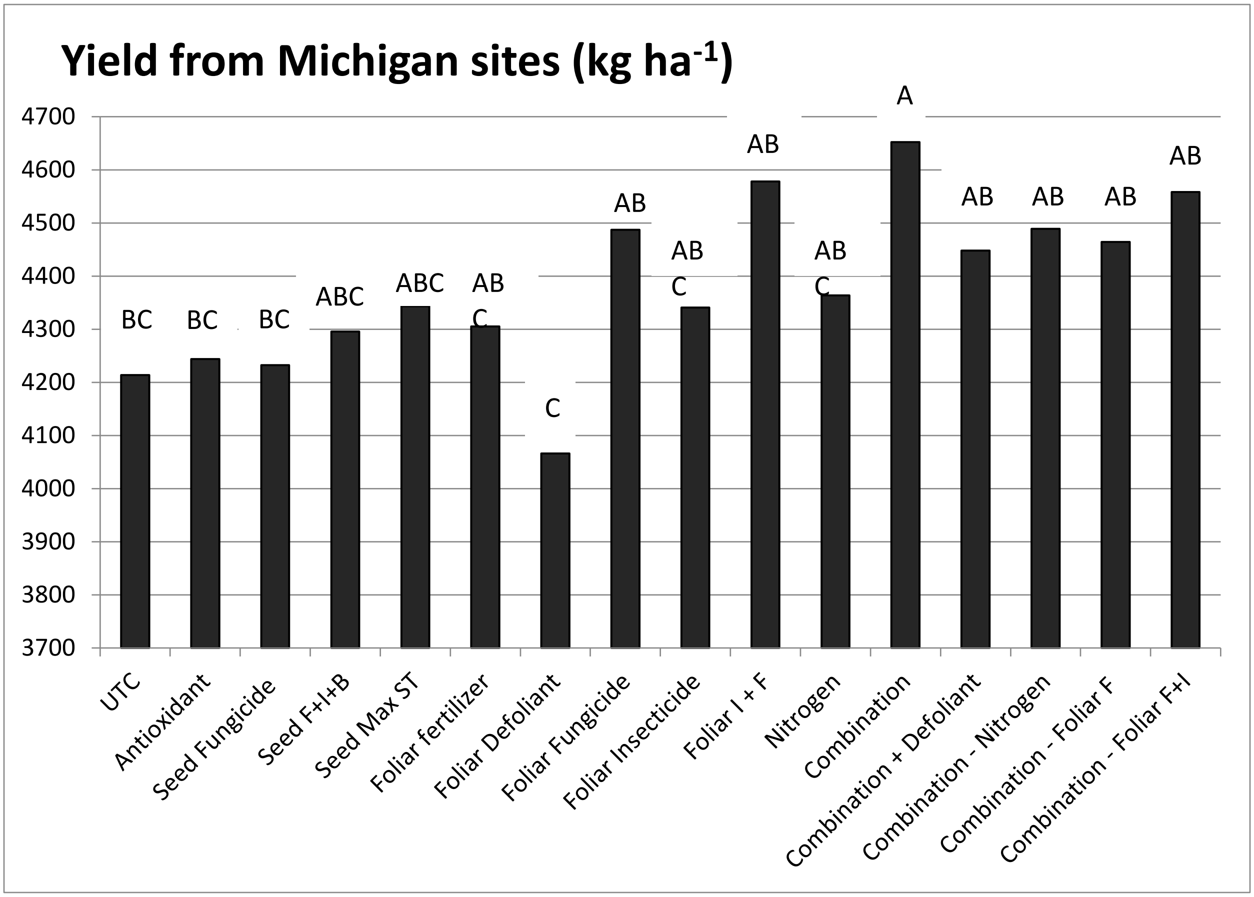
Table 1.3. Monthly precipitation (mm) and mean temperatures (°C) during the study years compared to the 30-year means (1984-2014). The 30-year averages were obtained from NOAA. Weather data were obtained via the Michigan State University Enviro-Weather Station.

Location	Cropping Month	Total precipitation (mm)				Mean Temperature (°C)			
		2012	2013	2014	30-yr Avg.	2012	2013	2014	30-yr Avg.
Breckenridge	May	43	116	80	86	16.5	16.4	14.4	14.1
Breckenridge	June	63	66	110	88	20.7	19.6	20.2	19.4
Breckenridge	July	141	31	131	78	24.1	21.5	19.4	21.6
Breckenridge	Aug	158	91	102	92	20.2	19.9	20.0	20.4
Breckenridge	Sept	26	33	66	82	16.0	16.1	15.7	16.1
Breckenridge	Oct	112	64	55	74	10.0	10.5	9.9	9.5
Breckenridge	Total/Avg	543	402	545	499	17.9	17.3	16.6	16.8
E. Lansing	May	62	84	74	85	16.8	16.1	14.5	14.3
E. Lansing	June	27	115	114	83	20.3	19.5	20.3	19.6
E. Lansing	July	37	56	60	79	24.4	21.6	19.2	21.6
E. Lansing	Aug	53	110	99	82	20.8	20.1	20.5	20.7
E. Lansing	Sept	55	18	80	82	16.3	16.1	15.8	16.4
E. Lansing	Oct	92	118	47	69	10.1	10.9	9.7	9.9
E. Lansing	Total/Avg	326	500	474	480	18.1	17.4	16.7	17.1

Soybean Seed Yield

When looking at all five site years together, there was a general trend for increased yield with increased inputs, but many were not statistically significantly different than the untreated control (Figure 1.1). Treatments 2-11, which included the antioxidant, all the seed treatments, and the individual foliar treatments, did not increase yield significantly. The combination treatment was the only one that showed significantly increased yield over the UTC when looking at all locations together. Lactofen lowered yield and was significantly different from the foliar fungicide, the foliar fungicide with insecticide, and all of the combination treatments.

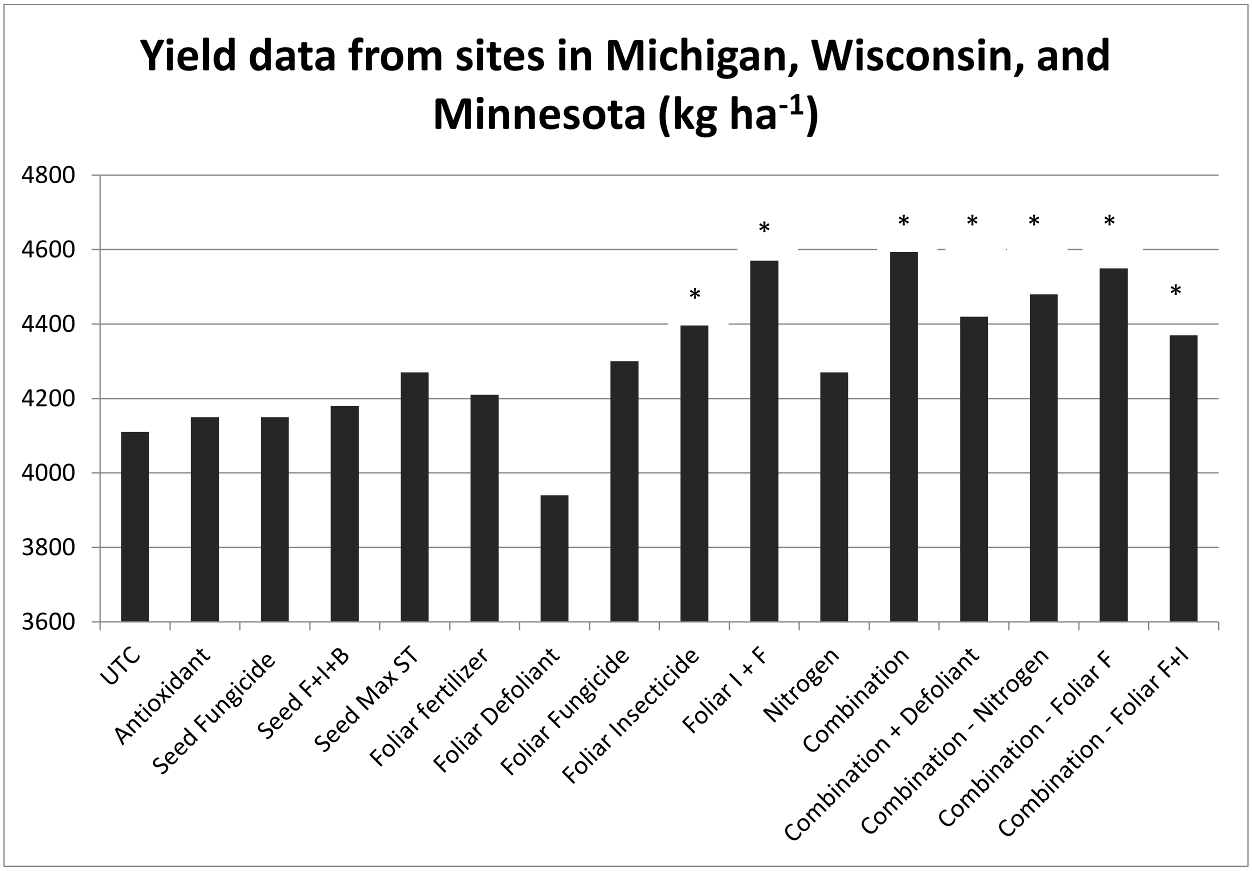
Figure 1.1. Summary of yield by treatments for Michigan 5 site years.



The data from Michigan is very similar to the data from the same Experiment grown in Wisconsin, Minnesota, and Michigan (Figure 1.2). The trends were almost identical and there were more treatments that showed significant differences when combining data from all northern locations. The foliar insecticide and foliar insecticide with fungicide, along with all combination treatments showed increased yield over the UTC.

In the central region, which included Iowa, Illinois, and Indiana, yield trends were the similar but there were no significant differences in any of the treatments compared to the UTC. The same is true for the southern region which included Kentucky, Kansas, and Arizona (data not shown).

