Cultivation and Nutritional Constituents of Virginia Grown Edamame

by

Luther C. Carson

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Horticulture

Josh H. Freeman, Chair
James R. Harris
Gregory E. Welbaum

April 28, 2010
Blacksburg, Virginia

Keywords: Cultural Practices, *Glycine Max* L. Merrill, Isoflavone, Planting Date, Soybeans

Cultivation and Nutritional Constituents of Virginia Grown Edamame

Luther C. Carson

ABSTRACT

Edamame's (*Glycine max* L. Merrill) consumption in the US has also been growing due to purported health benefits. Edamame grows well around the US, but few have measured the growth and yield in the mid-Atlantic region. The objective of these studies were to determine the potential yield of edamame, determine how yield components change with planting date and cultivar, and to measure total protein, lipids, antioxidant activity and isoflavone concentrations at harvest.. The five cultivars (BeSweet 292, BeSweet 2015, BeSweet 2001, Midori Giant and Sunrise) used in the cultivar evaluation trial and for nutritional constituents analysis were grown in Painter, Virginia in 2008 and 2009. The cultivar evaluation trial yielded between 5,600 and 8,400 kg per ha. Percent marketable pods ranged from 74.3 and 85.6% in the cultivar evaluation trial. There were significant differences in average seed size across cultivars in both years. Cultivar lipid content followed the same patterns in both years with 2009 having lower overall concentrations than 2008. Protein contents were similar in 2008 and 2009. Both years, 'BeSweet 2015' and 'BeSweet 2001' had high radical scavenging ability and Midori Giant had the lowest. In 2008, there were no significant differences in the ORAC assay. 'BeSweet 292' had significantly more reducing activity in the DPPH assay than Sunrise in 2009. Isoflavones were measured in 2008 and 2009 at harvest and temporally in 2009. Of all isoflavones, Malonyl genistin had the highest concentration. Edamame is a suitable crop for cultivation in Virginia, and is high in nutritional quality.

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Dr. Josh Freeman, for his guidance and direction that has gotten me to this point. Dr. Greg Welbaum and Dr. Roger Harris also deserve thanks for their guidance and knowledge that I used so often. Furthermore, I would like to thank Ursula Deitch for introducing me to plot work and completing many hours of data collection that I left behind when I returned to campus. I also need to thank Dr. Hyun Chung and Yumin Dai, of the Dr. Kevin Zhao Lab, who helped me through my laboratory work. I would also like to thank Dr. Jim Tokuhisa for teaching me how to use the HPLC, and for helping me through the sticky situations I found myself in so often.

I need to thank the Department of Horticulture at Virginia Tech for providing me funding to complete my work. I would also like to acknowledge Rupp Seed and Wannamaker Seed companies for providing some of the seed for my research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Introduction and Justification

Section 1.1 Statement of Problem, Rationale, and Significance

Virginia's need for alternative crops has been growing due to the declining area of tobacco production. From 1999 to 2009, United States tobacco production area declined by 46%. During the same time period Virginia tobacco production area decreased by 49% which amounts to a loss of 7,570 hectares (USDA, 2009). To fill the gap in tobacco production area, Virginia growers have been looking for alternative high-value crops.

Edamame or vegetable soybean (*Glycine Max* L. Merr.) is a high-value crop, especially for producers with regional and local markets (Duppong and Hatterman-Valenti, 2005). Edamame may also be well suited as a crop for vegetable bean producers and processors in Virginia. Much of the equipment used to process snap beans (*Phaseolus vulgaris* L.) can be used to process and freeze edamame. Bernick (2009) reported that contract growers of edamame in Ohio made on average $1480-1850 per hectare after direct costs. Ernst and Woods (2001) have sited much less favorable expected returns of $640 per hectare with a break-even of approximately $2.20 per kilogram for hand harvested beans.

The domestic sales of edamame rose from $18 million to $41 million between 2003 to 2009 (Bernick, 2009; Soyatech, 2010). The reason for edamame's increased popularity is likely due to the nutritional value and potential health benefits of eating soy-based foods (Duppong and Hatterman-Valenti, 2005; Zhang and Kyei-Boahen 2007). Although edamame's popularity has been increasing, the USDA considers edamame a minor crop and does not track the amount of edamame imported; it is included in the general category of soybeans (M.H. Blazejak, Personal Correspondence). However, it was estimated that 70% of the edamame consumed in the United States was imported from Asian countries such as Taiwan and China in 2001(Shurtleff and Lumpkin, 2001).

Consumers are selective when it comes to buying edamame and often look for specific quality criteria. Consumers in Japan, who are net bean importers, are selective and prefer large seeded beans with a minimum of 2 beans per pod with a "flower-like and beany flavor and texture", while Americans prefer to purchase their edamame shelled and frozen with a more buttery flavor (Duppong and Hatterman-Valenti, 2005; Lin, 2001; Sanchez et al., 2005). In a study by Young et al. (2000), American consumers indicated that their most important buying motivations are flavor, nutrition, and value.

Although some edamame is grown in the United States, there are no standard cultural practices for growing edamame to maximize bean quality characteristics. It is important to understand how agronomic practices affect the quality characteristics of edamame, before it can be grown as an alternative crop for export. According to Konovsky et al. (1994), yield is affected by between-row spacing more than inter-row spacing and the amount of pods with acceptable bean numbers declines as plant population increases. Sciarappa et al. (2007) planted organic edamame in staggered double rows on 76 cm plasticulture with 15 cm between plants, yet in other studies plants were spaced at 45, 60, and 75 cm between rows with populations up to 988,420 seeds per hectare (Duppong and Hatterman-Valenti, 2005; Lai et al., N.D.; Young et al., 2000). According to Duppong and Hatterman-Valenti (2005), cultural practices like planting date and density may affect flavor.

Section 1.2 Objectives

The objectives of this study were to:

1. Determine commercial cultivars that are suitable for cultivation in Virginia.
2. Determine the best planting date to maximize bean size, beans per pod, and yield.

3. Determine the nutritive characteristics of Virginia-grown edamame including: protein, lipids, antioxidant capacity and isoflavone percentages and content.
4. Determine isoflavones accumulate dynamics in the seeds during pod fill.

Section 1.3 Literature Review

Section 1.3.1. What is Edamame?

Edamame is similar to agronomic soybeans, but with many distinct characteristics. Edamame is a group of specialty-cultivars of vegetable-soybean that tends to have a green seed coat, but can range from black to yellow with a light brown or gray hilum. White pubescence on the pod is also an important varietal characteristic (Konovsky et al., 1994). Edamame has been selected for flavor, which is superior to agronomic soybeans, large seed size, green seed yield, and nutritional content (Johnson et al., 2001; Mebrahtu and Devine, 2008; Montri et al., 2006; Shurtleff, 2001; Young et al., 2000). Edamame is harvested when immature at 80% pod fill or the R6 stage of development when the pods have full size seeds at one of the four uppermost nodes (Fehr et al., 1971; Rao et al., 2002). Japanese consumers have very specific quality standards that they look for when purchasing edamame (Duppong and Hatterman-Valenti, 2005). They prefer large-seeded beans, two beans or more beans per pod, pods that are bright green in color, a flowery flavor, and prefer to purchase the beans in shell and often still attached to the stem (Kelley and Sanchez, 2005; Mebrahtu and Devine, 2008; Montri et al., 2006). American consumers are much less selective about the edamame they purchase; however, they tend to prefer a more buttery flavor (Kelley and Sanchez, 2005).

Section 1.3.2. Photoperiodism, Maturity Groups, and Harvest Timing

Reproduction in most soybeans is controlled by night length. As you go north from the equator, where, the day and night length are nearly equal, the night time gets shorter. Also, when moving through the seasons, from mid-winter to mid-summer the nights get shorter, then from mid-summer to mid-winter the nights get longer. Soybeans perceive night length using a ratio of phytochrome far red and phytochrome red. During the day, soybeans absorb both red light and infrared light. The sunlight provides more red light than far red light and causes the phytochrome red to turn into phytochrome far red. During the night the phytochrome far red decays back to phytochrome red. The ratio of phytochrome red to far red allows the plant to detemine how long the night is and it triggers flowering.

A system has been devised to classify geographic adaptability of soybean cultivars. This system allows producers to determine if a soybean cultivars' photoperiod fits their latitude. Soybeans have been divided into 12 maturity groups ranging from 00 to 10. If a soybean cultivar is in maturity group four and is moved north to latitudes that are suitable for maturity group two, the plant will likely not accumulate enough hours of darkness to flower. If it does receive the threshold night length to flower the plants may be killed by frost before they produce viable seed. In this situation, it took plants too long to accumulate dark hours. A cultivar can be moved from a maturity group two and planted in lower latitudes. Soybeans in this situation will flower earlier because the threshold night length will be reached sooner due to the longer nights in the south. Unfortunately, for a producer, this will mean a faster maturity and likely less total yield because the entire growing season would not be used to capture sunlight and produce photosynthate. In this situation, plants accumulated dark hours too soon and had insufficient vegetative growth.

There are conflicting views on the sensitivity to photoperiod in edamame. According to Konovsky et al. (1994), Japanese soybeans are classified into two types summer and fall. Most Japanese soybeans are temperature sensitive and are called summer type. Few types of soybeans are photoperiod sensitive and mature in the fall thus they are called fall type (Konovsky, 1994). However, Miles et al. (2000), states that soybeans mature differently according to photoperiod sensitivity which differs by cultivar. The difference between edamame in the United States and Japan may be due to the way breeders in the United States created new cultivars. Many of the new US edamame cultivars introduced have been bred by crossing Asian edamame cultivars with agronomic cultivars from the United States (Rao et al., 2002).

Research referring to edamame states that it is harvested at the R6 stage of development. The R6 stage of development is when one of the pods in the four uppermost nodes contains a full size bean, which can last from 9 to 30 days (Fehr et al., 1971; Naeve, 2005). It is important to harvest beans at the correct time. If the beans are harvested too early the grower will lose potential yield and quality. However, if the beans are harvested too late, the producer may reach yield goals, but will sacrifice quality. The most important quality characteristics in edamame are texture, color, nutrition, and size. Each of these characteristics are maximized at different times in the plant growth cycle creating a dynamic where the peak of one quality may not be achieved to reach a quality threshold in another area (Mbuvi and Litchfield, 1995). According to Konovsky (1994), the amount of sugar in the beans, which is the one of the most important flavor components, peaks days before edamame reaches R6 (Masuda, 2001). Mbuvi and Litchfield (1995) concluded that the weight per 100 seeds peaked at about 1710 growing degree days (GDD), color peaked at about 1380 GDD, and texture or hardness continued to increase until the seeds were harvested.

Section 1.3.3. Edamame Planting Date

It may seem intuitive that the earlier a grower plants, the better yields he or she can expect. Ray et al. (2008), reported planting in late May resulted in significantly greater yields than planting in late June in SC. Pederson and Lauer (2003) reported mixed results with significantly less yield in the late planting date at one of the two planting locations in WI. In a study from Indiana, Robinson et al. (2009) reported significantly greater yields in late-April and early-May plantings than in later plantings. A meta analysis of 28 planting date studies by Egli and Cornelius (2009) concluded that plantings delayed till after the end of May for the Midwest (Iowa, Nebraska, and Ohio) and Deep South (Alabalma, Florida, and Georgia), or early June in the Upper South (Arkansas, Missouri, Kentucky, and Tennessee) lost yield because of delayed planting. They also concluded that there was not clear advantage to planting in April or early-May. In a study conducted in New York, Cox et al. (2008) supported the meta-analysis reporting that there was not a significant difference in yield between the early and late May planting dates, but both yielded significantly more than the mid-June planting date. Planting dates in late-March and early-April have resulted in reduced yields because of less than optimum environmental conditions, which caused reduced emergence (Egli and TeKrony, 1995).

Edamame producers are looking for more than total seed yield. They must take into account quality factors such as seeds per pod and seed size. Mathew et al. (2000) reported that the maximum plant yield and seed size are genetic, however, they both can be slightly changed by modifying the environment. Robinson et al. (2009) reported that planting date had no significant effect on the number of seeds per pod but did significantly increase in seed size due to delayed planting. However, Pederson and Lauer (2004) observed increase from 2.40 to 2.46 seeds per pod with delayed planting in two years of a four year planting date study; no change in seed size was observed. Ray et al. (2008) found that there was an increase of 1.3 g per 100 seeds by delaying plantings from late-May to late-June.

It has been shown that soybean yield components adjust due to planting date and management system (Pedersen and Lauer, 2004). Later planting dates decrease the number of days to critical daylength when growth changes from vegetative to reproductive. Late planting dates often results in fewer reproductive nodes and fewer pods per square meter; however, the seed fill period is usually not reduced resulting in fewer seeds with a larger size given sufficient leaf area (Egli and Bruening, 2000; Egli, 1993). Pederson and Lauer (2004) reported that a seed's mass accumulated and reached its full size more quickly in later plantings than in the early plantings, however, there was no significant difference between the final seed size. Cox et al. (2009) observed that late-May and mid-June planting dates had few pods per plant than the mid-May planting date. They did not see an increase in seed size, but the late-May planting date had significantly more seeds per pod than both the mid-May and mid-June planting dates, which would account for the previously mentioned reduced yield in mid-June only.

Section 1.3.4. Edamame and Soybean Nutritive Values

One reason for the popularity of edamame is the great nutritional value as well as the potential human health benefits of antioxidants and isoflavones (Duppong and Hatterman-Valenti, 2005). Soybeans are considered low in fat and high in protein (Rao et al., 2002). They also contain all essential amino acids making them a particularly good meat substitute. According to Montri et al. (2006), edamame contains lower levels of trypsin inhibitors, fewer indigestible oligosaccharides, a greater amount of vitamins, and higher levels of phytic acid than agronomic soybeans, which makes edamame more nutritious than agronomic soybeans. Rao et al. (2002) determined that edamame oil content ranges between 130.7 and 155.8 g per kg on a dry weight basis (DWB), protein ranged from 333.2 and 389.0 g per kg DWB, and the moisture ranged from 539 to 561 g per kg on a fresh weight basis.

According to Hymowitz et al. (1972), protein content of agronomic soybeans ranged from 33.1 to 49.2% on a DWB. Similarly, Yin and Vyn (2005) and Hartwig and Kline (1991) found that soybeans averaged about 41% protein. Sikka et al. (1978) found that protein content of vegetable-type and agronomic-type soybeans is similar. The most common method for analyzing the amount of protein in plants material is the Kjeldahl method (AOAC International, 2005). Protein, in most cases, is 16% nitrogen. The Kjeldahl method provides the amount of nitrogen in the plant sample, which can then be multiplied by the percent nitrogen to determine amount of crude protein. Kjeldahl does not allow for analysis of individual amino acids because it is a destructive technique. In soybean, protein and lipid contents are inversely related (Hartwig and Kilen, 1991; Hymowitz et al., 1972; Rao et al., 2002; Shanmugasundaram et al., 2001).

Soybeans are considered an oil seed because they have a high fat content. Oil content in agronomic soybeans and edamame is between 145 to 230 g per kg and 130.7 and 155.8 g per kg, respectively, on a dry weight basis (Hymowitz et al., 1972; Rao et al. 2002). According to Sikka et al. (1978), vegetable soybeans contain less fat than agronomic soybeans, which agrees other reports cited earlier. The lipid content of soybeans is one of the reasons they are useful in industry and for biofuels. Variations in oil content can change the flavor of edamame; which according to Young et al. (2000), oily flavor in edamame negatively affects eating quality.

Environmental factors can affect the oil content in soybean. Pedersen and Lauer (2003) reported that late-May planting dates reduced soybean oil content in two locations, although the differences were significant in only one location. Ray et al. (2008) presented similar findings with late planting dates decreasing oil content in soybeans by 5 g per kg. Piper and Boote (1999) reported that oil concentration in the seed increased with temperature up to 28 ºC, after which it decreased.

Soybeans have become popular for many reasons and perhaps one of the biggest is due to the potential health benefits. Soybeans contain isoflavones and other antioxidants. Antioxidants are beneficial because they prevent negative effects of free radicals in the body (Achouri et al., 2005; Patel et al., 2001). Dietary intake of antioxidants has been associated with reduced incidence of atherosclerosis, cancer risk, neurodegenerative diseases, and improved immune system (Kumar et al., 2009; Patel et al., 2001) Isoflavones are phytohormones, which some studies have shown reduced prostate and breast cancer, reduced cholesterol, and lighter menopausal symptoms (Messina, 2001). Therefore, including soybeans in the human diet may have beneficial effects. In the plant, isoflavones assist in plant antifungal response, in deterring insect feeding, and in nodulation of legumes (Berger et al., 2008; Burden and Norris, 1992; Graham, 1990). Isoflavones are also good antioxidants of ultraviolet (UV) light induced oxidation (Wang and Murphy, 1994).

Isoflavones are produced in the phenyl propanoid pathway and are related to a large family of flavonoids (Berger et al., 2008; Kim and Chung, 2007; Rice-Evans et al., 1997). There are three major isoflavones in soybean, each with four types of conjugates that can be split up into categories: aglycone (genistein, glycitein, and daidzein), 7-O-glucosides (genistin, glycitin, and daidzin), 6-acetyl-O-glucosides, and 6-malonyl-O-glucosides (Prabhakaran et al., 2006). Acetyl conjugates tend to be the least plentiful in soybean seeds and malonyl genistein and malonyl daidzein tend to have the highest concentrations in the seed. The addition of a sugar, usually glucose, to the isoflavone aglycone (usually at the 7-hydroxyl group) makes it a glucoside (Rice-Evans, 1997). When a malonyl or acetyl group is bound to the "6" carbon on the sugar molecule, it becomes a malonyl-O- or acetyl-O- glucoside (Wang and Murphy, 1994). Isoflavones found in mature beans tend to be the glucoside and the malonyl conjugates (Wang and Murphy, 1996). However, malonyl glycosides easily convert to their respective glucoside due to weak linkages between the malonyl group and the sugar (Kim and Chung, 2007).

The concentration of isoflavones in bean seeds can be affected by year, environment, location, genetics, irrigation, and the assay used for analysis (Bennett et al., 2004; Dhaubhadel et al., 2007; Eldridge and Kwolek, 2002; Hoeck et al., 2000; Mebratu et al., 2004). According to Wang and Murphy (1994), year had more of an influence on isoflavone content than location. Tsukamoto et al. (1995) reported, soybeans that go though isoflavone accumulation during high temperatures, have lower concentrations than those who accumulate isoflavones during cooler conditions. Similarly, Lozonvay et al. (2005) showed that isoflavone accumulation is negatively correlated with temperature during seed fill. Irrigation was shown to increase isoflavones by 1.6 to 2.8 fold over non-irrigated beans (Bennett et al., 2004). Kim and Chung (2007) have shown that total isoflavone content in the beans was positively correlated with growth stage and that isoflavone concentrations peaked at R7 or physiological maturity. Berger et al. (2008) reported that isoflavone accumulation began in the hypocotyl first. After the hypocotyl concentrations plateaued, accumulation in the cotyledons began.

Before isoflavones can be measured using high performance liquid chromatography (HPLC), they must first be extracted. According to Achouri et al. (2005), there are several solvents including, methanol, ethanol, and a mixture of acetonitrile and hydrochloric acid, that can be used to extract isoflavones from soy. The HPLC works using a highly polar column. By applying a solvent gradient to the column, different substances can pass through at different times to be measured individually.

