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Effect of Pod Maturity and Plant Spacing on Isoflavone Content and Harvest Force of Edible Soybeans [*Glycine max* (L.) Merrill]

Allison E. Stewart
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To the Graduate Council:

I am submitting herewith a thesis written by Allison E. Stewart entitled "Effect of Pod Maturity and Plant Spacing on Isoflavone Content and Harvest Force of Edible Soybeans [*Glycine max* (L.) Merrill]." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Carl E. Sams, Major Professor

We have read this thesis and recommend its acceptance:

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Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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A Thesis Presented for
the Master of Science Degree
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Allison E. Stewart
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Abstract

In recent years consumer interest in edamame increased in part due to reported health benefits associated with vegetable soybeans. Consequently, interest in soybean production has increased steadily. This research consists of three related studies. A spacing and planting date study was performed to compare four soybean lines. This experiment utilized four plant spacings and three planting dates. The lines were grown at the Plateau Research and Education Center in Crossville, TN in 2005 and 2006. Seeds were harvested at the R6 and R8 reproductive stages. Samples were analyzed by HPLC for isoflavone content. Data from the R6 harvest confirmed previously published reports of high isoflavone concentration in line 5601T. At the R8 harvest there was a significant difference among lines for the isoflavone daidzein.

The maturity and force studies were planted together in four row plots during the 2006 and 2007 growing seasons at the East Tennessee Research and Education Center, Knoxville, TN. Isoflavones were extracted from freeze-dried soybeans and measured by HPLC. Samples for the maturity study were harvested from the leftmost inner row. Samples for the force study were harvested from the rightmost inner row. The maturity study measured the concentration of isoflavones in the seed at reproductive stages R4.5, R5, R5.5, R6, R7, and R8. Reproductive stage was significantly different for 9 isoflavones and for total isoflavone concentration. There were differences between lines for 4 isoflavones and for total isoflavone concentration. Line 5601T had the highest isoflavone concentrations. There were also differences in growth stage by line interactions in 3 isoflavones and total isoflavone concentration.

The force study investigated the amount of force needed to remove a pod from the plant in the weeks prior to maturity. Pod removal force was measured using two Imada PS gauges with maximum measurements of 2 kg and 5 kg. The reproductive stages sampled were R5, R5.2, R5.4, R5.6, R5.8, R6, R7, and R8. Pod removal force ranged from 0.055 kg to 2.6 kg. Pod removal force increased linearly from the R5 to R7 stage. There were significant differences due to reproductive stage, line, and combinations of the two (P <.0001).

Table of Contents

List of Tables

List of Figures

PART I: INTRODUCTION

Introduction

The soybean, *Glycine max* (L.) Merrill, is a legume native to Asia. The plant grows to a height of 75 to 125 cm. It may be sparsely or densely branched, depending on climate and the genetics of the line. Mature leaves are distichous and trifoliate. The shape may be oblong to lanceolate. Leaves are typically 4-20 cm long and 3-10 cm wide.

The root system consists of lateral and fibrous roots. Individual roots may reach a length of 250 cm. Most lateral roots emerge from the stem within the upper 15 cm of the soil. These roots generally grow horizontally for some distance, angle slightly downward for 40 to 74 cm, then turn sharply and grow straight downward (Lersten and Carlson, 2004).

Soybean flowers are small and white or purple. They are found in clusters at the nodes of the stems. The flowers typically self-pollinate. Shortly thereafter a hairy pod will begin to form. Each pod contains 2-4 seeds. Fehr and Caviness (1977) described stages as the pod matures. These reproductive stages began at R1, when the flowers bloom. At R4 the pod is 2 cm long. By R5 the seed is 3 mm long. At the R6 stage the seeds are green and fill the cavity of the pod. The final reproductive stage is R8 at which point the pod is mature, dry and either brown or grey. For conventional production, the pods remain on the plant until completely dry (R8). For vegetable soybeans the beans are harvested at the R6 stage.

The taxonomic classification of the soybean is as follows (USDA Plants Database):

Kingdom Plantae
Division Magnoliophyta
Class Magnoliopsida

Order Fabales
Family Fabaceae
Genus and species *Glycine max* (L.) Merr.

The soybean was domesticated in northwestern China around 1500 -1100 BC. By the first century AD, soybeans were grown in much of China and Japan. By the mid 1700's, soybeans had been imported in England and the United States. Early records indicate that the first soybeans in the United States were grown in Georgia (Wilcox, 2004). It is likely that soybeans were first grown as a curiosity or perhaps even as an ornamental in the United States.

Today soybean production in the United States is second only to corn. In 2002 8.6 billion bushels of corn were harvested for grain, compared with 2.7 billion bushels of soybean (USDA NASS). Farmers in the United States produced 83.4 million metric tons of soybeans in 2005/06. This was 37% of all soybeans grown worldwide. Brazil was the second leading producer with 25%, Argentina produced 18% and China was fourth in production with only 7.4% of worldwide soybean production (Wilcox, 2004). The huge quantity of soy produced in the United States is used to make numerous products from food to fuel to fabric.

In the United States the first commercial use for soybeans was animal feed. While much of the soybeans produced are still used for that purpose, they are also used to make a variety of other foods. Mature, dried soybeans can be hydrated then roasted and eaten as 'soynuts.' The marketing of soynuts is similar to peanuts; they are often sold salted or coated in chocolate. More often, soybeans are ground and processed into tofu, soymilk, miso, or soy sauce. Soy protein is found in processed foods from granola bars

and protein shakes to dog food. Soybean oil is the predominant vegetable cooking oil and is also found in many prepared foods.

Soybean oil has numerous non-food uses as well. It has been investigated as a spray for fruit trees (Moran et al., 2003). Soy oil is often used as an industrial lubricant. Acidulated soybean oil soapstock, a byproduct of the refining process, has been spread on gravel roads as an alternative to petroleum oil (Indiana Soybean Board). Soybean oil can be used to make biodiesel, an alternative fuel that is becoming more prevalent in the United States (Schmidt, 2007.)

In addition to the industrial and dietary applications, the leaves and stems of soy plants can be processed to extract fibers. Once these fibers are further treated and mixed with more usual synthetic fibers, they produce a soft yarn.

Fuel and fiber aside, the primary purpose of the soy grown in the United States is still for human and animal feed. Dietary consumption of soy in the United States has increased in the past decade. This is likely due in part to the fact that soy is an inexpensive protein source that is commonly used in processed foods. There is a dietary shift taking place as the media spotlights the declining health and increasing girth of the average American, leading to an increase in the number of vegetarian and vegan dieters. Another possibility might be the increase in the Asian population of the United States over the past 40 years. As they became established and began to accumulate wealth, they may have used their increasing buying power to demand more traditional Asian foods. There is also a contingent of average Americans who simply like to try new foods. Thus, tofu and soy sauce have become common in grocery stores in the United States.

There is, however, another soy food item, one that is so new that crop production does not keep pace with demand. This product is edamame, the vegetable soybean. Edamame is the Japanese word for the seeds of a soybean harvested and eaten while still green. The United States, the worldwide leader in soy production, has to import most of its edamame consumed from the countries of China and Taiwan. The varieties used for edamame production are typically larger seeded and sweeter than those used for commodity soy production (Born, 2006).

Edamame has great potential for growers in Tennessee. Both early and late maturing varieties are available. Since edamame is harvested green, it can be harvested two to four weeks earlier than conventional soybeans. This makes it very useful in a double cropping system. As a legume, edamame is a good addition to a crop rotation cycle. Finally, edamame is a novelty crop that sells for higher prices without higher production costs.

In the past two decades, researchers have been investigating the use of soy as a source for isoflavones. Isoflavones are plant secondary metabolites that have similar chemical structures as endogenous mammalian estrogens. Because of this structural similarity, isoflavones appear to interact with pathways in mammals that respond to estrogens. This is of interest in research related to migraines (Burke et al., 2002), cancer, and hormone replacement therapy (Burke et al., 2002; Dhaubhadel, 2003; Heimler et al., 2004; Ososki and Kennelly, 2003).

As a valuable commodity and a growing part of the American diet, it is important to know what compounds are consumed when soybeans are on the menu. Specifically, given the potential dietary effects of isoflavones, the composition and relative

concentrations of isoflavones found in commercial soybeans and edamame should be known. Previous research has measured isoflavone content at one or two reproductive maturity stages and in processed food products, but a comprehensive evaluation of isoflavone concentration as the seed matures has not yet been attempted.

The purpose of the research presented here is to clarify the effects of within-row plant spacing, planting date, and maturity on the isoflavone content of soybeans grown for edamame and to determine a baseline level of force required to remove pods from the plant. The data collected will add valuable information to the isoflavone literature regarding the effects of competition among plants on deposition of isoflavones in the seed and clarify the timeline for deposition of isoflavones in the seed as it matures. Finally, this research will provide valuable data on the amount of force needed to harvest soybean at the stages used for edamame. This information will be useful for mechanizing edamame production and for use as a baseline for future research in chemical treatments, such as ethylene, to increase harvest efficiency by weakening the connection between the pod and the stem.

Literature Review

Isoflavones

There are three groups of isoflavones found in soybeans: genistein, daidzein and glycitein. Genistein was identified from soy in 1941 (Walter, 1941) and glycitein was extracted from soybean in 1973 (Naim et al., 1973). Isoflavones are stress related secondary metabolites produced via the phenylalanine pathway (Figure I.1). Production of all three isoflavones begins with phenylalanine. The simplest forms of each

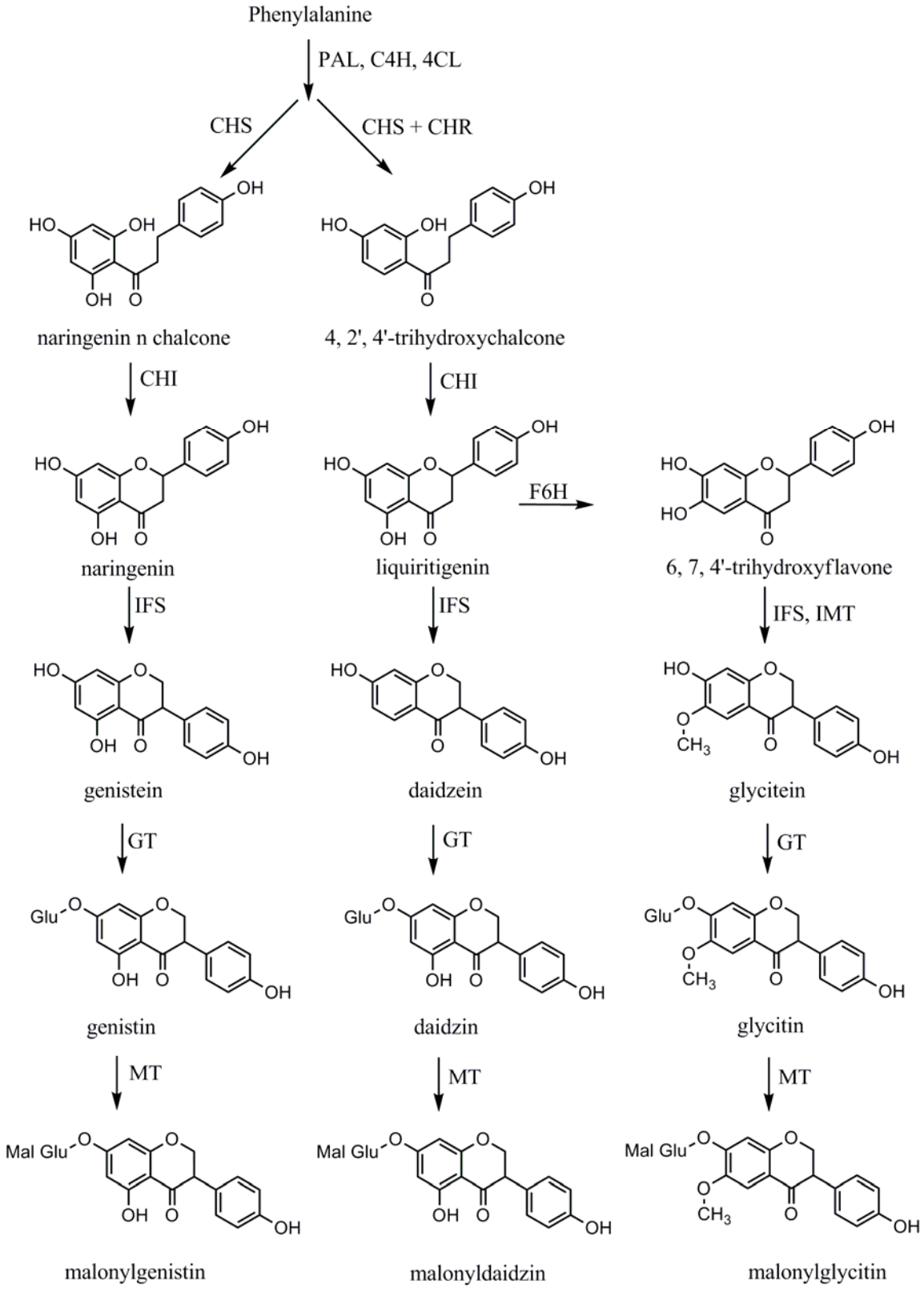

Figure I.1. Phenylalanine pathway from Dhaubhgadel et al. (2003).

isoflavone, daidzein, genistein, and glycitein, are produced first. Further modification of these isoflavones adds glucose to each structure. After the glucose is added, a malonyl or an acetyl group may be bound to the glucose. Glycitin differs from the other isoflavones in this. Thus far, acetyl glycitein has not been found in soybeans. Addition of the glucose to the structure is a large structural change that can be expected to affect the binding affinity of the glucosides relative to the aglucone (Figure I.2). This may be important because isoflavones strongly resemble mammalian estrogens (Figure I.3). Because of their similarity to estrogen, most isoflavones are able to bind to estrogen receptors in mammals (Heimler et al., 2004).

The potential medical consequences of consuming isoflavones are the subject of many studies (Naciff et al., 2004, Stauffer et al., 2006, Burke et al., 2002, Setchell et al., 2005, Ali et al., 2004, Song et al., 2006a, Song et al., 2006b.) Medical studies on the effects of soy phytoestrogens on humans and animal models have been mixed (Messina et al., 2006; Ososki and Kennelly, 2003). One study (Naciff, 2004) looked at the effect of a high soy diet on the reproductive organs of female rats. There was no effect on organ size or weight. Microarray analysis of cRNA from ovaries and uterus of individuals indicated a significant effect on the regulation of 29 genes. Though significant, the changes were generally not large and few of these genes were known to be regulated by estrogen. The authors concluded that it is unlikely that the levels of isoflavones found in standard rodent diets would adversely influence reproductive research in rodents. They also inferred that phytoestrogen levels found in a normal human diet would not activate estrogen regulated pathways or cause any harmful effects. This second inference seems dangerously broad. To equate a rodent diet that never varies

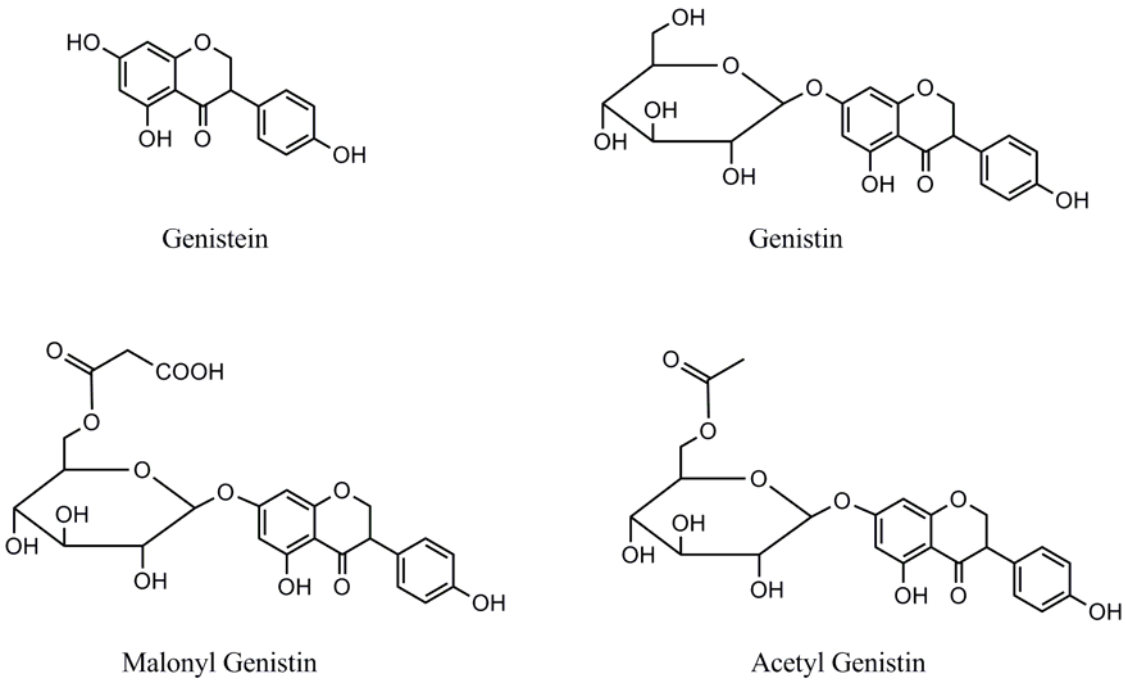

Figure I.2. Structure of Genistein and its glucones (Ososki and Kennelly, 2003).