Figure 1.2 Summary of yield by treatments for 18 site years in the northern region which includes Minnesota, Wisconsin, and Michigan (Data from Orlowsi Thesis 2015).



*indicate statistically greater than UTC at $p \leq 0.05$

When looking at site years individually, there were some site years that showed much more response than others (Table 1.4). The Breckenridge site in 2012 only showed response to the combination of inputs, and the East Lansing site in 2014 only showed response to the combination minus foliar fungicide and insecticide. There was no significant response to the individual treatments of antioxidant, foliar fertilizer, the defoliant, of foliar fungicide or any of the seed treatments. At the Breckenridge site in 2013, foliar insecticide showed 5.33% increase of the UTC, and Nitrogen showed 5.45% increase over the UTC. The East Lansing site in 2013 showed 10.14% increase with foliar fungicide and insecticide. When looking at the combination of inputs in treatments #10-16, there were many treatments that showed a yield response. The combination treatment showed a positive yield response in 4 of the 5 site years in Michigan.

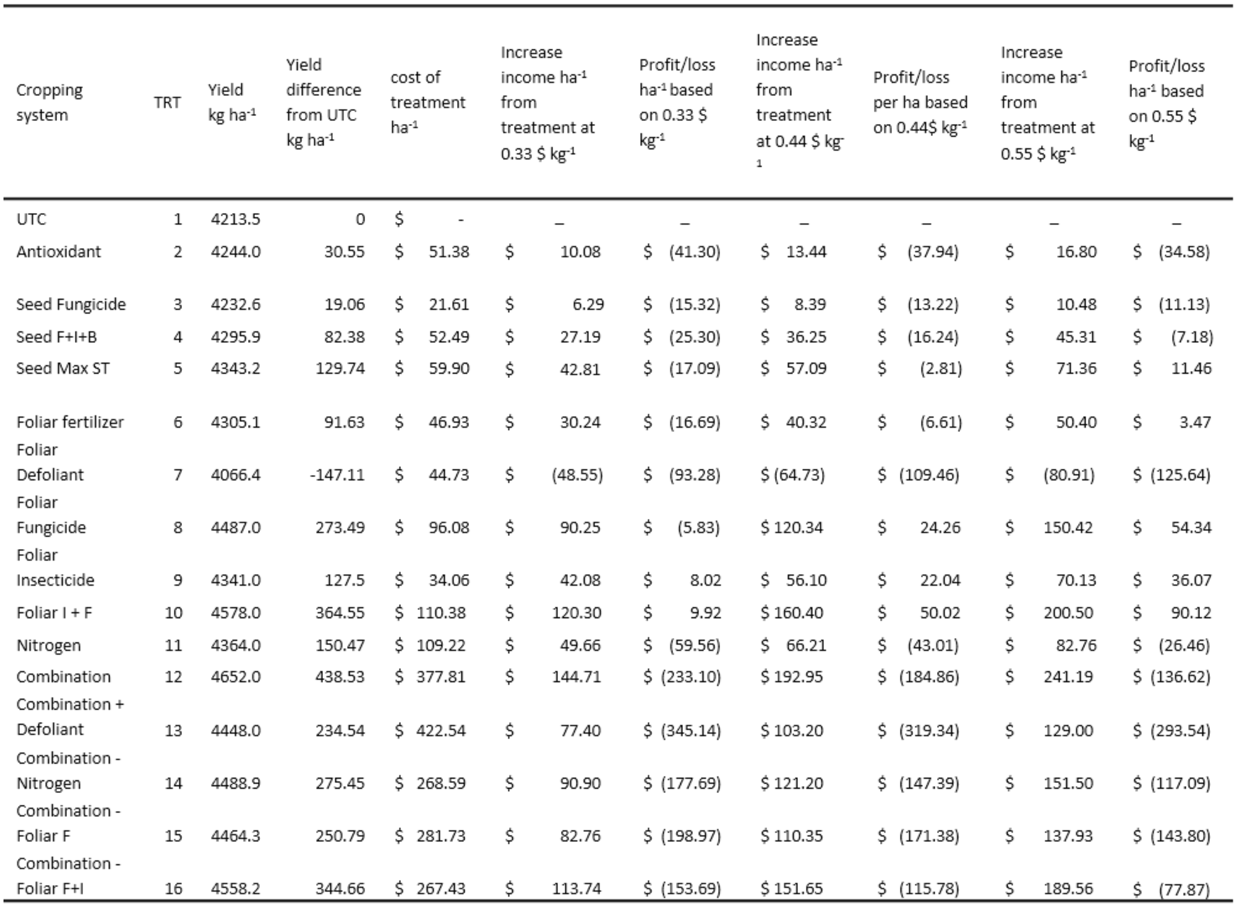
Table 1.4 Yield increase of treatments compared to the Untreated Control (UTC) by site year. Only data that is statistically different is shown in chart.

		% increase in yield over UTC				
Trt		Breck 2012	Breck 2013	Breck 2014	MSU 2013	MSU 2014
1	UTC					
2	Antioxidant					
3	Seed Fungicide					
4	Seed F+I+B					
5	Seed Max ST					
6	Foliar fertilizer					
7	Foliar Defoliant					
8	Foliar Fungicide					
9	Foliar Insecticide		5.33%			
10	Foliar I + F			13.74%	10.14%	
11	Nitrogen		5.45%			
12	Combination	9.00%	7.17%	13.91%	11.02%	
13	Combination + Defoliant		6.02%			
14	Combination - Nitrogen			15.23%		
15	Combination - Foliar F		7.05%		11.89%	
16	Combination - Foliar F+I		6.10%	11.12%	11.83%	7.34%

In a concomitant multi-state study which included these Michigan locations in the compiled analysis, all inputs had no effect or increased yields except lactofen, where there was a yield reduction. In the northern region of this study which included Michigan, Minnesota, and Wisconsin data in the analysis, the complete seed treatment increased seed yield by 3.9%, nitrogen increased seed yield by 3.9%, and foliar fungicide increased seed yield by 4.6%, when compared to the Untreated Control (UTC). Supporting the data in our Michigan study, lactofen decreased seed yield, and all combination cropping systems increased seed yield in the regional data (Orlowski 2016). In another accompanied multi-state study which included these Michigan locations, the management input combination cropping system had greater yield compared to the untreated check in 29 of 53 site-years (55%). The same management system minus the foliar fungicide showed greater yield than the untreated check in 23 of 53 site-years (43%) (Marburger 2016).

Although many of the treatments increased yield, the added income did not usually pay for the extra expense. Yields were calculated as the increase (or decrease) from the Untreated Control (UTC) (Table 1.5). Treatment expenses were calculated using public sources and input from industry representatives and application costs were included for some inputs (Orlowski thesis 2016). The amount of revenue gained was calculated using three grain prices (\$0.33 kg^{-1}, \$0.44 kg^{-1}, and \$0.55 kg^{-1}). Cost of treatment is listed in \$ ha^{-1} over the cost of the untreated control. Calculations are using the insecticide and fungicide that were used in 2013 and 2014.

Table 1.5 Income gain or (loss) for each treatment based on 3 different soybean grain prices.



Cropping system	TRT	Yield kg ha^{-1}	Yield difference from UTC kg ha^{-1}	cost of treatment ha^{-1}	Increase income ha^{-1} from treatment at 0.33 \$ kg^{-1}	Profit/loss ha^{-1} based on 0.33 \$ kg^{-1}	Increase income ha^{-1} from treatment at 0.44 \$ kg^{-1}	Profit/loss per ha based on 0.44\$ kg^{-1}	Increase income ha^{-1} from treatment at 0.55 \$ kg^{-1}	Profit/loss ha^{-1} based on 0.55 \$ kg^{-1}
UTC	1	4213.5	0	\$ -	–	–	–	–	–	–
Antioxidant	2	4244.0	30.55	\$ 51.38	\$ 10.08	\$ (41.30)	\$ 13.44	\$ (37.94)	\$ 16.80	\$ (34.58)
Seed Fungicide	3	4232.6	19.06	\$ 21.61	\$ 6.29	\$ (15.32)	\$ 8.39	\$ (13.22)	\$ 10.48	\$ (11.13)
Seed F+I+B	4	4295.9	82.38	\$ 52.49	\$ 27.19	\$ (25.30)	\$ 36.25	\$ (16.24)	\$ 45.31	\$ (7.18)
Seed Max ST	5	4343.2	129.74	\$ 59.90	\$ 42.81	\$ (17.09)	\$ 57.09	\$ (2.81)	\$ 71.36	\$ 11.46
Foliar fertilizer	6	4305.1	91.63	\$ 46.93	\$ 30.24	\$ (16.69)	\$ 40.32	\$ (6.61)	\$ 50.40	\$ 3.47
Foliar Defoliant	7	4066.4	-147.11	\$ 44.73	\$ (48.55)	\$ (93.28)	\$ (64.73)	\$ (109.46)	\$ (80.91)	\$ (125.64)
Foliar Fungicide	8	4487.0	273.49	\$ 96.08	\$ 90.25	\$ (5.83)	\$ 120.34	\$ 24.26	\$ 150.42	\$ 54.34
Foliar Insecticide	9	4341.0	127.5	\$ 34.06	\$ 42.08	\$ 8.02	\$ 56.10	\$ 22.04	\$ 70.13	\$ 36.07
Foliar I + F	10	4578.0	364.55	\$ 110.38	\$ 120.30	\$ 9.92	\$ 160.40	\$ 50.02	\$ 200.50	\$ 90.12
Nitrogen	11	4364.0	150.47	\$ 109.22	\$ 49.66	\$ (59.56)	\$ 66.21	\$ (43.01)	\$ 82.76	\$ (26.46)
Combination	12	4652.0	438.53	\$ 377.81	\$ 144.71	\$ (233.10)	\$ 192.95	\$ (184.86)	\$ 241.19	\$ (136.62)
Combination + Defoliant	13	4448.0	234.54	\$ 422.54	\$ 77.40	\$ (345.14)	\$ 103.20	\$ (319.34)	\$ 129.00	\$ (293.54)
Combination - Nitrogen	14	4488.9	275.45	\$ 268.59	\$ 90.90	\$ (177.69)	\$ 121.20	\$ (147.39)	\$ 151.50	\$ (117.09)
Combination - Foliar F	15	4464.3	250.79	\$ 281.73	\$ 82.76	\$ (198.97)	\$ 110.35	\$ (171.38)	\$ 137.93	\$ (143.80)
Combination - Foliar F+I	16	4558.2	344.66	\$ 267.43	\$ 113.74	\$ (153.69)	\$ 151.65	\$ (115.78)	\$ 189.56	\$ (77.87)

With a soybean grain price of \$0.33 kg^{-1}, the increased revenue from increased yield was not high enough to pay for the treatments except foliar insecticide and foliar fungicide with insecticide which had an increase of \$8.02 ha^{-1} and \$9.02 ha^{-1} respectively, from the UTC. At a grain market price of \$0.44 kg^{-1}, the foliar fungicide showed an increase of \$24.26 ha^{-1}. Even at a soybean market price of \$0.55 kg^{-1}, only these 5 treatments were profitable. These were the 3 treatments mentioned plus the maximum seed treatment with an increase of \$11.46 kg^{-1}, and foliar fertilizer with a small increase of \$3.47 kg^{-1}.