There are several antioxidants in plants including: flavonoids, vitamin C and E, caffeic acid, chlorogenic acid, ferulic acid, tannins, and proanthocyanidins (Rice-Evans et al.,1997;

Mohamed et al., 2001; Chung et al., 2008). These antioxidants scavenge free-radicals and inhibit lipid oxidation (Patel et al., 2001; Malencic et al., 2007). Membranes in the plants and human body are made of phospholipids bi-layers among other macromolecules. Oxidation of these lipids can shorten polyunsaturated lipids, causing increased fluidity of the membrane and loss of effectiveness (Moller, 2006). The Diphenylpicrylhydrazyl (DPPH) assay and the Oxygen Radical Absorbing Capacity (ORAC) assay determine antioxidant content. DPPH is a stable free radical, which is purple in color. The purple color changes to yellow once the DPPH has been reduced. By creating a serial dilution of an antioxidant with a known reducing power (Trolox), adding DPPH, and measuring the absorbance, a standard curve can be obtained. Adding DPPH to a sample with antioxidants and measuring the absorbance, the reducing or antioxidant activity can be obtained by comparison to the standard curve. The ORAC test uses trolox, 2-2'-azobis(2-amidino-propane) dihydrochloride (AAPH), and fluorescein (FL). The ORAC assay measures the ability of the sample's antioxidants to compete with a peroxyl radical generator (AAPH) and protect fluorescein from degradation. Fluorescein is an organic dye, which when mixed with AAPH, is degraded and loses it bright yellow color. Samples containing antioxidants should protect the dye from degradation, allowing antioxidant content to be quantified.

Chapter 2: Cultivar Evaluation Trial

Section 2.1 Introduction

Edamame or vegetable soybean (*Glycine max* L. Merrill), is a group of special cultivars of soybean. When harvested at immature green stage, the flavor of edamame have been described as nutty, sweet, buttery, and beany (Wszelaki et al., 2005). High sucrose content is particularly important for a sweet flavor (Masuda and Harada, 2001). Edamame is harvested green at the R6 stage of development or about 80% pod fill and is therefore considered a vegetable (Fehr et al., 1971). Consumers of edamame look for bright green pods, two or more beans per pod, and large seed size (Montri et al., 2006).

Edamame is a popular vegetable in Asian countries, and is becoming increasingly popular in the United States. The increase in popularity can be attributed to soybeans high fat and protein content, as well as, its beneficial phytonutrients (Duppong and Hatterman-Valenti, 2005; Zhang and Kyei-Boahen, 2007). The amount of edamame purchased in the United States went from $18 million in 2003 to $41 million in 2009 (Bernick, 2009; Soyatech, 2010). Edamame is eaten steamed in the pod as an appetizer or can be added to dishes such as salads and stir-fry (Konovsky et al., 1994).

Despite its gain in popularity, most edamame is imported into the United States. There have been several reports that edamame grows well in the United States (Duppong and Hatterman-Valenti, 2005; Johnson, 1999; Rao et al., 2002; Sanchez et al., 2005; Sciarappa et al., 2007; Zhang and Kyei-Boahen, 2007). Although there have been substantial edamame breeding efforts in Virginia, there has been little work to evaluate commercially-available cultivar yield potential for the mid-Atlantic region. Virginia has a robust snap bean industry that may be able to incorporate edamame into their business model. Growing edamame in Virginia may help replace some of the 7,570 hectares of tobacco production area that was lost between 1999-2009 (USDA, 2009). Charles Fry of the Sweet Bean Company in Medina, Ohio reported that growers producing for their cooperative could expect net returns of $1480-1850 per hectare (Bernick, 2009). Ernst and Woods (2001) have reported much less favorable expected returns of $640 per hectare with a break-even of approximately $2.20 per kilogram for hand-harvested beans. The purpose of this study was to determine and evaluate suitable cultivars for cultivation on Virginia's Eastern Shore.

Section 2.2 Material and Methods

On 7 May, 2008 and 22 May, 2009, five commercially-available cultivars were planted in Painter, Virginia on Bojac Sandy Loam (Thermic Typic Hapludults) in a randomized complete block design with four replications. In both 2008 and 2009, three cultivars BeSweet 292 (BS292), BeSweet 2015 (BS2015), and BeSweet 2001 (BS2001), were obtained from Rupp Seed Company of Wauseon, Ohio, and two cultivars, Midori Giant (MG) and Sunrise (SR) were obtained from Wannamaker Seed Company of St. Matthews, South Carolina. Experimental plots consisted of four rows in 2008 and 2 rows in 2009. Plots were 7.62 m long with between-row spacing of 0.91 m and in-row spacing of 7.62 cm. Phosphorus and potassium were added pre-plant based on soil test results and pest management measures were based on cooperative extension recommendations (Virginia Cooperative Extension, 2000). Time to 50% flower was collected before harvest. At harvest, August 8, 2008 and August 20, 2009, the plant height was measured, and a plant maturity rating was given. Plant height was calculated by taking three random measurements per plot. Experimental plots were harvested using a single row Pixall harvester. Yield data was obtained from a single row (interior row in 2008). A one-hundred pod subsample was taken and the pods containing 1, 2 and 3 beans were counted and weighed to determine percent marketable pods. The beans were shelled and the weight of 100 seeds was recorded. A harvest efficiency rating was given after harvest based on the number of pods remaining on the plant. The number of pods left on 20 harvested plants were counted and compared to the number of beans on 5 plants in the remaining unharvested test row.

Data for each experiment was analyzed using the fit model platform in JMP (version 8.0; SAS Institute, Cary, NC.). Cultivar, year, and cultivar × year were considered main effects. The arcsine tranformation of percent marketable pod and harvest efficiency was used to determine statistical differences. Blocks were considered random. Means were separated using Tukey's HSD ($P \leq 0.05$).

Section 2.3 Results

There were no interactions between year and cultivar (P=0.079), so the yield data were pooled (Table 2.1). The cultivars produced between 5,606 to 8,430 kg of pods per ha (Table 2.2). 'MG' yielded 8,430 kg/ha, which was significantly greater than only 'BS2015' at 5,606 kg/ha. 'BS2001' was not significantly different from any cultivars with yield of 6,820 kg/ha.

For percent marketable pods data, there were no significant interactions between year and cultivar (P=0.73), so the data was pooled for analysis (Table 2.1). Any pod containing two or more large size beans were considered marketable. Flat pods and those with only one seed were considered unmarketable. The percent marketable pods ranged from 74.3 to 85.6% (Table 2.2). 'SR' had the highest percent marketable pods at 85.6%; however, there were no significant differences between cultivars.

There were significant interactions between year and cultivar (P=0.0032), so 100-seed weight data is present by year (Table 2.1). The range of seed weights in 2008 was from 50.1 g to 71.0 g per 100 seeds (Table 2.2). In 2009, the range of seed weight was 47.5 g to 68.0 g per 100 seeds. In 2008, 'SR' had the highest seed weight at 71.0 g per 100 seeds which was significantly greater than all but 'MG' at 66.2 g per 100 seeds. 'MG' had the highest seed weight in 2009 at 68.0 g per 100 seeds. 'BS2015' had the lowest seed weight in both years with a weight of 50.1 g and 47.5 g per 100 seeds in 2008 and 2009, respectively. Overall, with the exception of 'MG', the seed weights in 2009 were lower than in 2008.

Harvest efficiency was measured by comparing the number of pods left on unharvest plants to the amount left on harvested plants. There were significant interactions between year and cultivar (P=0.0062), so the results are reported separately (Table 2.3). Overall, with the exception of 'MG', harvest efficiency was greater in 2008 than 2009. There were no significant differences between harvest efficiencies in 2008, which ranged from 90.2 to 95.9% in 'MG' and 'BS292', respectively. In 2009, the harvest efficiency ranged from 82.3% for 'BS292' to 95.4% for 'MG'. 'MG' had significantly greater harvest efficiency than both 'BS292' and 'BS2001', but not significantly greater than 'BS2015' or 'SR' with harvest efficiencies of 87.3 and 88.8%, respectively.

The average plant height was measured prior to harvest. 'BS2015' was the tallest cultivar in 2008 with a height of 62.0 cm and the second tallest cultivar in 2009 at 59.2 cm; however, it was not significantly shorter in 2009 than the tallest cultivar (Table 2.3). In 2009, 'SR' was the tallest cultivar with a plant height of 67.1 cm. The shortest cultivar in both 2008 and 2009 was 'MG' with a height of 46.5 and 49.3 cm, respectively.

The five edamame cultivars were once-over harvested and did not mature at the same time, so a maturity rating was given. The maturity rating ranges from one to five. A rating of one meant pods were mature beyond the R6 stage of development with leaves starting to yellow and all seeds were visually mature. A rating of five meant all leaves were green and some pods at the top of the plant contained seeds of less than marketable size. At harvest in 2008, ‘SR’ was the most mature with a rating of 1.0 and appeared to mature about five days prior to the other cultivars (Table 2.3). However, in 2009, ‘SR’ was less mature than ‘MG’ with a rating of 2.75 and 1.25, respectively. ‘BS2015’ and ‘BS2001’ were the least mature cultivars in both years.

Section 2.4 Discussion

The objective of this study was to determine potential yield and yield components of commercially available cultivars. The five cultivars of edamame yielded comparable to snap beans on Virginia’s Eastern Shore (Phillips et al., 2002). When compared to other edamame studies from Georgia and North Dakota, the yields obtained were less than previously published amounts; although 0.61 m inter-row spacing was used in North Dakota and 0.76 m between-row spacing was used in Georgia (Duppong and Hatterman-Valenti, 2005; Rao et al., 2002).

A plant’s yield is genetically determined but changes in environment and cultural practices can affect the maximum. In soybeans, plant population and planting date can influence the number of beans per pod, the average seed size, and yield (Ray et al., 2008; Penderson and Lauer, 2004). In this study, cultivar and year did not interact significantly with respect to percent marketable pods but did so with average seed size. It is difficult to determine if there was a difference between the number of pods with two or three beans because it would not have affected the percent marketable pods. The difference in seed size is most likely a function of relative maturity at harvest. The beans were slightly less mature at harvest in 2009 than in 2008, which allowed less time for seed fill (Table 2.3). According to Hanway and Weber (1971), soybean seeds can accumulate 98.6 kg of dry matter per hectare per day.

Few researchers have looked at the amount of force required to remove a green soybean pod from the stem. Showalter (1969) reported that it takes more force to remove a snap bean as it matures. Maturity was related to the size of the bean, which was correlated with a larger pedicel. Most of the pods left on the soybean plants after harvest were at the bottom of the plant and missed by the reel on the harvester. Smith et al. (1961) reported that losses could be expected if conventional soybeans were set lower than 13 to 15cm above the soil. According to Beaver and Johnson (1981), narrower rows increased lowest pod height, which could increase the lowest pod height to above the 13 to 15 cm threshold. Although this height is for a conventional combine, raising the lowest bean height could increase the harvest efficiency with a snap bean harvester. Mean plant height differences between years were a function of environmental differences between years (Weather data attached in Appendix A).

2.5 Conclusion

With yields similar to snap beans, edamame appears to be a viable alternative crop for Virginia from a productivity standpoint. Although there is room for more domestic edamame production, there needs to be a market established where growers can sell their crop. The R6 stage of development is a wide time frame and more research is needed to define the best harvest window to maximize yield, seed size, texture and other quality characteristics. Quality of edamame can diminish quickly after the peak has been occurred. Investigations of planting patterns effects on harvest efficiency need to be made; however they need to fit into growers existing framework of snap bean production to possibly increase acceptance to a new crop.

Table 2.1. P-values of yield, percent marketable pods, 100 seed weight, harvest efficiency, and plant height by factor for five edamame cultivars, with four replications each, planted in a randomized complete block pattern. The edamame was grown in Painter, VA during 2008 and 2009.

Source	Yield	Percent Marketable Pods	100 Seed Weight	Harvest Efficiency	Plant Height
Cultivar	0.0066	0.0078	<0.0001	0.0003	<.0001
Year	0.0322	<0.0001	<0.0001	0.1484	0.2757
Cultivar × Year	0.0797	0.7318	0.0032	0.0062	0.0135

Table 2.2. Yield, marketable pods and 100-seed weight of five edamame cultivars grown in Painter, VA during 2008 and 2009.

		Percent Marketable	100 Seed Weight (g)	
Cultivar	Yield (kg/ha)	Pods	2008	2009
Midori Giant	8,430 a	80.4 ns	66.2 ab	68.0 a
BeSweet 292	7,938 a	78.5	62.4 b	54.8 b
Sunrise	7,831 a	85.6	71.0 a	54.3 b
BeSweet 2001	6,810 ab	74.3	61.3 b	52.5 b
BeSweet 2015	5,606 b	80.6	50.1 c	47.5 b

NS= Not significantly different

Numbers not followed by the same letter, within a column, are significantly different at $P \leq 0.05$ by Tukey's HSD.

Table 2.3. Harvest efficiency, foliar disease rating, maturity rating, and average plant height of five edamame cultivars grown in Painter, VA in 2008 and 2009. Means are average of four samples for harvest efficiency and maturity rating. Plant height is the average of three measurements per replication with four replications per cultivar (n=12).

Cultivar	Harvest Efficiency (%)		Maturity Rating[z]		Height (cm)	
	2008	2009	2008	2009	2008	2009
BeSweet 292	95.9 ns	82.3 b	1.8	2.25	52.8 b	52.8 b
Midori Giant	90.2	95.4 a	2.5	1.25	46.5 b	49.3 b
Sunrise	94.1	88.8 ab	1.0	2.75	54.6 b	67.1 a
BeSweet 2015	95.6	87.3 ab	3.5	3.25	62.0 a	59.2 ab
BeSweet 2001	90.7	84.5 b	3.0	4.25	51.6 b	51.8 b

ns = no significant difference
Numbers not followed by the same letter within a column are significantly different at $P \leq 0.05$ by Tukey's HSD
[z] Maturity Rating is a score of 1 to 5. A score of one has leaves starting to yellow down and large seeds. A score of 5 the leaves are all green with some pods at the top of the plant having less than full size seeds.

Chapter 3: Planting Date

Section 3.1 Introduction

Over centuries of soybean cultivation, human selection has separated soybeans into two categories, agronomic and food grade (Young et al., 2000). Edamame is a food grade soybean that has specific characteristics that are important for quality standards. These characteristics include large seed size, flavor, two or more beans per pod, and bright green pods (Duppong and Hatterman-Valenti, 2005; Konovsky et al., 1994; Tsou and Hong, 2001). Edamame is harvested at the R6 stage of development and is considered a vegetable crop (Fehr and Caviness, 1971; Rao et al., 2002). The R6 stage of development can range from 9-33 days long, and is not the most accurate indicator of harvest readiness (Fisher and Fanta, 2004). Unfortunately, not all of the quality characteristics peak at the same point, so the peak quality in some areas may be missed to meet other quality standards (Mbuvi and Litchfield, 1995).

Many studies have reported that in soybeans, earlier planting dates have produced larger yields to a certain point (Cox et al., 2008; Robinson et al., 2009). A meta-analysis of 28 experiments by Egli and Cornelius (2009), concluded that plantings delayed until after the end of May for the Midwest (Ohio, Iowa, Nebraska) and Deep South (Alabama, Florida, Georgia, Louisiana, and Mississippi), or early June in the Upper South (Arkansas, Missouri, Kentucky and Tennessee) reduced yield due to delayed planting. They also concluded that when using the conventional soybean system or using full season beans (not the Early Soybean Production System), there was not a clear advantage to planting in late-April or early-May. The early soybean production system is a method for growing earlier maturing soybeans to produce a crop earlier and lessening the impact of fall drought. For example planting a maturity group four soybean where a maturity five or six is usually planted (Heatherly, 1998). However, growers are concerned about other edamame quality factors that may be affected by planting date, including the number of seeds per pods and large seed size. Soybeans can show a compensation effect to planting dates with increased seed size (Robinson et al., 2009). When investigating soybean yield components, there are several factors including the number of pods per plant, number of beans per pod, average seeds size, and number of plants per unit area that help build yield. Late planted beans tend to be shorter, have fewer reproductive nodes, and have fewer pods per plant or fewer pods per square meter (Cox et al., 2008; Pedersen and Lauer, 2003; Ray et al., 2008; Robinson et al., 2009). Cox et al. (2008) reported more seeds per pod in late-May plantings than in mid-May or mid-June plantings. Pedersen and Lauer (2004) reported that seeds per pod were only significantly affected in two years of a four year trial. Robinson et al. (2009) reported planting dates having no influence on the number of seeds per pod. Reports on the effect of planting date on seed size have been mixed. Pedersen and Lauer (2004) and Cox et al. (2008) reported no change in seed size due to planting date. Ray et al. (2008) and Robinson et al. (2009) observed an increase in average seed size with later planting date, but reported some cultivars that had relatively little change in seed size.

Determining the correct time to harvest can be difficult for producers. Harvesting too early reduces yield due to insufficient time accumulating photosynthate, however, harvesting too late can cause deterioration in texture, flavor, and color (Mbuvi and Litchfield, 1995). According to Masuda (2001), sucrose is the most important sugar in the beans flavor. Determining when sucrose peaks in the soybean may help identify when beans are the sweetest and ready to harvest. Soluble solids concentration can be measured to track the sugar concentration in the bean as the percent sugar in solution.

The accumulation of heat units, which can be used to measure soybean growth, may allow growers to calculate the correct harvest time. There are several methods used to model soybean growth including iterative regression analysis, which takes into account hourly temperatures and day length, or calculating heat units without base temperature (Major et al., 1975a; Major et al., 1975b). One method often used, due to its simplicity, is to calculate growing degree days with a base temperature of 10 ºC. After a general number of heat units is reached, the crop is near or ready for harvest.

The purpose of this study was to determine how planting date affects the yield and the percent marketable pods in two commercially available edamame cultivars. Also, to determine if soluble solids concentration, heat units, days after flowering, or days after planting can be used to more easily quantify a harvest window.

Section 3.2 Material and Methods

This trial was conducted in 2008 and 2009 in Painter, VA on Bojac Sandy Loam (Thermic Typic Hapludults). Two cultivars [BeSweet 292 (BS292) and Midori Giant (MG)] were planted on three different planting dates: planting date 1 (PD1), planting date 2 (PD2) and planting date 3 (PD3). In 2008, edamame was planted on 6 May, 23 May, and 11 June. In 2009 planting dates were 22 May, 12 June, and 26 June. Plots were arranged in a randomized complete block design with four replications. Each replication contained one plot of each cultivar. Experimental plots consisted of four rows in 2008 and two rows in 2009, 7.6 meters long with between-row spacing of 0.76 m and in-row spacing of 7.62 cm. A plot was considered an experimental unit. Phosphorus and potassium were added pre-plant based on soil test results and pest management measures were based on cooperative extension recommendations (Virginia Cooperative Extension, 2000). Plot yield was determined by harvesting a single row (a middle row in 2008). A 200 bean random subsample was collected and analyzed for beans per pod. Soluble solids concentration was measured and growing degree days calculated for each planting date.

Soluble solids concentration was measured twice weekly in 2008 and 2009 starting July 30 and ending September 4 and 24, respectively. Starting and ending dates of each planting are listed in (Table 3,1). One edamame pod was harvested randomly from ten different soybean plants throughout the plot. The pod was taken from the first (bottom most) node bearing pods. Pods were put in cheese cloth and placed in boiling water for 6 minutes. After 6 minutes, the edamame was removed and placed in a beaker filled with ice water until cool. Edamame was then removed from the ice water and left to drain. Each pouch was cut open and the edamame was shelled and patted dry. The beans were juiced with a garlic press using constant but slow pressure. Soluble solids concentration was measured using an Atago hand refractometer (model number PAL-1).

Growing degree days (GDD) were calculated using the following equation:
[(Daily Minimum Temperature + Daily Maximum Temperature)/2 – Base Temperature = GDD] All temperatures used are in Fahrenheit. The base temperature used was 10 ºC.

A Randomized complete block design was used to determine statistical differences between planting date yields. Factors were year, planting date, cultivar, year x planting date, planting date x cultivar, year x cultivar, and year x planting date x year. Blocks were considered random variables. The arcsine transformation of percent marketable pods was used to determine statistical differences. For yield and percent marketable pod data, the fit model platform in JMP (version 8.0; SAS Institute, Cary, NC.) was used. Analysis of soluble solids concentration used general linear mixed models procedure of SAS (version 9.2; SAS Institute, Cary, NC).. Fixed effects for soluble solids concentration were cultivar, sample date, and variety by sample date. Means were separated using Tukey's HSD ($P \leq 0.05$).