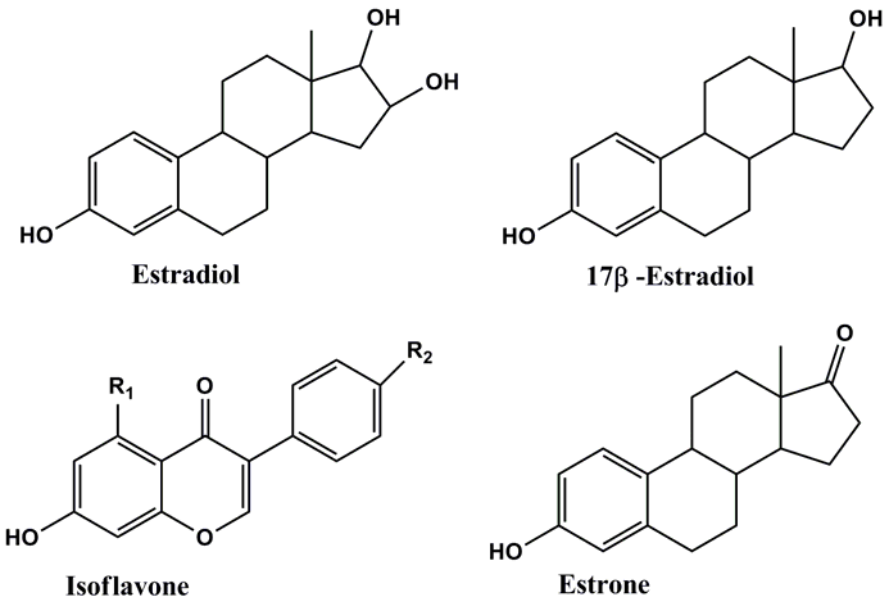

Figure I.3. Mamalian estrogens estradiol, 17β-estradiol and estrone share a similar ring structure with isoflavones. (Ososki and Kennelly, 2003).

with a human diet that may change frequently based on availability and fads is questionable. The authors seem unconcerned by the genes that were upregulated by the phytoestrogens.

Ali et al. (2004) studied the effects of probiotics and isoflavone supplementation on the SHR/N-cp rat model. This rat model will spontaneously become obese and develop hypertension. Probiotics are living microorganisms ingested as dietary supplements. They are rumored to decrease LDL cholesterol and have "a beneficial effect in obesity" (Ali et al., 2004). This study evaluated the effects of isoflavones on plasma glucose, plasma triglycerides, three liver enzymes related to liver function and two enzymes related to kidney function. Results showed that dietary supplementation of isoflavones decreased plasma glucose and triglyceride levels in lean mice by altering kidney and liver functions that lead to the production of glucose and triglycerides. However, this effect was only present in lean mice, not obese mice.

Soy isoflavones may not be benign when ingested by males. Male and female mice (and humans) do not present the same symptoms of heart disease. Stauffer et al. (2006) compared male and female mice fed soy based diets and milk protein (casein) based diets. The mouse model used in this study produces a mutant myosin heavy chain so that it mimics the gender differences in heart disease found in humans. The hearts of male mice on the soy diet were larger in size and showed a decrease in contractile function, as well as thinner walls and dilation of the left ventricle. The female soy fed mice only exhibited the increase in heart size. The control, casein fed mice were generally larger and did not exhibit the symptoms of heart disease.

Another study indicated a positive benefit related to soy consumption in adult women. Burke et al. (2002) ran a clinical trial in which women suffering from menstrually triggered migraines were given a placebo or a pill containing a combination of soy isoflavone, don quai extract, and black cohosh extract. The treatment resulted in a 75% decrease in migraines. Unfortunately, this experiment did not test the effects of the three compounds individually or in pairs. It is unknown if the effect was caused by one ingredient alone or some combination of the three.

Setchell et al. (2005) investigated the use of a slow-release isoflavone formulation in post-menopausal women. The plasma content of isoflavone and the isoflavone metabolites equol and *O*-desmethylangolensin of the study subjects was determined by GC-MS. The aim of this study was to determine the effect of a slow-release formulation of isoflavones on plasma isoflavone content. This study showed that a slow release formula was achievable and lead to a reversal of the usual proportion of daidzein to genistein. A net result of the slow-release formula was more steady concentrations of isoflavones in the plasma throughout the day. With normal soy consumption this is only achievable by repeatedly ingesting soy products throughout the day. Steady blood isoflavone levels are preferred when using isoflavones as a medicinal compound.

The research conducted to date implies a gender specific response. In men, who have naturally lower levels of estrogen, the increase in estrogenic activity caused by isoflavones may cause more harm than good. In women, whose bodies already produce significant quantities of estrogen, the benefits may be increased.

A pair of studies released by Song et al. (2006a, 2006b) were more positive. Their research tested the use of extracted soy isoflavones as a protectant for mice treated

with radiation. Radiation treatments cause oxygen free radicals to form in the body. Free radicals are extremely reactive, interacting with and damaging DNA, proteins, and cell membranes. In one experiment (2006a) mice were given extracted soy isoflavones in soybean oil, followed by a radiation treatment seven days later. There were three treatment groups of six mice randomized by body weight. Control mice were given corn oil, but no isoflavones or radiation. A second group of mice received radiation and corn oil, and a third received both isoflavones in soybean oil and radiation. Mice from all treatment groups were killed two days after the radiation. Their livers were flash frozen, and RNA was extracted from six mice, pooled, then run on microarray. The microarray data from the control mice had upregulated genes related to cellular damage. The isoflavone treatment group had almost normal gene expression for genes relating to cellular damage.

A second radiation experiment (Song et al. 2006b) involved treating mice with three dosage levels of extracted soy isoflavones, then subjecting the mice to whole body radiation. The dosages were 50, 100, and 400 mg isoflavone per kg of mouse body weight, and the composition of isoflavones was 16.4% daidzin, 1.0% daidzein, 22.6% genistin, and 1.5% genistein. The isoflavone injections began seven days before the radiation treatment and ended either two or seven days after the radiation treatments ended, when the mice were killed. Three blood parameters, white blood cell count, red blood cell count, and reticulocyte count, and two liver enzymes were measured to evaluate potential protective effects of the isoflavones. Medium doses of soy isoflavones appeared to aid in recovery from radiation treatments by slowing the decrease in white blood cell count. The medium and high isoflavone groups had better liver enzyme

activity seven days after the radiation treatments. Curiously, the high isoflavone treatment appeared more protective when biomarkers were compared, but the medium dosage appeared more protective when physical liver damage was compared. Taken together, the two Song et al. (2006a and 2006b) studies seem to indicate a protective effect from radiation damage at medium to high doses of soy isoflavones.

Soybean Response to Drought, Heat, and CO_2

Plant physiology is a dynamic process that allows the plant to adjust to changes in its environment. Plant responses must change to accommodate or counteract environmental stresses. In a hot, dry summer, heat stress damage can be expected to be increased by drought, because transpiration is used to cool the leaves. Less water will lead to less cooling and more foliar damage will occur more quickly. Sometimes one stressor can ameliorate the effects of another stressor. Such appears to be the case with CO_2 concentration and drought.

In general, elevated atmospheric CO_2 leads to higher biomass and increased carbon content in the plant (Uprety and Mahalaxmi, 2000). The extra CO_2 is transported to the chloroplast where it is used to increase photosynthesis. Serraj et al. (1999) investigated the effects of increased CO_2 concentration and drought stress on soybean. They proposed that the increased CO_2 would result in a change in the plant's response to soil water content. The elevated CO_2 resulted in decreased water loss, increased photosynthesis, and greater leaf area. This result is promising, because as atmospheric CO_2 concentrations increase, crop production and water use efficiency should improve (Serraj et al. 1999).

Environmental factors like CO_2 and drought are likely to influence the production of stress-related secondary metabolites like isoflavones. A dwarf soybean line used in growth chamber studies further clarified the effects of CO_2, temperature, and drought on isoflavone concentration (Caldwell et al., 2005). The unidentified dwarf soybean was determinate. The CO_2 levels were 700 and 400 ppm. Temperature treatments were 23°C and 28°C. Seeds were harvested as the pods matured and analyzed for isoflavone content. They found that the increased CO_2 would slightly increase isoflavone content, but that a 5°C increase in temperature decreased isoflavone content by far more (a max of 8% increase versus a 35-60% decrease). There was also a change in the proportions of the different isoflavones. It is interesting to note that slight drought conditions increased the isoflavone content. Slight drought combined with the higher CO_2 level (700ppm) at the lower temperature of 23°C, was able to counteract the high temperature and resulted in isoflavone concentrations similar to the low temperature control group. This study confirmed previous results indicating irrigation to combat the dry, hot conditions of a southern summer was able to significantly increase isoflavone content of the soybeans (Bennett, et al., 2004).

Tsukamoto, et al. (1995) published a study comparing the isoflavone content of seven varieties of edamame grown in Japan. Four of these had naturally lower isoflavone concentrations. Three had higher isoflavone content. The plants were planted on three different dates in two fields. The results were mixed in that the later planting dates from one field produced more isoflavones. But, in the other field the earlier dates yielded higher isoflavone concentrations. This experiment is usually interpreted to show that higher temperatures during seed filling produce lower isoflavone concentrations. Similar

results from a multi-year study of six genotypes (Hoeck et al., 2000) confirmed that seasonal and genotypic variation influenced isoflavone content among genotypes and seasons.

Another study evaluated soil moisture and air temperature effects on isoflavone concentration (Lozovaya et al., 2005). This study used greenhouse grown soybeans. They were two French and three American varieties, all in maturity group II. Large differences in isoflavone concentration were found among cultivars. The most interesting result was that the ranking from most to least isoflavones was consistent. They concluded that isoflavone synthesis is largely genetically controlled.

Isoflavone concentration differs among maturity groups (Bennett, et al., 2004). This is unsurprising given that previous research indicated variation in isoflavone concentration due to year, genotype and air temperature (Wang and Murphy, 1994; Tsukamoto, et al., 1995; Hoek, et al., 2000; Lozovaya et al., 2005). Plants from different maturity groups will not share the same genotypes and may experience vastly different air temperatures and drought levels as the pods mature. These conditions may be helpful in determining the genetic influences that control isoflavone concentration in soybeans. Comparing the genetics of individual lines from different maturity groups may be useful in parsing out the specific effects of each environmental factor.

There are other environmental stresses that may influence isoflavone concentration. Air temperature and water availability affect isoflavone concentration. Both of these factors are influenced by planting density. If higher isoflavone concentration is desirable, less dense plant spacing may be advantageous.

Materials and Methods

This study had three components. The first was a spacing and planting date experiment. This experiment investigated the effect of plant spacing on the isoflavone content of soybeans. The second experiment evaluated the isoflavone content of soybeans during pod filling and maturity. Previous research has involved harvesting pods at different stages, but a comprehensive analysis of isoflavone concentration in different lines as the pods mature has not been published.

The final experiment measured the force needed to harvest the pods at the maturity stages just before and including the stages relevant to edamame production.

Germplasm Utilized In These Studies

Four soybean lines were used in these experiments. They were TN00-60, TN03-349, 5601T, and Gardensoy-43. TN00-60 is a line that was bred from the cross MD92-5769 x Fillmore. TN03-349 is a new cultivar developed in Tennessee for edamame production. It was created by crossing TN93-99 x PI 416937. This cultivar has been released under the name NUTRIVEG Soy6407. The cultivar 5601T (Pantalone et al., 2003) is a USDA check cultivar for the Southern Uniform MG V tests. Gardensoy-43 is a commercially available edamame cultivar developed by the USDA at the University of Illinois. The lines Gardensoy-43, TN00-60, 5601T, and TN03-349 have relative maturities of 4.3, 4.8, 5.6, and 6.0, respectively (Carpenter 2007). Gardensoy-43 seed was acquired from Dr. Dick Bernard of USDA-ARS (Urbana, IL). Seed for the other lines was provided by Dr. Vincent Pantalone, University of Tennessee (Knoxville, TN).

Isoflavone Extraction and Analysis

Isoflavone data was collected for both the spacing and maturity studies. Pods were collected from each plot then shelled, counted and weighed. These samples were freeze-dried at -40 °F. Following drying, the samples were ground with a Knifetec 1095 Sample Mill (Foss Tecator, Hoganas, Sweden). The ground samples were extracted using a method modified from Griffith and Collison (2001). This method used 200 mg of soybean meal, 2.0 mL acetonitrile, 1.9 mL H_2O, and 100 µL apigenin internal standard. Samples were extracted for 2 hours at room temperature, then filtered and stored at -80°C until analyzed by HPLC. Data from 2005 were analyzed on an Agilent 1050 HPLC. In late 2006, mechanical problems necessitated moving the analysis to a different instrument. Data from 2006 and 2007 was analyzed on an Agilent 1200 RRHPLC. The column used with this instrument is an XDB-C18 reverse phase column (1.8 µm, 4.6 x 50 mm). The method used on this instrument was a faster version of the method used on the Agilent 1050 HPLC, which was based on the method by Griffith and Collison (2001). All three methods used an injection volume of 5 µm, and a column temperature of 40 °C. The solvents remained the same in each modification: solvent A is 1% (v/v) acetic acid in deionized water, solvent B is 1% (v/v) acetic acid in acetonitrile. The flow rates, ratios of solution A to solution B, and length of time for each run were changed (Table I.1).

Table I.1. Modifications to the HPLC Methods

	Griffith and Collison (2001) [a]	Agilent 1050	Agilent 1200
Flow Rate (mL/min)	0.65	8	0.8
First Gradient	0% to 30% for 60 min	12% to 24% for 34 min	12% to 88% for 15 min
Second Gradient	NA	NA	24% to 76% for 5 min
Column Wash	90% for 3 min	90% for 5 min	90% for 5 min
Column Equilibration	10% for 10 min	10% for 3 min	10% for 3 min
Total time per run	73 min	50 min	28 min

[a] The gradients and column washes are stated in percentage of solution B.