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CHAPTER 2

DETERMINATION OF ISOFLAVONE (GENISTEIN AND DAIDZEIN) CONCENTRATION OF SOYBEAN SEED AS AFFECTED BY ENVIRONMENT AND MANAGEMENT INPUTS

ABSTRACT

Isoflavones, such as genistein and daidzein, are produced in soybean seed (*Glycine max* (L.) Merr.) and may be associated with health benefits in the human diet. More research is required to determine the effect of agronomic soybean treatments on isoflavone concentration. In this study from 2012 to 2014 at Michigan State University and Breckenridge locations, we evaluated agronomic input management systems which are marketed to increase or protect potential soybean grain yield, including: nitrogen fertilization; herbicide-defoliant; foliar applied fertilizer; a biological-based foliar application; foliar applied fungicide; foliar applied insecticide; a seed applied fungicide; and a maximized seed treatment that included fungicide and insecticide as well as an inoculant and lipo-chitooligosaccharide nodulation promoter; for their effect on soybean seed genistein and daidzein concentrations.

Paired comparisons were made between treatments receiving a designated management input and those without the input. Year and location had a significant effect on isoflavone concentrations. Agronomic management inputs impacted soybean seed daidzein concentrations in 15 of 48 field observations and genistein concentrations in 11 of 48 observations. The research supports findings that soybean seed isoflavone levels exhibit a location specific response, and the temporal variability experienced between years appears to influence changes in soybean isoflavone levels more than location.

INTRODUCTION

The chemical constituents of the soybean (*Glycine max* (L.) Merr.) plant can have a significant effect on human and animal health. Soybean responds to growing conditions and various inputs in many ways including changes in crop quality, crop yield, plant height, and plant structure and architecture. Environmental stress during soybean seed fill can alter the chemical composition of the seed and reduce yield, viability and vigor (Dornbos et al., 1992).

Isoflavones are a group of phytochemicals in soybean and other legumes that are thought to contribute to human health (He F-J, Chen J-Q, 2013). Soybean is an important component of the human diet and is a leading source of isoflavones among major food crops (Bhagwat et al., 2008). Isoflavones are phenolic compounds used to prevent and treat chronic diseases (Barnes et al., 1999) and represent the most common group of phytoestrogens, which are structurally similar to estradiol-17β, the most potent mammalian estrogen (Setchell 1998). Isoflavones exhibit three aglycone structures, which enter into three-glycoside conjugates, each with a corresponding acetyl and malonyl glycoside conjugate (Teekachunhatean et al., 2013). Daidzein and genistein are isoflavone aglycones having a 3-phenylchroman skeleton, and are mainly found in soybean and soy products (Penalvo et al., 2004; Murphy, Barr et al., 2005) as well as in other species of the Fabaceae family (Liggins et al., 2000; Umphress et al., 2005). Since glycitein and its glycoside conjugates account for less than 5–10% of the total isoflavones in soy-based products, most studies have focused on daidzein and genistein and their respective glycoside conjugates (Song, Barua 1998; Jung et al., 2008).

Isoflavone content in soybean depends on both genetic and environmental factors including climate, planting location, crop year, planting dates within a given crop year, and storage conditions (Zhu et al., 2005). In a comprehensive literature review, Bhagwat et al. (2008) reported average total global isoflavone concentrations in raw mature soybean seed of 0.8 mg g^{-1} genistein and 0.6 mg g^{-1} daidzein, although levels varied considerably among multiple sources. Hoeck et al. (2000) showed that genotype, genotype by year, genotype by location, and genotype by year by location interactions were all significant for both total and individual isoflavone concentrations. Isoflavone concentrations were lower in soybean seeds that developed under high field temperatures during seed filling than those in seeds exposed to low temperatures during the seed filling period (Kitamura etal., 1991; Tssukamoto et al., 1995). Soybean grain stored for a long period of time resulted in a decrease in soybean isoflavones, in particular malonyl conjugates and beta-glucosides (Kim et al., 2005). Isoflavone concentrations were increased by low temperatures at maturation, and three malonyglucoisides were easily converted into glucoside groups that were unstable under high heat (Tsukamoto et al., 1995). However, to date, little is known regarding field-level management input effects on soybean isoflavone levels.

The objective of this study was to evaluate the isoflavone (genistein and daidzen) content of soybean grain as affected by location and various field applied agricultural management input systems.

MATERIALS AND METHODS

Field Experiments

Field experiments were established in 2012, 2013, and 2014 at Breckenridge, MI (N 43° 29' 27.6'', W 84° 24' 30.24'') and in 2013 and 2014 at East Lansing, MI (N 42° 42' 35.64'', W 84° 28' 14.16'). The East Lansing location was established at the Michigan State University Agronomy Research Farm in Ingham County on a Capac Loam soil (fine-loamy, mixed, mesic, Aeric Ochraqualfs). The Breckenridge location was in Midland County, MI and has a Parkhill Loam soil (fine-loamy, mixed, semiactive, nonacid mesic Mollic Epiaquepts). Each study consisted of 4 replications of each treatment in a randomized complete block design. The cultivar used at Breckenridge in year 2012 was Asgrow AG2731 and Asgrow AG2431 in years 2013 and 2014, and at Michigan State University the cultivar was Asgrow AG2731 for both years.

Eight agronomic inputs were evaluated for their effect on soybean seed genistein and daidzein levels: nitrogen fertilization; lactofen herbicide-defoliant; foliar-applied fertilizer; a biological-based foliar application (Bio Forge); foliar applied fungicide; foliar applied insecticide; a seed-applied fungicide; and a maximized seed treatment that included fungicide, insecticide, an inoculant and lipo-chitooligosaccharide (LCO) nodulation promoter. Agronomic inputs were applied as single stand-alone inputs and also in combination with other inputs to reflect common grower practices, resulting in fifteen cropping system field treatments and an untreated control. Individual products were combined as part of high-yield management systems and are referred as “Combination” treatments. Inputs used were commercially available products marketed to increase or protect potential grain yield.

Three separate seed treatment combinations were used in this study. The first seed treatment was the fungicide package with pyraclostrobin, (0.031 mg a.i. per seed), metalazul (0.049 mg a.i. per seed), and fluxapuroxad (0.0161 mg a.i. per seed) marketed as Acceleron. (Table 1). The second seed treatment was pyraclostrobin with the addition of an insecticide imidacloprid (N-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl}nitramide) at 0.2336 mg a.i. per seed, clothianidin [1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine] at 0.13 mg a.i. per seed, and Bacillus firmus at 0.026 mg a.i. per seed. The third seed treatment was Max seed treatment and contained the same products as the fungicide and insecticide system, with the addition of a nematacide and biologicals (*Bradyrhizobium japonicum*) and lipo-chitooligosaccharide (LCO) at an application rate of 1.83 mL per kg seed and included a foliar applied LCO at a rate of 292 mL ha^{-1}. The nitrogen treatment consisted of 84 kg ha^{-1} of urea plus an urease inhibitor (Agrotain, Koch Agronomic Services, Wichita, KS) at 82 ml Mg-1, and 84 kg ha^{-1} of polymer coated urea (44-0-0) at soybean growth stage V4. A defoliant, lactofen, was applied at 240 g a.i. ha^{-1} with 1% crop oil concentrate at growth stage V4. Foliar fertilizer, (11-8-5-0.1-0.05-0.04-0.02-0.00025-0.00025% N-P_2O_5-K_2O-Fe-Mn-Zn-B-Co-Mo) was applied at 4676 ml ha^{-1} at growth stage R1. An antioxidant, N,N'-diformyl urea, was applied at 1169 mL ha^{-1} at growth stage R3. A foliar fungicide pyraclostrobin was applied in 2012 at 108 g a.i. ha^{-1} at growth stage R3. In the years 2013 and 2014 the foliar fungicide was a combination product containing pyraclostrobin applied at 194 g a.i. ha^{-1} and fluxapyroxad at 97 g a.i. ha^{-1}. Foliar insecticide lambda cyhalothrin was used in 2012 at 35 g a.i. ha^{-1} while in the years 2013 and 2014 the foliar insecticide was a combination product containing lambda cyhalothrin applied at 31 g a.i. ha^{-1} and thiamethoxam at 41 g a.i. ha^{-1} at growth stage R3. All field treatments are provided in Table 2.1.

Table 2.1. Description of agronomic management inputs used in the study in the years 2012 to 2014.