Section 3.3 Results

For yield data, interactions between year and planting date ($P<0.0257$), so data are presented by year (Table 3.2). There were no significant interactions between cultivar and planting date in ($P=0.9804$), so the yield data for the two cultivars were pooled for analysis. In 2008, PD1 yielded 8,055 kg per ha, which was significantly greater than PD 2 and PD 3 with yields of 6,664 and 5,696 kg per ha, respectively (Fig. 3.1). There was no significant difference between PD 2 and PD 3 with respect to yield. There were no significant differences in yield between the planting dates in 2009. PD2 had the highest yield followed by PD3 and PD1 with yields of 6,286, 6,062, and 5,854 kg per ha, respectively.

There was a significant two way interaction between year and planting date ($P=0.0003$), so marketable pods is present by year (Table 3.2). However, marketable pods per cultivar were pooled together because there were no interactions between cultivar and planting date ($P=0.2484$). In 2008, PD2 had significantly greater percent marketable pods than PD1 and PD3; there were no significant differences between planting dates one and three (Fig. 3.2). Planting date three had the highest percent marketable pods in 2009, but was only significantly greater than PD1.

Soluble solids concentration could not be compared by planting date or year because of uneven sample dates, so each planting date is presented separately. There were no interactions between cultivar and sample date. The 2008 samples had a tendency to show increasing soluble solids concentration with time (Fig. 3.3). In 2009, the soluble solids concentration generally increased and plateaued or decreased at later sample dates (Fig. 3.4). In both years, none of the planting dates showed a clear enough pattern to be reliable. The soluble solids concentrations generally increased overtime but were somewhat sporadic with large changes during development.

Heat unit accumulation from planting and fifty percent flower to harvest is shown in (Table 3.3). In both years, the number of heat units accumulated from planting to harvest and fifty percent flower to harvest were similar amoung all planting dates with differences of 13.1 and 19.2 %, respectively.

Section 3.4 Discussion

In 2008, yield data was similar to previously reported findings with the earliest planting date yielding greater than later planting dates. Egli and Cornelius (2009) reported that plantings in early-May yield better than those in mid-June in the upper-south. However, they also stated that planting dates between early- and late-May should have similar yields. Difference in yield could be attributed to the number of heat units accumulated; however, in 2008, the edamame produced 6.2, 5.3, and 4.8 kg of pods per ha per heat unit for the first, second, and third planting dates, respectively (Table 3.3). Differences in yield per heat unit between planting dates shows that building yield requires more than accumulating heat units (Major et al., 1975a). The difference is likely due to the later planting dates having less time to build a canopy before switching from vegetative to reproductive growth and subsequently having less ability to produce photosynthate during pod fill (Egli and Cornelius, 2009). Unfortunately leaf area, plant height, and branching data were not collected to make this determination. In 2009, the earliest planting date did not yield greater than the later planting dates. This is likely attributed to increased weed competition due to a loss of control from the herbicide program. Eaton et al. (1976) reported that weeds reduced soybean yields up to 1010 kg/ha. Abusteit (1993) found that weed competition reduced yields between 14.9 and 51.1% depending on the length of the competitive period.

Percent marketable pods and planting date had interactions similar to those reported by Cox et al. (2008). Cox et al. (2008) observed similar results to 2008, with a mid-May planting date having significantly more seeds per pod than either early-May or early-June planting dates. In 2009, the first planting date's lower percent marketable pods may be attributed to weed competition (Eaton et al., 1976).

Soluble solids concentration is used in many fruit to determine quality. Renquist and Reid (1998) reported that soluble solids concentration is an important factor in determining quality of tomatoes used in paste, and that it changes over time in the tomato. However, they went further to say that soluble solids concentration is simply one quality factor to be considered when harvesting. Similar to Renquist and Reid, Johnson (1999) stated that the edamame processing industry requires soluble solids concentration of 8.5% as one factor in determining acceptability. In 2008, only a few of the measurements at the beginning of seed fill fell below this threshold. In 2009, many of the measurements fell below this benchmark. Soluble solids concentration in edamame does not allow accurate determinations of when to harvest but is simply one quality factor.

Heat units or growing degree days have been the subject of several modeling and research studies. The number of growing degrees required from emergence to maturity changes by cultivar. Day length and temperature can also influence the length of time soybeans take to move from one growth stage to the next (Cober et al., 2001). However, the data show that when days after 50% flower or growing degree days after 50% flower offer at least a window for growers to closely monitor crop progress in order to harvest in a timely manner. Growers of edamame need to collect this data each year to get a better idea of how cultivars react to specific locations.

Section 3.5 Conclusion

This study shows the importance of planting early. Using the 2008 results, even with the lower percent marketable pods, the net marketable yield would be greater by planting early. It also shows the importance of weed control in plantings. Soluble solids concentration is not a reliable indicator for timing of harvest. More work may be done with soluble solids concentration or flavor to determine harvest readiness. Accumulated heat units appear to offer a reasonable method to determine when harvest is nearing. Critical heat units will vary by cultivar and location and will need to be tuned to the growers' location and cultivars.

Table 3.1. Start and end dates of soluble solids concentration sampling of edamame grown in Painter, VA during 2008 and 2009.

2008			2009		
Planting Date	Start	End	Planting Date	Start	End
6-May	30 July	18 Aug.	22 May	30 July	27 Aug.
23-May	7 Aug.	28 Aug.	12 June	13 Aug.	10 Sept.
11-June	18 Aug.	4 Sept.	26 June	8 Sept.	24 Sept.

Table 3.2. P-values of from an edamame planting date study of three planting dates with two cultivars (Midori Giant and BeSweet 292) grown in Painter, VA during 2008 and 2009.

Source	Yield	Percent Marketable Pods
Cultivar	0.072	0.0143
Planting Date	0.08	<.0001
Year	0.0587	0.0546
Cultivar x Planting Date	0.9804	0.2484
Cultivar x Year	0.9766	0.0896
Planting Date x Year	0.0257	0.0003
Cultivar x Planting Date x Year	0.4857	0.0449

Table 3.3. Number of days after planting, 50% flower, heat units, and yield per heat unit accumulated at harvest in five edamame cultivars grown in Painter, Virginia during 2008 and 2009.

Planting Date		Days After Planting to Harvest		Days After 50% Flower to Harvest		Heat Units Accumulated (degree hours)				Yield Accumulation per Heat Unit (Kg of Pods/Heat Unit/Ha)			
						From Planting		After 50% Flower		From Planting		After 50% Flower	
2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
6-May	22-May	90	90	42	51	2147	2390	1298	1439	3.8	2.4	6.2	4.1
23-May	12-Jun	83	84	44	48	2187	2260	1255	1335	3.0	2.8	5.3	4.7
11-Jun	26-Jun	78	82	43	48	2100	2163	1189	1299	2.7	2.8	4.8	4.7

Figure 3.1. Yield of edamame planted at three planting dates conducted during 2008 and 2009 in Painter, VA. Columns calculated using pooled samples of two cultivars (Midori Giant and BeSweet 292) with four replications each (n=8). Means with the same letter are not significantly different according to Tukey's HSD (P≤0.05). Planting date in 2009 were not statistically (NS) different.

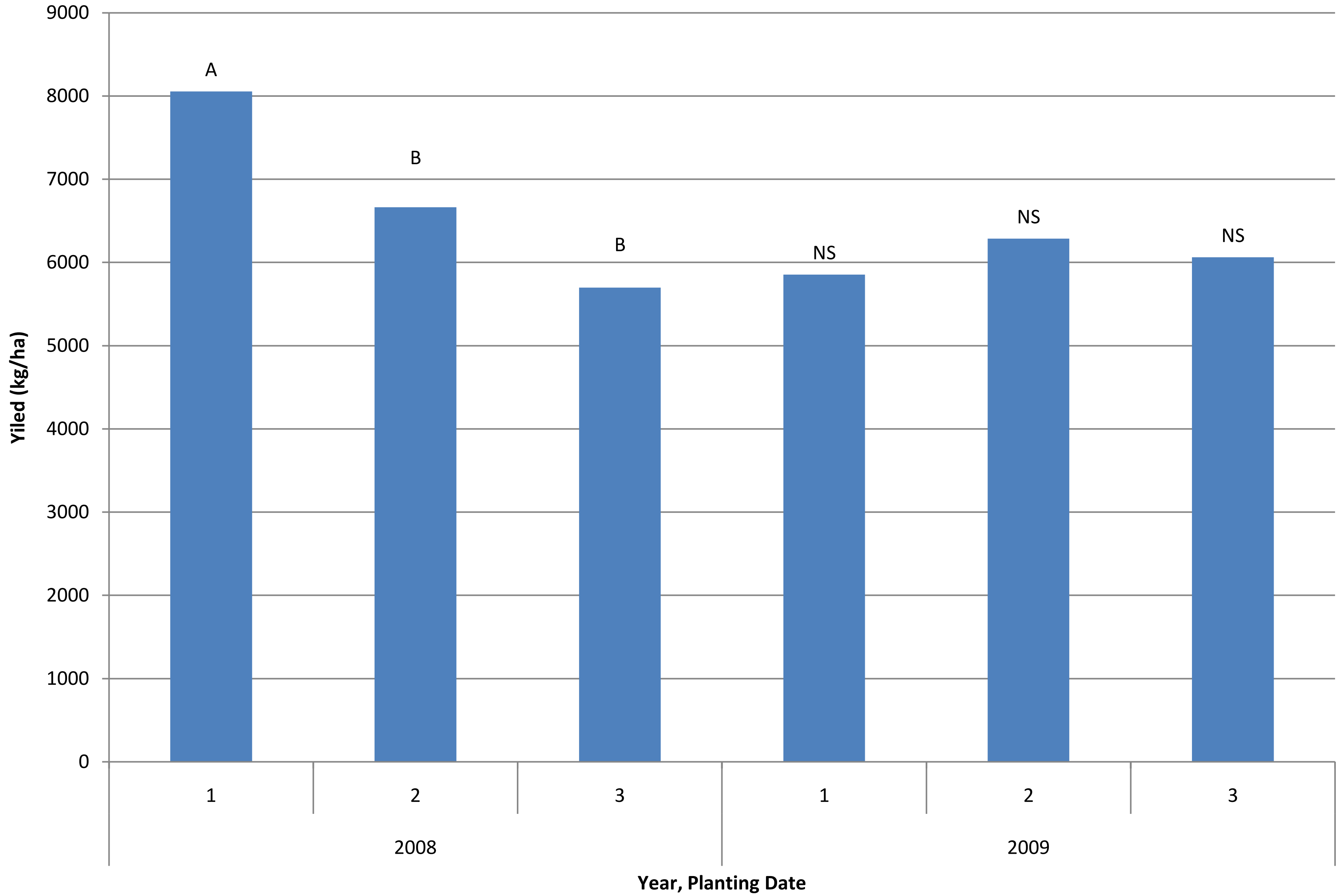


Figure 3.2. Percent marketable pods of edamame planted at three planting dates, grown during 2008 and 2009 in Painter, VA. Columns are calculated from pooled samples two cultivars (Midori Giant and BeSweet 292) with four replications each (n=8). Columns with the same letters are not statistically different according to Tukey's HSD ($P \leq 0.05$).

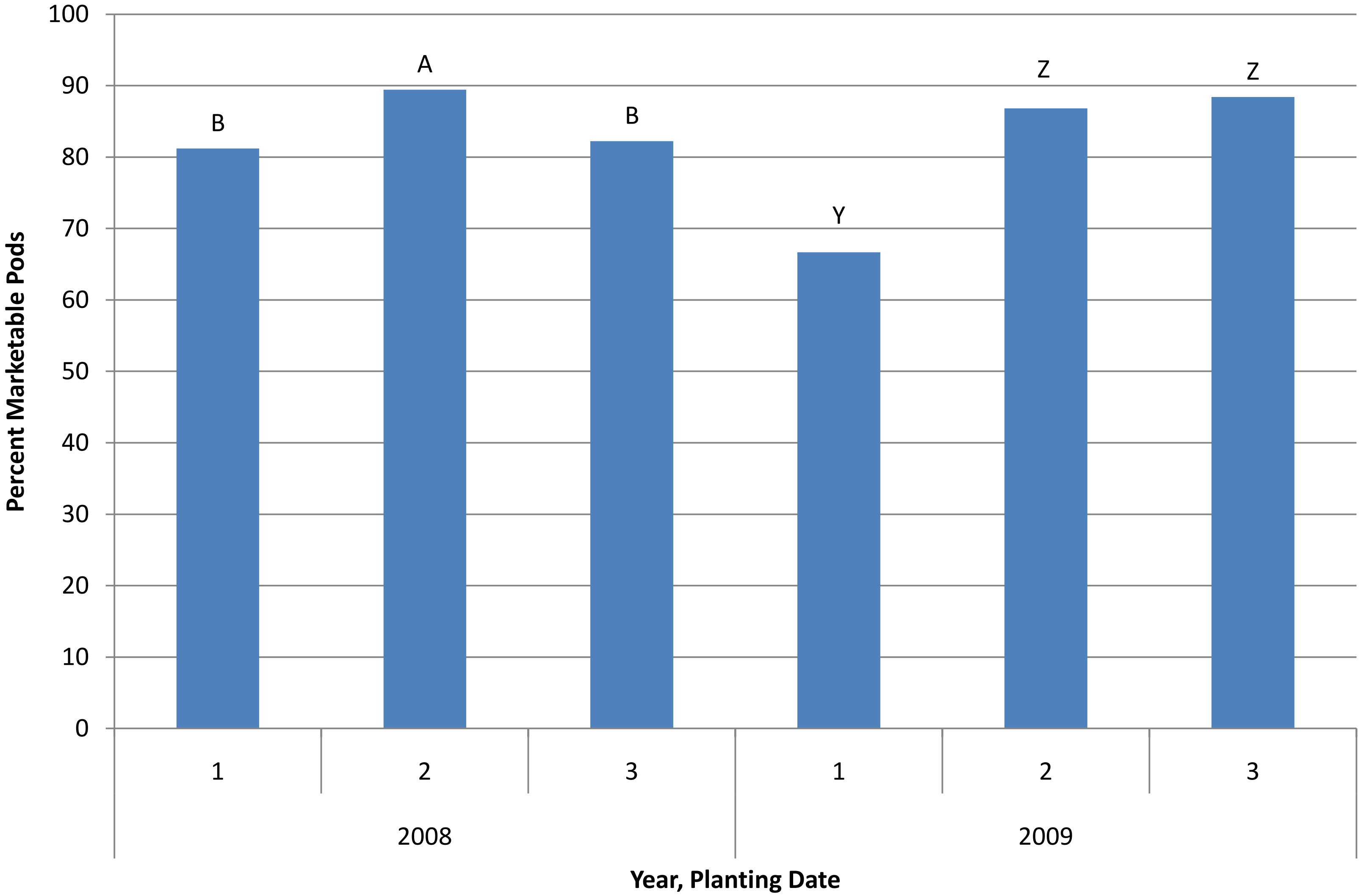


Figure 3.3. Soluble solids measurements from edamame planted at three dates grown in 2008 in Painter, VA. Each data point is the average of the soluble solids content of two cultivars (Midori Giant and BeSweet 292) with four replications each (n=8). The bars represent the standard error.

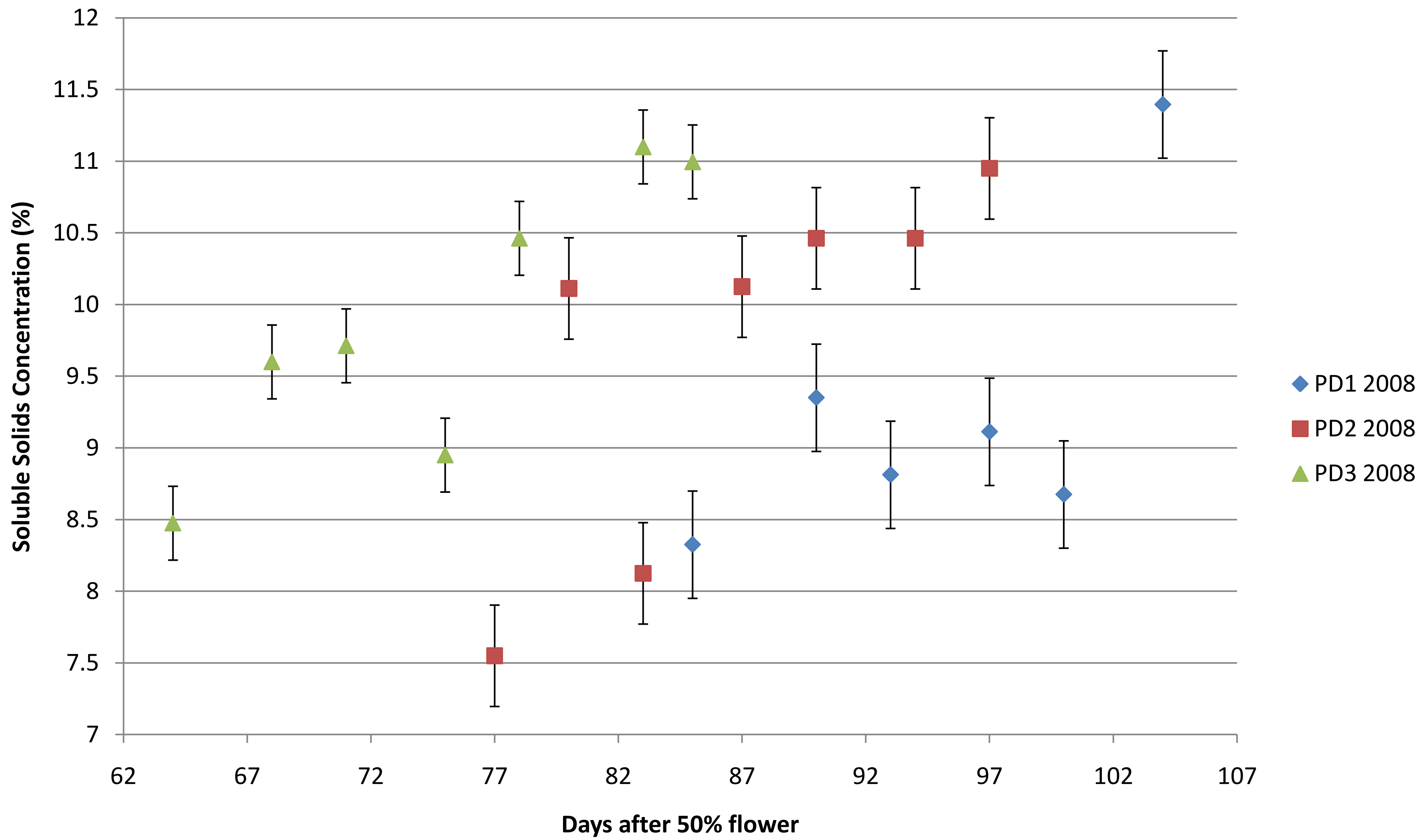
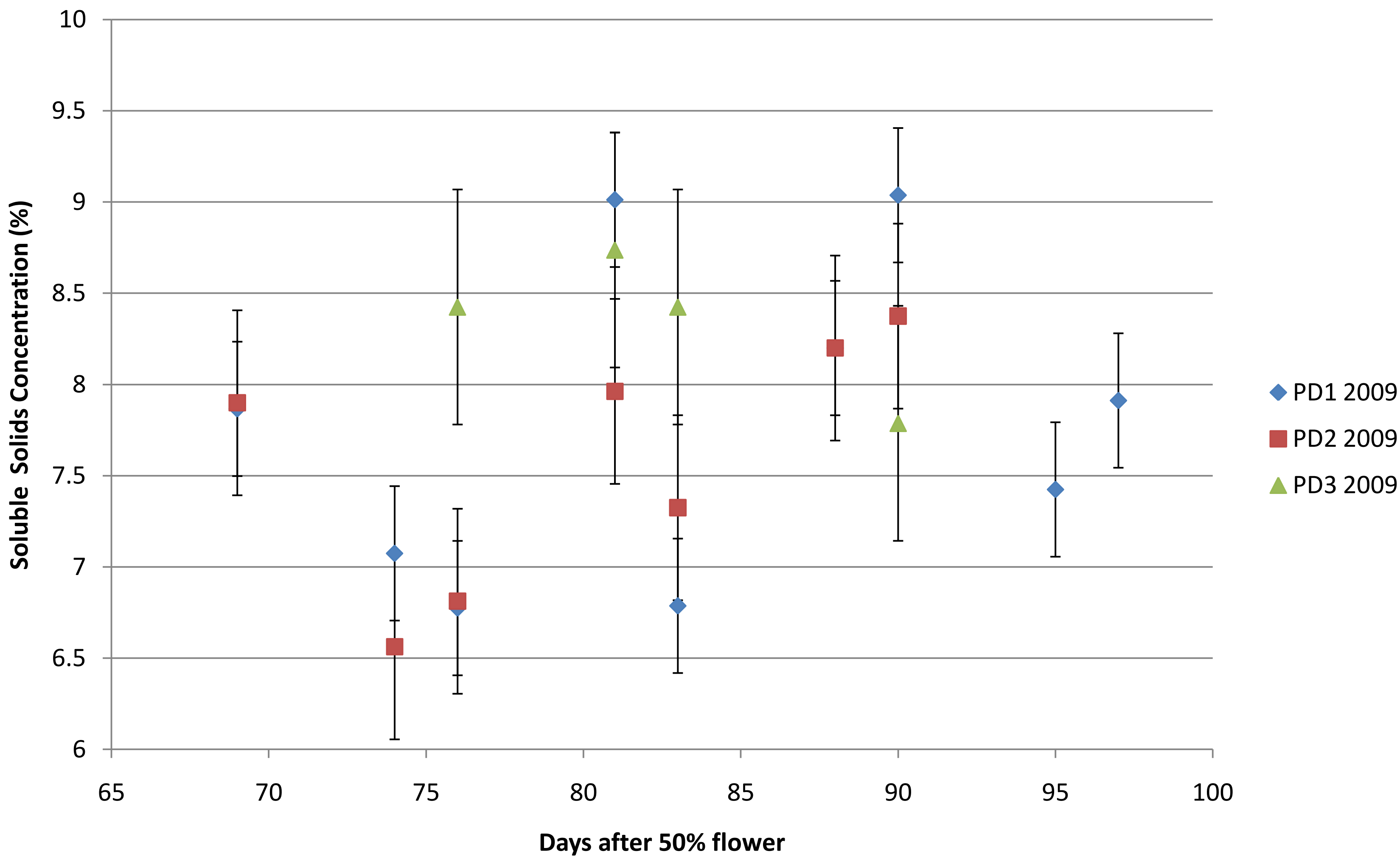