Data Analysis and Experimental Design

Comparison of isoflavone content and harvest force was analyzed using mixed model analysis of variance with SAS v9.1.3. The spacing and planting date study and the maturity study were randomized complete block designs. The force study was a randomized complete block design with subsampling.

Objectives of This Research

I. Spacing and Planting Date

This experiment was used to determine the effect of plant spacing and planting date on isoflavone content of the seeds. It tested the null hypothesis that all soybean lines produced the same isoflavones in the same concentrations regardless of planting date or spacing.

II. Isoflavone Concentration During Pod Maturation

The goal of this study was to determine the sequence of isoflavone deposition in the soybean seed during maturation. This experiment tested the null hypothesis that there is no peak in isoflavone content during maturity and that there were no differences among soybean lines in isoflavone content or concentration at different stages of pod maturity.

III. Harvest Force Measurement

This experiment evaluated the force required to remove pods from the soybean plant. The experiment tested the null hypothesis that there is no difference in force required to remove a pod at different pod maturities and that all soybean lines require the same amount of force for pod removal.

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PART II: PLANT SPACING AND GENOTYPIC INFLUENCES ON ISOFLAVONE CONCENTRATION IN EDIBLE SOYBEANS

Plant Spacing and Genotypic Influences on Isoflavone Concentration in Edible Soybeans

Abstract

In recent years a marked increase in consumer interest in edamame has occurred, in part due to reported health benefits associated with edible vegetable soybeans. Consequently interest in edamame production has increased steadily. A spacing and planting date study was performed to compare four soybean lines grown at three different within row plant spacings, planted on three different dates at the Plateau Research and Education Center in Crossville, TN in 2005 and 2006. The lines used were 5601T, TN00-60, Gardensoy-43, and TN03-349. The soybeans were planted using four different within-row planting densities (2.5 cm, 5.0 cm, 10 cm, and 20 cm), and three planting dates (May 24, June 14, and July 5). The same planting dates were used in both years. Soybeans were harvested at two stages, R6 and R8. Plots for the R6 harvest were planted on all three dates. Plots for the R8 harvest were planted on May 24. Samples were analyzed by HPLC for isoflavone content. Data from the R6 harvest confirmed previously published differences between lines. The isoflavone malonyl genistin was found to be significantly higher in line 5601T (43,229 μg/g). The two edamame lines, TN03-349 and Gardensoy-43, yielded significantly less malonyl genistin than 5601T but were not different from each other (17,123 μg/g and 13,690 μg/g respectively). The fourth line, TN00-60 was not different from any of the other lines (28,654 μg/g). The R8 harvest indicated a significant difference among lines for the isoflavone daidzein. In this case, 5601T, TN00-60, and Gardensoy-43 were not different (296 μg/g, 284 μg/g, and 315 μg/g, respectively). TN03-349 was different from all other lines with 193 μg/g.

Introduction

Soybean is an extremely important crop worldwide. It is a highly versatile crop that is processed into a number of food items. In recent years there has been increased interest in soybeans due to their isoflavone content. Isoflavones are phytoestrogens, plant secondary metabolites that are similar in structure to mammalian estrogen. This similarity allows them to interact with pathways in the mammalian body that are influenced by estrogen. This presents a number of possible health benefits from consumption of soy products (Ososki and Kennelly, 2003; Messina et al., 2006). While the amount of soy consumed in the United States is increasing, it is not known if the increase is sufficient to confer the benefits of soy onto people consuming an average American diet. To that end, there is interest in increasing the isoflavone content of soybeans so that a person could gain the health benefits of soy without drastic diet alterations.

Previous research has shown that isoflavone concentration in soybeans is influenced by line (Eldridge and Kwolek, 1983; Wang and Murphy, 1994; Tsukamoto et al., 1995; Hoeck et al., 2000; Charron et al., 2005), planting date (Hoeck et al., 2000), and planting location (Eldridge and Kwolek, 1983; Tsukamoto et al. 1995; Charron et al., 2005). Lozovaya et al. (2005) found that air temperature and soil water content during seed filling could also influence isoflavone concentration in soy. Light was found to influence isoflavone concentration in soy (Kim, et al. 2006; Kirakosyan, et al., 2007).

Soybeans are typically planted at a density of 26 – 40 plants per meter (2.5 – 3.8 cm between plants.) This dense planting rate is likely to lead to high competition among individual plants for water. The close proximity of other plants and the formation of a

canopy will affect air temperature and light availability, as well. Therefore planting density is likely to influence isoflavone concentration produced. This experiment attempted to determine if within-row planting density and planting date affected isoflavone concentration in edamame grown in Tennessee. The differences between the lines used will also be discussed.

Materials and Methods

Four lines were used in these experiments. They were Gardensoy-43, TN00-60, 5601T, and TN03-349. TN00-60 is a new line. TN03-349 is a recently released cultivar developed in Tennessee for edamame production. 5601T is a USDA check cultivar for the Southern Uniform MG V tests. Gardensoy-43 is a commercially available edamame cultivar. These lines have relative maturities of 4.3, 4.8, 5.6, and 6.0, respectively. Gardensoy seed was acquired from Dr. Dick Bernard of USDA-ARS (Urbana, IL). Seed for the other lines was provided by Dr. Vincent Pantalone, UTK (Knoxville, TN).

The spacing experiment was planted in 2005 and 2006. This experiment had three replications of three planting dates. The lines were planted in four rows strips. Each strip was 0.45 m apart (Carpenter 2007). This experiment was a split plot randomized complete block design (RBD).

Samples were collected from each plot at the appropriate maturity date for that line. The soybeans were freeze-dried at -40 °F. Following drying, the samples were ground using a water-cooled grinder for 20 sec (Knifetec 1095 Sample Mill, Foss Tecator, Hoganas, Sweden). The samples were kept on ice before grinding, water-cooled during grinding, and returned to the ice after grinding to decrease isoflavone degradation.

Isoflavone concentrations in the soybeans were determined by HPLC analysis followed by extraction with acetonitrile. The extraction procedure was modified from Griffith and Collison (2001). The modified procedure differed in the flow rate and rate of change to the gradient over the course of each run. Column temperature and solutions remained the same. Data from 2005 were analyzed on an Agilent 1050 HPLC. In late 2006, mechanical problems necessitated moving the analysis to a different instrument. Data from 2006 was analyzed on an Agilent 1200 RRHPLC.

Results

There were no measurable differences between years. Mechanical difficulties required moving the isoflavone analysis from an older Agilent1050 HPLC to a newer and more sensitive Agilent 1200 RRHLC. It is possible that the lack of measurable differences was due to the change to a more sensitive instrument.

Reproductive Stage 6.0

There were no significant effects found in the combined year data for year, planting date, or plant spacing. There was a significant effect of soybean line on the isoflavone malonyl genistin. The line 5601T had the highest mean concentration of 43,229 ± 7,717 μg g^{-1}, followed by TN00-60 with 28,654 ± 8,096 μg g^{-1}. The edamame lines, Gardensoy-43 and TN03-349 had the lowest means, 13,690 ± 7,673 μg g^{-1} and 17,123 ± 7,505 μg g^{-1}, respectively. The two edamame lines were not significantly different from one another (Table II.1). There were no significant interactions for the combined year data.

In 2005, there was a significant difference among plant spacings for the isoflavone malonyl genistin ($P < 0.05$). The 5.0 cm spacing yielded average malonyl genistin concentrations of 42,304 ± 8,247 μg g^{-1}. The 2.5 cm, 10 cm, and 20 cm spacings yielded 11,080 ± 7,923 μg g^{-1}, 17,130 ± 8,710μg g^{-1}, and 11,315 ± 7,924 μg g^{-1}, respectively. There was also a significant interaction between planting date and line for the isoflavone malonyl genistin ($P < 0.05$). The June planting of 5601T was higher than all other lines at both planting dates (Table II.2). The May planting of 5601T, Gardensoy-43, TN00-60, and TN03-349 yielded 63720 ± 12528 μg g^{-1}, 6223.61 ± 12528 μg g^{-1}, 13,284 ± 12528 μg g^{-1}, and 18050 ± 11206 μg g^{-1}, respectively. The June planting yielded 11667 ± 11206 μg g^{-1}, 12441 ± 10729 μg g^{-1}, 27048 ± 10729 μg g^{-1}, and 11,226 ± 11,206 μg g^{-1} malonyl genistin, respectively.

In 2006, there were no significant differences among plant spacings. There were differences among lines for the isoflavones daidzin, malonyl daidzin, genistin, malonyl genistin, malonyl glycitin, and total isoflavone ($P < 0.01$ for all except malonyl daidzin, $P < 0.001$). Line TN00-60 experienced poor germination in 2006, so was not included in the analysis. For all significant isoflavones and total isoflavone, 5601T had the highest concentrations (Table II.3). This supports previous findings of high isoflavone concentration in that line (Charron et al., 2005). The two edamame lines were not significantly different from one another, but were both less than 5601T. There was an interaction between spacing and line for the 2006 planting in the isoflavones daidzin, malonyl daidzin, genistin, malonyl genistin, and total isoflavone ($P < 0.05$). There was a significant difference among planting dates for the isoflavones genistin ($P < 0.01$) and malonyl genistin ($P < 0.05$) (Table II.4). There was an additional interaction between

Table II.1. Isoflavone Concentration in Soybeans Harvested at the R6 Stage, Grown at Crossville Research and Education Center

	isoflavones (μg g^{-1}) [a, b, c]									
Line	Daidzein	Daidzin	Malonyl Daidzin	Acetyl Daidzin	Genistein	Genistin	Malonyl Genistin	Glyctin	Malonyl Glycitin	Total
5601T	24 a	1494 a	54473 a	3 a	11 a	1260 a	43229 a	2118 a	9157 a	112 a
Gardensoy-43	89 a	1173 a	23489 b	bd	1 a	406 b	13690 b	1818 a	6063 a	47 b
TN00-60	98 a	1699 a	46066 ab	22 a	160 a	950 ab	28654 ab	2800 a	7928 a	89 ab
TN03-349	79 a	626 a	22476 b	bd	18 a	740 ab	17123 b	1690 a	7366 a	50 b

[a] Isoflavone concentrations are reported on a dry weight basis. [b] Acetyl Genistin and Glycitein were below detection in all samples. Acetyl daidzin was below detetction (bd) for Gardensoy-43 and TN03-349. [c] LSD means followed by the same letter within a column are not significantly different at the 0.05 probability level.

Table II.2. Isoflavone Concentrations in Soybeans Grown at the Crossville Research and Education Center in 2005

		isoflavones (μg g^{-1}) [a, b]			
Date	Line	Malonyl Daidzin	Geinstin	Malonyl Genistin	Total Isoflavone
May	5601T	17621 ab	383 b	11667 b	39 b
	Gardensoy-43	42557 ab	470 b	12441 b	72 ab
	TN00-60	66195 ab	781 ab	27048 b	120 ab
	TN03-349	22018 ab	716 ab	11226 b	44 ab
June	5601T	87686 a	1704 a	63720 a	169 a
	Gardensoy-43	9932 b	115 b	6224 b	23 b
	TN00-60	24264 ab	446 b	13284 b	45 ab
	TN03-349	27801 ab	559 b	18050 b	60 ab

[a] Isoflavone concentrations are reported on a dry weight basis. [b] LSD means followed by the same letter within a column are not significantly different at the 0.05 probability level.

Table II.3. Variation in Isoflavone Concentrations Among Soybean Lines Grown at the Plateau Research and Education Center in 2006

	Isoflavones (μg g^{-1}) [a, b]					
Line	Daidzin	Malonyl Daidzin	Geinstin	Malonyl Genistin	Malonyl Glycitin	Total Isoflavone
5601T	1626 a	58331 a	1495 a	49819 a	9519 a	123000 a
Gardensoy-43	626 b	18883 b	495 b	17240 b	4136 b	43000 b
TN03-349	709 b	20553 b	842 b	19535 b	5873 b	49000 b

[a] Isoflavone concentrations are reported on a dry weight basis. [b] LSD means followed by the same letter within a column are not significantly different at the 0.05 probability level.

Table II.4. Variation in Isoflavone Concentrations Among Soybean Lines Grown at Different Spacings at the Plateau Research and Education Center in 2006

		isoflavone (μg g^{-1}) [a, b]				
Spacing (cm)	Line	Daidzin	Malonyl Daidzin	Geinstin	Malonyl Genistin	Total Isoflavone
2.5	5601T	1386 ab	55021 abcd	1347 ab	46620 abc	115000 abcd
	Gardensoy-43	627 b	15825 cd	451 b	15260 c	37000 cd
	TN00-60	.	.	.	.	.
	TN03-349	592 b	17408 cd	708 b	17242 c	42000 cd
5	5601T	1450 b	51362 bc	1221 b	42726 bc	107000 bc
	Gardensoy-43	408 b	11157 d	275 b	10499 c	26000 d
	TN00-60	1244 b	40244 cd	1073 b	30897 c	80000 cd
	TN03-349	584 b	17089 cd	730 b	15798 c	40000 cd
10	5601T	1018 b	33081 cd	953 b	28624 c	70000 cd
	Gardensoy-43	658 b	21549 cd	516 b	17010 c	46000 cd
	TN00-60	2501 a	83346 ab	2417 a	70868 ab	171000 ab
	TN03-349	718 b	19677 cd	822 b	18701 c	47000 cd
20	5601T	2651 a	93858 a	2458 a	81307 a	198000 a
	Gardensoy-43	812 b	27000 cd	739 b	26189 c	61000 cd
	TN00-60	1086 b	35876 cd	1035 b	28819 c	73000 cd
	TN03-349	940 b	28040 cd	1106 b	26397 c	66000 cd

[a] Isoflavone concentrations are reported on a dry weight basis. [b] LSD means followed by the same letter within a column are not significantly different at the 0.05 probability level.

date and line for the isoflavones malonyl daidzin, genistin, malonyl genistin, and total isoflavone ($P < 0.05$) (Table II.5).

Reproductive Stage 8.0

There were no significant differences in isoflavone content among soybean lines, and plant spacings at the R8.0 stage when compared over two years. The overall mean concentrations of the 11 isoflavones at the R8 stage vary from zero to nearly 170,000 μg g^{-1}. While there were no significant differences between within-row plant spacings, there is an interesting trend. Daidzin, malonyl daidzin, genistin, malonyl genistin, glycitin, and malonyl glycitin showed noticeable (but not significant) increases in mean concentration for the 20 cm spacing at the R8 stage.

Discussion

This research confirmed previous reports of differences in isoflavone concentration between lines and high isoflavone concentration in the cultivar 5601T (Charron, et al., 2005). The influence of genotype on isoflavone concentration is well established (Eldridge and Kwolek, 1983; Tsukamoto, et al., 1995; Hoek et al., 2000; Swanson, et al., 2004; Charron, et al., 2005). This information will be useful in evaluating lines for use in determining what genes influence isoflavone concentration.

Prior research indicated that isoflavone concentration was influenced by air temperature, soil water content, potassium and CO_2 availability, and irradiance (Tsukamoto, et al., 1995; Hoek et al., 2000; Vyn, et al.,2002; Kim, et al., 2006; Kirakosyan, et al., 2007). All of these factors should be affected by planting density. Therefore, planting density should influence isoflavone concentration in soybeans. That

conclusion was not supported by this research. Planting density did not result in a significant effect on isoflavone concentration for any density or genotype evaluated. Perhaps the variation within the planting densities was not great enough to influence the isoflavone concentrations.

Total Isoflavones at R6.0 and R8.0

There is an interesting trend in the data for total isoflavone at the R6 and R8 stages. At the more dense plant spacings, total isoflavone concentration was higher at the R6.0 stage than at the R8.0 stage. The R8 growth stage yielded the highest isoflavone concentration at the least dense, 20 cm spacing. This is an interesting trend. A future study specifically addressing the interactions between planting density and pod maturity at harvest could be worth investigating. If this trend is verified by future studies, growers seeking higher (or lower) total isoflavone concentration may wish to adjust their planting density depending on the intended harvest stage (Figure II.1).