Cropping system Number	Cropping System Description	Seed Applied		Foliar Applied					
		F	Max	N	Defoliant	Fertilizer	Antioxidant	F	I
1	UTC	-	-	-	-	-	-	-	-
2	Antioxidant	-	-	-	-	-	+	-	-
3	Seed Fungicide	+	-	-	-	-	-	-	-
4	Seed F+I+B	+	-	-	-	-	-	-	-
5	Seed Max ST	+	+	-	-	-	-	-	-
6	Foliar fertilizer	-	-	-	-	+	-	-	-
7	Foliar Defoliant	-	-	-	+	-	-	-	-
8	Foliar Fungicide	-	-	-	-	-	-	+	-
9	Foliar Insecticide	-	-	-	-	-	-	-	+
10	Foliar I + F	-	-	-	-	-	-	+	+
11	Nitrogen	-	-	+	-	-	-	-	-
12	Combination	+	+	+	-	+	+	+	+
13	Combination + Defoliant	+	+	+	+	+	+	+	+
14	Combination - Nitrogen	+	+	-	-	+	+	+	+
15	Combination - Foliar F	+	+	+	-	+	+	-	+
16	Combination - Foliar F+I	+	+	+	-	+	+	-	-

B: Biologicals
F: Fungicides
I: Insecticides
Max: Maximum seed treatment
N: Nitrogen treatment
ST: Seed treatment
UTC: Untreated control

All studies were planted at 432,250 seeds ha^{-1}, in 38 cm rows. Plot size was 3 by 12 m. The Breckenridge site was planted on May 21, 2012; May 9, 2013, and May 25, 2014. Planting dates at East Lansing were May 9, 2013 and May 22, 2014. Tillage, weed control, and pest management operations were performed according to best management practices of Michigan State University. Plots were machine harvested and soybean grain yield was measured with calibrated weight scales and adjusted to 13% moisture.

Determination of Isoflavones

Soybean seeds were collected at harvest from the experimental field locations. Concentrations of daidzein and genistein in soybean were determined using a modified protocol from Franke et al., (1995) using High Performance Liquid Chromatography (HPLC). Finely ground soybean seed (0.4g) was weighed in 15 ml centrifuge tubes and 4ml of 53% acetonitrile was added to each tube. These tubes were shaken in an incubator at relative centrifugal force 7 x g for 2 hrs at ambient temperature and then centrifuged at relative centrifugal force 956 x g for 10 minutes. One ml supernatant was transferred into a 50 ml tube and diluted with 50X deionized water. This experiment was replicated three times. An aliquot (1ml) of the solution was transferred into 96 well HPLC trays for analysis. Isoflavone standards were purchased from Chromadex (Irvine, CA). Isoflavone analytics were done on a Mass Spectrometer (Quatro Premier XE, Waters Company, Milford, MA) and HPLC system (Acquity Ultra Performance Liquid Chromatography (UPLC), Waters Company, Milford, MA) using the following instrumental conditions: Capillary voltage was 3kV, with source temperature 120^{o}C and desolvation temperature 350^{o}C. Desolvation gas flow was 600 L /hr and collision gas flow was 0.15 ml / minute. Ionization mode was ESI negative and function type was MRM mode. MRM channels were: 206.93 > 191.87 (7,8-dihydroxy-6-methoxycoumarin); 268.85 > 116.29 (Apigenin); 253.07 > 223.03 (Daidzein); 269.02 > 132.18 (Genistein). The UPLC column was Ascentis Express C18, 50mm x 2.1mm, with particle size 2.7 µM, and the column temperature was set to 50^{o}C. The mobile phase consisted of two solvents, 0.1% aqueous formic acid (phase A) and 100% HPLC grade Methanol (phase B). The flow rate was set to 0.3 ml/minute, column temperature was 50^{o}C, gradient program set for 5 minutes, and injection volume was 10µL. Recovery was monitored by the addition of a recovery standard, coumarin, to the sample prior to hydrolysis. Using this program, daidzein (retention time (RT) at 2.33 minutes) and genistein (RT at 2.64 minutes were well separated near the beginning of the run. The Mass Lynx software (version 4.0) associated with the Waters HPLC instrument was used to generate linear calibration curves (1:1, 1:1.5, 1:3, 1:6, 1:15, 1:30) based on peak areas for both the isoflavone standards run with each sample batch. Resulting peak areas were used to quantitatively analyze the isoflavone concentrations.

Statistical Analysis

Data were analyzed using PROC GLIMMIX in SAS 9.4 (SAS Institute, Cary NC). Contrast statements were run to compare all treatments containing a certain agronomic input against treatments that did not contain the respective input. Regression analysis (PROC REG) was calculated to check for the correlation between genistein and daidzein levels and among grain yield and the isoflavone content.

RESULTS AND DISCUSSION

Climatological Summary

Monthly precipitation (cm) and mean temperatures (°C) during the study years was compared to the 30-year means (1984-2014). The 30-year averages were obtained from National Oceanic and Atmospheric Administration (NOAA) and weather data were obtained via the Michigan State University Enviro-Weather Station. Mean monthly air temperatures were higher in 2012 and 2013 compared to the 30-year average for both locations (Table 2.2). Temperatures for 2014 were lower than the 2012 and 2013 years and the 30-year average. Despite drought conditions throughout much of Michigan in 2012, the Breckenridge location received some timely rains and ended the season with 4.4 cm over the 30-year average. The Breckenridge location received nearly the same rainfall in 2014 as 2012, but was 9.8 cm lower than the 30-year average in 2013. Rainfall at the East Lansing location in the years 2013 and 2014 was very near the 30-year average. Quality component levels in soybean are generally considered to be influenced by late season weather during the grain fill period (Tsukamoto et al., 1995). Rainfall was considerably below the 30-year average in 2013 for both locations during the critical grain fill month of September. The Breckenridge and East Lansing locations received only 40% and 22% of the 30-year average rainfall in the month of September respectively.

Table 2.2. Monthly precipitation (mm) and mean temperatures (°C) during the study years compared to the 30-year means (1984-2014). The 30-year averages were obtained from NOAA. Weather data were obtained via the Michigan State University Enviro-Weather Station.

Location	Cropping Month	Total precipitation (mm)				Mean Temperature (°C)			
		2012	2013	2014	30-yr Avg.	2012	2013	2014	30-yr Avg.
Breckenridge	May	43	116	80	86	16.5	16.4	14.4	14.1
Breckenridge	June	63	66	110	88	20.7	19.6	20.2	19.4
Breckenridge	July	141	31	131	78	24.1	21.5	19.4	21.6
Breckenridge	Aug	158	91	102	92	20.2	19.9	20.0	20.4
Breckenridge	Sept	26	33	66	82	16.0	16.1	15.7	16.1
Breckenridge	Oct	112	64	55	74	10.0	10.5	9.9	9.5
Breckenridge	Total/Avg	543	402	545	499	17.9	17.3	16.6	16.8
E. Lansing	May	62	84	74	85	16.8	16.1	14.5	14.3
E. Lansing	June	27	115	114	83	20.3	19.5	20.3	19.6
E. Lansing	July	37	56	60	79	24.4	21.6	19.2	21.6
E. Lansing	Aug	53	110	99	82	20.8	20.1	20.5	20.7
E. Lansing	Sept	55	18	80	82	16.3	16.1	15.8	16.4
E. Lansing	Oct	92	118	47	69	10.1	10.9	9.7	9.9
E. Lansing	Total/Avg	326	500	474	480	18.1	17.4	16.7	17.1

Isoflavones

Daidzein was slightly more responsive to the applied management inputs than genistein (Tables 2.3 and 2.4) and levels were within reported estimations of soybean seed genistein and daidzein levels according to Bhagwat et al., (2008). Statistical analysis showed a significant treatment effect on daidzein seed concentration (g kg^{-1}) in 15 of 48 contrast observations and on genistein (g kg^{-1}) levels in 11 of 48 observations. When a significant response was observed to management inputs it generally resulted in an increase in isoflavone levels (21 of 26 observations). We also observed a significant variation between site years in genistein and daidzein levels (Fig 2.1). Daidzein ranged from an average low concentration in soybean of 1.03

g kg^{-1} in East Lansing 2014, to 5.24 g kg^{-1} in East Lansing 2013. Similarly, genistein ranged from an average concentration in soybean grain of 2.07 g kg^{-1}at East Lansing 2013 to 11.02 g kg^{-1}at Breckenridge 2013. The wide range of genistein and daidzein soybean grain concentrations is consistent with previous reports in the literature (Bhagwat et al., 2008).

Table 2.3. Summary of ANOVA showing the treatment effect on daidzein level in soybean seed at Breckenridge (Breck) and Michigan State University, E. Lansing (E.L.) locations. The difference in daidzein level (g kg^{-1}) is the number shown in the table.

Daidzein	All	2012	2013		2014	
Management Input Contrast	Sites	Breck	Breck	E.L.	Breck	E.L.
Nitrogen vs. no Nitrogen	NS	NS	.26*	NS	NS	NS
Lactofen vs. no Lactofen	-.28**	NS	NS	-1.7**	.35**	NS
Foliar Fertilizer vs. no Foliar Fert.	NS	NS	.38**	NS	.13*	NS
Bio-Forge vs. no Bio-Forge	NS	NS	.42**	NS	NS	NS
Foliar Fung. vs. no Foliar Fung.	.15*	NS	.44**	NS	.12*	NS
Foliar Insect. vs. no Foliar Insect.	.14*	-.16*	.55**	NS	.18**	NS
Seed Fung. vs. no Seed Fung.	NS	.12**	.40**	NS	.14**	NS
Seed Comp. vs. no Seed Comp.	NS	NS	.40**	NS	NS	NS

** Significant at the 0.05 probability level.
* Significant at the 0.1 probability level.
NS Not Significant

Table 2.4. Summary of ANOVA showing the treatment effect on genistein level in soybean seed at Breckenridge (Breck) and Michigan State University, E. Lansing (E.L.) locations. The difference in genestein level (g kg^{-1}) is the number shown in the table.