Figure 3.4. Soluble solids concentration of edamame planted on three dates during 2009 in Painter, VA. Data points are the pooled samples of two cultivars (Midori Giant and BeSweet 292) with four replications each (n=8). The bars represent the standard error.



Chapter 4: Nutritional Constituents

Section 4.1 Introduction

Edamame or vegetable soybeans are specialty cultivars of soybean harvested just before maturity while the pods are still green or at R6 stage of development (Fehr et al., 1971; Mebrahtu and Devine, 2008). They tend to have a green seed coat, but may range from yellow to black; with gray or light color hilum, white pubescence on the pods, and large seed size (Konovsky et al., 1994). Edamame also has important flavor characteristics that and have been described as sweet, nutty, flowery, and buttery; they have a much less beany flavor than typical agronomic beans (Duppong and Hatterman-Valenti, 2005; Wszelaki et al., 2005).

Edamame has been consumed in Asian countries for centuries and has recently been gaining popularity in the United States. The reason for this gain in popularity is likely due to the nutritional quality of edamame, the purported human health benefits, and flavor (Kelley and Sanchez, 2005, Wszelaki et al., 2005). Edamame contains all essential amino acids and can be a meat substitute in the human diet (Sikka et al. 1978). It also contains polyphenolics such as tannins, flavonoids, and proanthocyanidins that provide a relatively high antioxidant capacity (Malencic et al., 2007). One particular species of these polyphenolic compounds are isoflavones, which have been extensively studied and have been linked to reduced prostate cancer, reduced menopausal symptoms, and lower heart disease risks (Messina, 2001). To quantify the gain in popularity, in 2003 and 2008 it was estimated that $18 million and $41 million worth of edamame was sold, respectively (Bernick, 2009; Soyatech, 2010).

Soybeans are recognized as oil seeds because of their high lipid content. This lipid content is one factor that makes soybeans useful in industry. High oil contents can give edamame an oily flavor, which is considered undesirable (Konovsky et al., 1994; Wszelaki et al., 2005; Young et al., 2000). According to Hymowitz et al. (1972), agronomic soybeans' lipid content ranges from 145 to 230 g per kg of beans. Rao et al. (2002) reported edamame having between 130.7 and 155.8 g lipids per kg of beans. These numbers agree with Sikka et al. (1978), who reported that vegetable soybeans contain less fat than agronomic type soybeans.

Soybeans' high protein content makes them a particularly desirable vegetable. Hymowitz et al. (1972) reported that soybeans contain between 33.1 and 49.2% protein on a dry weight basis. Other studies have found that agronomic soybeans average around 41% protein (Hartwig and Kline, 1991; Yin and Vyn, 2005). Sikka et al (1978) reported that edamame and agronomic soybeans contain similar amounts of protein.

Isoflavones are a class of polyphenolic compounds in soybeans. Soybeans use isoflavones to communicate with nodulating mycorrhiza and as a deterrent to insect feeding (Burden and Norris, 1992; Graham, 1991). There are twelve isoflavone conjugates; three aglycones (genistein, glycitein, and gaidzein), three 7-O- glucosides (genistin, glycitin, and daidzin), three 6-O- malonyl glucosides, and three 6-O-acetyl glucosides (Wang and Murphy, 1994). It is thought that the health benefits from isoflavones are due to their estrogenic effects and their antioxidant capacity (Lee et al., 2004; Murphy et al., 1997)

The antioxidant capacity in the soybeans is given by many different compounds including flavonoids, phenolics, tannins, proanthocyanides and other polyphenolics (Malencic et al., 2007). Soybeans antioxidant capacity varies by cultivar, postharvest storage, growing environment, and type (Lee et al., 2003). Many studies have measured the antioxidant capacity of soybeans (Chung et al., 2008; Lee et al., 2004; Xu and Chang, 2007). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) quench assay measures the ability of the sample to reduce free radicals (Molyneux, 2004). DPPH has a free electron that has the capability to absorb a hydrogen ion. The Oxygen

Radical Absorbing Capacity (ORAC) measures the ability of the sample to protect a fluorescent probe from degradation by peroxyl radicals (Chung et al., 2008; Zhou et al., 2004). The purpose of these trials are to determine expected values and differences of lipids, protein, and isoflavone contents, as well as, antioxidant capacity of typical commercially-available edamame cultivars.

Section 4.2 Material and Methods

The five edamame cultivars [BeSweet 292, (BS292); BeSweet 2015, (BS2015); BeSweet 2001, (BS2001); Midori Giant, (MG); Sunrise, (SR)] for these trials were grown as part of an edamame cultivar evaluation in Painter, VA in 2008 and 2009. Refer to Chapter 2.2 for more through growing methods. After the 2008 harvest, a subsample of beans was collected from each plot and frozen in shell until analysis. In 2009, the subsample of beans was collected from each plot, shelled, and frozen.

Sample Preparation

The shelled beans were placed in a freeze dryer and dried until they were a constant weight. The dry beans were milled to pass through a 20 mesh size sieve using a Mini-Mill (Thomas Wiley, Swedesboro, NJ) in 2008. In 2009, the dry bean samples were ground to a fine powder using a coffee grinder. The beans were placed in airtight bags and stored in the freezer until analysis.

Lipids

The lipid content was measured using the official methods of the AOAC International (1995) ether extraction method. The extraction cups, filter paper, and thimbles were dried for 30 minutes at 90°C and cooled in desiccators. A two gram sample was weighed onto Whatman #4 filter paper, which was then folded and placed into the thimble. The thimbles were placed into the extraction unit (Soxtec System HT 1045 and 1043, FOSS Tecator, Eden Prairie, MN) to be extracted. The weighed extraction cups were filled with 50 – 70 mL of petroleum ether and placed in the machine. The samples were boiled for 2 hours in the petroleum ether, then allowed to rinse for one hour. The rinsing action of the extraction system was turned off and ether was evaporated from the ether cup for 30 minutes. The extraction cups were removed from the extractor and allowed to cool in desiccators for 30 minutes. The weight of the cups was taken and the percent of lipids was obtained as: [(weight of the cup with lipids- weight of the dry cup)/weight of the sample extracted*100].

Protein

Solutions needed for analysis:

1) Boric Acid Solution, 4%. 0.55 g of methyl Red and 0.78 g of bromocresol green in 100 mL of 95% ethyl alcohol. Forty grams of boric acid was dissolved in distilled water to which 1 mL of the alcohol solution was added then diluted to 1000 ml.
2) Sodium hydroxide (10N) – Sodium thiosulfate solution. Dissolved in water was 400 g sodium hydroxide and 25 g sodium thiosulfate. The solution was then diluted to 1000 mL.

The percent protein was determined using a modified AOAC International method. For each of the cultivars, a one gram sample was placed on a Whatman #541 no-nitrogen filter paper, folded and placed in a digestion tube. Kelmate 'N Kjeldahl Digestion Mixture, 20 mL of concentrated sulfuric acid (36N), and 3.0 mL of hydrogen peroxide (30%) was added to the tube. The tubes were then placed in the block digester preheated to $350^{\circ}C$ and covered with an exhaust manifold. After the digestion and frothing slowed, the block heater was turned to $410^{\circ}C$. Once the digest turned clear blue in color the heating continued for 30 more minutes. Fifty mL of distilled water was added to the tubes after the digestion was complete and the tubes cooled for 5 to 10 minutes. The digestion tube was placed into the receiver of a prepared Rapid Still II (Labconco Corporation, Kansas City, Missouri) along with a receiving flask containing 25 mL of boric acid solution. The Rapid Still had been prefilled with the solution of sodium hydroxide-sodium thiosulfate solution and a boiling flask with distilled water. To the digestion tube in the distillation unit 75 mL of sodium hydroxide-sodium thiosulfate solution was added and steam drove the nitrogen gas into the receiving flask. The receiving flask was titrated with hydrochloric acid (0.2 N) until the green color turned to clear. The percent nitrogen was calculated using the following equation %N= [(Volume of HCL to titrate the sample – the Volume of HCL to titrate the blank) * 1.4007 * 0.2] / (weight of glycine * 18.65). The test was run in duplicate for each cultivar.

Antioxidant Capacity

Antioxidants were measured using two assays. The antioxidants were extracted for both assays using 50% acetone in a 1:10 w/v ratio. Two grams of dry defatted beans were placed in a 30 mL test tube with 20 mL of 50% acetone. The test tube was vortexed for one minute every 30 minutes for two hours. The test tubes were incubated in the refrigerator for 24 hours.

The first assay was DPPH Radical Scavenger Assay. A serial dilution of trolox solution was prepared as 0, 5, 10, 20, 40, 80, and 100 µM in 50% acetone. In a clear well plate, 100 µL 0.2mM DPPH was mixed with 100 µL of Trolox serial dilution or bean solution diluted 3 fold, in triplicate. The absorbance was measured at 515 nm by a Victor3 plate reader (Perkin Elmer, Waltham, Mass). The reading was taken every 5 minutes for 30 minutes (Chung et al., 2008). Results were reported in trolox equivalence (TE).

The second assay was the Oxygen Radical Absorbing Capacity (ORAC) Assay. A trolox standard was prepared as 0, 5, 10, 20, 40, 80 and 100 µM trolox in 50% acetone. In a black 96 well plate, 40 µL of the trolox standard or 40 µL of bean solution diluted 100 fold was added to 200 µL of 158 uM fluoroscein. The well plate was incubated for 20 minutes at 37℃ in a Victor3 plate reader. While the plate was incubating, 0.36M 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) solution was prepared. After the plate was incubated, 35 µL of AAPH solution was added to each well. The plate was read in the plate reader every minute using excitation wavelength of 485 nm and emission wavelength of 535 nm for 30 minutes. The data was saved and an Excel Macro was used to analyze the data. The results were expressed as micromoles Trolox Equivalents (TE) per gram sample (Chung et al., 2008).

Isoflavones

The isoflavones were extracted from dry-milled beans using 0.1N hydrochloric acid, acetonitrile, and distilled water (2:7:3, v/v/v) (Chung et al., 2008). In a flask, 12 mL of extraction solution was added to 0.5 g bean sample. The samples were vortexed for 1 minute and shaken overnight. The effluent was poured from the flask into a test tube the centrifuged for 5 minutes. The supernatant was filtered using Whatman #6 filter paper. Four milliliters of bean solution was dried in a rotary evaporator in a 10 mL test tube. The residue was reconstituted with 1 mL of methanol and filtered with a 0.45 um Whatman disk filters. Isoflavone profile of the soybean extract was performed using a C18 column (250mm x 4.6mm, particle size 5um).

Data were analyzed using analysis of variance. Year, cultivar, and respective nutritional constituent were main effects. No blocking was used. The arcsine transformation was used for percentage data. The fit model platform in JMP (version 8.0; SAS Institute, Cary, NC.) was used for statistical analysis. Means were separated using Tukey’s HSD ($P \leq 0.05$)

Section 4.3 Results

For all tests in this study, there were significant interactions between year and cultivar, so all the data are present by year (Tables 4.1-4.2). All data is given on a dry weight basis.

In 2008, the lipid content of the bean cultivars ranged from 14.0 g to 18.3 g per 100 g sample (Fig. 4.1). In 2009, the lipid contents were lower ranging from 12.9 g to 15.3 g per 100 g sample. The lipid contents of the cultivars followed the same trend in both years. ‘SR’ had the greatest lipid contents in both 2008 and 2009 with 18.3 and 15.3 g per 100g sample, respectively. ‘BS2015’ had the least amount of lipids in both 2008 and 2009 with 14.0 and 12.9 g per 100g respectively,. ‘SR’ was significantly greater than other cultivars in both years, except ‘MG’ in 2008 (Fig. 4.1).

The protein contents ranged from 35.7 g to 39.5 g per 100 g sample across both years (Fig. 4.2). In 2008, ‘BS2001’ had the greatest protein content with 38.3 g per 100 g, but was only significantly greater than ‘BS292’ at 36.1 g per 100 g sample. ‘SR’ had the highest protein content in 2009 at 39.5 g per 100 g, which is significantly greater than all but ‘BS2015’ at 37.1 g protein per 100 g beans, respectively.

The DPPH Quench measures the ability of a sample to reduce peroxyl radicals. In 2008, ‘SR’ had the highest numerical free radical scavenging ability with 19.5 µg TE per g sample, but was only significantly greater than ‘MG’ at 13.0 µg TE per g sample (Fig. 4.3). ‘BS2015’ and ‘BS2001’ had the largest scavenging ability in 2009 with 18.0 and 16.1 µg TE per g sample, respectively; both had significantly greater scavenging ability than the remaining three cultivars.

In 2008, there were no significant differences found in the cultivars ability to absorb oxygen radicals. However, there were large numerical differences with ‘BS2015’, ‘SR’ and ‘BS292’ having the highest numerical values of 37.2, 37.1 and 35.1 µmol TE per g sample and

‘BS2001’ and ‘MG’ having the lowest values at 22.6 and 20.4 µmol TE per g sample (Fig.4.4). In 2009, ‘BS292’ had the highest ORAC value of 40.2 µmol TE per g sample but only significantly greater than ‘SR’ at 26.0 µmol TE per g sample.

In all cultivars except ‘SR’ the total isoflavone concentration was lower in 2009 than in 2008 (Table 4.3). Total isoflavones ranged from 144.6 to 529.2 µg per g sample in 2008 and 127.2 to 315.5 µg per g sample in 2009 (Table 4.3). The isoflavone malonyl genistin was greatest in concentration in all cultivars and both years except for ‘SR’ in 2008. Glucosides had a greater concentration than aglycones in all but three samples (‘BS292’ in both years and ‘SR’ in 2009). Genistein was not found in 2008 and was found in ‘BS292’ and ‘MG’ in 2009. Glycitein was found in all five cultivars in 2008, but found in only ‘SR’ in 2009.

Section 4.4 Discussion

Lipids followed the same pattern both years with 2009 having an overall lower lipid content than 2008. According to Piper and Boote (1999), warmer temperatures during seed fill, which starts about two weeks after flowering, relates to increased lipid content. When temperature during seed fill (Appendix A) was reviewed, the average air temperature was 0.47 ºC higher in 2009 than 2008. However, the seed lipid content decreased. In the cultivar evaluation trial, weed competition became a problem before harvest in 2009. Weeds can cause two problems, water stress and shading stress. According to Champolivier and Merrien (1996), drought stress during seed fill caused reductions of seed oil content in rape (*Brassica napus* L. var. *oleifera*). Johnson (1971) reported reduced oil content in sunflowers due to weed competition.

Although there were differences in the protein content, four of the cultivars had less than one percent difference between years. Only ‘SR’ had a year-to-year difference larger than one percent, which was 3.2%. Many sources have a negative correlation between protein and oil concentration (Hartwig and Kilen, 1991; Hymowitz et al., 1972; Kumudini et al., 2005). With the reduced lipid contents, it seems the protein concentration should have been greater in 2009, which did not occur in three of the five cultivars.

All cultivars but ‘MG’ had a lower TE in the DPPH assay in 2009 than in 2008. One of the main functions of antioxidants is to protect macromolecules from degradation. Isoflavones have been linked to reduced plasma membrane lipid oxidation (Patel et al., 2001). However, antioxidant capacity was not correlated with either protein or lipid contents. According to Dolde et al. (1999) and Marwede et al. (2004), there has been no correlation found between total lipid content and total antioxidant content in soybean or canola. There are many compounds that may account for the concentration of soybeans’ antioxidant capacity (Kumar et al., 2009). Compounds with associated antioxidant content have been shown to change in concentration with maturity at harvest, growing environment, and location (Dolde et al., 1999; Kumar et al. 2009).

Three cultivars followed a similar pattern between years in the ORAC assays as the DPPH assay. The ORAC assay measures antioxidant capacity differently than the DPPH assay, so different antioxidant capacities are expected (Chung et al., 2008). Few antioxidant studies have been conducted testing soybean antioxidant activity across environments and temperatures to see how ORAC values change and if they should be similar to DPPH changes; most ORAC assays are genotype based. However, Whent et al. (2009) found environment to be the most important main effect describing 55.8% of the variation in the ORAC assay.

Isoflavone differences between growing seasons are well documented (Eldridge and Kwolek, 1983; Hoeck et al., 2000; Riedl et al., 2009). Eldridge and Kwolek (1983) reported that yearly environment accounted for 16.6 to 400% of the difference in isoflavone concentration. Tsukamoto et al. (1995) found that seeds maturing under cooler day and night temperatures had a 14-16 fold higher isoflavone concentrations. However, extremes in air temperature, not what would be typically found in a field setting, were used to show the effects of temperature. The average daily temperature was 0.47 ºC warmer in 2009 than 2008 (Appendix A). The difference in temperature likely contributed little difference. Weed competition may have had an effect on the lower concentrations in 2009. However, Caldwell et al. (2005) and Sakthivelu et al. (2008) both found that drought stress increases total isoflavone contents in soybean seeds. Swinny and Ryan (2005) found that growing red clover (*Trifolium pratense*) under reduced ultraviolet (UV) light resulted in fewer isoflavones, flavonoids and caffeic acid. Shading could reduce the concentration of isoflavones in the soybeans, especially when considering that one of the purposes of isoflavones is to ameliorate the effects of UV damage to the plant (Wang and Murphy, 1994). Shading would have made a larger impact than drought on isoflavone content because of irrigation.