Table II.5. Significant Isoflavones for the Interaction Between Planting Date and Soybean Line

		isoflavones (μg g^{-1}) [a, b]			
Planting Date	Line	Malonyl Daidzin	Geinstin	Malonyl Genistin	Total Isoflavone
May	5601T	63623 a	1573 ab	54489 a	135000 a
	Gardensoy-43	16360 b	469 c	13563 c	36000 c
	TN00-60	.	.	.	.
	TN03-349	16524 b	631 c	14878 c	39000 c
June	5601T	53038 a	1417 ab	45150 ab	110000 ab
	Gardensoy-43	21406 b	521 c	20916 c	49000 c
	TN00-60	63083 a	2017 a	54927 a	129000 a
	TN03-349	24583 b	1052 bc	24191 bc	58000 bc

[a] Isoflavone concentrations are reported on a dry weight basis. [b] LSD means followed by the same letter within a column are not significantly different at the 0.05 probability level.

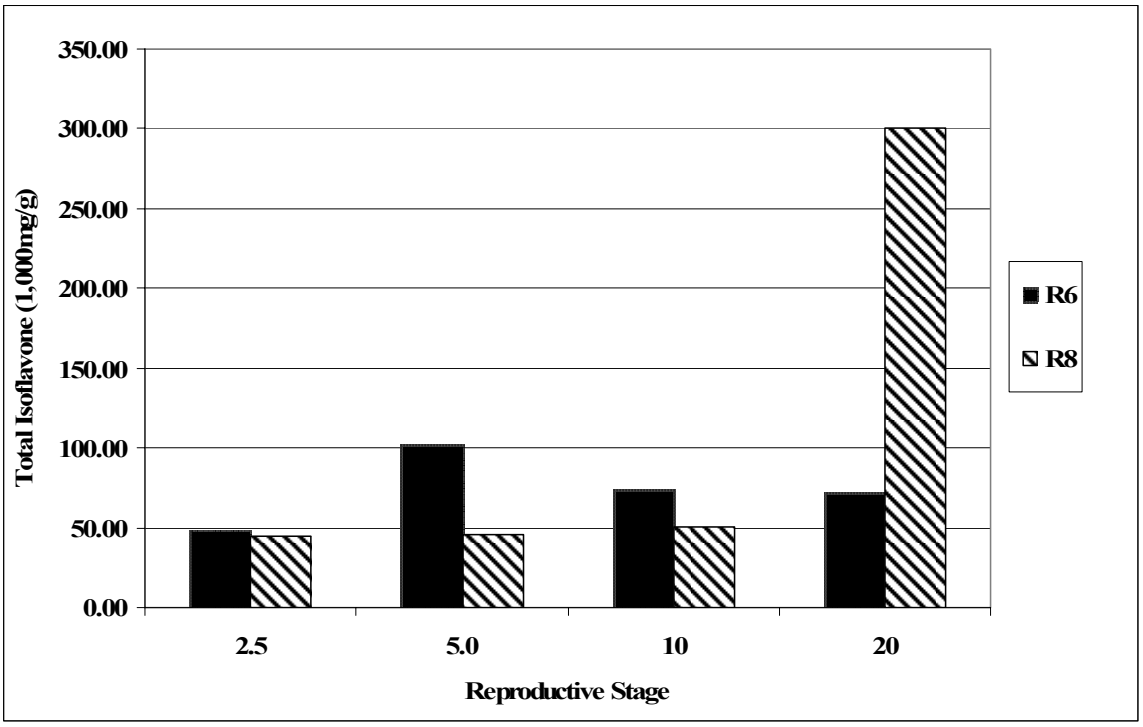

Figure II.1. Total isoflavone at the R6 and R8 stages in soybeans grown at the Plateau Research and Education Center were highest at the 5.0 cm plant spacing when harvested at the R6 growth stage. Soybeans harvested at the R8 growth stage yielded the highest levels

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PART III: ISOFLAVONE CONCENTRATION IN FOUR SOYBEAN LINES CHANGES AS THE SEED MATURES

Isoflavone concentration in four soybean lines changes as the seed matures

Abstract

Edamame is a short season soybean grown for consumption as a green bean. Soybeans are a common source for isoflavones, plant secondary metabolites which may have beneficial health effects. Four soybean lines, two conventional and two edamame lines, were grown in Tennessee during the 2006 and 2007 seasons. Pods were harvested at reproductive stages 4.5, 5.0, 5.5, 6.0, 7.0, and 8.0. Samples were freeze-dried, ground, and extracted with acetonitrile. Isoflavone content of the seeds at each stage was determined using RRHPLC. Reproductive stage showed significant differences for 9 of the 11 isoflavones investigated and for total isoflavone. There were differences between lines for the isoflavones daidzein, malonyl daidzin, acetyl genistin, malonyl glycitin and total isoflavone. There were differences in interactions among growth stages and lines for isoflavones malonyl daidzin, malonyl genistin, acetyl genistin and total isoflavones. Mean daidzein concentration for all lines was 907 μg g^{-1} at R6 and 497 μg g^{-1} at R8. The mean daidzin concentration was 699 μg g^{-1} at R6 and 1,132 μg g^{-1} at R8. Malonyl daidzin increased from 11,589 μg g^{-1} at R6 to 18,461 μg g^{-1} at R8. Mean genistin concentration increased from 184 μg g^{-1} at R6 to 645 μg g^{-1} at R8. Malonyl genistin varied from 5,167 μg g^{-1} at R6 to 16,603 μg g^{-1} at R8. Malonyl glycitin yielded means of 5,376 μg g^{-1} at R6 and 4,170 μg g^{-1} at R8. Genistein means varied very little from a mean of 3 μg g^{-1} at R6 to 57 μg g^{-1} at R8. Total isoflavone concentration was 26,000 μg g^{-1} at R6 and 43,000 μg g^{-1} at R8. Daidzein, malonyl daidzin, acetyl genistin, malonyl glycitin and total isoflavone content were significantly different among the four soybean lines.

Line 5601T had the highest isoflavone concentrations (736 μg g^{-1} daidzein, 14,574 μg g^{-1} malonyl daidzin, 100 μg g^{-1} acetyl genistin, 5,301 μg g^{-1} malonyl glycitin, and 36,000 μg g^{-1} total isoflavones).

Introduction

Soybean is extremely important worldwide because it is a highly versatile crop that is processed into a great number of products. In recent years there has been increased interest in soybeans due to their isoflavone content. Isoflavones are phytoestrogens, plant secondary metabolites that are similar in structure to estrogen. This similarity allows them to interact with pathways in the mammalian body that are influenced by estrogen. Thus, consumption of phytoestrogens may lead to a number of health benefits (Ososki and Kennelly, 2003; Messina et al., 2006). While the amount of soy consumed in the United States is increasing, it is not known if the increase is sufficient to confer the potential benefits of soy onto people consuming an average American diet. To that end, there is interest in increasing the isoflavone content of soybeans so that an individual could gain the health benefits of soy without drastic diet alterations.

Most soy grown in the United States is harvested at the R8 stage, when the seeds are mature and the pods are dry (Fehr and Caviness, 1977). Consumption of green soybeans, called edamame is increasing. Edamame are soybeans harvested at the R6 stage, when seeds are mature and have filled the pod but not begun to dry. The United States currently imports most edamame consumed here from China (Born, 2006).

Edamame has advantages over conventional soybeans. The pods can be harvested two to four weeks earlier than conventional soybeans depending on the weather. Conventional soybeans are typically highly processed to extract the oils and isoflavones or turn them into tofu. Processing edamame can be a much simpler process. Often, the beans are sold fresh or frozen while still in the pod. A relatively short growing season and minimal processing make edamame an appealing crop for growers in the United States.

Previously published studies investigated isoflavone concentration at either the R6 or R8 reproductive stage, not both. It is potentially valuable to know how isoflavone concentration changes during pod maturation and if the deposition of isoflavone in the seed differs between conventional and edamame lines. This experiment was designed to determine how the isoflavone concentration changes over time as the seed matures in two edamame and two conventional soybean lines.

Materials and Methods

Four soybean lines were used in these experiments. They were TN00-60, TN03-349, 5601T, and Gardensoy-43. TN00-60 is a new line. TN03-349 is a new cultivar developed in Tennessee for edamame production. 5601T (Pantalone et al., 2003) is a USDA check cultivar for the Southern Uniform MG V tests. Gardensoy-43 is a commercially available edamame cultivar. These cultivars have relative maturities of 4.8, 6.0, 5.6, and 4.6, respectively (Carpenter, 2007). Gardensoy-43 seed was acquired from Dr. Dick Bernard of USDA-ARS (Urbana, IL). Seed for the other lines was provided by Dr. Vincent Pantalone, UTK (Knoxville, TN).

The pod maturation experiment was designed as a split plot arrangement of randomized block design. The lines were planted in strips consisting of seven plots. Each plot was 3.0 m long, with a 2.1 m alley between plots. The plots were four rows wide, with ten plants per row and 0.8 m between rows. Reproductive maturity stage for isoflavone content was randomly assigned as a treatment to each plot. Pods for this experiment were harvested from the second row of the four row plot (left inner row).

The reproductive stages sampled for isoflavone concentration were R4.5, R5.0, R5.5, R6.0, R7.0, and R8.0 (Fehr and Caviness, 1977). Ten pods per plant were harvested from a minimum of 4 plants and a maximum of eight plants per plot for stages R6.0, R7.0, and R8.0. A larger number of pods per plant were harvested from the earlier stages to have enough seeds for analysis. Pod count, seed count, fresh seed weight and dry seed weight were recorded for each plot.

Isoflavone concentration in the soamples was determined by HPLC following extraction with acetonitrile. Pods from each plot were shelled, counted, and weighed. These samples were freeze-dried at -40 °F. Following drying the samples were ground (Knifetec 1095 Sample Mill) for 20 sec, in two 10 sec pulses with a 10 second pause between pulses. The samples were kept on ice before grinding, water-cooled during grinding, and returned to the ice after grinding to decrease isoflavone degradation.

Extraction of the isoflavones was accomplished using a procedure from Griffith and Collison (2001), modified by Charron (2005) and further modified for use on an RRHPLC. HPLC was used to determine the isoflavone content of each sample. Data from 2005 were analyzed on an Agilent 1050 HPLC. The sample injection size was 5 μm. The column used for the HPLC data analysis was an XDB-C18 reverse phase

column (1.8 μm, 4.6 x 50 mm). This method used a flow rate of 0.8mL per min and a column temperature of 40°C. Two solvents were used. Solvent A was 1% acetic acid in deionized water. Solvent B was 1% acetic acid in acetonitrile. At injection the solvent ratio was 12%B to 88%A. This ratio increased after 15 min to 24%B and 76%A. At 20 min the column was washed with 90% solution B for 5 min then equilibrated with 12% solution B for 3 min. The total elapsed time from one sample to the next was 28 min. Samples were evaluated for the presence of 11 isoflavones, daidzein, daidzin, malonyl daidzin, acetyl daidzin, genistein, genistin, malonyl genistin, acetyl genistin, glycitein, glycitin, and malonyl glycitin. Total isoflavone concentration was determined as well.

Data Analysis

Comparison of isoflavone content was analyzed using mixed model analysis of variance with SAS v9.1.3.

Results

There were no effects due to years or replications. Reproductive stage showed significant differences for all but two of the isoflavones analyzed. Acetyl daidzin and glycitein were not significant for reproductive stage. There were significant differences among lines for the isoflavones malonyl daidzin, malonyl glycitin and total isoflavone ($P < 0.01$), daidzein ($P < 0.001$), and acetyl genistin ($P < 0.0001$). In all cases, 5601T had the highest mean concentration. There were significant interactions between reproductive stage and line for malonyl daidzin, malonyl genistin, and total isoflavone ($P < 0.05$). Lines differed significantly for the isoflavones daidzein, ($P < 0.001$) malonyl

daidzin, malonyl glycitin, ($P < 0.005$) acetyl genistin, ($P < 0.0001$) and total isoflavones ($P < 0.01$).

Line 5601T was the only line with significant differences among reproductive stages. There were three trends in the data for this line. Three isoflavones were not significant: daidzin, malonyl daidzin, malonyl glycitin. Four isoflavones (genistein, genistin, malonyl genistin, and acetyl genistin) were lower at the earlier stages and peaked at R7 or R8. Total isoflavone followed this pattern as well. Glycitin and glycitein showed the reverse trend. These two isoflavones were much more concentrated in the young pod and disappeared as the pod matured.

Trends in isoflavone content over time—Daidzein and its glucosides

Three of the daidzein series isoflavones were significant at different reproductive stages. There were two trends in the significant isoflavones for this series. Daidzein was lowest at the earliest stage, highest at R6, then decreased to a medium level (Figure III.1). The remaining two significant isoflavones in this series, daidzin and malonyl daidzin, were highest at stages R7 and R8.

The isoflavone daidzein was highest at R6 with a predicted mean of 907 ± 98 μg g^{-1} (Figure III.1). Reproductive stage 4.5 had the lowest daidzein mean concentration of 137 ± 109 μg g^{-1}. Stages R5, 5.5, 7 and 8 fell in the middle with predicted means of 399 ± 116 μg g^{-1}, 309 ± 100 μg g^{-1}, 449 ± 99 μg g^{-1}, 497 ± 97 μg g^{-1}, respectively.
The isoflavone daidzin was high at the first sampling stage, R4.5 and the last two stages, R7 and R8 (Figure III.2). The means for these stages were 837 ± 139 μg g^{-1}, 1050 ± 128 μg g^{-1}, and 1132 ± 124 μg g^{-1}, respectively. Stage 5.5 yielded the lowest mean

concentration of daidzin, 289 ± 128 μg g^{-1}. The remaining two stages, R5 and R6 were not significantly different for any stage except R8. The R5 stage had a mean of 625 ± 147 μg g^{-1}. The R6 stage had a mean of 699 ± 109 μg g^{-1}.

The isoflavone malonyl daidzin yielded the highest predicted mean concentrations at the R7 and R8 stages. The means for this isoflavone at all stages from R4.5 to R8 were 4703 ± 5794 μg g^{-1}, 7138 ± 5869 μg g^{-1}, 4202 ± 5697 μg g^{-1}, 11589 ± 5684 μg g^{-1}, 21030 ± 5693 μg g^{-1}, 18461 ± 5662 μg g^{-1}, respectively (Figure III.3).

There was a significant interaction among reproductive stage and line for the isoflavone malonyl daidzin (Figure III.4). At R4.5, 5, and 5.5 all four lines performed the same. At reproductive stage 6 the means were slightly higher than the previous stages, but all lines performed the same. At R7, there was a significant difference among lines.