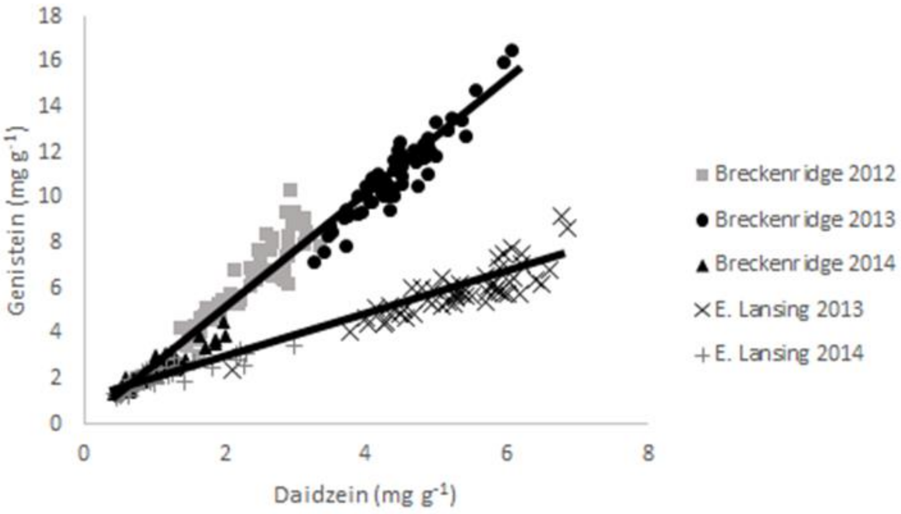
Genistein Management Input Contrast	All Sites	2012 Breck	2013 Breck	 E.L.	2014 Breck	 E.L.
Nitrogen vs. no Nitrogen	NS	.61**	.18*	NS	NS	NS
Lactofen vs. no Lactofen	NS	NS	NS	-1.4**	.49**	NS
Foliar Fertilizer vs. no Foliar Fert.	NS	NS	.94**	NS	NS	NS
Bio-Forge vs. no Bio-Forge	NS	NS	1.0**	NS	NS	NS
Foliar Fung. vs. no Foliar Fung.	NS	NS	1.1**	NS	NS	NS
Foliar Insect. vs. no Foliar Insect.	NS	NS	1.5**	NS	NS	NS
Seed Fung. vs. no Seed Fung.	NS	NS	1.0**	NS	NS	-.16*
Seed Comp. vs. no Seed Comp.	NS	NS	1.1**	NS	NS	NS

** Significant at the 0.05 probability level.
* Significant at the 0.1 probability level.
NS Not Significant

Figure 2.1. Regression of genistein:daidzein across all site years.

At Breckenridge 2012-14, genistein = 2.5 daidzein -0.04; R^2 = 0.96; N = 192.

At East Lansing 2013-14, genistein = 0.9 daidzein + 1.1; R^2 = 0.95; N = 128.



Location and year had an effect on the response of soybean seed isoflavone levels to the field applied management inputs (Tables 2.3 and 2.4). Both daidzein and genistein concentration in soybean grain were especially responsive to management inputs during the 2013 growing season at Breckenridge with all input systems except the application of the soybean herbicide-defoliant increasing isoflavone levels. Additionally, the Breckenridge location in 2014 resulted in daidzein concentrations in soybean grain increasing in response to five of eight management input systems.

Application of a soybean herbicide-defoliant (lactofen) had an inconsistent effect on soybean grain daidzein levels, resulting in an increase at Breckenridge 2014, and a decrease in East Lansing 2013, (Table 2.3). Similarly, plots treated with a foliar applied insecticide had lower concentrations of daidzein in soybean grain at Breckenridge in 2012 but increased daidzein levels at Breckenridge in 2013, and 2014, while having no effect at the East Lansing in the 2013, and 2014, study years. Soybean seed fungicide was the most consistent agricultural input, eliciting an increase in daidzein levels at Breckenridge in all three site years. Foliar applied fungicide and foliar applied fertilizer both increased soybean grain daidzein levels at Breckenridge in 2013 and 2014, but had no effect at East Lansing or at Breckenridge in 2012. Although very little information exists in the literature regarding management effects on soybean isoflavone levels, Vyn et al., (2002) reported a positive response in soybean isoflavone levels to potassium fertilization in soils testing low for potassium. However, the 2013 and 2014 Breckenridge location which had the positive response to foliar fertilization was above optimal for soil potassium levels having 220 and 193 ppm soil K respectively for 2013 and 2014. Nitrogen application and the complete seed treatment application did not affect soybean grain daidzein levels in four of five site years but did increase daidzein levels at Breckenridge in 2013.

At the 2013 Breckenridge location all input systems except the defoliant increased soybean grain genistein levels. Conversely, at other four site years, only one of the eight agricultural management input systems had a significant effect on soybean grain genistein levels. In 2012 at Breckenridge, nitrogen applications increased genistein levels; at East Lansing in 2013, the defoliant application decreased soybean genistein levels; at Breckenridge in 2014, the same defoliant application increased genistein concentrations in soybean seed, suggesting an environmental interaction. Finally, at East Lansing in 2014, the seed applied fungicide decreased genistein concentration in soybean grain, as opposed to the increase in genistein levels observed in response to seed applied fungicide at the 2013 Breckenridge location.

It appears that the dryer than normal August through October precipitation levels in 2013 may have contributed to the increased effect of management practices on isoflavone levels at Breckenridge. A similar level of response to management inputs was not observed at East Lansing during a similar dry September experienced there in 2013, although August and October had more normal precipitation levels at East Lansing. Nevertheless, the trial averages for both genistein and daidzein were the highest at both respective locations during 2013 (Fig 2.1). Kitamura et al., (1991) and Tsukamoto et al., (1995) reported that isoflavone concentrations were lower when seed fill occurred during high temperatures relative to lower temperatures. This appears to conflict with our observations in 2013, but the Breckenridge and East Lansing locations also experienced lower than normal precipitation during the grain fill period which may have interacted with temperature to cause an increase in observed isoflavone levels. The overall wide variability in soybean grain isoflavone levels observed in this study was consistent with the findings of Vyn et al., (2002) on Indiana soybeans grown across varying field conditions.

Genistein and daidzein isoflavone levels were positively and significantly correlated but exhibited a bimodal response to location (Fig 2.1). This suggests that local field conditions had a significant influence on the relative concentrations of the two major isoflavones. Also, Wang and Murphy, 1994 reported that differences in soybean grain isoflavone concentrations of Vinton 81 soybeans seemed to vary more by year than by location. At Breckenridge, the slope function relationship of genistein to daidzein across all study years was 2.5, while at East Lansing it was 0.9. Interestingly, despite clear differences in genistein and daidzein concentrations between years, the slope function between the two major soybean isoflavones stayed remarkably consistent for each location, with a correlation coefficient (R^2) of 0.95 or higher for each location. We did not observe a significant correlation of daidzein or genistein concentration with soybean grain yield (data not shown). This contrasts with the findings of Vyn et al., (2002) who reported a positive correlation between isoflavone concentrations and seed yield (Table 2.5).

Soybean Seed Yield

In this study, management inputs showed differences in seed yield (Table 2.5). In an accompanied multi-state study which included these Michigan locations, all inputs had no effect or increased yields except lactofen, where there was a yield reduction (Orlowski et al., 2016). In the northern region of this study which included Michigan, Minnesota, and Wisconsin, the complete seed treatment increased seed yield by 3.9%, nitrogen increased seed yield by 3.9%, and foliar fungicide increased seed yield by 4.6%, when compared to the Untreated Control (UTC). Supporting the data in our Michigan study, lactofen decreased seed yield, and all combination cropping systems increased seed yield in the regional data (Orlowski et al., 2016). In another accompanied multi-state study which included these Michigan locations, the management input combination cropping system had greater yield compared to the untreated check in 29 of 53 site-years (55%). The same management system minus the foliar fungicide showed greater yield than the untreated check in 23 of 53 site-years (43%) (Marburger et al., 2016). Michigan experienced low rainfall and higher temperature in the early part of the growing season in the year 2012 (Table 2.2) which caused stress during the application timings and may have lowered the management input effects. In the year 2012 at Breckenridge, there were no differences in the eight management inputs except foliar fungicide vs. no foliar fungicide.

Table 2.5. Summary of ANOVA showing the treatment effect on soybean seed yield at Breckenridge (Breck) and Michigan State University, E. Lansing (E.L.) locations. The difference in the grain yield (g kg^{-1}) is the number shown in the table.

Grain Yield Input Contrast	All Sites	2012 Breck	2013 Breck	E.L.	2014 Breck	E.L.
Nitrogen vs. no Nitrogen	229**	NS	202**	511**	135*	175**
Lactofen vs. no Lactofen	-148**	NS	-87*	NS	-249**	NS
Foliar Fert. vs. no Foliar Fert.	188**	NS	182**	282**	188**	175**
Bio-Forge vs. no Bio-Forge	188**	NS	121**	343**	195**	175**
Foliar Fung. vs. no Foliar Fung.	229**	3.1*	94*	296**	296**	215**
Foliar Insect. vs. no Foliar Insect.	202**	NS	141**	289**	249**	188**
Seed Fung. vs. no Seed Fung.	114**	NS	74*	NS	121*	135**
Seed Comp. vs. no Seed Comp.	215**	NS	128**	363**	222**	195**

** Significant at the 0.05 probability level.
*Significant at the 0.1 probability level.
NS Not Significant

CONCLUSIONS

In summary, agricultural inputs did not always impact isoflavone concentrations and daidzein was more responsive to agricultural management inputs than genistein (daidzein responsive in 15 of 48 field observations) and genistein (11 of 48 observations). When a soybean seed isoflavone concentration response to management inputs was observed it generally resulted in an increase in isoflavone concentrations (21 of 26 observations) which demonstrates that managing for high yield is not adversely affecting soybean isoflavone concentrations. The research confirms an interaction between the field environment and management inputs on soybean isoflavone concentrations. At one particular site year, Breckenridge 2013, all management inputs, with the lone exception of the defoliant, increased daidzein and genistein concentrations when contrasted with systems not having that particular input. The research supports findings that soybean seed isoflavone concentrations exhibit a location specific response, and the temporal variability experienced between years appears to influence changes in soybean isoflavone concentrations more than in location.