Section 4.5 Conclusion

Although statistical differences were found, protein and lipids both appear to remain fairly constant across cultivars and years. All five edamame cultivars had numerically similar contents of all of these nutritive components. The primary differences between cultivars are the differences in the antioxidant capacities and isoflavones concentrations. Many factors influence the amount of antioxidants and isoflavones in the plants. If practices to optimize antioxidants, isoflavones, and yield could be linked, then both consumers and growers would benefit.

Table 4.1. P-value of lipid and protein contents, and antioxidant capacity measured by DPPH and ORAC assay. The edamame used in these assays was grown in Painter, VA during 2008 and 2009.

Source	Lipids	Protein	DPPH Quench	ORAC
Cultivar	<.0001	0.0403	0.0276	0.1567
Year	<.0001	0.0073	<.0001	0.0289
Cultivar x Year	<.0001	0.0014	0.0012	0.013

Table 4.2. P-values of isoflavone factors and interactions of five edamame cultivars grown in Painter, VA during 2008 and 2009.

Whole Model	Isoflavone
Cultivar	<.0001
Year	<.0001
Cultivar x Year	<.0001
Isoflavone	<.0001
Cultivar x Isoflavone	<.0001
Year x Isoflavone	<.0001
Cultivar x Year x Isoflavone	<.0001

Figure 4.1. Lipid content of five edamame cultivars grown during 2008 and 2009 in Painter, VA. Columns are the average of two samples. Columns connected by the same letter are not statistically different according to Tukey's HSD ($P \leq 0.05$).

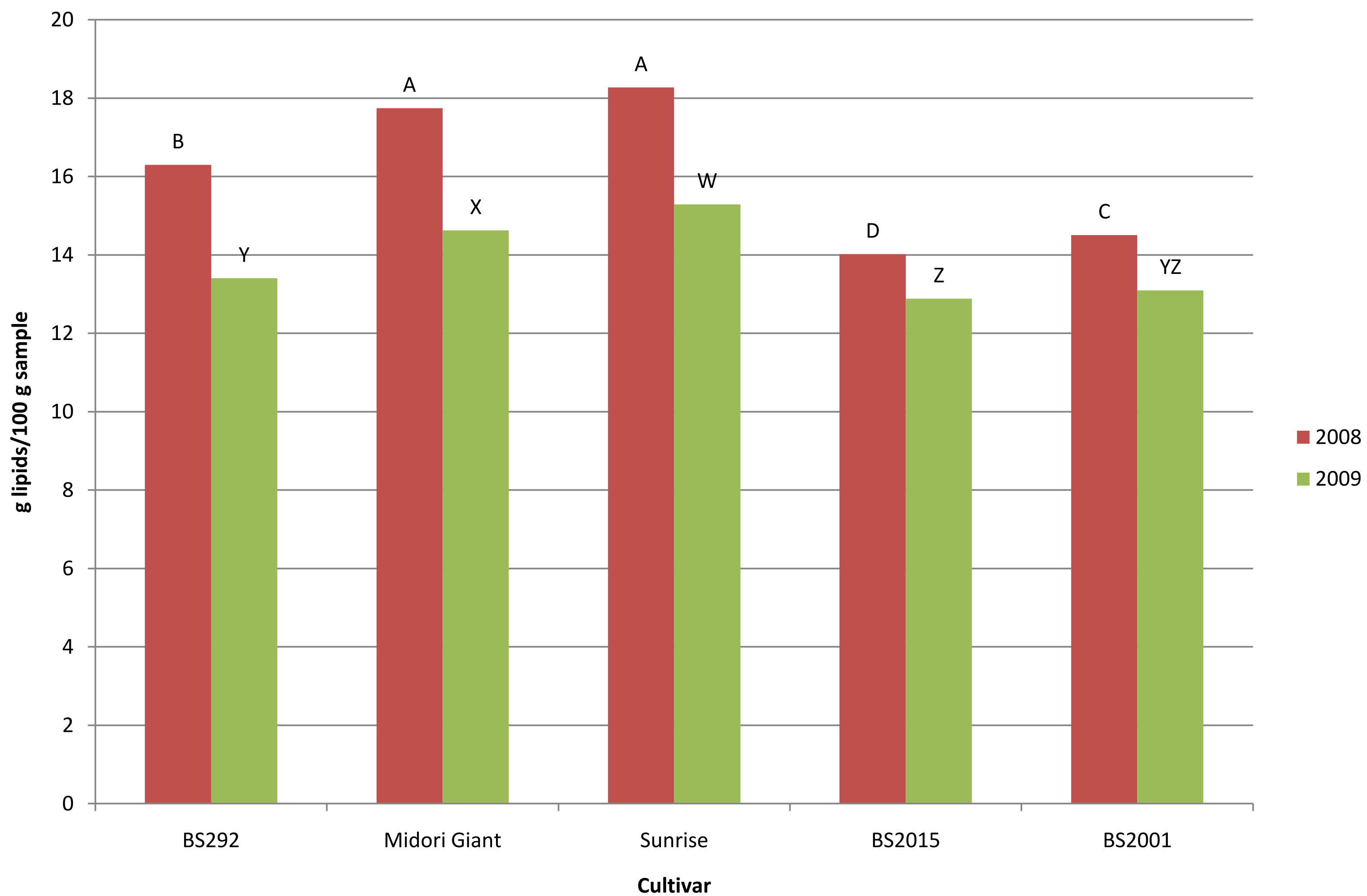


Figure 4.2. Protein content of five edamame cultivars determined by Kjeldahl method. The edamame used in these tests were grown during 2008 and 2009 in Painter, VA. Means are the average of two samples. Means with the same letter are not significantly different according to Tukey's HSD ($P \leq 0.05$).

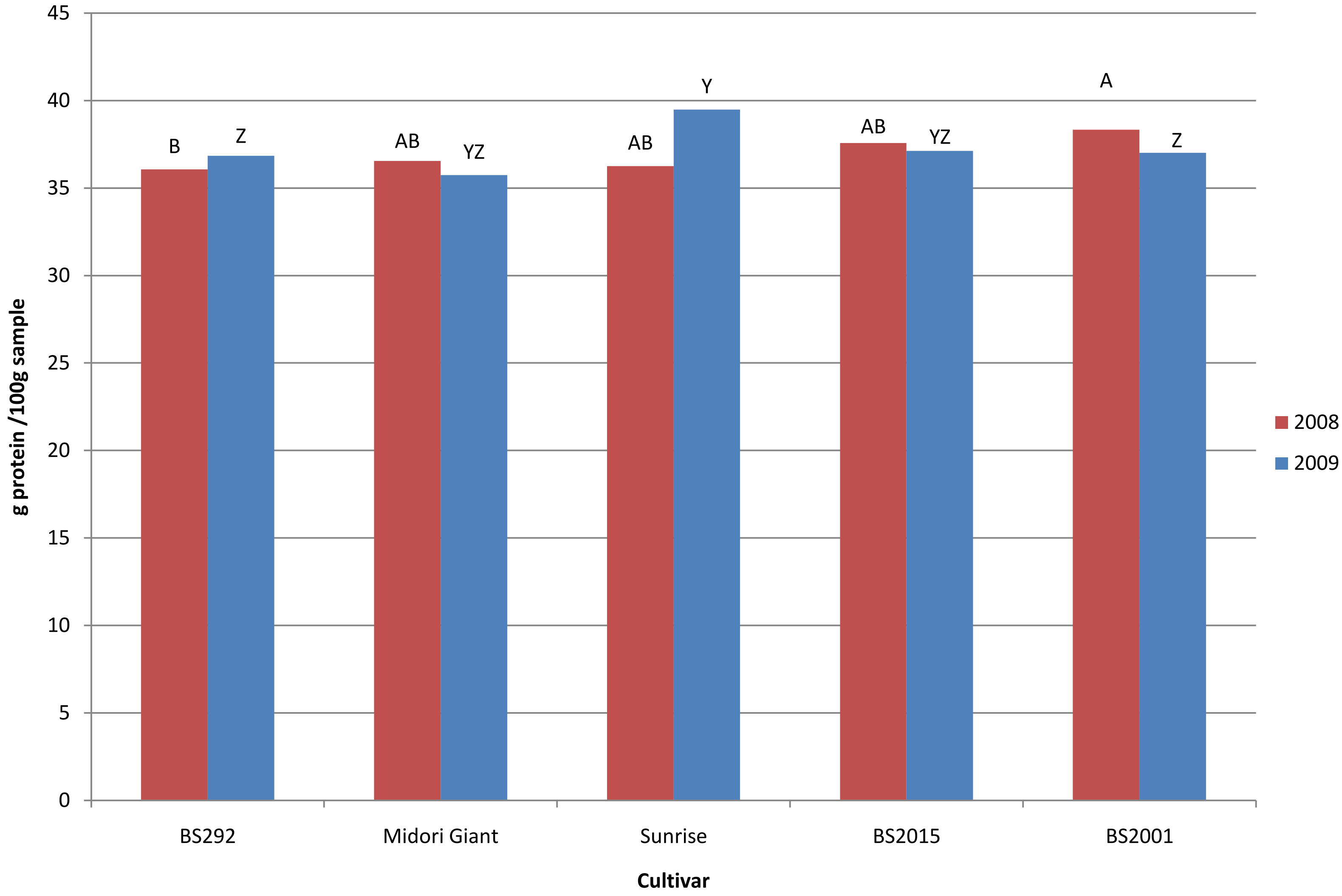


Figure 4.3. DPPH Quench assay was used to determine radical scavenging ability of five edamame cultivars. The five edamame cultivars were grown during 2008 and 2009 in Painter, VA. The values are given in µg trolox equivalence per gram sample. Columns are the average of four samples (n=4). Columns connected by the same letter are not different ($P \leq 0.05$) according to Tukey's HSD.

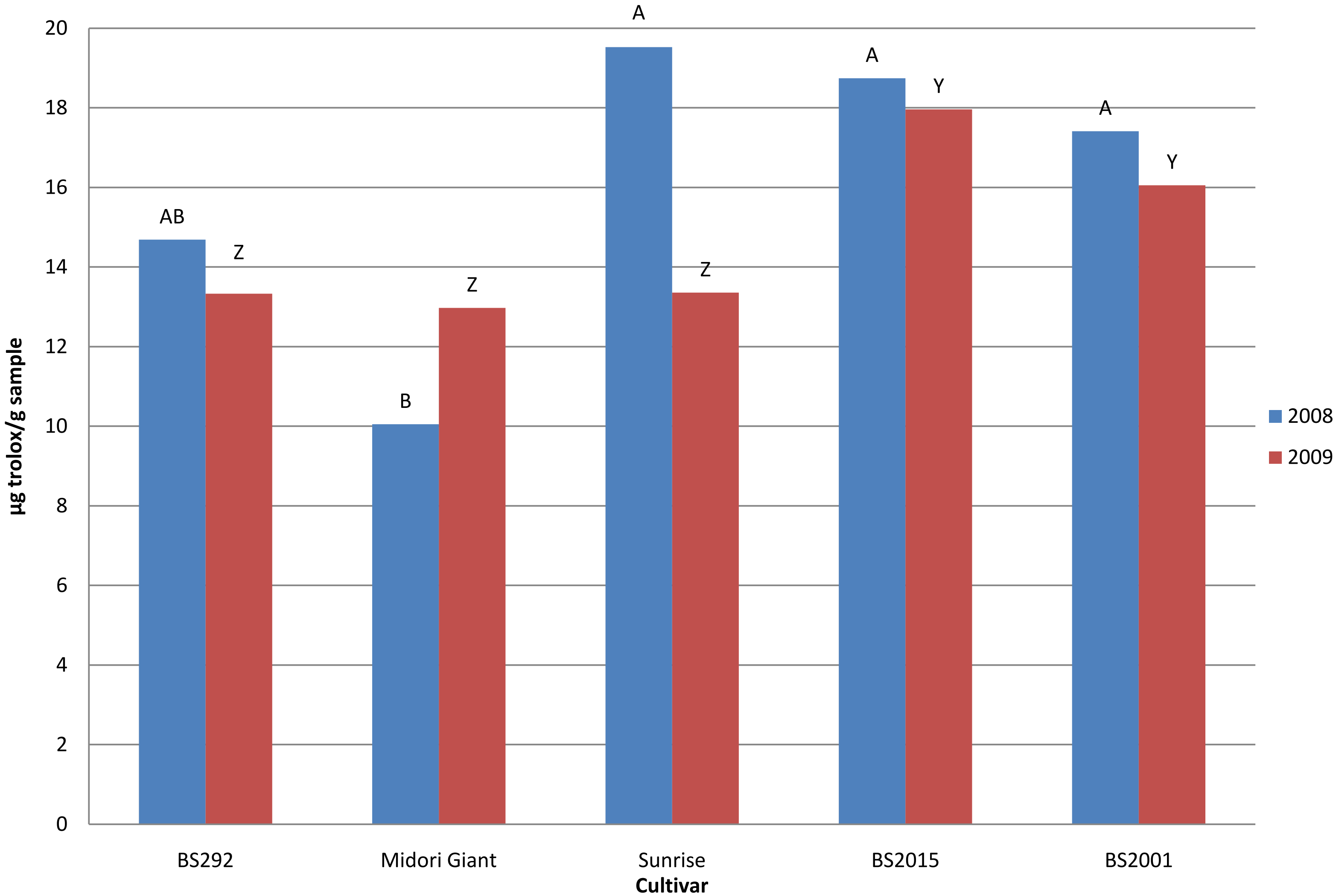


Figure 4.4. Oxygen radical absorbing capacity (ORAC) assay was performed on five edamame cultivars. The cultivar evaluation trial was conducted in 2008 and 2009 in Painter, VA. The values are given as µmol of trolox equivalent per gram sample. Columns are the average of three samples. Columns connected by the same letter are not different ($P \leq 0.05$) according to Tukey's HSD. NS= Not significantly different.

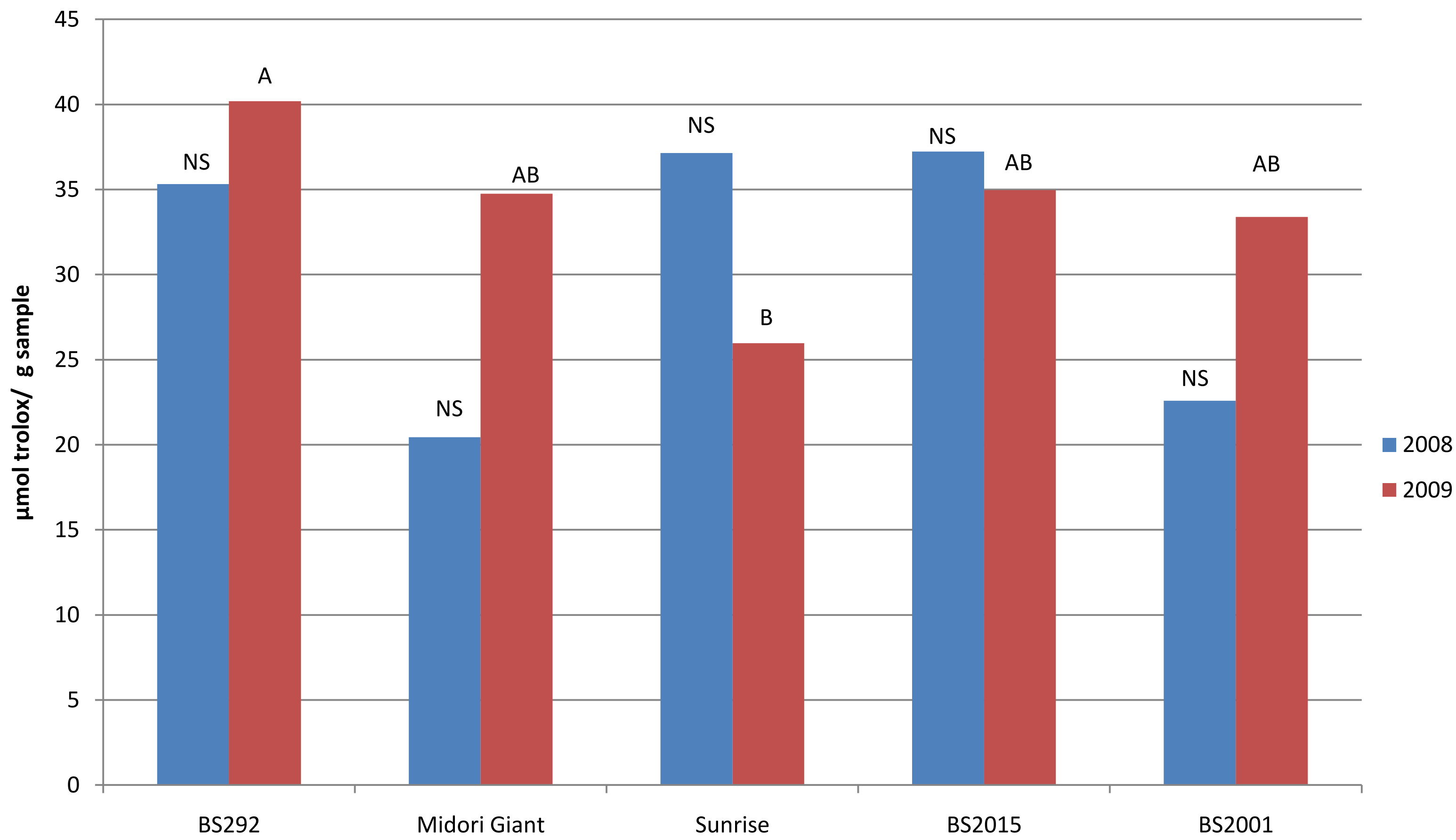


Table 4.3. Isoflavone concentration of an edamame cultivar evaluation trial by year and cultivar. The cultivar evaluation trial was grown in Painter, VA during 2008 and 2009.

		Glucosides		Aglycone			Malonyl	
Year	Cultivar	Daidzin	Genistin	Daidzein	Glycitein	Genistein	Genistin	Total
2008	BeSweet 292	4.2 ± 5.9	4.0 ± 0.3	0.0 ± 0.0	16.6 ± 3.3	0.0 ± 0.0	265.5 ± 8.6	290.3 ± 18.1
2009	BeSweet 292	0.0 ± 0.0	0.0 ± 0.0	3.1 ± 0.1	0.0 ± 0.0	1.4 ± 0.1	200.3 ± 8.1	204.9 ± 2.5
2008	Midori Giant	17.9 ± 0.9	12.4 ± 1.9	3.0 ± 1.2	26.3 ± 0.2	0.0 ± 0.0	469.7 ± 5.4	529.2 ± 9.1
2009	Midori Giant	2.7 ± 0.6	2.7 ± 0.3	2.5 ± 0.1	0.0 ± 0.0	2.1 ± 0.0	293.6 ± 18.7	315.5 ± 33.4
2008	Sunrise	77.8 ± 3.9	31.4 ± 0.6	0.0 ± 0.0	8.9 ± 1.1	0.0 ± 0.0	26.5 ± 0.4	144.6 ± 3.9
2009	Sunrise	2.4 ± 0.1	2.3 ± 0.0	2.9 ± 0.0	28.1 ± 0.1	0.0 ± 0.0	177.9 ± 8.4	213.6 ± 14.3
2008	BeSweet 2015	50.6 ± 3.0	24.9 ± 0.9	0.0 ± 0.0	13.9 ± 0.3	0.0 ± 0.0	73.5 ± 1.4	162.8 ± 2.3
2009	BeSweet 2015	2.1 ± 0.2	1.5 ± 0.1	2.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	119.3 ± 3.2	127.2 ± 5.7
2008	BeSweet 2001	44.4 ± 1.2	16.2 ± 0.3	0.0 ± 0.0	19.7 ± 1.5	0.0 ± 0.0	76.5 ± 1.6	156.4 ± 2.2
2009	BeSweet 2001	3.7 ± 0.1	1.7 ± 0.0	3.6 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	126.6 ± 0.9	135.6 ± 0.8

Chapter 5 – Temporal Isoflavones

Section 5.1 Introduction

Interest in soybeans has been increasing mostly due to their nutritive value and phytochemical content (Kelley and Sanchez, 2005; Wszelaki et al., 2005). The consumption of edamame, a group of large-seeded, specialty soybean cultivars has increased from $18 million to $41 million between 2004 and 2009 (Bernick, 2009; Soyatech, 2009). Edamame is harvested as a vegetable in a mature green state that correlates as 80% pod fill or the R6 stage of development (Fehr et al., 1971; Rao et al., 2002).