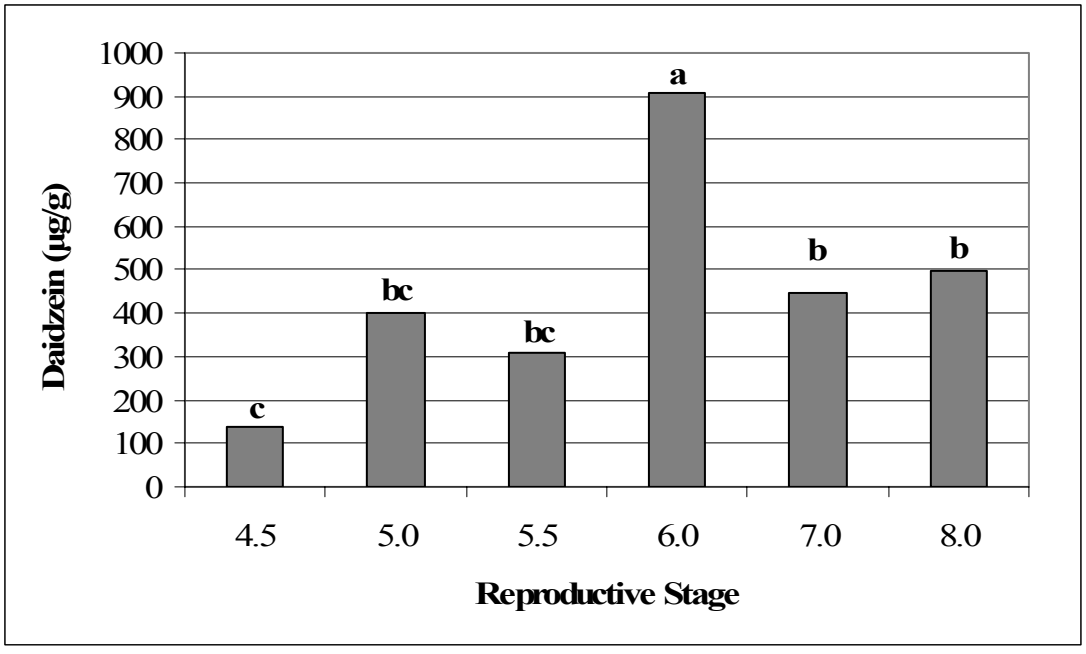

Figure III.1. Daidzein Concentration in Soybeans as the Seed Matures. LSD means with the same letter are not significantly different at the 0.0001 probability level.

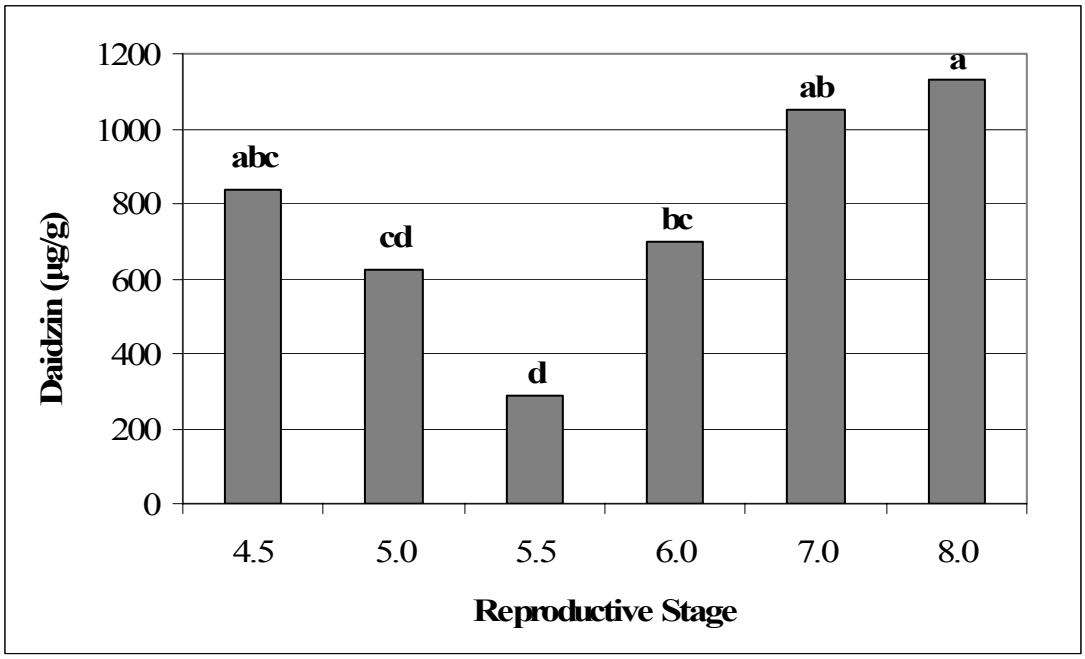

Figure III.2. Daidzin Concentration in Soybeans as the Seed Matures. LSD means with the same letter are not significantly different at the 0.0001 probability level.

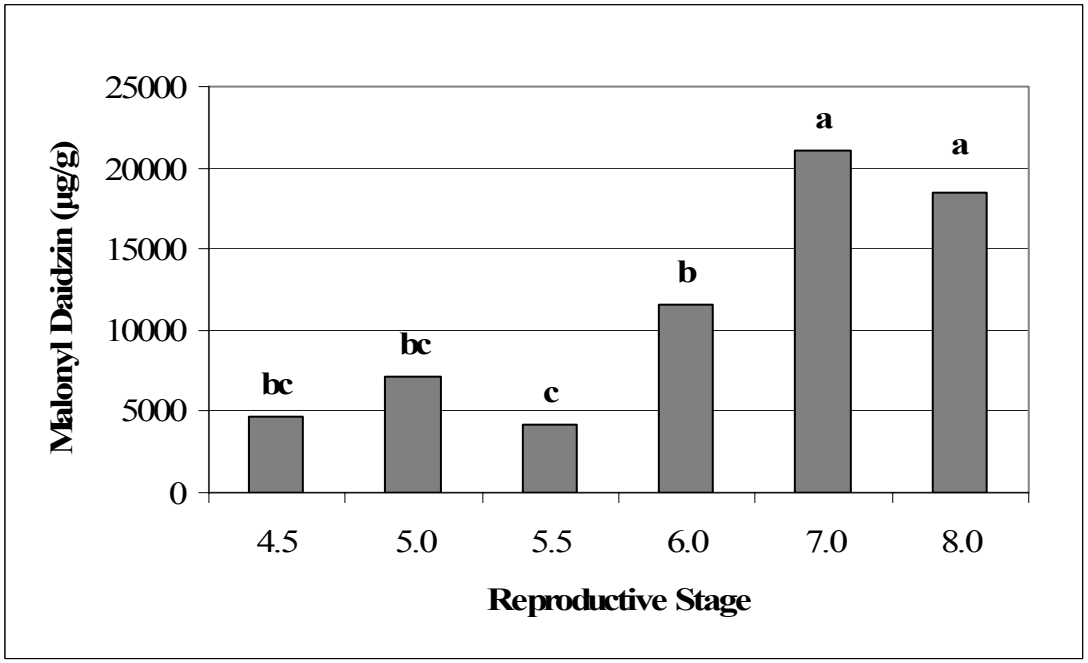

Figure III.3. Malonyl Daidzin in Soybean Seeds as the Seeds Mature. LSD means with the same letter are not significantly different at the 0.0001 probability level.

Line 5601T produced the highest mean of any line or stage, 41,082 ± 6,975 μg g^{-1}. TN00-60 yielded 24,067 ± 7,6125 μg g^{-1} malonyl daidzin which was significantly less than 5601T. The two edamame varieties yielded less malonyl daidzin than either of the commodity lines.

The mean concentrations of Gardensoy-43 and TN03-349 were 8,071 ± 6,975 μg g^{-1} and 10,902 ± 6,772 μg g^{-1}, respectively. At the R8 stage, the mean concentrations of 5601T and Gardensoy decreased to 17,312 ± 7,612 μg g^{-1} and 27344 ± 6772 μg g^{-1}, respectively. The means of TN00-60 and TN03-349 increased to 7,238 ± 6,772 μg g^{-1} and 21952 ± 6772 μg g^{-1}, respectively.

Trends in isoflavone content over time—Genistein and its glucosides

All four of the genistein series isoflavones were significantly different as the seed

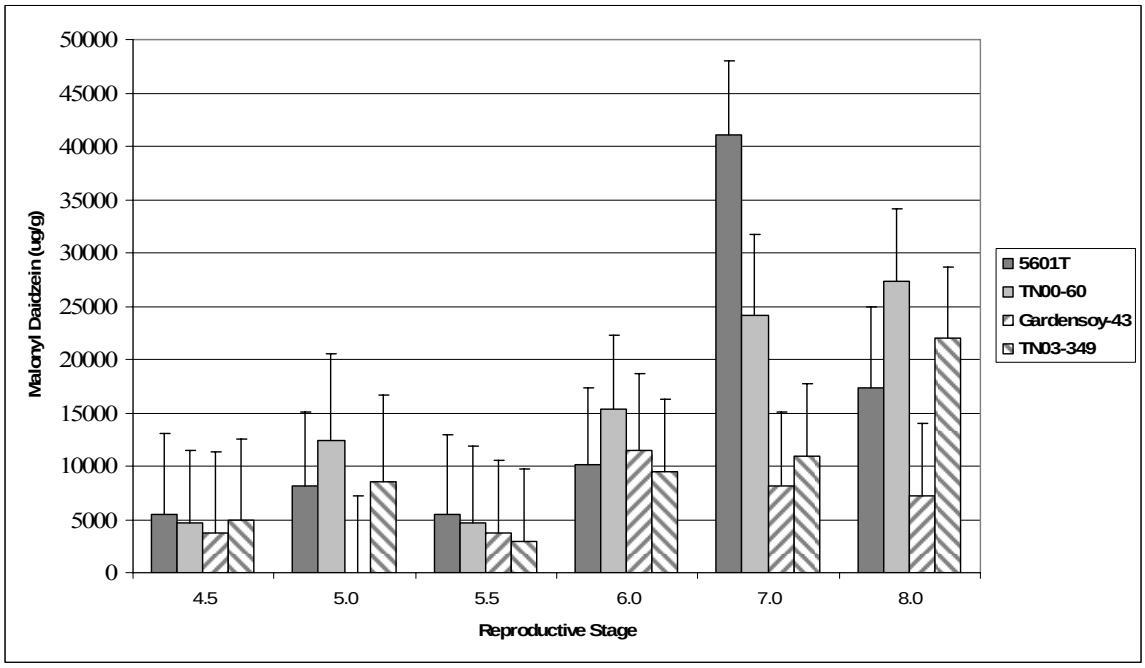

Figure III.4. Malonyl Daidzin Concentration in Four Soybean Lines as the Seed Matures in Soybeans Grown at ETREC in 2006 and 2007. LSD means with the same letter are not significantly different at the 0.0001 probability level.

matured. For genistin, the four earliest stages were not different, with means of 4 ± 25 μg g^{-1}, 8 ± 26 μg g^{-1}, 2 ± 25 μg g^{-1}, and 3 ± 25 μg g^{-1}, respectively (Figure III.5) The last two stages, R7 and R8, were higher with means of 39 ± 25 μg g^{-1} and 57 ± 24 μg g^{-1}, respectively.

The isoflavone acetyl genistin followed a pattern similar to genistein with the predicted means for earliest three stages being 0 ± 15 μg g^{-1}, 0 ± 16 μg g^{-1}, and 16 ± 14 μg g^{-1}, respectively (Figure III.5). At the fourth stage, R6, the mean increased to 45 ± 14 μg g^{-1}. At the R7 stage the predicted mean increased again to 60 ± 14 μg g^{-1}. At the last stage, R8, the mean decreased slightly to the same level as the R6 stage. The predicted mean at the R8 stage was 41 ± 13 μg g^{-1}.

For genistin, the four earliest stages were not different with means of 84 ± 168 μg g^{-1}, 165 ± 170 μg g^{-1}, 38 ± 166 μg g^{-1}, and 184 ± 165 μg g^{-1}, respectively (Figure III.6). The last two stages, R7 and R8, were higher with means of 614 ± 166 μg g^{-1} and 645 ± 165 μg g^{-1}, respectively.

The last isoflavone in this series, malonyl genistin, was also significantly different as the seed matured. The first four stages, R4.5 to R6 had predicted means of 2,342 ± 5,273 μg g^{-1}, 3,107 ± 5,321 μg g^{-1}, 1,602 ± 5,211 μg g^{-1}, and 5,167 ± 5,203 μg g^{-1} (Figure III.7). The last two stages, R7 and R8, were not different from one another, but were significantly higher than all other stages. The predicted mean for R7 was 16,876 ± 5,208 μg g^{-1}. The predicted mean for R8 was 16,603 ± 5,189 μg g^{-1}.

There were significant interactions between reproductive stage and line for malonyl genistin (Figure III.8). Predicted means for all lines were not significantly different for stages 4.5, 5, 5.5, and 6. The predicted means increased for all lines at the

R7 stage. The means for the two commodity lines, 5601T and TN00-60, were 31,939 ± 6,050 μg g^{-1}, and 14,437 ± 6,481 μg g^{-1}, respectively. The two edamame lines, Gardensoy-43 and TN03-349, yielded predicted means of 9,825 ± 6,050 μg g^{-1}, and 11,302 ± 5,913 μg g^{-1}, respectively. At the R8 stage mean malonyl genistin concentration for 5601T decreased to 14,304 ± 6,481 μg g^{-1}. Mean concentration for Gardensoy-43 increased slightly to 9,951 ± 5,913 μg g^{-1}, while TN00-60 decreased to 19,044 ± 5,913 μg g^{-1}. The predicted mean for TN03-349 increased to 23,112 ± 5,913 μg g^{-1}.

There was also a significant interaction between reproductive stage and line for acetyl genistin (Figure III.9). The predicted means for this isoflavone were essentially zero for TN00-60 and TN03-349 at all stages. The predicted means for 5601T and Gardensoy-43 were essentially zero for R4.5 and R5. At R5.5, 5601T and Gardensoy-43 produced predicted means of 60 ± 32 μg g^{-1} and 5 ± 25 μg g^{-1}. At R6, Gardensoy-43 was

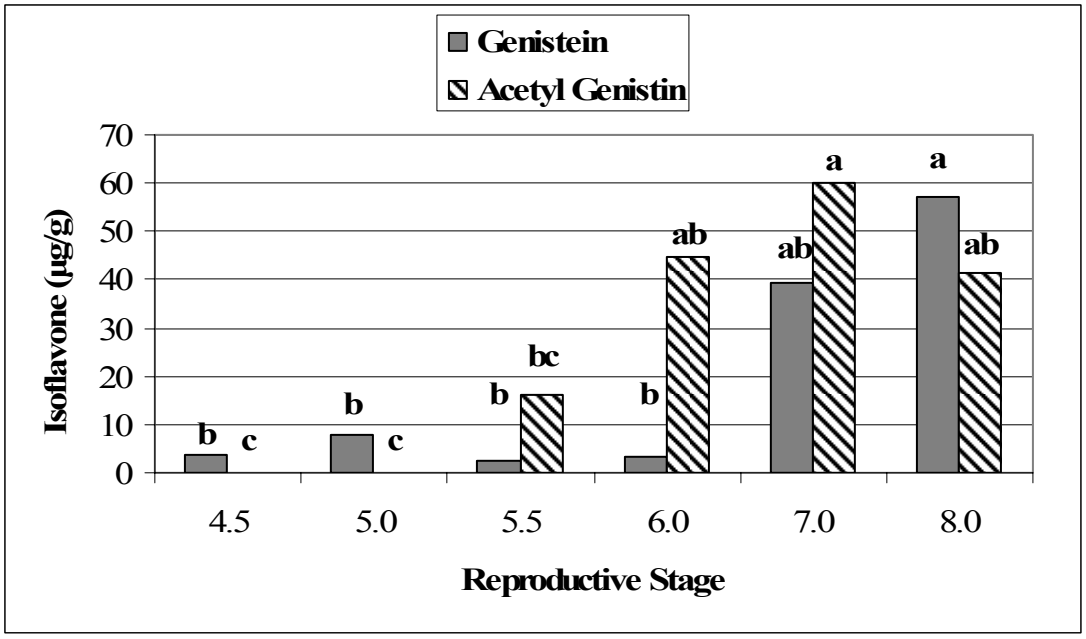

Figure III.5. Genistein and Acetyl Genistin in Soybean Seeds as the Seeds Mature. LSD means with the same letter for genistein and acetyl genistin are not significantly different at the 0.05 probability level.