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CHAPTER 3

DETERMINATION OF THE FATTY ACID PROFILE OF SOYBEAN AS AFFECTED BY ENVIRONMENT AND MANAGEMENT INPUTS

ABSTRACT

Many agronomic products are sold to soybean growers that are used to help protect or increase soybean yield. There has been little research on these products to identify what effects they have on the quality of the soybean. The purpose of this study was to investigate the effects of agronomic inputs on the fatty acid profile of soybean grain. A research trial was conducted at 2 locations in Michigan in the years 2012-2014 with various agronomic inputs, as well as the combination of inputs, applied to soybean plots. Harvested grain was analyzed for fatty acid profile and total oil content. Total oil content was not greatly affected although there was a slight negative correlation of total oil with soybean yield over all locations. Management inputs were consistent in altering the fatty acid profile of soybean oil. There were significant differences in all five fatty acids in relation to agronomic practices in individual site years but the effects were not consistent between sites. There were very few significant differences in 2012 or either location in 2014 for any of the fatty acids, but in 2013 there were several contrasts showing differences. These were greatest for oleic and linoleic acids, two that also showed greatest correlation to temperature. All five fatty acids were significantly correlated to yield. Palmitic, oleic, and linolenic acids were positively correlated with yield, while stearic and linoleic acids were negatively correlated with yield. This study failed to find evidence that management for high yield adversely affects soybean fatty acid quality.

INTRODUCTION

Soybean (*Glycine max*) is a large part of the diet of many people. Processed soybean is the world's largest source of animal protein feed and the second largest source of vegetable oil. The United States is the leading producer and exporter of soybean, and soybean accounts for about 90% of the US oil seed production (USDA 2017). Soybean produces fatty acids, which have recently gained more interest for health reasons.

Fatty acids are a carboxylic acid of a hydrocarbon chain and a terminal carboxyl group. They can be saturated or unsaturated. The five major fatty acids that are produced in soybean are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and alpha – linolenic acid (C18:3). Essential fatty acids, linoleic and linolenic, cannot be synthesized by animals and must be consumed. Hydrogenation of unsaturated fatty acids increases the shelf life of processed foods containing soybean oil. It also prolongs longevity of soybean oil used in frying before going rancid. If the hydrogenation process is not complete, the result is some level of trans fat. Partially hydrogenated soybean oils are claimed to be one of the causes of heart disease. (Booyens et al., 1988). Oils with high oleic acid have longer shelf life and, therefore, do not need to be hydrogenated.

The internal makeup of the soybean plant can have a large effect on human and animal health. Soybean responds to growing conditions and various inputs in different ways, changing crop quality, yield, plant height, and plant structure (branching). Environmental stress during seed fill can alter the chemical composition of the grain and reduce yield, viability and vigor (Dornbos and Mullen 1992). Delayed planting was found to increase protein content and linolenic acid levels and reduce total oil content and oleic acid levels, but had little or no influence on palmitic, stearic, or linoleic acid levels. The higher seed-fill temperatures associated with early planting were strongly correlated with increased oil content and oleic acid levels and reduced linolenic acid levels (Kane 1995). A study of four management systems including high and low chemical inputs showed little influence on total oil content, and oleic acid (O) or linoleic acid (L) compositions. Soybean grown under a no-till management system had an equal or higher palmitic acid composition than the other three management systems; similarly, conventional tillage treatments had as low or lower linolenic acid composition in soybean when compared with the other three management systems (Gao 2008).

A study comparing fertilizer, no fertilizer and manure in canola found fertilizer applications often increased total saturated fatty acid content and decreased the ratio of oleic/(linoleic+linolenic). (Gao et al., 2009). This suggests that fertilizer may change the fatty acid profile of soybean as well. A study comparing soybean seed oil with varying levels of manganese in the soil found that low manganese in the leaf tissue correlated to higher percentages of linoleic, palmitic, linolenic, and stearic acids and a lower percentage of oleic acid (Wilson et al., 1982). This suggests that soil and/or foliar fertilizers may influence fatty acid levels.

Farmers growing soybean have a wide variety of commercially available inputs, which are most often chosen for agronomic purposes, not for grain quality. To date, there have been very few studies testing the effect of agronomic inputs on soybean oil composition. This study looked at total oil levels and the concentration of five major fatty acids in soybean grown under several different agronomic management input systems.

The objective of this study was to evaluate the total oil content and fatty acid composition of soybean grain as affected by various field applied agricultural management inputs and grain yield.

MATERIALS AND METHODS

Collection of samples: Field Experiments

Field experiments were established in 2012, 2013, and 2014 at Breckenridge, MI (N 43∘ 29′ 27.6′′, W 84∘ 24′ 30.24′′) and in 2013 and 2014 at East Lansing, MI Michigan (42∘ 42′ 35.64′′ N; 84∘ 28′ 14.16′′ W). The East Lansing location was at the Michigan State University Agronomy Research Farm in Ingham County on a Capac Loam soil (fine-loamy, mixed, mesic, Aeric Ochraqualfs). The Breckenridge location was in Midland County, MI on a Parkhill Loam soil (fine-loamy, mixed, semiactive, nonacid mesic Mollic Epiaquepts). Each study consisted of four replications of each treatment in a randomized complete block design. The cultivar used at Breckenridge was Asgrow AG2731 in 2012 and Asgrow AG2431 in 2013 and 2014; while Asgrow AG273 was used both years at Michigan State University.

Eight agronomic inputs, alone or in combinations, were evaluated for their effect on soybean fatty acid levels: nitrogen fertilization; lactofen herbicide-defoliant; foliar applied fertilizer; a biological-based foliar application; foliar applied fungicide; foliar applied insecticide; a seed applied fungicide; and a maximized seed treatment that included fungicide and insecticide as well as an inoculant and lipo-chitooligosaccharide (LCO) nodulation promoter. Agronomic inputs were applied as single stand-alone inputs and also in combination with other inputs to reflect common grower practices, resulting in fifteen field treatments and an untreated control. Individual products were combined as part of high-yield management systems and are referred as “combination” treatments. All field treatments and application rates are provided in Table 1. Similar field treatments were applied at both locations all three years.

All studies were planted at 432,250 seeds ha^{-1}, in 38 cm rows; and the plot size was 3 by 12 m. The Breckenridge site was planted on May 21, 2012; May 9, 2013, and May 25, 2014. Planting dates at East Lansing were May 9, 2013 and May 22, 2014. The MSU location in 2012 received 15.47 cm less precipitation than the 30-year average, showed severe drought symptoms, and endured a significant spider mite infestation and therefore was not used for analysis. Tillage, weed control, and pest management operations were performed according to university best management practices. Plots were machine harvested and soybean grain yield was measured with calibrated weight scales and adjusted to 13% moisture. Soybean seeds were collected at harvest from the experimental field locations for quality analysis.

The first seed treatment was a fungicide, pyraclostrobin, applied at 0.031mg a.i. per seed, metalazul applied at 0.049mg a.i. per seed, and fluxapuroxad applied at 0.0161mg a.i. per seed marketed as Acceleron (Table 1). The second seed treatment was pyraclostrobin with the addition of an insecticide imidacloprid (N-{1-[(6-chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl} nitramide) at 0.2336mg a.i. per seed, clothianidin [1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine] at 0.13mg a.i. per seed, and Bacillus firmus at 0.026mg a.i. per seed. The third seed treatment was Max seed treatment and contained the same products as the fungicide and insecticide system, with the addition of a nematacide and biologicals (Bradyrhizobium japonicum) and lipo chitooligosaccharide (LCO) at an application rate of 1.83 mL kg−1 seed and included a foliar applied LCO at a rate of 292 mL ha^{-1}. The nitrogen treatment consisted of 84 kg ha^{-1} of urea plus an urease inhibitor (Agrotain; Koch Agronomic Services, Wichita, KS, USA) at 82 mL Mg^{-1}, and 84 kg ha^{-1} of polymer-coated urea (44-0-0) at soybean growth stage V4. A defoliant, lactofen, was applied at 240 g a.i. ha^{-1} with 1% crop oil concentrate at growth stage V4. Foliar fertilizer (11-8-5-0.1-0.05-0.04-0.02-0.00025-0.00025% N-P2O5-K2O-Fe-Mn-Zn-B-Co-Mo) was applied at 4676 mL ha−1 at growth stage R1. An antioxidant, N,N'-diformyl urea, was applied at 1169 mL ha−1 at growth stage R3. A foliar fungicide, pyraclostrobin, was applied in 2012 at 108 g a.i. ha−1 at growth stage R3. In the years 2013 and 2014 the foliar fungicide was a combination product containing pyraclostrobin applied at 194 g a.i. ha^{-1} and fluxapyroxad at 97 g a.i. ha^{-1}. The foliar insecticide lambda cyhalothrin was used in 2012 at 35 g a.i. ha^{-1} while in the years 2013 and 2014 the foliar insecticide was a combination product containing lambda cyhalothrin applied at 31 g a.i. ha^{-1} and thiamethoxam at 41 g a.i. ha^{-1} at growth stage R3. All field treatments are provided in Table 3.1.

Table 3.1. Description of agronomic management inputs used in the study.