One group of phytochemicals that have been studied extensively because of their health benefits is isoflavones. Isoflavones are part of a larger family of flavoniods that are synthesized in the phenylpropanoid pathway (Kim and Chung, 2007). In soybeans, there are three isoflavones with four chemical structures: aglycones (genistein, glycitein, and daidzein), 6-O glucosides (genistin, glycitin, and daidzin), 7-O malonyl glucoside, and 7-O acetyl glucoside (Wang and Murphy, 1994). According to the USDA (2007), mature agronomic and food grade soybeans average about 6 times more isoflavones than immature edamame type beans. Soybeans have also been shown to have different isoflavone concentrations due to temperature, irrigation, location, and storage duration (Bennett et al., 2004; Lee et al., 2003; Mebratu et al., 2004; Tsukamoto et al., 1995; Yin and Vyn, 2005).

Soybean maturity at harvest plays an important role in the concentration of isoflavones in the bean. Some studies have been conducted regarding how isoflavones accumulate in soybeans. Kumar et al. (2009) conducted experiments on one cultivar and found that total isoflavone content increased 5.1-fold from R5 to maturity with the largest increase between R5 and R6.

Kim and Chung (2007) reported that largest increase in isoflavone concentration happens between R6 and R7 stages of development.

Berger et al. (2008) looked at how isoflavones accumulate in different seed fractions. They found that isoflavones accumulate first in the hypocotyl. After accumulation in the hypocotyl has plateaued, accumulation begins in the cotyledons. Tsukamoto et al. (1995) found that hypocotyls had higher concentrations of isoflavones than the cotyledons.

Few of the isoflavone studies have looked at vegetable soybeans. Edamame has a larger seed size and tends to have fewer isoflavones than agronomic type soybeans, possibly because they are harvested when immature. With the importance of edamame increasing and the focus on the phytochemicals, the accumulation of isoflavones in the bean seeds could have important implications on cultivar selection and harvest timing. The purpose of this study is to determine when isoflavones accumulate in 6 edamame cultivars over time.

Section 5.2 Material and Methods

Three cultivars from each Wannamaker Seed Company [Midori Giant, (MG); Mojo Green, (Mojo); and Sunrise, (SR)] and Rupp Seed Company [BeSweet 292, (BS292); BeSweet 2015, (BS2015); and BeSweet 2001, (BS2001)] were planted on 22 May, 2009 on Bojac Sandy Loam (Thermic Typic Hapludults) in Painter, VA. Soybeans were harvested twice weekly starting 30 July, 2009 for a total of 9 harvest dates and were frozen at harvest until they could be freeze dried. The beans were freeze dried using a Labconco freeze dryer to a constant weight then fractured using a paint shaker and ball bearings. Additional grinding was conducted, with a mortar and pestle, as needed. A 0.5 g bean sample was placed in a 15 mL test tube and extracted using 12 mL of solvent, consisting of 0.1N hydrochloric acid, acetonitrile, and distilled water (2:7:3, v/v/v) (Chung et al. 2008). The samples were vortexed for 1 minute and shaken overnight at 45 RPM on a reciprocal shaker. After extraction, the test tubes were centrifuged for 5 minutes at 4500 RPM. A 1.0 ml subsample of bean solution was dried with a rotary evaporator in a 1.5 ml micro centrifuge tube. The residue was reconstituted with 0.5 mL of methanol and filtered with a 0.45 µm Whatman disk filters. Soybean isoflavone profiles and standards profiles were performed on an Agilent 1200 series HPLC equipped with a photodiode array detector. A Varian Lichrospher C18 Column (250mm x 4.6mm, particle size = 5µm) was used for isoflavone separation with a mobile phase containing solvent A (0.1% glacial acetic acid in deionized water) and solvent B (acetonitrile). The solvent B gradient was programmed to follow a gradient between time points (Table 5.1). The column temperature was maintained at 25 ºC during analysis and the samples were cooled to 15 ºC in the autosampler tray.

Statistical analysis was conducted using the fit model platform in JMP (version 8.0; SAS Institute, Cary, NC.). Cultivar and sample date were the main effects for the Seed Dry Matter Accumulation. Cultivar, isoflavone, sample date and isoflavone by cultivar were considered main effects for isoflavone analysis. There was no comparison between individual isoflavones or isoflavone by sample date. Tukey's HSD was used for separation of means ($P \leq 0.05$).

Section 5.3 Results

Seed Dry Matter Accumulation

Seed dry matter accumulation was measured and there were no significant interactions between sample date and cultivar (P=0.1102), so the cultivars were pooled for analysis. The seeds accumulated 6.8 mg dry weight per day (Fig. 5.1).

Isoflavones

There was a three way interaction present between cultivar, sample date, and isoflavone type, which was expected because the concentration of isoflavone by type was the data of interest. There were also two way interactions between sample date and cultivar, so each sample date was analyzed individually for differences between cultivars (Table 5.2). Sample date and cultivar interactions were also expected because the isoflavones were expected to accumulate as the season progressed.

Total Isoflavone Accumulation

Average total seed isoflavone content increased with time (Fig 5.2.) The times of peak total isoflavone accumulation for ‘MG’, ‘Mojo’, and ‘BS292’ were between 88 and 90 days after planting (DAP) and 90 to 95 DAP (Table 5.3). For ‘SR’, ‘BS2015’ and ‘BS2001’ the peak accumulation of total isoflavones was between 95 and 97 days. Total isoflavone accumulation is predominated by malonyl genistin accumulation, which at its peak makes up between 61.4% of total isoflavones in ‘BS2015’ and ‘BS2011’ and 76.5% of total isoflavone contents in ‘SR’ (Table 5.4). Daidzin is the second most abundant isoflavone with concentrations at the last sample date greater than any of the other isoflavones except malonyl genistin (Fig. 5.3). At sampling initiation, the agylcones and glucosides make up 100% of the total isoflavone content (Table 5.4). For example, daidzein ranges from 36.1 to 55.9% of the total isoflavone content in ‘Mojo’ and ‘SR’ at 69 DAP. However, by 97 days after harvest, in the same cultivars, daidzein makes up only 1.4 and 0.4% of total isoflavones, respectively. Although daidzein’s percent of total isoflavone decreased, its concentration in the bean, remained relatively stable with less than 2.5 µg per bean (Fig. 5.3).

Individual Isoflavone Accumulation

Daidzein appeared to have two areas of peak accumulation. All six cultivars shared one peak area of accumulation between 74 and 76 days, and in four cultivars (SR, BS292, BS2015, and BS2001), daidzein’s second peak periods of accumulation was between 81 and 83 DAP

(Table 8). In ‘MG’ and ‘Mojo’ the 2nd peak area of accumulation was between 88 and 90 DAP, and 95 and 97 DAP, respectively. The other aglycones all had areas of increasing and decreasing accumulation, and although no clear pattern emerged, glycitein and genistein were more abundant at 97 DAP than at 69DAP (Figs. 5.4-5.5).

With the exception of ‘BS2015’ and ‘BS2001’, glucosides tended to accumulate more quickly between 88 and 95 DAP (Table 5.3). For example, ‘MG’ accumulated daidzin, glycitin, and genistin at a rate of 1.5, 0.57, 1.4 µg of the respective isoflavone per day. The week with the next highest accumulation, without overlap, is between 74 and 81 DAP for daidzin and glycitin with a rate of 0.12 and 0.13 µg per day and between 76 and 88 DAP for genistin with accumulation of 0.10 µg accumulated per day. ‘BS2015’ accumulated the glucosides at the highest rate between 90 and 97 DAP. ‘BS2015’s’ peak accumulation varied depending on the glucoside. The glucosides order of abundance in the seed was daidzin, genistin, and glycitin (Figs. 5.6-5.8).

Malonyl genistin accumulated most quickly the last week of sampling, unless total malonyl genistin content started to decline, then accumulation took place most quickly just before the decline at the end of sampling (Table 5.3). For instance, ‘Mojo’ lost 21.9 µg of malonyl genistin per bean between 95 and 97 DAP (Fig. 5.9). The time of peak malonyl genistin accumulation was between 88 and 95 DAP with 14.05 µg accumulated per day.

Section 5.4 Discussion

Total isoflavone increased with days after planting (Fig. 5.2 and 5.10). The edamame was harvested at 90 DAP. All six cultivars continued to accumulate isoflavones between 90 and 97 DAP (Fig. 5.10). ‘Mojo’ and ‘MG’ showed the lowest and highest changes in total isoflavone concentration after harvest with increases of 1.3 and 2.9 fold, respectively. Kim and Chung

(2007), and Kumar et al. (2009) both showed an increase between R6 and R7 stages of development which supports the findings that isoflavones continue to accumulate after edamame's peak harvest. In edamame, harvest should occur while the pods are still bright green. One of the characteristics of the R7 stage of development is yellowing of plants, which would not allow for delayed harvesting because the beans would become unmarketable.

Kim and Chung (2007) found that malonyl glucosides and glucosides made up the majority of total isoflavones in the seed, which our results are in agreement. Malonyl genistin was responsible for the majority of the isoflavone content in this study (Fig. 5.2). They also found that total daidzein predominated at early stages of seed development, but reduced relative to total genistein. In this study, daidzein contributed a major portion of the total isoflavone then reduced in respect to other isoflavones, however the concentration per bean remained relatively constant throughout the growing season (Table 5.4, and Fig. 5.3). However, genistin was never in higher concentration than daidzin (Table 5.4).

Although other legumes contain isoflavones, soybeans and soy-foods have the greatest isoflavone content. According to USDA, alfalfa sprouts, peanuts, and snap beans all have isoflavones with a range of total content being 0.02 mg per 100 gram for snap beans to 0.26 mg per 100 g for peanut (USDA, 2008). However, the USDA reported the mean edamame and agronomic soybean isoflavone content at 48.95mg per 100gs and 154.5 mg per 100g, respectively (USDA, 2008).

Section 5.5 Conclusion

Although isoflavone content is important, quality of edamame would be greatly reduced if harvest is delayed for isoflavone accumulation. As others have found, our results indicated that malonyl genistein and other glucosides predominate.

.

Table 5.1. Solvent B gradient used for HPLC analysis of isoflavones

Time	% Solvent B
0	15
10	18
12	22
42	35.5
44.5	100
47	100
48	15
50	15

Table 5.2. Significance table of six edamame cultivars grown in Painter, VA during 2009. Significant differences between edamame cultivars by sample date (days after planting) and isoflavone.

Sample Date (DAP)	Glucoside		Malonyl	Aglycone			
	Daidzin	Genistin	Genistin	Daidzein	Glycitein	Genistein	Total
69	*	NS	NS	**	**	*	***
76	NS	***	NS	NS	NS	NS	NS
76	*	*	*	NS	*	NS	NS
81	NS	**	**	NS	NS	*	**
83	**	NS	*	NS	*	NS	*
88	*	NS	*	**	NS	**	***
90	NS	NS	**	NS	NS	NS	NS
95	**	NS	NS	NS	NS	NS	NS
97	**	NS	NS	NS	NS	NS	NS

NS, *, **, *** - Not Significant, or significant using Tukey's HSD at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 5.3. The changes in isoflavone concentration between samples dates (days after planting) of six edamame cultivars grown in Painter, VA during 2009.

DAP	Cultivar	Daidzin	Glycitin	Genistin	Malonyl Genistin	Daidzein	Glycitein	Genistein	Total
69-74	Midori Giant	0.03	0.04	0	0	0.1	0.04	0.04	0.17
74-76	Midori Giant	0.03	0.14	0.05	2.08	-0.09	0.12	0.12	2.58
76-81	Midori Giant	0.15	0.13	0.07	0.6	0.81	0.01	0.01	1.78
81-84	Midori Giant	-0.12	-0.06	-0.1	0.05	-2	-0.09	-0.09	-2.68
83-88	Midori Giant	0.41	0.11	0.18	2.52	0.19	0.1	0.1	3.5
88-90	Midori Giant	1.67	0.75	0.81	8.65	0.36	0.13	0.13	12.5
90-95	Midori Giant	1.46	0.5	1.57	16.4	0.69	0.09	0.09	20.71
95-97	Midori Giant	1.32	-0.3	-0.73	-9.36	-0.19	-0.17	-0.17	-9.39
69-74	Mojo Green	-0.04	-0.06	0	0	0.09	-0.04	-0.04	-0.16
74-76	Mojo Green	0.22	0.37	0.05	1.06	-0.02	0.24	0.24	2.27
76-81	Mojo Green	0.36	0.23	0.08	1.46	0.64	0.21	0.21	3.05
81-84	Mojo Green	0.54	0.74	0.25	4.86	-0.88	-0.37	-0.37	4.71
83-88	Mojo Green	0.33	0.08	0.14	2.03	0.08	0.09	0.09	2.78
88-90	Mojo Green	2.55	0.87	1.33	14.05	0.84	0.21	0.21	19.81
90-95	Mojo Green	0.36	-0.09	0.46	5.38	0	-0.04	-0.04	5.99
95-97	Mojo Green	0.62	0.41	-0.71	-10.95	-0.34	-0.16	-0.16	10.92
69-74	Sunrise	-0.01	-0.02	0	0	0.17	0.02	0.02	0.01
74-76	Sunrise	-0.03	0.05	0	0.26	-0.31	0.14	0.14	0.38
76-81	Sunrise	0.07	0.06	0.03	0.42	0.13	0.01	0.01	0.59
81-84	Sunrise	0.13	0.11	-0.02	-0.13	-0.21	-0.03	-0.03	-0.01
83-88	Sunrise	0.24	0.16	0.13	2.4	0.13	0.16	0.16	3.18
88-90	Sunrise	0.47	0.15	0.79	7.89	-0.04	-0.24	-0.24	8.9
90-95	Sunrise	1	0.38	0.34	6.47	0.25	0.09	0.09	8.55
95-97	Sunrise	1.45	0.14	1.39	10.63	-0.16	-0.05	-0.05	13.38
69-74	BeSweet 292	-0.02	0.01	0.01	0.12	0.17	0.07	0.07	0.42
74-76	BeSweet 292	0.03	0.03	0	0.11	-0.42	0.06	0.06	-0.03
76-81	BeSweet 292	0.01	0.02	0.01	0.22	0.1	0.08	0.08	0.38
81-84	BeSweet 292	0.32	0.12	0.07	2.18	0.17	0.08	0.08	3.13
83-88	BeSweet 292	0.46	0.08	0.04	0.75	0.35	0.1	0.1	1.85
88-90	BeSweet 292	0.8	0.62	0.58	4.88	-0.5	0.13	0.13	6.45
90-95	BeSweet 292	0.32	0.29	0.28	2.71	0.2	0.02	0.02	3.71
95-97	BeSweet 292	-0.3	-0.43	-0.84	-8.81	-0.54	0.02	0.02	10.85
69-74	BeSweet 2015	0.06	0.02	0.01	0.14	0.11	0.06	0.06	0.49
74-76	BeSweet 2015	-0.05	0.03	0	0.03	-0.21	0.02	0.02	0.04
76-81	BeSweet 2015	0.09	0.01	0.01	0.11	0.26	0.03	0.03	0.45
81-84	BeSweet 2015	0.01	0.15	0.04	0.66	-0.44	0.13	0.13	0.77
83-88	BeSweet 2015	0.28	0.07	0.05	0.74	0.1	0.07	0.07	1.33
88-90	BeSweet 2015	0.17	0.04	0.19	1.25	-0.14	0.18	0.18	1.52
90-95	BeSweet 2015	0.48	0.05	0.01	0.24	0.05	-0.05	-0.05	0.75
95-97	BeSweet 2015	-0.78	-0.01	0.32	2.91	-0.05	0.09	0.09	2.65
69-74	BeSweet 2001	0.08	-0.01	0.01	0.09	0.12	0.06	0.06	0.44
74-76	BeSweet 2001	-0.07	0.06	0	0.11	-0.19	0.07	0.07	0.2
76-81	BeSweet 2001	0.1	-0.02	0	0.08	0.09	0.04	0.04	0.27
81-84	BeSweet 2001	0.02	0.2	0.07	0.85	-0.14	0.02	0.02	1.17
83-88	BeSweet 2001	0.63	0.23	0.07	0.94	0.18	0.19	0.19	2.35
88-90	BeSweet 2001	0.61	-0.06	0.1	0.56	0.12	-0.07	-0.07	0.71
90-95	BeSweet 2001	0.47	0.23	0.11	1.24	0.16	0.03	0.03	2.33
95-97	BeSweet 2001	3.69	1.18	1.87	19.84	1.39	0.22	0.22	28.2

Table 5.4. Individual isoflavones as a percent of total isoflavones in six edamame cultivars grown in Painter, VA during