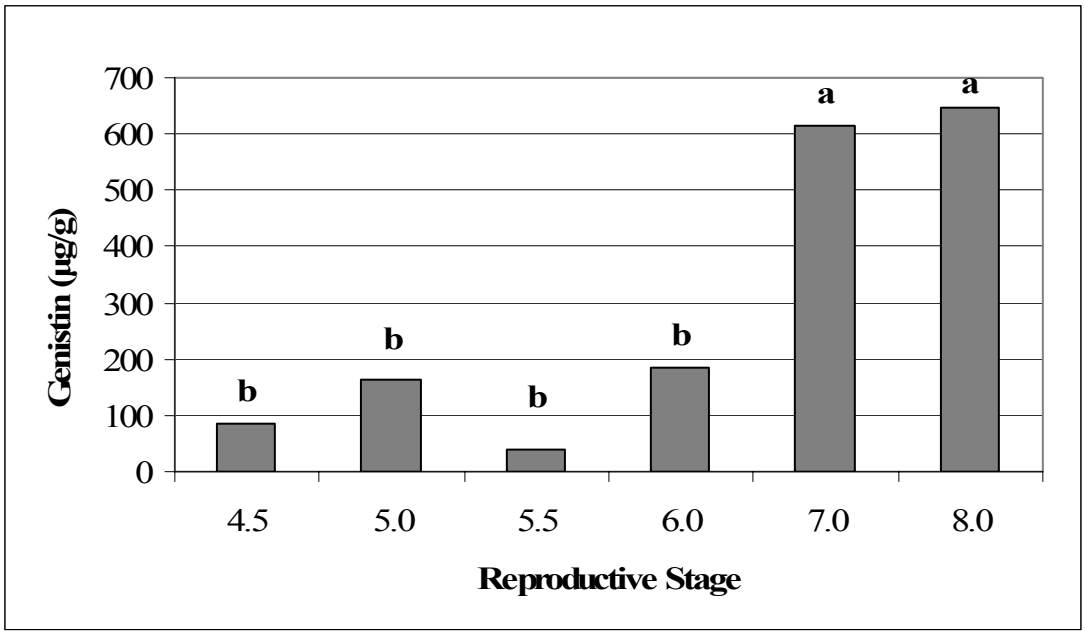

Figure III.6. Genistin in Soybean Seeds as the Seeds Mature. LSD means with the same letter are not significantly different at the 0.0001 probability level.

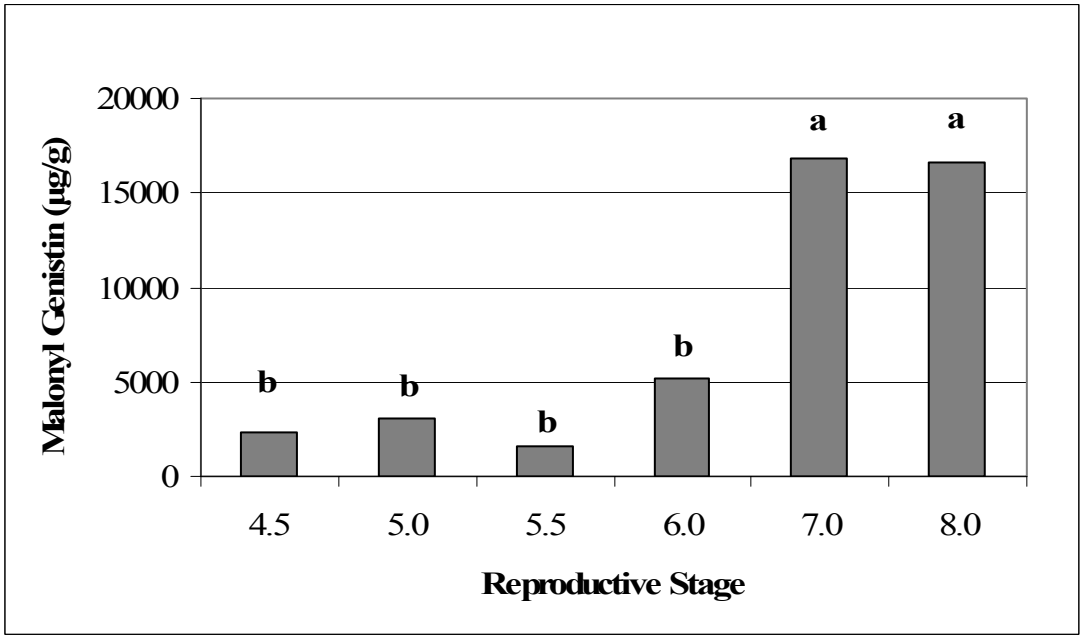

Figure III.7. Malonyl Genistin in Soybean Seeds as the Seeds Mature. LSD means with the same letter are not significantly different at the 0.0001 probability level.

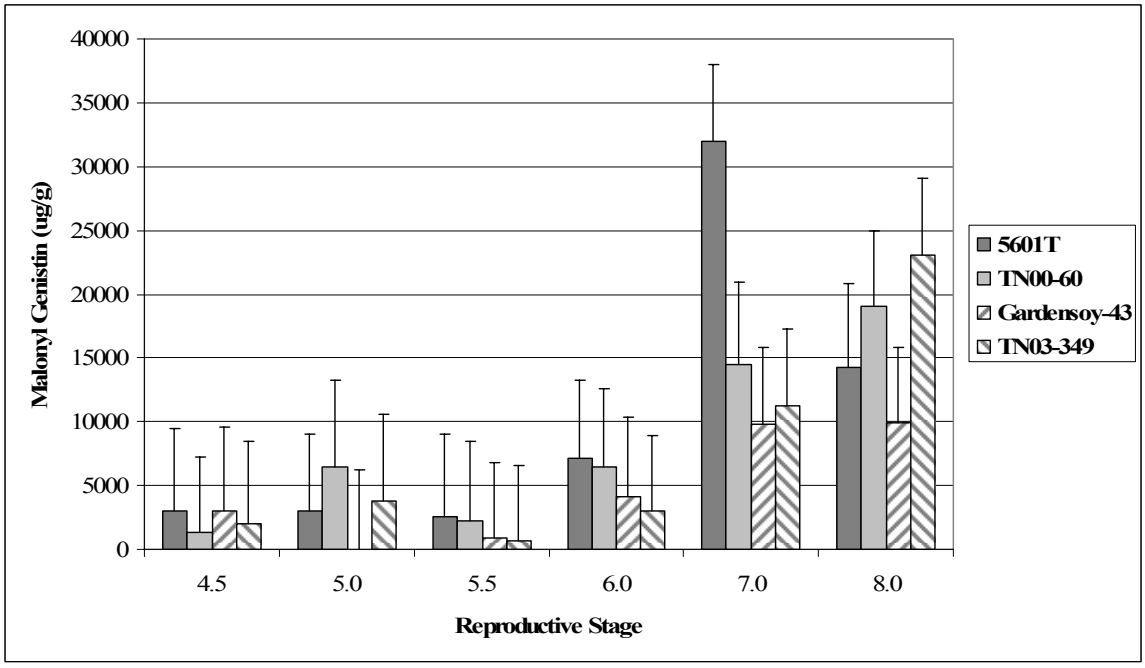

Figure III.8. Malonyl Genistin Concentration in Four Soybean Lines as the Seed Matures in Soybeans Grown at ETREC in 2006 and 2007. LSD means with the same letter are not significantly different at the 0.05 probability level.

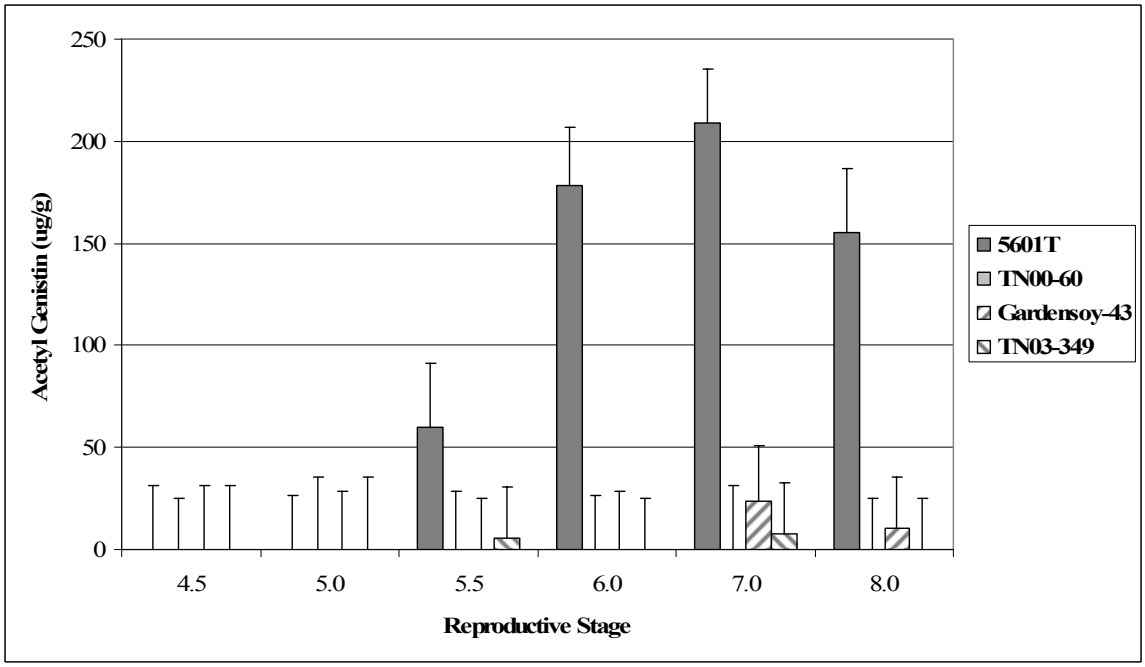

Figure III.9. Acetyl Genistin Concentration in Four Soybean Lines as the Seed Matures in Soybeans Grown at ETREC in 2006 and 2007. LSD means with the same letter are not significantly different at the 0.005 probability level.

again, essentially zero, but 5601T had increased to a mean of 178 ± 29 μg g^{-1}. At R7, 5601T produced its highest mean for this isoflavone, 209 ± 27 μg g^{-1}, while Gardensoy-43 produced 24 ± 27 μg g^{-1}. TN03-349 yielded a predicted mean of 8 ± 25 μg g^{-1} for this stage, but that is essentially zero. At the last stage, 5601T and Gardensoy-43 were again the only two lines with positive predicted means of 155 ± 32 μg g^{-1}, and 10 ± 25 μg g^{-1}.

Trends in isoflavone content over time—Glycitein and its glucosides

There were two isoflavones in the glycitein series with significant differences among reproductive stages. The isoflavone glycitin was highest at the R4.5 and R5 stages with mean concentrations of 1873 ± 281 μg g^{-1} and 1693 ± 295 μg g^{-1} (Figure III.10). At R5.5 the predicted mean decreased to 623 ± 265 μg g^{-1}. The mean increased at R6 to 1439 ± 262 μg g^{-1}. After R6 the predicted mean decreased again to 956 ± 263 μg g^{-1} at R7 and 970 ± 258 μg g^{-1} at R8.

Malonyl glycitin concentration did not change appreciably as the seed matured, except at the R5.5 stage. Predicted means for stages 4.5, 5, 6, 7, and 8 were 3,730 ± 1,266 μg g^{-1}, 3,535 ± 1,293 μg g^{-1}, 5,376 ± 1,226 μg g^{-1}, 4,228 ± 1,230 μg g^{-1}, 4,170 ± 1,218 μg g^{-1}, respectively. At the R5.5 stage the predicted mean was 1541 ± 1233 μg g^{-1}.

There were no significant interactions between reproductive stage and line for any of the glycitein series isoflavones.

Trends in isoflavone content over time—Total Isoflavone

Total isoflavone increased over time as the seed matured (Figure III.11). The first three stages sampled had means of 14,669 ± 13,285 μg g^{-1}, 16,877 ± 13,432 μg g^{-1}, and 8,772 ± 13,097 μg g^{-1}. These means were not significantly different. Total isoflavone concentration decreased at R6 to 25,545 ± 13,071 μg g^{-1}, which was significantly more than at R5.5, but not different from R4.5 or R5. The last two stages, R7 and R8, were significantly higher than the earlier stages, but not different from one another. The mean at R7 was 47,487 ± 13,088 μg g^{-1}. The mean at R8 was 43,281 ± 13,029 μg g^{-1}.

There was a significant interaction between reproductive stage and line for total isoflavone (Figure III.12). There were no differences between lines or reproductive stages for R4.5, R5, R5.5, or R6. At R7 the two edamame lines were still statistically

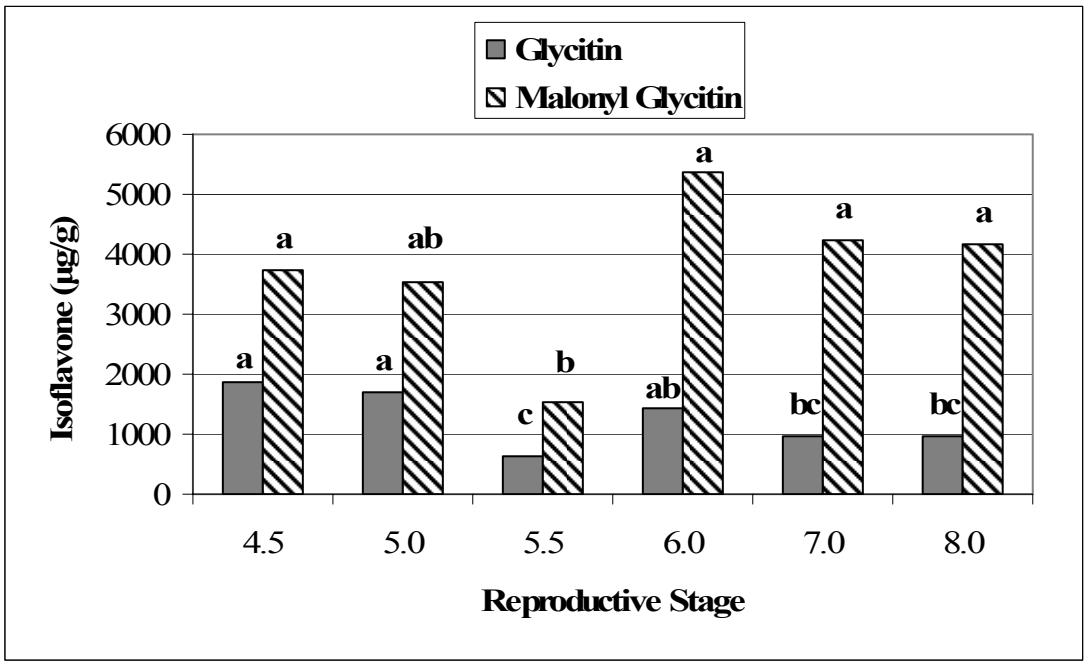

Figure III.10. Glycitin and Malonyl Glycitin in Soybean Seeds as the Seeds Mature. LSD means with the same letter for glycitin are not significantly different at the 0.001 probability level. LSD means with the same letter for malonyl glycitin are not significantly different at the 0.05 probability level.