Cropping system Number	Cropping System Description	Seed Applied		Foliar Applied					
		F	Max	N	Defoliant	Fertilizer	Antioxidant	F	I
1	UTC	-	-	-	-	-	-	-	-
2	Antioxidant	-	-	-	-	-	+	-	-
3	Seed Fungicide	+	-	-	-	-	-	-	-
4	Seed F+I+B	+	-	-	-	-	-	-	-
5	Seed Max ST	+	+	-	-	-	-	-	-
6	Foliar fertilizer	-	-	-	-	+	-	-	-
7	Foliar Defoliant	-	-	-	+	-	-	-	-
8	Foliar Fungicide	-	-	-	-	-	-	+	-
9	Foliar Insecticide	-	-	-	-	-	-	-	+
10	Foliar I + F	-	-	-	-	-	-	+	+
11	Nitrogen	-	-	+	-	-	-	-	-
12	Combination	+	+	+	-	+	+	+	+
13	Combination + Defoliant	+	+	+	+	+	+	+	+
14	Combination - Nitrogen	+	+	-	-	+	+	+	+
15	Combination - Foliar F	+	+	+	-	+	+	-	+
16	Combination - Foliar F+I	+	+	+	-	+	+	-	-

B: Biologicals
F: Fungicides
I: Insecticides
Max: Maximum seed treatment
N: Nitrogen treatment
ST: Seed treatment
UTC: Untreated control

Total Oil Content using ASE

Soybean grain total oil content was measured by following the method of Matthaus, and Bruhl (2001). Soybean seeds were ground using a Foss water – cooled Knifetec 1095 sample mill (Tecator AB, Hoganas, Sweden) for 10 seconds. Moisture content was taken for each sample using a moisture analyzer (A & D Company Limited). Ground soybean sample (1.0g) was used for total oil extraction using an accelerated solvent extractor ASE 200 (Dionex, Idstein, Germany) with an 11ml stainless steel extractor cell size. Glass fiber filter (Cat. No. 600004-2129-DB, Environmental express) was placed in the bottom of the extractor cell, and the dead volume of the extractor cell was filled with Ottawa sand (Fisher Scientific, Rochester, NY). The following conditions were set on the ASE system: preheat for 6 min, heat for 6 min, oven temperature at 105^{o}C, static time for 10 min, flush volume 70%, and purge time for 60S, 2 static cycles, and extraction pressure at 1000psi. After processing, the extraction solvent, hexane, was evaporated by purging oxygen-free compressed nitrogen (AGA gas) above the surface. The residual hexane was removed by drying the cells in an oven at 50°C for at least 2 hrs. Prior weight and dry weight of the extractor cell was used to calculate the oil content of the soybean sample. Each field sample was analyzed in triplicate in the lab. The total oil content was calculated by the equation as follows.

$$C = 100 \times Ow / (W \times (1- \text{moisture }\%))$$

Ow (g) is the total oil extracted from ground sample, W (g) is the weight of ground sample, and moisture% is the moisture percentage of the ground sample measured by A & D Moisture analyzer.

Fatty Acid Analysis using GC-MS

Fatty acid composition was measured using gas chromatography-mass spectrometry (GC-MS). Each crushing tray was filled by 2 soybean seeds in each well for each sample. The seeds were crushed at 2.812 metric tonnes cm^{-1} by a seed crusher using a hydraulic press. 400ul n-Hexane was added in to each well, then the trays were closed with glass plates, to prevent evaporation of the hexane. Soybean seeds were soaked for at least 2 hours. From each well, 100ul of liquid was transferred into a labeled glass vial and 500ul of 1N Sodium methoxide was added. Vials were shaken in the plastic tray for 10 minutes on the counter top or until the oil was diluted. For transesterification to occur, the trays were incubated at room temperature for an hour with intermediate shaking after every 10 minutes. Once the oil droplets disappeared, 150ul deionized water and 1250ul of n-Hexane were added into each vial. The samples were analyzed by gas chromatography, carried out with an Agilent 6890N Network GC system combined with an Agilent 7683 Network Mass Selective Detector (GC-MS).

Statistical Analysis

Data were analyzed using in SAS 9.4 (SAS Institute, Cary NC) as appropriate for a randomized complete block design. Analysis of variance (ANOVA) was conducted, and mean separations were accomplished using Fisher's protected LSD test. Probability levels lower than 0.05 and 0.1 were categorized as significantly different. Contrast statements were run with glimmix to compare all entries with a certain agronomic application against entries that did not contain the respective application. Regression analysis was calculated also using glimmix to check for the correlation among the five fatty acids with total oil and grain yield.

RESULTS AND DISCUSSION

Climatological Summary

Mean monthly air temperatures were generally higher in 2012 and 2013 compared to the 30-year average for both locations (Table 3.2). Temperatures for 2014 were lower than the 2012 and 2013 years and slightly lower than the 30-year average. Despite drought conditions throughout much of Michigan in 2012, the Breckenridge location received some timely rains and ended the season with 4.4 cm over the 30-year average. The Breckenridge location received almost the same rainfall in 2014 as 2012, but was 9.8 cm lower than the 30-year average in 2013. Rainfall at the East Lansing location in the years 2013 and 2014 was very near the 30-year average.

Table 3.2. Monthly precipitation (mm) and mean temperatures (°C) during the study years compared to the 30-year means (1984-2014). The 30-year averages were obtained from NOAA. Weather data were obtained via the Michigan State University Enviro-Weather Station.

Location	Cropping Month	Total precipitation (mm)				Mean Temperature (°C)			
		2012	2013	2014	30-yr Avg.	2012	2013	2014	30-yr Avg.
Breckenridge	May	43	116	80	86	16.5	16.4	14.4	14.1
Breckenridge	June	63	66	110	88	20.7	19.6	20.2	19.4
Breckenridge	July	141	31	131	78	24.1	21.5	19.4	21.6
Breckenridge	Aug	158	91	102	92	20.2	19.9	20.0	20.4
Breckenridge	Sept	26	33	66	82	16.0	16.1	15.7	16.1
Breckenridge	Oct	112	64	55	74	10.0	10.5	9.9	9.5
Breckenridge	Total/Avg	543	402	545	499	17.9	17.3	16.6	16.8
E. Lansing	May	62	84	74	85	16.8	16.1	14.5	14.3
E. Lansing	June	27	115	114	83	20.3	19.5	20.3	19.6
E. Lansing	July	37	56	60	79	24.4	21.6	19.2	21.6
E. Lansing	Aug	53	110	99	82	20.8	20.1	20.5	20.7
E. Lansing	Sept	55	18	80	82	16.3	16.1	15.8	16.4
E. Lansing	Oct	92	118	47	69	10.1	10.9	9.7	9.9
E. Lansing	Total/Avg	326	500	474	480	18.1	17.4	16.7	17.1

Temperature and precipitation can greatly affect soybean total oil and fatty acid content. Tsukamoto et al., 1995 found the isoflavone content, together with the ratio of linoleic plus linolenic acid to total fatty acid, significantly decreased in the seeds harvested after growth at a high temperature for all soybean varieties tested. Gao et al found oleic and linoleic were influenced by seasonal precipitation level (2008). Quality component levels in soybean are generally considered to be influenced by late season weather during the grain fill period. Warm temperature after first pod increased oil concentration up to a maximum mean temperature of 28°C. (Piper and Boote, 1999) Rainfall was considerably below the 30-year average in 2013 for both locations during the critical grain fill month of September. The Breckenridge and East

Lansing locations received only 40% and 22% of the 30-year average rainfall in the month of September respectively.

Total Oil

The effect of management practices on total oil content was negligible (Table 3.3). When data were averaged over all site years, none of the management practices tested resulted in a change in soybean seed total oil levels. However, there were a several site-year specific instances where a management practice had an effect on oil levels. At Breckenridge in 2014, plots that received foliar applications of both insecticides and fungicides had lower levels of total oil content in soybean seed. Conversely in East Lansing 2014, foliar applied insecticide had higher levels of total oil content. Also, at Breckenridge 2013, soybean seed oil content increased in response to lactofen (Cobra) application.

Table 3.3. Summary of ANOVA showing the treatment effect on soybean grain oil content. The difference in oil content (g/g) is shown in parentheses.

Oil Content	All	2012	2013		2014	
Input Contrast	Sites	Breck	MSU	Breck	MSU	Breck
Nitrogen vs. no Nitrogen	NS	NS	NS	NS	NS	NS
Lactofen vs. no Lactofen	NS	NS	NS	*(3.52)	NS	NS
Foliar Fertilizer vs. no Foliar Fertilizer	NS	NS	NS	NS	NS	NS
Bio-Forge vs. no Bio-Forge	NS	NS	NS	NS	NS	NS
Foliar Fungicide vs. no Foliar Fungicide	NS	NS	NS	NS	NS	*(-1.33)
Foliar Insecticide vs. no Foliar Insecticide	NS	NS	NS	NS	*(1.11)	*(-1.33)
Seed Fungicide vs. no Seed Fungicide	NS	NS	NS	NS	NS	NS
Seed Complete vs. no Seed Complete	NS	NS	NS	NS	NS	NS

** Significant at 95% confidence
* Significant at 90% confidence
NS not significant

A slight negative correlation of total oil with soybean yield over all locations was observed (Table 3.4). Differences in environment can result in soybean oil levels. Bellaloui et al. (2015) showed that early planting resulted in higher soybean seed oil and oleic acid, but lower protein and linolenic acid concentrations. The late planting resulted in higher protein and linolenic acid. Dombos and Mullen (1992) found that higher air temperature during seed fill resulted in higher protein and lower oil levels. Severe drought caused oil content to lower by 2.9%. It is likely that environment could be a factor in the slight differences in soybean seed total oil content observed in this study. However, overall, differences in soybean oil content were minimal throughout the study. There was no significant correlation observed between fatty acids and total oil (Table 3.4).

Table 3.4. Correlation of total oil (g/g) and fatty acids (%) with soybean grain yield and regression of fatty acids with total oil.

Regression	Pr>/t/	Slope
Total oil: grain yield	*	(-) 0.04
Palmitic acid: grain yield	***	(+) 0.02
Stearic acid: grain yield	***	(-) 0.02
Oleic acid: grain yield	*	(+) 0.03
Linoleic acid: grain yield	***	(-) 0.04
Linolenic acid: grain yield	*	(+) 0.1
Palmitic acid: total oil	NS	
Stearic acid: total oil	NS	
Oleic acid: total oil	NS	
Linoleic acid: total oil	NS	
Linolenic acid: total oil	NS	

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

Fatty Acid Profile

Significant differences were not observed in profiles among the treatments when comparing all site-years together for palmitic, stearic, and linolenic acids, but were observed for oleic and linoleic acids. Significant differences in soybean seed fatty acid profiles between the treatments could be observed when looking at each site-year separately. (Tables 3.5-3.9).