Sample	DAP	Cultivar	Daidzin	Glycitin	Genistin	Malonyl Genistin	Daidzein	Glycitein	Genistein
1	69	BeSweet 292	19.6	11.1	0.0	0.0	54.2	9.0	6.1
2	74	BeSweet 292	6.0	6.7	1.1	16.1	32.2	26.2	11.7
3	76	BeSweet 292	7.8	8.2	1.2	22.3	41.3	4.0	15.1
4	81	BeSweet 292	5.9	6.9	1.5	33.8	23.9	11.1	16.9
5	83	BeSweet 292	8.2	5.2	1.8	52.6	14.7	8.1	9.4
6	88	BeSweet 292	15.4	4.8	1.9	47.4	10.0	12.9	7.6
7	90	BeSweet 292	14.3	6.6	4.5	58.1	5.9	5.0	5.5
8	95	BeSweet 292	12.3	7.1	5.6	63.3	2.9	5.1	3.7
9	97	BeSweet 292	18.9	9.2	4.1	50.9	5.2	5.2	6.5
1	69	BeSweet 2001	9.7	20.1	0.0	0.0	54.8	7.9	7.4
2	74	BeSweet 2001	14.9	6.4	1.3	13.8	31.4	20.2	11.9
3	76	BeSweet 2001	9.4	9.0	1.3	18.3	39.5	8.4	14.1
4	81	BeSweet 2001	16.6	5.1	1.0	21.3	26.2	15.1	14.6
5	83	BeSweet 2001	11.9	8.8	2.5	37.2	22.3	6.6	10.7
6	88	BeSweet 2001	21.1	9.4	2.7	38.9	11.6	7.3	9.1
7	90	BeSweet 2001	25.6	8.2	3.4	41.6	5.4	8.0	7.8
8	95	BeSweet 2001	23.6	8.7	3.9	45.8	4.8	7.6	5.5
9	97	BeSweet 2002	16.9	5.9	5.7	61.4	1.8	5.9	2.5
1	69	BeSweet 2015	16.4	19.4	0.0	0.0	49.2	3.4	11.7
2	74	BeSweet 2015	13.3	7.7	1.4	21.3	26.6	17.7	12.0
3	76	BeSweet 2015	9.7	9.2	1.6	22.7	39.2	4.6	13.1
4	81	BeSweet 2015	14.0	6.6	1.7	23.4	17.8	26.1	10.4
5	83	BeSweet 2015	11.2	9.3	2.4	36.9	20.3	8.1	11.9
6	88	BeSweet 2015	15.9	7.2	3.0	46.1	11.2	7.8	8.8
7	90	BeSweet 2015	15.0	6.3	4.7	52.6	7.2	4.7	9.4
8	95	BeSweet 2015	24.0	6.4	4.2	48.9	5.1	5.0	6.4
9	97	BeSweet 2015	13.1	5.0	5.8	61.4	5.4	3.6	5.7
1	69	Midori Giant	17.3	10.8	0.0	0.0	42.5	9.9	19.6
2	74	Midori Giant	16.7	14.1	0.0	0.0	25.1	23.0	21.1
3	76	Midori Giant	7.2	8.8	1.2	49.4	15.6	6.7	11.0
4	81	Midori Giant	7.8	8.1	2.6	41.4	7.9	26.7	5.6
5	83	Midori Giant	9.3	10.6	2.1	60.8	5.4	5.3	6.6
6	88	Midori Giant	10.8	6.1	3.9	67.5	2.0	5.4	4.4
7	90	Midori Giant	12.0	6.1	5.1	68.2	1.6	4.2	2.9
8	95	Midori Giant	8.7	3.7	6.7	75.4	0.6	3.6	1.3
9	97	Midori Giant	11.8	3.7	6.6	72.1	0.7	3.9	1.2
1	69	Mojo Green	17.9	18.3	0.0	0.0	36.1	12.2	15.4
2	74	Mojo Green	16.3	14.2	0.0	0.0	29.8	26.6	13.1
3	76	Mojo Green	12.6	15.2	1.3	25.6	22.0	11.5	11.8
4	81	Mojo Green	12.2	10.2	2.2	39.9	9.3	17.6	8.6
5	83	Mojo Green	12.0	11.8	3.1	58.0	3.9	7.3	3.9
6	88	Mojo Green	12.0	9.2	3.7	62.5	2.9	6.0	3.7
7	90	Mojo Green	12.4	7.0	5.1	66.4	1.5	5.2	2.5
8	95	Mojo Green	10.8	4.8	5.7	72.4	0.8	3.9	1.7
9	97	Mojo Green	14.6	6.8	5.6	66.0	1.4	4.0	1.7
1	69	Sunrise	10.2	8.0	0.0	0.0	55.9	7.4	18.5
2	74	Sunrise	8.2	4.3	0.0	0.0	25.1	40.2	22.2
3	76	Sunrise	4.8	6.5	0.0	15.6	35.0	12.5	25.6
4	81	Sunrise	7.9	7.9	2.5	41.5	8.4	17.2	14.5
5	83	Sunrise	12.1	11.4	1.8	37.7	13.0	10.5	13.5
6	88	Sunrise	8.9	6.9	3.4	64.9	2.6	5.9	7.4
7	90	Sunrise	7.3	4.6	5.8	75.5	0.8	3.1	2.9
8	95	Sunrise	9.5	4.5	4.9	75.6	0.5	3.0	1.9
9	97	Sunrise	9.8	3.7	6.2	76.5	0.4	2.0	1.4

Figure 5.1. Average dry matter accumulation rate of six edamame cultivar grown in Painter, VA during 2009. Data points are the mean of six cultivar means, each the mean of two samples (n=12).

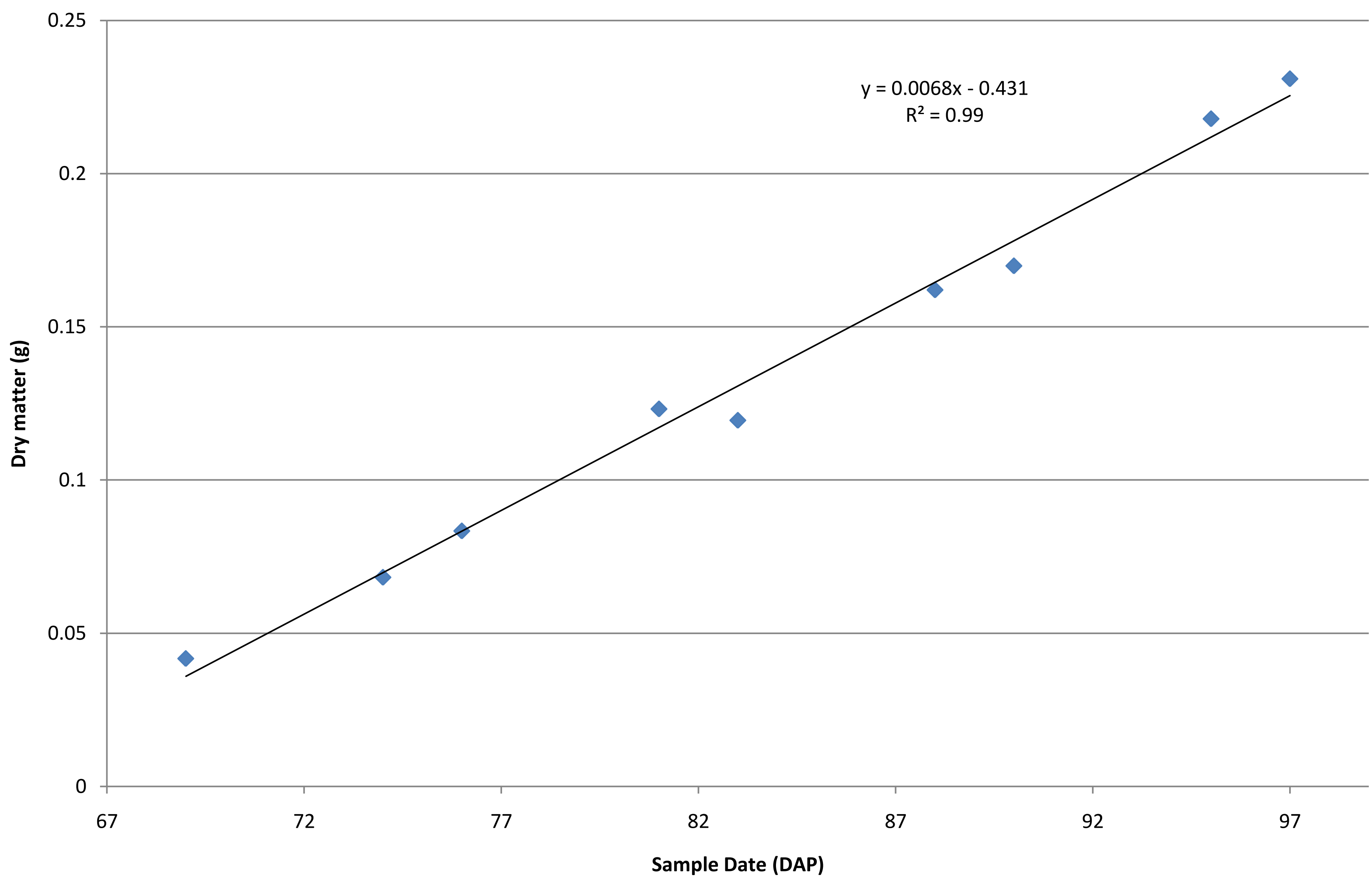


Figure 5.2. Average isoflavone content (µg/bean) of six edamame cultivars across nine sample dates (days after planting). Means were calculated using two samples per cultivar. The edamame was grown in Painter, VA during 2009.

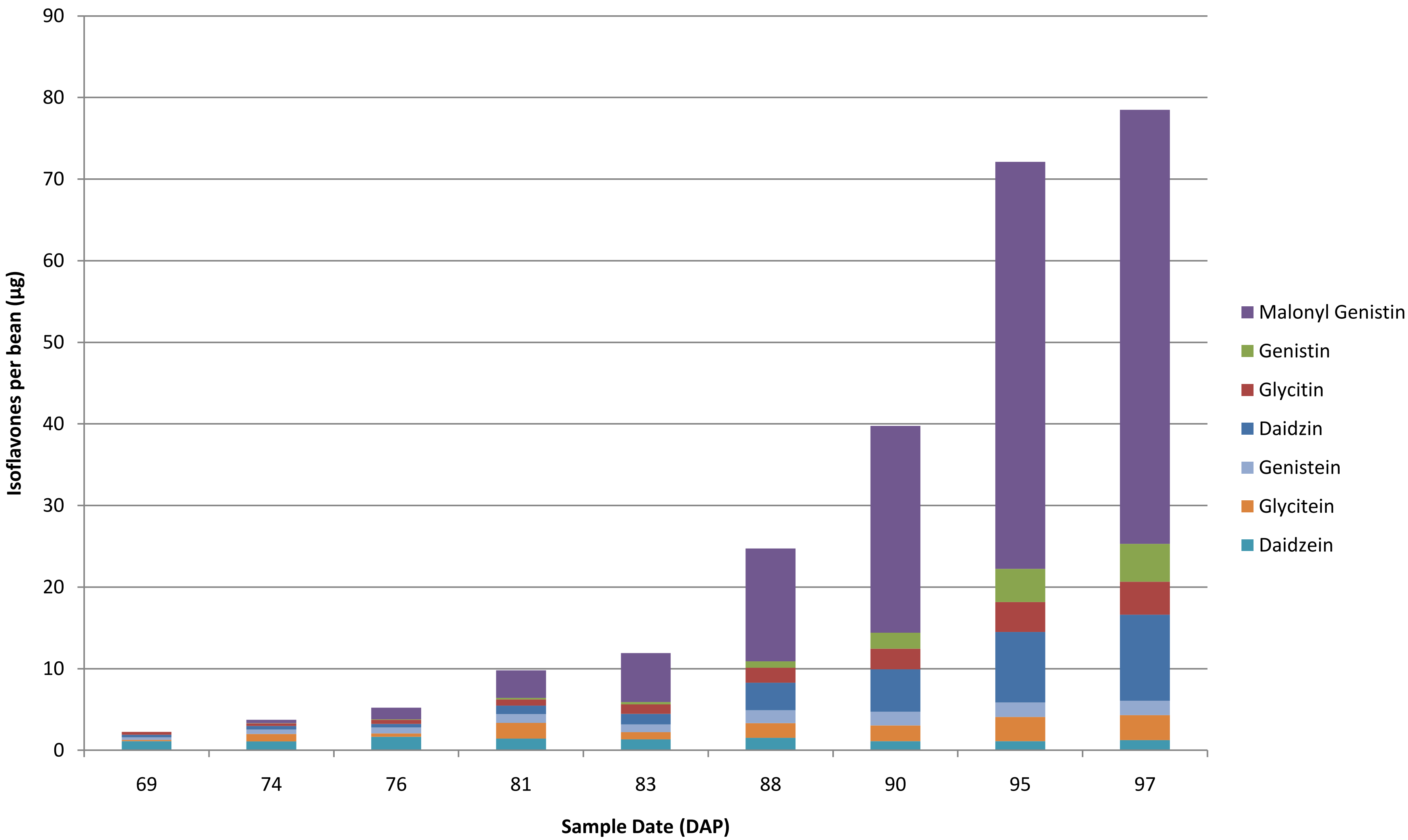


Figure 5.3. Diadzein content of six edamame cultivars sampled nine times (days after planting) during the 2009 growing season in Painter, VA. Isoflavone concentrations reported in µg per bean. Data points are the mean of two samples per cultivar per sample date. The vertical line represents when the beans were ready for harvest.

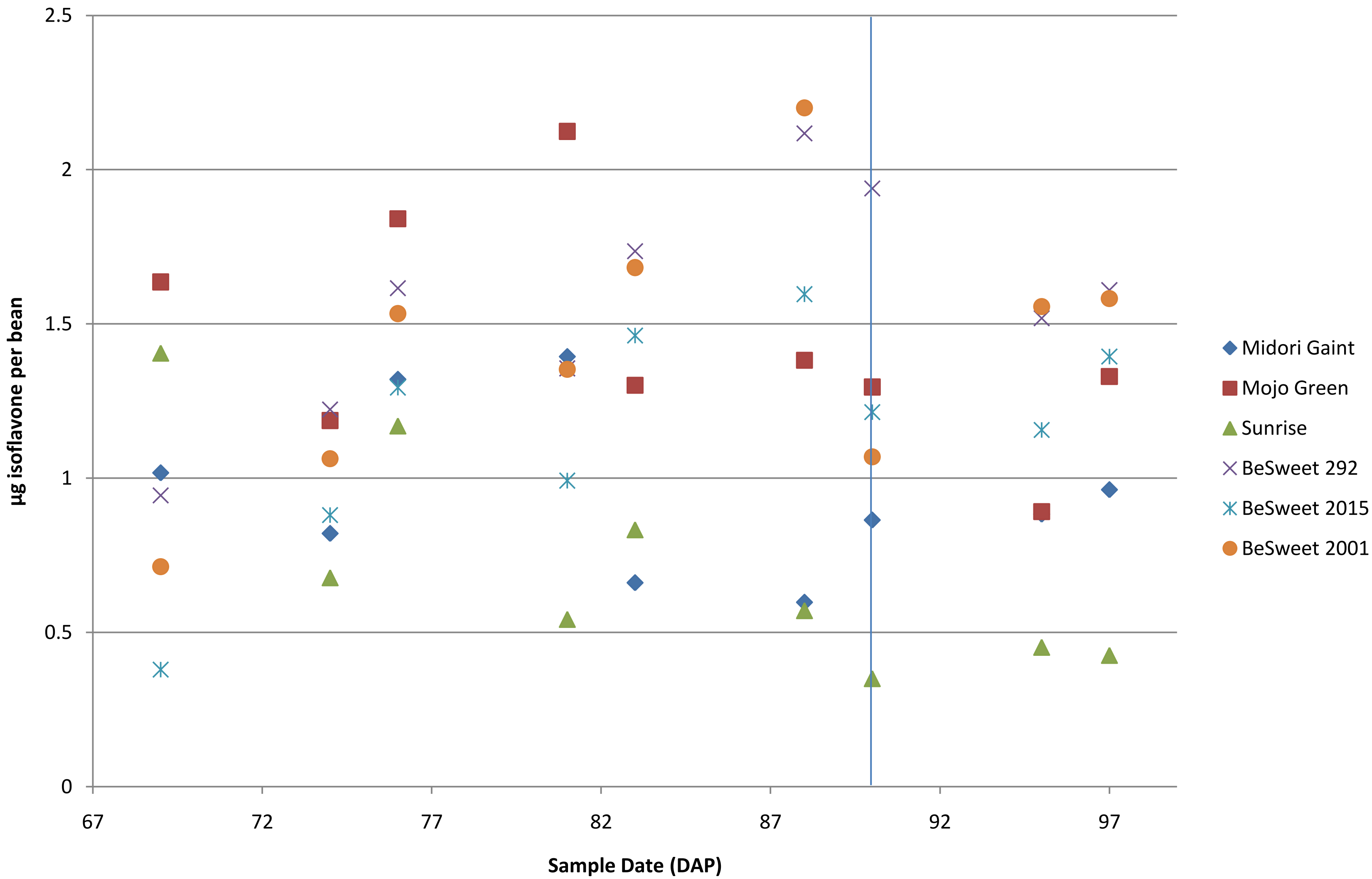


Figure 5.4. Glycitein concentration (µg per bean) of six edamame cultivars. The edamame cultivar evaluation trial was conducted during 2009 in Painter, VA. The vertical line represents when the beans were ready for harvest.

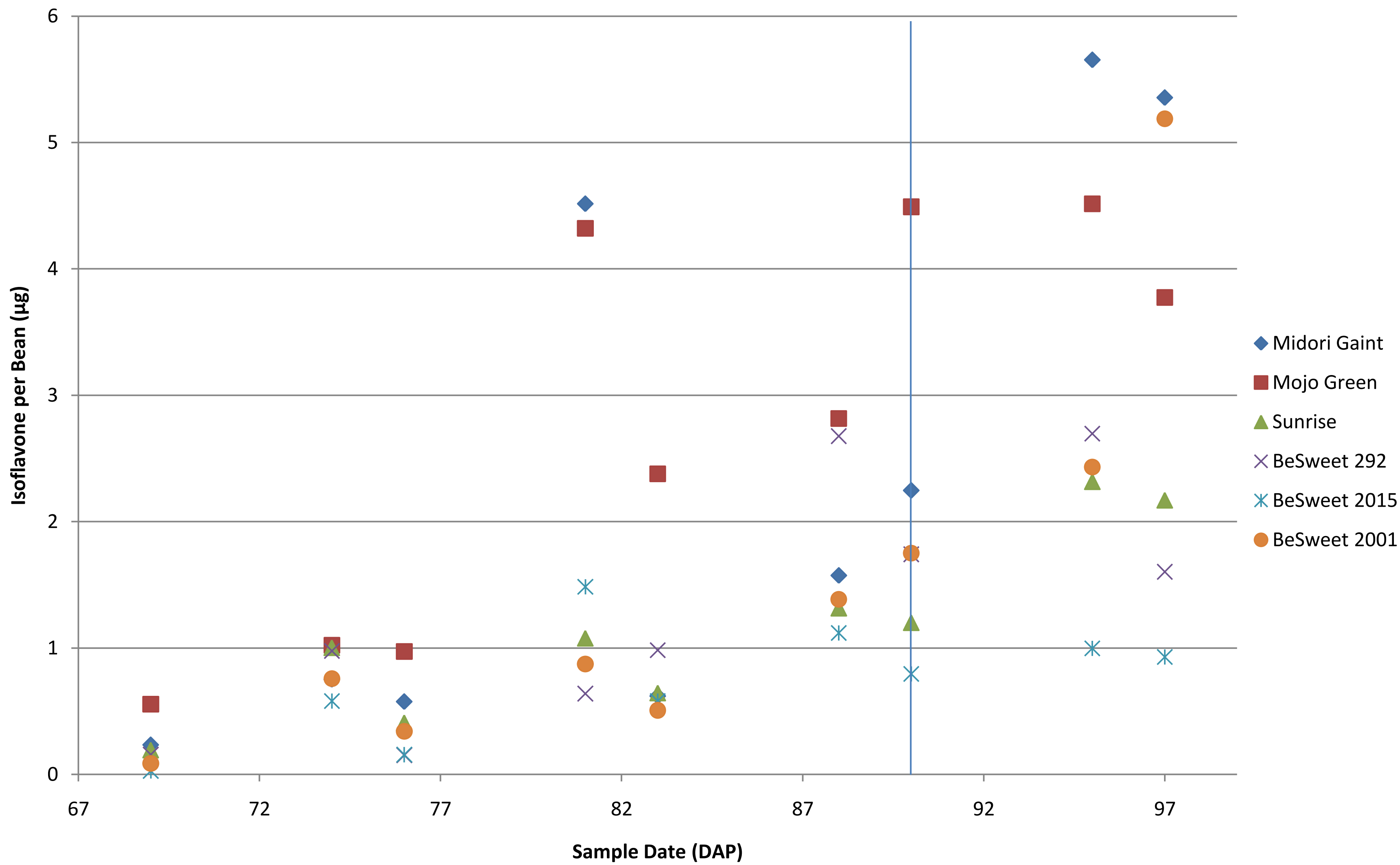


Figure 5.5. The concentration (µg per bean) of genistein in six edamame cultivars sampled across nine times (days after planting). The edamame was grown in Painter, VA during 2009. Data points are the mean of two samples. The vertical line represents when the beans were ready for harvest.