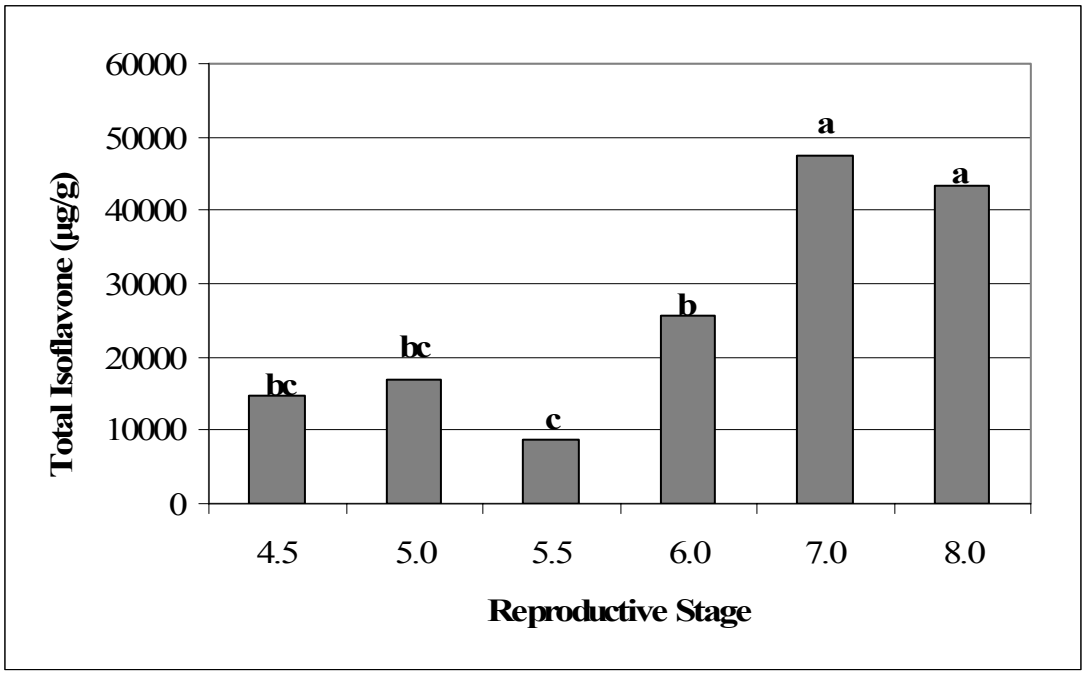

Figure III.11. Total Isoflavone in Soybean Seeds as the Seeds Mature. LSD means with the same letter are not significantly different at the 0.0001 probability level.

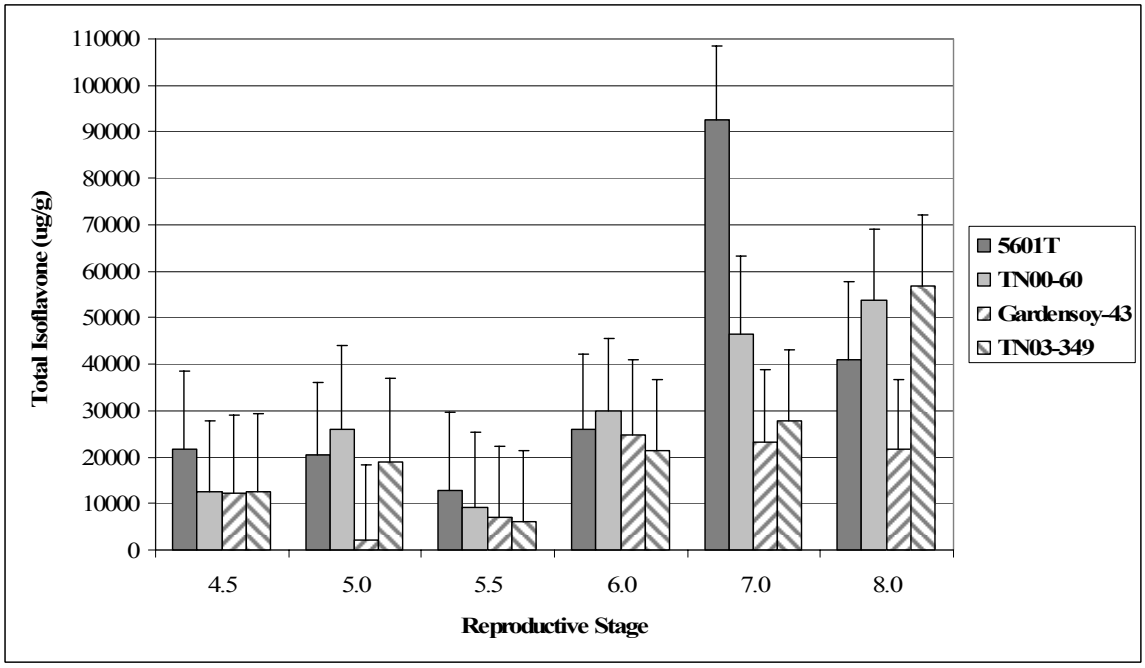

Figure III.12. Total Isoflavone Concentration in Four Soybean Lines as the Seed Matures in Soybeans Grown at ETREC in 2006 and 2007. LSD means with the same letter are not significantly different at the 0.05 probability level.

similar to the earlier stages. The two commodity lines were not. The mean for 5601T increased at R7 to 92,695 ± 15,628 μg g^{-1}. At the same stage, TN00-60 yielded a predicted mean of 46,395 ± 16,909 μg g^{-1}. At R8, Gardensoy-43 had a mean of 21,594 ± 15,221 μg g^{-1}. This is not different from any other stage for this line. The mean of line 5601T decreased to 40,820 ± 16,909 μg g^{-1}, which is significantly less than R7. TN00-60 and TN03-349 were not different at the R8 stage. The predicted mean for TN00-60 and TN03-349 at the R8 stage were 53,921 ± 15,221 μg g^{-1} and 56,788 ± 15,221 μg g^{-1}, respectively.

Discussion

Isoflavone concentration is greatly influenced by genetic and environmental influences. Thus, differences were expected between both years and lines. There were no detectable differences in isoflavone concentration between the two years. This was extremely surprising given the differences found by Tsukamoto, et al. (1995). The environmental conditions in 2007 were far drier than in 2006. The field was irrigated both years. It is possible that the irrigation mitigated the dry conditions resulting in no detectable differences between years.

The data confirmed expected differences between lines. Line 5601T is a high isoflavone producing line. This was previously reported by Charron, et al. (2005). At every stage 5601T produced higher concentrations of every isoflavone. The three remaining lines, TN00-60, Gardensoy-43, and TN03-349 were not significantly different at any stage, for any isoflavone. Edamame lines are generally sweeter and larger seeded than commodity lines (Born, 2006). It would be unsurprising to find the genetic selection

that lead to a sweeter flavor and larger bean size would also produce a difference in isoflavone concentration. That was not the case. The background of the two edamame lines used in this study may provide an explanation. Both Gardensoy-43 and TN03-349 were bred from commodity soybean lines. Conversely, the minimal differences in isoflavone concentration between the commodity and edamame lines may indicate that an environmental factor was more influential than genetics in determining the isoflavone yield in this study.

Isoflavones were believed to increase as the seed matures, with the highest concentrations found at the R8 stage. Most isoflavones followed this trend. However, nine isoflavones showed a marked decrease at stage R5.5. This is unlikely to be environmentally related because each line reached this stage at different times. Gardensoy-43 matured fastest, so it reached R5.5 2 weeks before TN00-60 and 5601T. The two commodity lines matured at slightly different rates but reached R5.5 within the same week. TN03-349 was the slowest to mature, reaching to R5.5 6 weeks after Gardensoy-43.

This decrease was significant for the isoflaovnes daidzin, glycitin, malonyl glycitin, and total isoflavones. Three isoflavones and total isoflavones were highest at the R7 stage rather than R8. Malonyl daidzin, acetyl genitin, malonyl genistin, and total isoflaovnes produced markedly higher means at R7. For malonyl genistin and total isoflavone this was due to line 5601T. Additionally, three daidzein, glycitin, and malonyl glycitin were found in the highest concentrations at the R6 stage. This may be important if the different isoflavones are found to have different biological activities. The reproductive stage at harvest will affect the relative isoflavone concentrations in the

resulting product. Ingesting soybean harvested at earlier stages may produce a different effect. Additionally, the harvest date could be adjusted if a higher yield of a specific isoflavone was desired.

Four isoflavones had different trends in deposition. Total isoflavone did not change significantly in Gardensoy-43 at any time as the seed matured. The isoflavone glycitin was highest at R4.5, then decreased over time. R5.5 yielded the lowest means. The means at R6, R7, and R8 were slightly higher than R5.5, but were significantly lower than the mean at R4.5. The trend does not mirror the trends in the malonyl or acetyl forms of glycitin.

Daidzein and daidzin show opposite trends. Daidzein concentration was lowest at the earliest stages and peaked at R6. The last two stages, R7 and R8, were not different from R5 and R5.5. Daidzin was higher at the early and late stages, with a significant decrease at R5.5. The highest mean concentration occurred at R8, but this mean was not different from the means at R4.5 and R7. The mirrored trends in daidzein and its glycosylated form, daidzin might indicate that daidzein is being converted to daidzin in the seed. The highest mean concentrations of both isoflavones are approximately 1,000 $\mu g\ g^{-1}$. For daidzein that high mean only occurs at R6. For daidzin that high mean occurred at R7 and R8. At those stages daidzein decreased to ~ 450 $\mu g\ g^{-1}$.

Trends in total isoflavone concentration over time were not the same for all lines. TN00-60 and Gardensoy-43 were not significantly different at any stage. TN00-60 did produce the highest mean concentration of total isoflavone at for that line at R8. Gardensoy-43 was the same at all stages. TN03-349 followed the expected trend of increasing total isoflavone concentration over time, with the highest mean R8. This mean

was not significantly different from the R7 stage, but it was significantly higher than stages R4.5 to R6. Total isoflavone means for line 5601T not significantly different at any stage except R7. At R7, 5601T yielded a mean isoflavone concentration that was significantly higher than any other stage or line.

This research has revealed some intriguing avenues for future research. That nine out of 11 isoflavones decrease at stage R5.5 was interesting. It is likely to have a genetic cause. The fate of the isoflavones that were deposited in the seed prior to R5.5 could be investigated. They could be processed into different isflavones or other compounds. Or, perhaps they are simply degraded and replaced. The high isoflavone concentrations reported for line 5601T by this research and previously published studies make it a favorable line for use in future genetic studies investigating isoflavone synthesis.

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PART IV: POD REMOVAL FORCE INCREASES LINEARLY AS PODS APPROACH MATURITY IN SOYBEAN [*Glycine max*]

Pod removal force in increases linearly as pods approach maturity in soybean [*Glycine max*]

Abstract

Harvesting soybean [*Glycine max* (L.) Merr.] for edamame production requires that the entire pod be removed from the plant, intact. This study evaluated the strength of the pod attachment to the stem beginning when the pod is only 2 cm long (R5.0) until the seeds are mature (R8.0). Single pods were removed from the plant with a push-pull force gauge. The change in pod removal force was linear from reproductive stage 5.2 to 7.0. This change can be described by the equation $y = 0.141x + 0.1623$ ($R^2 = 0.9789$.) There were significant effects due to reproductive stage, line, and interactions among lines and reproductive stages ($P < 0.0001$). Mean pod removal force at the R6 stage was 1.28 kg, 1.15 kg, 1.02 kg and 0.89 kg for soybean lines Gardensoy-43, TN00-60, TN03-349 and 5601T, respectively.

Introduction

Soybean [*Glycine max* (L.) Merr.] is a highly versatile crop that is processed into a number of food items. The ubiquity of soy oils, proteins, and fibers in consumer products makes soybean an extremely important crop worldwide. The many uses of soy and its potential health benefits (Ososki and Kennelly, 2003) have lead to increased production in recent years.

Edamame are edible green soybeans [*Glycine max* (L.) Merr.] harvested when the seeds fill the pod but have not yet begun to dry. Despite the fact that the United States is

the top producer of soy worldwide, most edamame consumed in the United States is imported from China (Wilcox, 2004). Edamame is a commercially valuable product (Wilcox, 2004). Perhaps the most advantageous quality of edamame is the short growing season. The earliest maturing lines can be harvested in August in Tennessee. Edamame can be sold fresh or frozen, shelled or in the pod, decreasing the amount of processing needed. Edamame fit well within a double cropping system, increasing the potential profits from a single field.

Edamame are typically harvested with a green bean harvester. Commodity soybeans, harvested at a dry, mature stage, are harvested with a combine. At both stages, pod removal force is very important. Too much force may shatter the dry commodity beans or crush the valuable pods of edamame. Differences in pod removal force may potentially affect harvest efficiency and yield.

This study measured pod removal force in soybean as the pod grew and matured. Comparisons were made among soybean lines, pod maturity, and combinations of the two. The baseline pod removal force data provided by this study will be useful in evaluating new lines and comparing them to existing lines. This data will be useful in evaluating potential chemical or hormonal treatments to improve harvest efficiency.

Materials and Methods

Four soybean lines were used in these experiments. They were TN00-60, TN03-349, 5601T, and Gardensoy-43. TN00-60 is a new line. TN03-349 is a new cultivar developed in Tennessee for edamame production. 5601T (Pantalone et al., 2003) is a USDA check cultivar for the Southern Uniform MG V tests. Gardensoy-43 is a

commercially available edamame cultivar. These lines have relative maturities of 4.8, 6.0, 5.6, and 4.6, respectively (Carpenter 2007). Gardensoy-43 seed was acquired from Dr. Dick Bernard of USDA-ARS (Urbana, IL). Seed for the other lines was provided by Dr. Vincent Pantalone, UTK (Knoxville, TN).

The experiment was designed as a randomized block design with a split plot arrangement and sub-sampling. The lines were planted in strips consisting of seven plots. Each plot was 3.05 m long, with a 2.13 m alley between plots. The plots were four rows wide with ten plants per row and 0.76 m between rows. Reproductive maturity stages for harvest were randomly assigned as a treatment to each plot. For this experiment, pods were harvested from the third row of each four row plot (the right-most inner row).

The reproductive stages for harvest force in 2006 were R5.0, R5.2, R5.4, R5.6, R5.8, and R6.0 (Fehr and Caviness, 1977). In 2007, R7.0 and R8.0 were added. These two stages were not done in 2006 because test measurements at that stage exceeded 2kg per pod. Force measurements were determined using two push-pull gauges (Imada, Northbrook, IL.) The smaller gauge had a maximum force of 2kg. This gauge was used for stages R5.0 to R6.0. Data for R7.0 and R8.0 was collected using a gauge with a maximum force of 5 kg. A gauge with a maximum measureable force of 5kg was acquired for the 2007 harvest. Six pods per plant were harvested from a minimum of 4 plants per plot for each plot. The amount of force required to remove a single pod from the plant was measured in kg.

Data Analysis

Comparison of harvest force was analyzed using mixed model analysis of variance with SAS v9.1.3.

Results

This experiment provided baseline data on the amount of force needed to harvest soybeans at reproductive stages R5 to R8. The force needed to remove a single pod increased linearly as the plant matured from the R5 to the R7 stage (Figure IV.1). The change in force can be described by a line with the equation $y = 0.1443x + 0.2044$ ($R^2 = 0.9817$). From the R7 stage to the R8 stage the force necessary to remove a pod from the plant decreased to a level similar to the R5.8 stage (Figure IV.2). There was no significant difference between years.

Mean pod force was lowest at the R5.0 stage, 0.4116 ± 0.06298 kg. Pod force increased linearly to 0.4866 ± 0.0616 kg at R5.2, 0.5954 ± 0.06087 kg at R5.4, 0.7235 ± 0.0618 kg at R5.6, 0.9159 ± 0.06107 kg at R5.8, 1.0856 ± 0.06059 kg at R6.0, and 1.2522 ± 0.06852 kg at R7.0. Pod force decreased from R7.0 to R8.0. The force measurement at the final mature stage was 0.9368 ± 0.06868 kg (Figure IV.2).

There were differences among lines in this experiment. Gardensoy-43 was always the most difficult to harvest with a mean over all maturity stages of 1.0437 ± 0.06266 kg. TN00-60 was the next most tenacious with a mean of 0.8076 ± 0.06341 kg. TN03-349 and 5601T were the easiest to harvest with means of 0.6880 ± 0.06223 kg and 0.6645 ± 0.06405 kg, respectively. TN03-349 and 5601T were not significantly different.