Table 3.5. Summary of ANOVA showing the treatment effect on palmitic acid in soybean grain. The difference in palmitic acid (%) is shown in parentheses.

Palmitic Acid	All	2012	2013		2014	
Input Contrast	Sites	Breck	MSU	Breck	MSU	Breck
Nitrogen vs. no Nitrogen	NS	NS	NS	NS	NS	NS
Lactofen vs. no Lactofen	NS	** (0.24)	NS	NS	NS	NS
Foliar Fertilizer vs. no Foliar Fertilizer	NS	NS	NS	NS	NS	NS
Bio-Forge vs. no Bio-Forge	NS	NS	NS	NS	NS	NS
Foliar Fungicide vs. no Foliar Fungicide	NS	NS	NS	*(0.11)	NS	*(-0.55)
Foliar Insecticide vs. no Foliar Insecticide	NS	NS	NS	*(0.13)	*(0.17)	NS
Seed Fungicide vs. no Seed Fungicide	NS	NS	*(-0.08)	NS	NS	NS
Seed Complete vs. no Seed Complete	NS	NS	NS	NS	NS	NS

** Significant at 95% confidence
* Significant at 90% confidence
NS not significant

Table 3.6. Summary of ANOVA showing the treatment effect on stearic acid in soybean grain. The difference in stearic acid (%) is shown in parentheses.

Stearic Acid	All	2012	2013		2014	
Input Contrast	Sites	Breck	MSU	Breck	MSU	Breck
Nitrogen vs. no Nitrogen	NS	NS	NS	NS	NS	NS
Lactofen vs. no Lactofen	NS	NS	NS	*(-0.37)	NS	NS
Foliar Fertilizer vs. no Foliar Fertilizer	NS	NS	NS	NS	NS	NS
Bio-Forge vs. no Bio-Forge	NS	NS	NS	NS	NS	NS
Foliar Fungicide vs. no Foliar Fungicide	NS	NS	NS	*(-0.18)	*(-0.20)	NS
Foliar Insecticide vs. no Foliar Insecticide	NS	NS	NS	NS	NS	NS
Seed Fungicide vs. no Seed Fungicide	NS	NS	*(0.09)	NS	NS	NS
Seed Complete vs. no Seed Complete	NS	NS	NS	NS	NS	NS

** Significant at 95% confidence
* Significant at 90% confidence
NS not significant

Table 3.7. Summary of ANOVA showing the treatment effect on oleic acid in soybean grain. The difference in oleic acid (%) is shown in parentheses.

Oleic Acid	All	2012	2013		2014	
Input Contrast	Sites	Breck	MSU	Breck	MSU	Breck
Nitrogen vs. no Nitrogen	NS	*(0.60)	NS	NS	NS	NS
Lactofen vs. no Lactofen	*(-0.75)	NS	NS	*(-0.67)	NS	*(-1.54)
Foliar Fertilizer vs. no Foliar Fertilizer	NS	NS	NS	NS	NS	NS
Bio-Forge vs. no Bio-Forge	NS	*(0.56)	*(0.77)	NS	NS	NS
Foliar Fungicide vs. no Foliar Fungicide	NS	NS	NS	**(-.82)	NS	NS
Foliar Insecticide vs. no Foliar Insect.	NS	*(0.54)	NS	**(-.67)	NS	NS
Seed Fungicide vs. no Seed Fungicide	NS	**(0.83)	NS	*(-0.45)	*(0.88)	NS
Seed Complete vs. no Seed Complete	NS	**(0.88)	NS	NS	NS	NS

** Significant at 95% confidence
* Significant at 90% confidence
NS not significant

Table 3.8. Summary of ANOVA showing the treatment effect on linoleic acid in soybean grain. The difference in linoleic acid (%) is shown in parentheses.

Linoleic Acid	All	2012	2013		2014	
Input Contrast	Sites	Breck	MSU	Breck	MSU	Breck
Nitrogen vs. no Nitrogen	NS	*(-0.55)	**(-0.78)	NS	NS	NS
Lactofen vs. no Lactofen	*(0.76)	NS	NS	**(0.97)	NS	NS
Foliar Fertilizer vs. no Foliar Fertilizer	NS	NS	*(-0.53)	NS	NS	NS
Bio-Forge vs. no Bio-Forge	NS	*(-0.51)	**(-0.76)	NS	NS	NS
Foliar Fungicide vs. no Foliar Fungicide	NS	NS	NS	**(0.78)	NS	NS
Foliar Insecticide vs. no Foliar Insect.	NS	NS	NS	**(0.51)	NS	NS
Seed Fungicide vs. no Seed Fungicide	NS	**(-0.81)	NS	NS	NS	NS
Seed Complete vs. no Seed Complete	NS	**(-0.83)	*(-0.60)	NS	NS	NS

** Significant at 95% confidence
* Significant at 90% confidence
NS not significant

Table 3.9. Summary of ANOVA showing the treatment effect on linolenic acid in soybean grain. The difference in linolenic acid (%) is shown in parentheses.

Linolenic Acid	All	2012	2013		2014	
Input Contrast	Sites	Breck	MSU	Breck	MSU	Breck
Nitrogen vs. no Nitrogen	NS	NS	NS	NS	NS	NS
Lactofen vs. no Lactofen	NS	NS	NS	NS	NS	NS
Foliar Fertilizer vs. no Foliar Fertilizer	NS	NS	NS	NS	NS	NS
Bio-Forge vs. no Bio-Forge	NS	NS	NS	NS	NS	NS
Foliar Fungicide vs. no Foliar Fungicide	NS	NS	**(0.22)	NS	NS	NS
Foliar Insecticide vs. no Foliar Insecticide	NS	NS	**(0.19)	NS	NS	NS
Seed Fungicide vs. no Seed Fungicide	NS	NS	NS	NS	*(-0.22)	NS
Seed Complete vs. no Seed Complete	NS	NS	NS	NS	NS	NS

** Significant at 95% confidence
* Significant at 90% confidence
NS not significant

Treatments showed differences between the locations but there was no interaction between location and treatment. The saturated fats (palmitic and stearic acid) showed little response to any of the agronomic practices (Tables 3.6 and 3.7). Foliar fungicide showed some differences by lowering stearic acid levels at Breckenridge in 2013, and MSU in 2014. Foliar fungicide also lowered palmitic acid levels at Breckenridge in 2014 but raised palmitic levels at Breckenridge 2013. Foliar insecticide raised palmitic acid levels at Breckenridge in 2013 and MSU 2014.

Oleic acid and linoleic acid showed the greatest differences from the agronomic practices (Tables 3.8 and 3.9). When comparing all locations together, the defoliant (lactofen) caused oleic acid to decrease and linoleic acid to increase. The effect of the defoliant was particularly evident at the Breckenridge 2013 site year. Oleic acid showed the most differences in response to treatments at the Breckenridge location in 2012 and 2013 although management inputs caused oleic acid levels to consistently raise in 2012 and consistently lower in 2013. This result directly contrasts with linoleic acid levels which consistently increased in response to management inputs in 2013 and decreased in 2012. However, the observed reverse relationship is consistent with oleic and linoleic acid levels being naturally negatively correlated (Burton 1982). The MSU location in 2013 also had several differences and data was similar to the Breckenridge 2012 site where linoleic levels decreased in response to the management input applications. Linolenic acid showed little response to agronomic treatments (Table 3.10).

Soybean oil fatty acid profiles are strongly correlated with the temperature during seed development, particularly oleic, linoleic and linolenic acids (Wolf 1982). There were very few significant differences in 2012 or either location in 2014 for any of the fatty acids, but in 2013 there were several contrasts showing differences. These were greatest for oleic and linoleic acids, the two fatty acids which also showed the greatest correlation to temperature by Wolf, 1982.

All five fatty acids were significantly correlated to yield. Palmitic, oleic, and linolenic acids were positively correlated with yield, while stearic and linoleic acids were negatively correlated with yield (Table 3.4). Yina and Vyn (2004) found that oil concentration in seed decreased 4.2 g kg^{-1} with each megagram per hectare of increased seed yield, but they did not check individual fatty acids. Eleni Bachlava et al (2008) observed a significant negative correlation between yield and oleate content, and positive correlations between yield and linoleate, and linolenate and palmitate contents.

CONCLUSIONS

Common agronomic practices that were used in this study did not significantly increase or decrease total oil levels. In general, management inputs were not consistent in altering the fatty acid profile of soybean oil. Averaged across all site years, application of a defoliant (lactofen) decreased oleic acid levels and increased linoleic acid levels. However, the relationship was greatly influenced by one particularly responsive site-year. There were significant differences in all five fatty acids in relation to agronomic practices in individual site years but the effects were not consistent between sites. Oleic and linoleic acid levels showed the largest response to management inputs.

The differences that were observed, although sometimes statistically significant, were very small and likely insignificant to the soybean grain industry. Oleic acid levels in this trial averaged 23.7% of the total oil. High oleic lines are produced by a major seed companies that are over 75% oleic acid (Waltz 2010). Changes that were observed were less than 1%, which does not raise or lower fatty acid levels significant enough to change marketing options. Nevertheless, when compiled across a geographic region, even a small percentage increase in soybean grain total oil content or quality component level can have a significant effect for a soy processor.

This study provides information on soybean quality as affected by management inputs and finds evidence that managing for high yield does not adversely affect soybean food quality parameters such as fatty acid profile. This type of information is currently not widely known and the information will be specifically of value to Michigan growers but also to growers on a national basis and to the public in general. In addition, the quality component analytical results will increase the scientific literature knowledge base on how management inputs affect soybean crop quality.

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