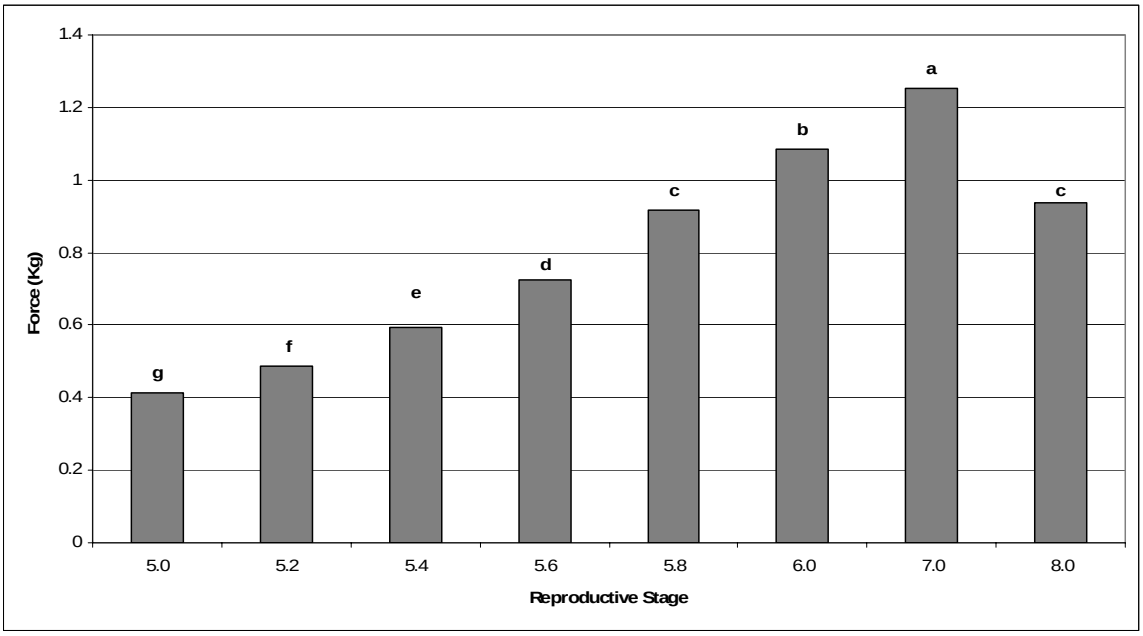

Figure IV.1. Pod Removal Force for Soybeans Grown at the East Tennessee Research and Education Center. LSD means with the same letter are not significantly different at the 0.0001 probability level.

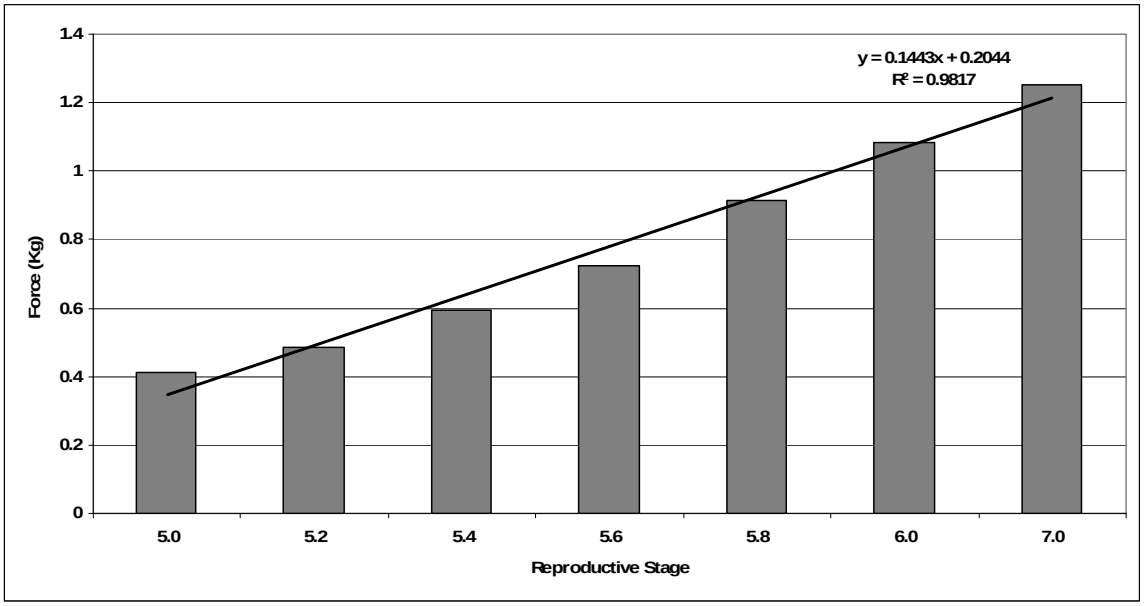

Figure IV.2. Linear Increase in Pod Removal Force for Soybeans Grown at the East Tennessee Research and Education Center.

The lines performed differently as the seeds matured (Figure IV.3). This difference was not noticeable in the earliest stages, when the seed is too small to be of agronomic value (average seed weight was 0.0001g at the R5.0 stage.) The pod force for all lines at the earliest stage, R5.0, was not significantly different. At R5.2, three lines performed similarly. 5601T had a mean of 0.4525 ± 0.08015 kg, TN00-60 had a mean of 0.4625 ± 0.07877 and TN03-349 had a mean of 0.4231 ± 0.07253 kg. Mean pod force for Gardensoy-43, was much higher at that stage with a mean of 0.6085 ± 0.06857 kg. This trend continued until R6.0 when TN00-60 was significantly higher than either 5601T or TN03-349 with means of 0.894 ± 0.076 kg, 1.152 ± 0.071 kg, and 1.018 ± 0.069 kg, respectively. Gardensoy-43 was still much higher than the other lines at R6.0 with a mean of 1.2791 ± 0.07228 kg. At R7.0, 5601T, Gardensoy-43, and TN00-60 were

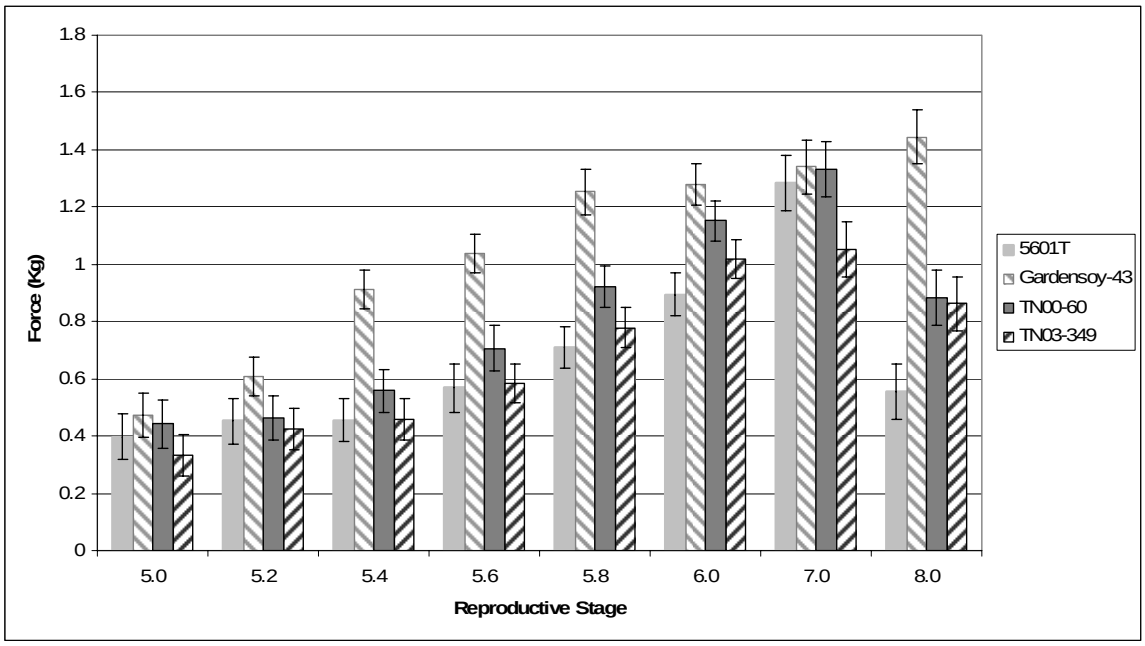

Figure IV.3. Variation Between Pod Removal Force in Soybeans Grown at the East Tennessee Research and Education Center. LSD means with the same letter are not significantly different at the 0.0001 probability level.

not different with means of 1.2843 ± 0.09619 kg, 1.34 ± 0.09544 kg, and 1.3334 ± 0.09581 kg. TN03-349 had a significantly lower pod removal force at R7.0, of 1.051 ± 0.09523 kg. Finally, at the R8.0 stage, pod force for all lines except Gardensoy-43 decreased to levels similar to the R5.8 stage. Pod force for Gardensoy-43 was highest at R8 than at any other stage, in any other line. Mean pod force at the R8.0 stage was 0.556 ± 0.096 kg, 0.884 ± 0.096 kg, 0.862 ± 0.095 kg, for 5601T, TN00-60, and TN03-349 respectively. Gardensoy-43 pods were much more strongly attached to the plant with a mean of 1.445 ± 0.096 kg.

Discussion

There were very significant differences between lines, reproductive stages, and interactions between lines and reproductive stage. The increase in pod removal force as the pod matures would provide a reproductive benefit to the plant. As the pod matures it consumes more resources from the plant. The connection to the plant becomes larger and more secure as it ages, until physiological maturity is reached at the R7.0 stage. Once the seed is mature and begins to dry, attachment to the plant becomes more tenuous and pod removal force decreases.

The differences between lines began at R5.2. Gardensoy-43 and the two commodity lines were statistically the same, but pod removal force for TN03-349 was significantly less than Gardensoy-43. Pod removal force for Gardensoy-43 continued to be higher than all other lines. Pod removal force for TN03-349 was statistically the same as for the two commodity lines at stages R5, 5.2, 5.4, 5.6, 5.8, and 6. At R7, TN03-349 required significantly less force than any of the three other lines. At R8, TN03-349 was

not different from TN00-60, but both were higher than 5601T. Gardensoy-43 was developed by the USDA at the University of Illinois. The two commodity lines and TN03-349 were developed at UTK. It would be interesting to perform this experiment on a few more lines from Illinois, to see if they respond similar to Gardensoy-43. Marked differences in pod removal force for lines developed by two different breeding programs may indicate a strong genetic component to pod removal force. That would be an expected influence, given the potential for pod attachment to directly influence seed yield by preventing the loss of immature pods.

A future study comparing the pods left on the plants in the field would be interesting. Given the marked differences in pod removal force among the lines , an effect on relative yield would be expected by line. Specifically, Gardensoy-43 should have a lower relative yield than TN03-349.

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PART V: CONCLUSION

Conclusion

Most soy grown in the United States is harvested at the R8 stage. Edamame has advantages over conventional soybeans. The pods can be harvested two to four weeks earlier than conventional soybeans depending on the weather. Conventional soybeans are typically highly processed to extract the oils and isoflavones or turn them into tofu. Processing edamame can be a much simpler process if the beans are sold while still in the pod. A relatively short growing season and minimal processing make edamame an advantageous crop for the United States.

The United States currently imports most edamame consumed here from China (Born, 2006). The demand for edamame in the fresh and frozen markets is increasing. There is great potential for growth in edamame production in the United States. To encourage and aid edamame production in the Southern states, these studies were designed to investigate the influences affecting isoflavone concentration in soy and evaluate the differences in edamame and commodity lines. The force study yielded baseline data that can be used to evaluate treatments intended to improve harvest efficiency.

Isoflavone concentration is greatly influenced by genetics, air temperature, soil water content, potassium and CO_2 availability, and irradiance (Tsukamoto, et al., 1995; Hoeck et al., 2000; Vyn, et al.,2002; Kim, et al., 2006; Kirakosyan, et al., 2007). All of these factors are affected by competition, so planting density should influence isoflavone concentration in soybeans. Planting density did not result in a significant effect on isoflavone concentration for any density or genotype evaluated. The isoflavone

concentrations were affected by line and maturity. Results from both the spacing and maturity studies confirmed previous reports of differences in isoflavone concentration between lines and high isoflavone concentration in the cultivar 5601T (Charron, et al., 2005). The influence of genotype on isoflavone concentration is well established (Eldridge and Kwolek, 1983; Tsukamoto, et al., 1995; Hoeck et al., 2000; Swanson, et al., 2004; Charron, et al., 2005).

Line 5601T produced the highest isoflavone concentrations in both the spacing and maturity studies. Total isoflavone growth stage means for line 5601T were not significantly different at any stage except R7. At R7, 5601T yielded a mean isoflavone concentration that was significantly higher than any other stage or line.

Trends in total isoflavone concentration over time were not the same for all lines. TN00-60 and Gardensoy-43 were not significantly different at any stage. TN03-349 followed the expected trend of increasing total isoflavone concentration over time, with the highest mean at R8. Total isoflavone concentration in line 5601T was highest at R7, but all other stages were statistically the same. The varying trends in isoflavone concentration seen for Gardensoy-43, 5601T, and TN03-349 would be useful in genetic comparisons aimed at determining which genes influence isoflavone concentration.

Most isoflavones increased as the seed matures, with the highest concentrations found at the R8 stage. Three isoflavones, malonyl daidzin, acetyl genitin, malonyl genistin, and total isoflaovnes produced markedly higher means at the R7 stage rather than R8. Three isoflaovnes, daidzein, glycitin, and malonyl glycitin were found in the highest concentrations at the R6 stage. These differences may be important if the individual isoflavones are found to have different biological activities

Nine isoflavones showed a marked decrease at stage R5.5. This is unlikely to be environmentally related because each line reached this stage at different times. Gardensoy-43 matured earliest, so it reached R5.5 two weeks before TN00-60 and 5601T. The two commodity lines matured at slightly different rates but reached R5.5 within the same week. TN03-349 was the slowest to mature, reaching to R5.5 six weeks after Gardensoy-43. This disparity in the dates when the lines reached R5.5 implies that the decrease is not caused by an environmental factor.

The decrease in isoflavone concentration at R5.5 was significant for the isoflaovnes daidzin, glycitin, malonyl glycitin, and total isoflavones. For malonyl genistin and total isoflavone this was due to line 5601T. The reproductive stage at time of harvest will affect the relative concentrations in the resulting product. Ingesting soybean harvested at earlier stages may produce a different effect. Additionally, the harvest date could be adjusted if a higher yield of a specific isoflavone was desired.

Future Studies

This research has revealed some intriguing areas for future research. It is very curious that nine out of 11 isoflavones decrease at stage R5.5. This is likely a genetic effect, since the lines reached this stage at such different times in the season. The fate of the isoflavones that were deposited in the seed prior to R5.5 could be investigated. It is possible that the isoflavones deposited prior to R5.5 were processed into another compound. Or, perhaps they are simply degraded and replaced. The high isoflavone concentrations reported for line 5601T by this research and previously published studies

make it a favorable line for use in future genetic studies investigating isoflavone synthesis.

The trend total isoflavone concentration at different planting densities and harvest maturities may also be interesting to pursue. At the more dense plant spacings, total isoflavone concentration was higher at the R6.0 stage than at the R8.0 stage. The R8 growth stage yielded the highest isoflavone concentration at the least dense, 20 cm spacing. A future study specifically addressing the interactions between planting density and pod maturity at harvest could be worth investigating. If this trend is verified by future studies, growers seeking higher (or lower) total isoflavone concentration may wish to adjust their planting density depending on the intended harvest stage.

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Vita

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