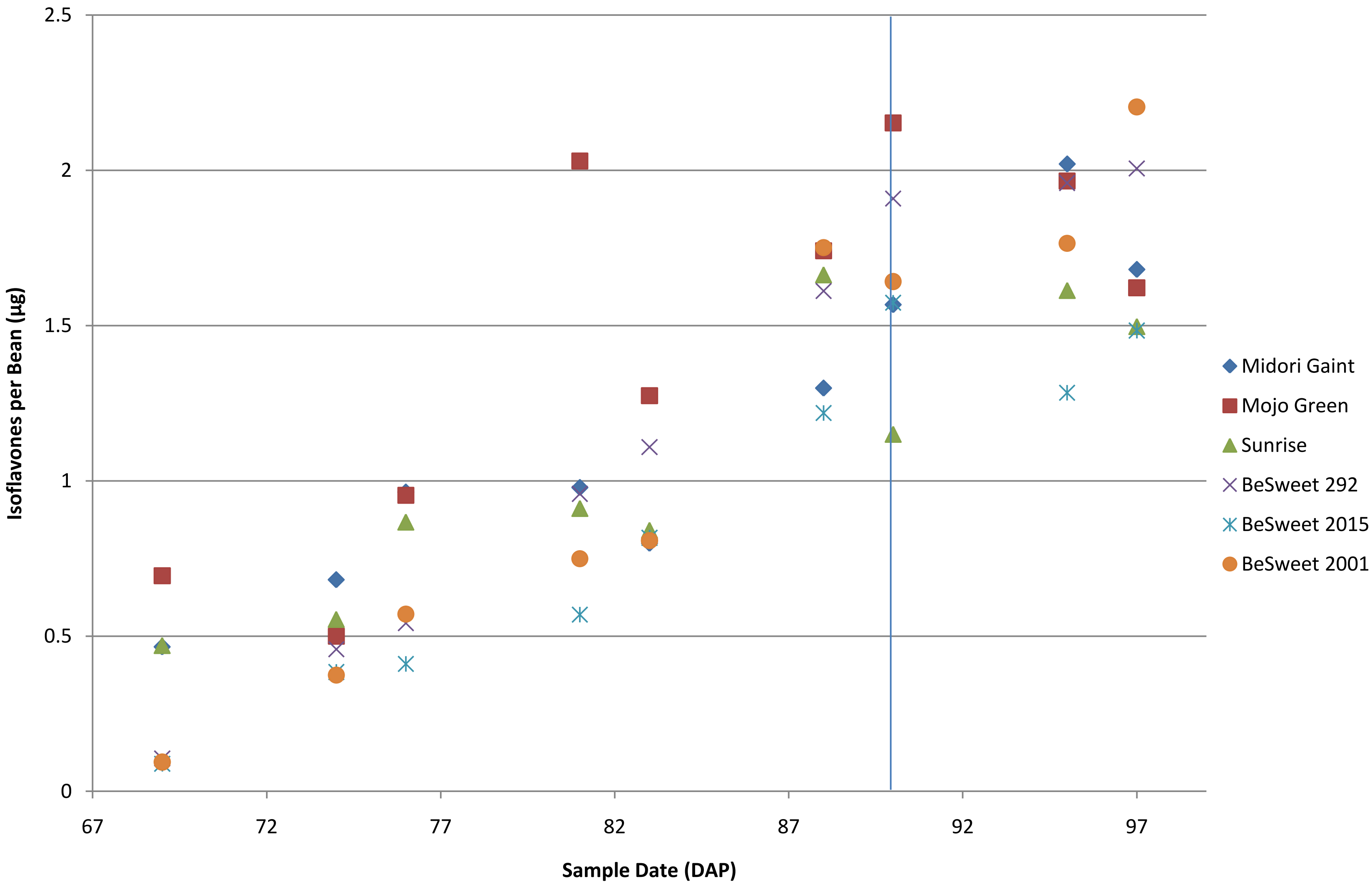


Figure 5.6. Daidzin concentration (µg per bean) at nine sample dates (days after planting) during the 2009 growing season. The six edamame cultivars were grown in Painter, VA. Data points are the mean of two samples. The vertical line represents when the beans were ready for harvest.

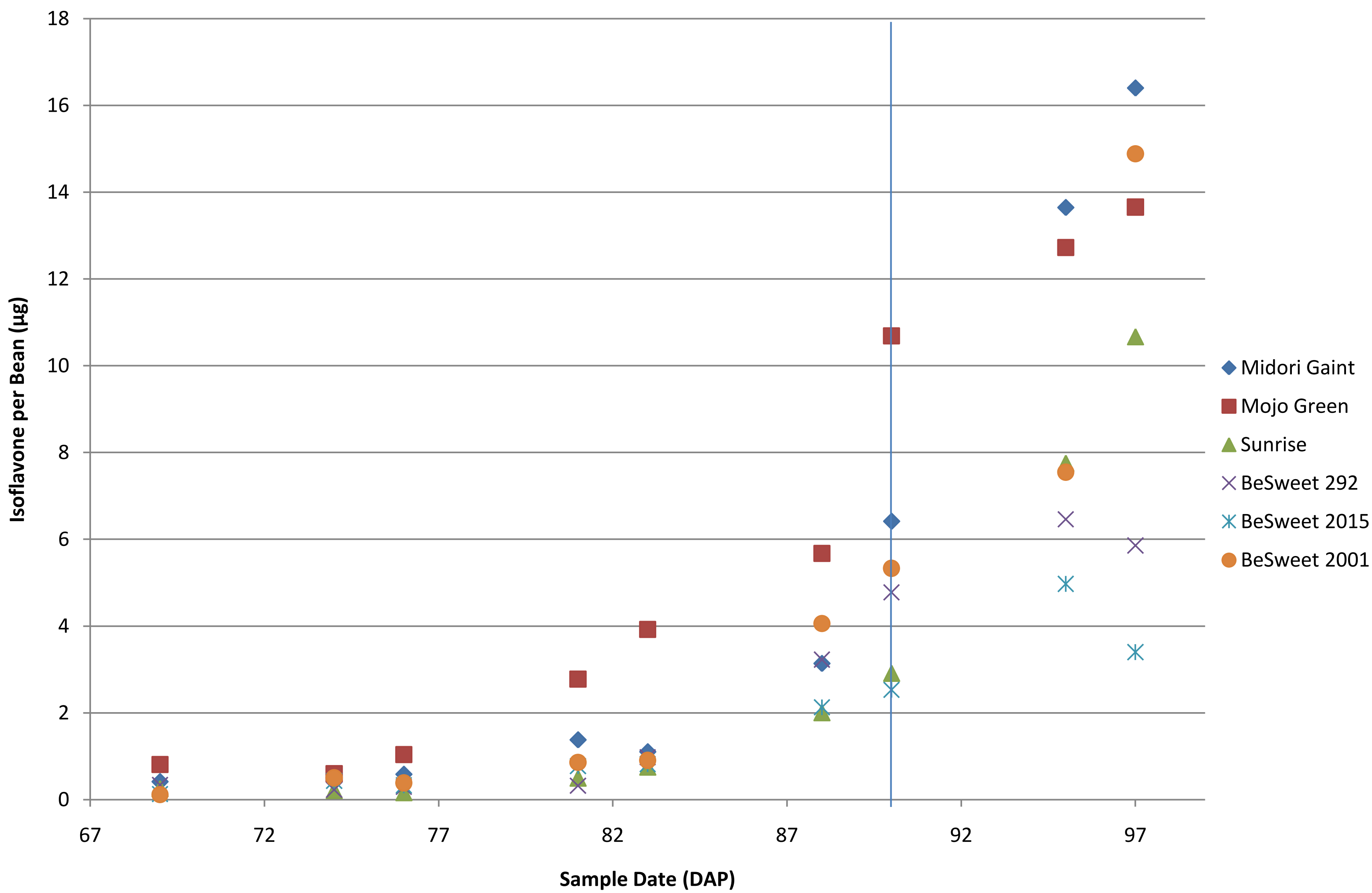


Figure 5.7 Genistin concentration (μg per bean) of six edamame cultivars grown in Painter, VA during 2009. The edamame was sampled nine times from 69 to 97 days after planting. Data points are the mean of two samples. The vertical line represents when the beans were ready for harvest.

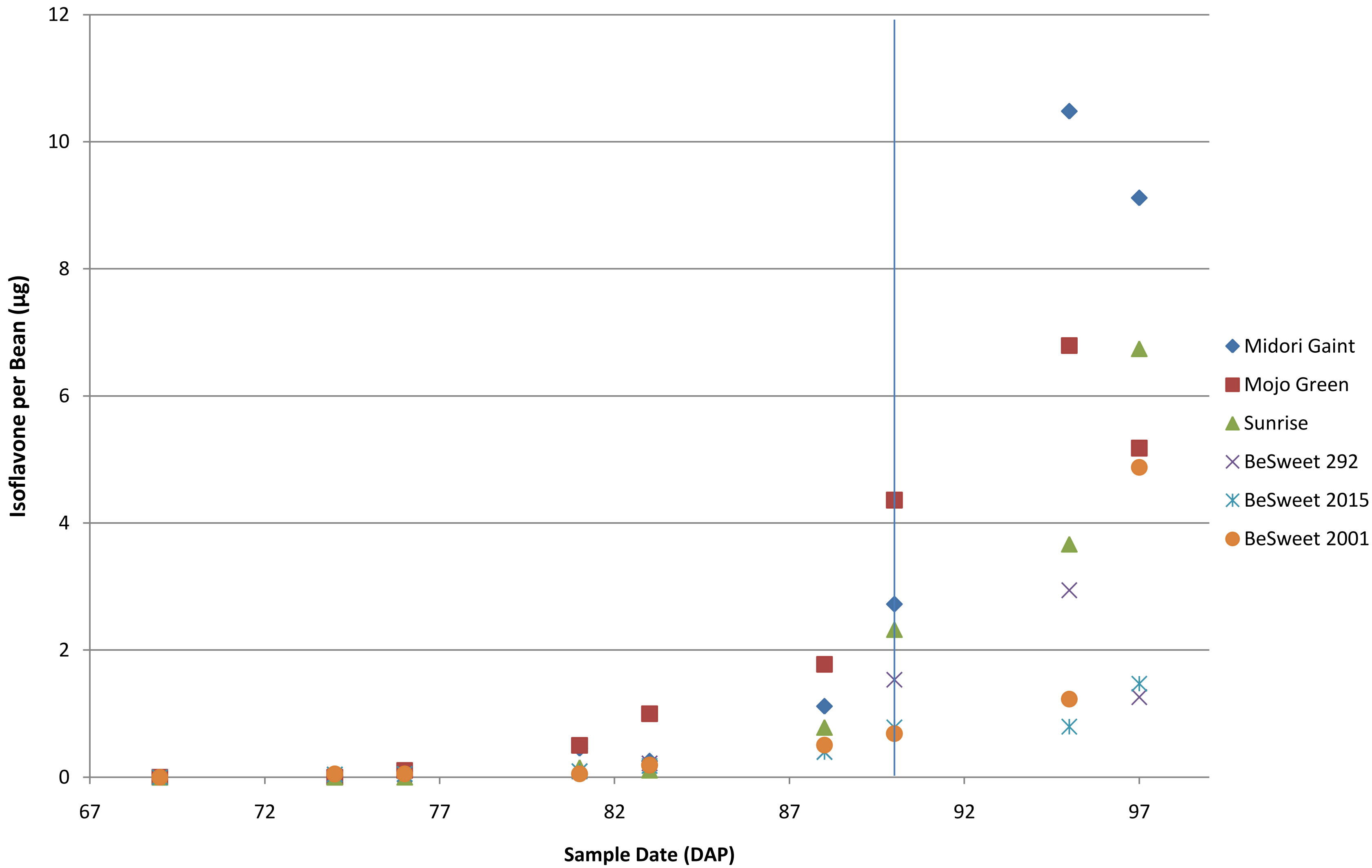


Figure 5.8. The concentration (µg per bean) of glycitin in six edamame cultivars sampled nine times from 69 days after planting to 97 days after planting during 2009. The edamame cultivar evaluation trial was grown in Painter, VA. Data points are the mean of two samples per cultivar per sample date. The vertical line represents when the beans were ready for harvest.

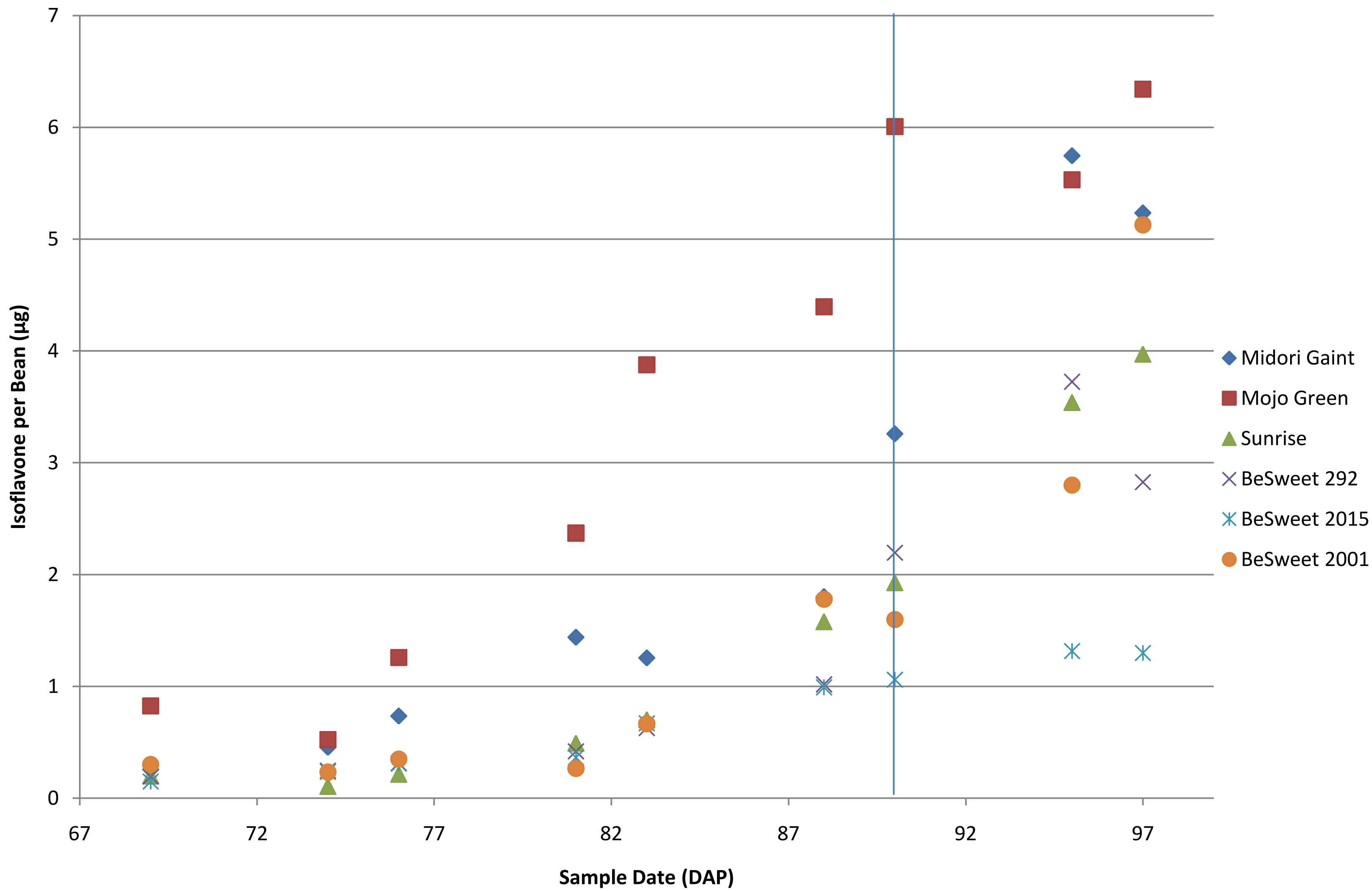


Figure 5.9. Malonyl genistin concentration (µg per bean) of six edamame cultivars sampled nine times from 69 to 97 days after planting. The edamame was grown in Painter, VA during 2009. Data points are the mean of two samples for each cultivar at each sample date. The vertical line represents when the beans were ready for harvest.

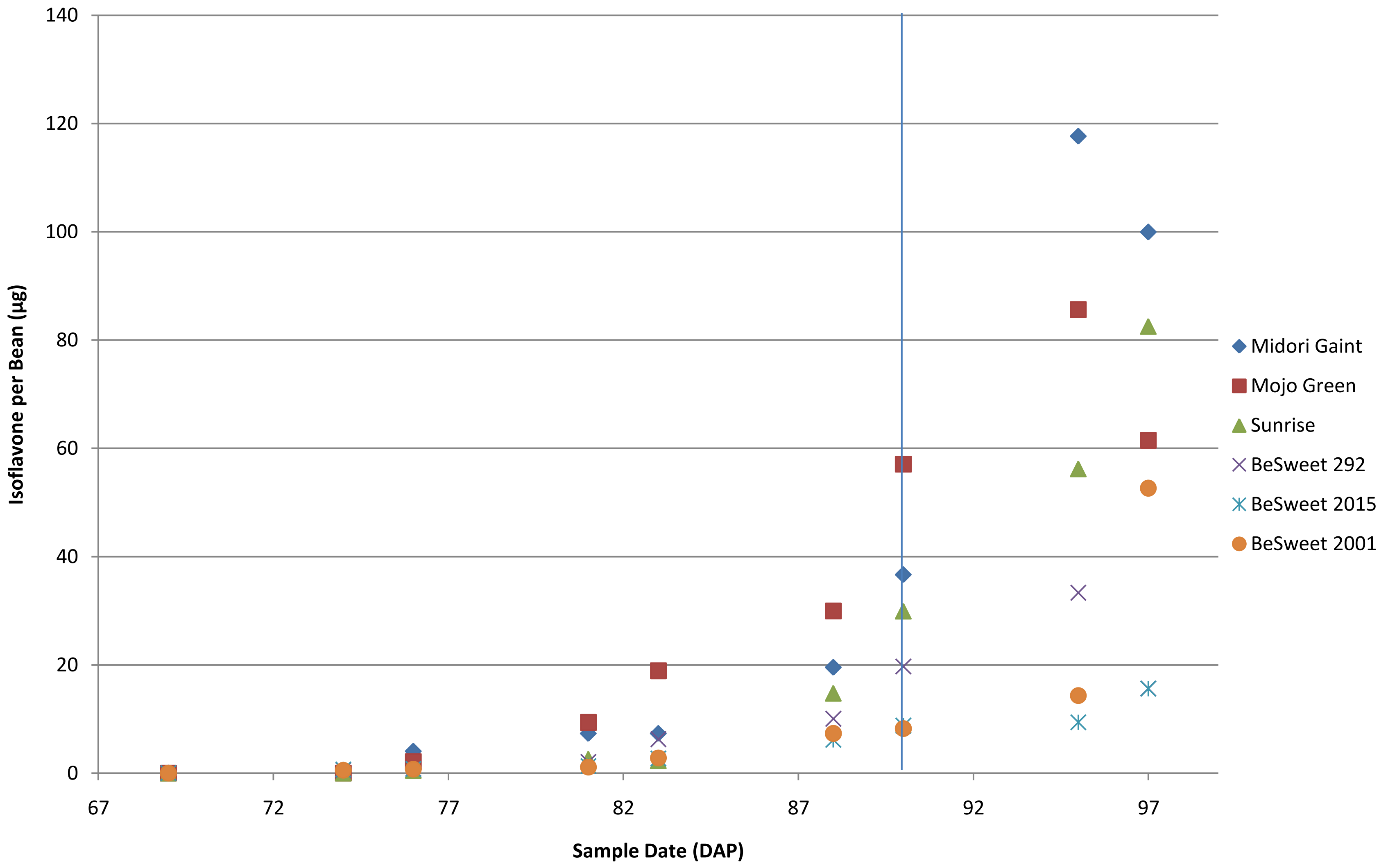
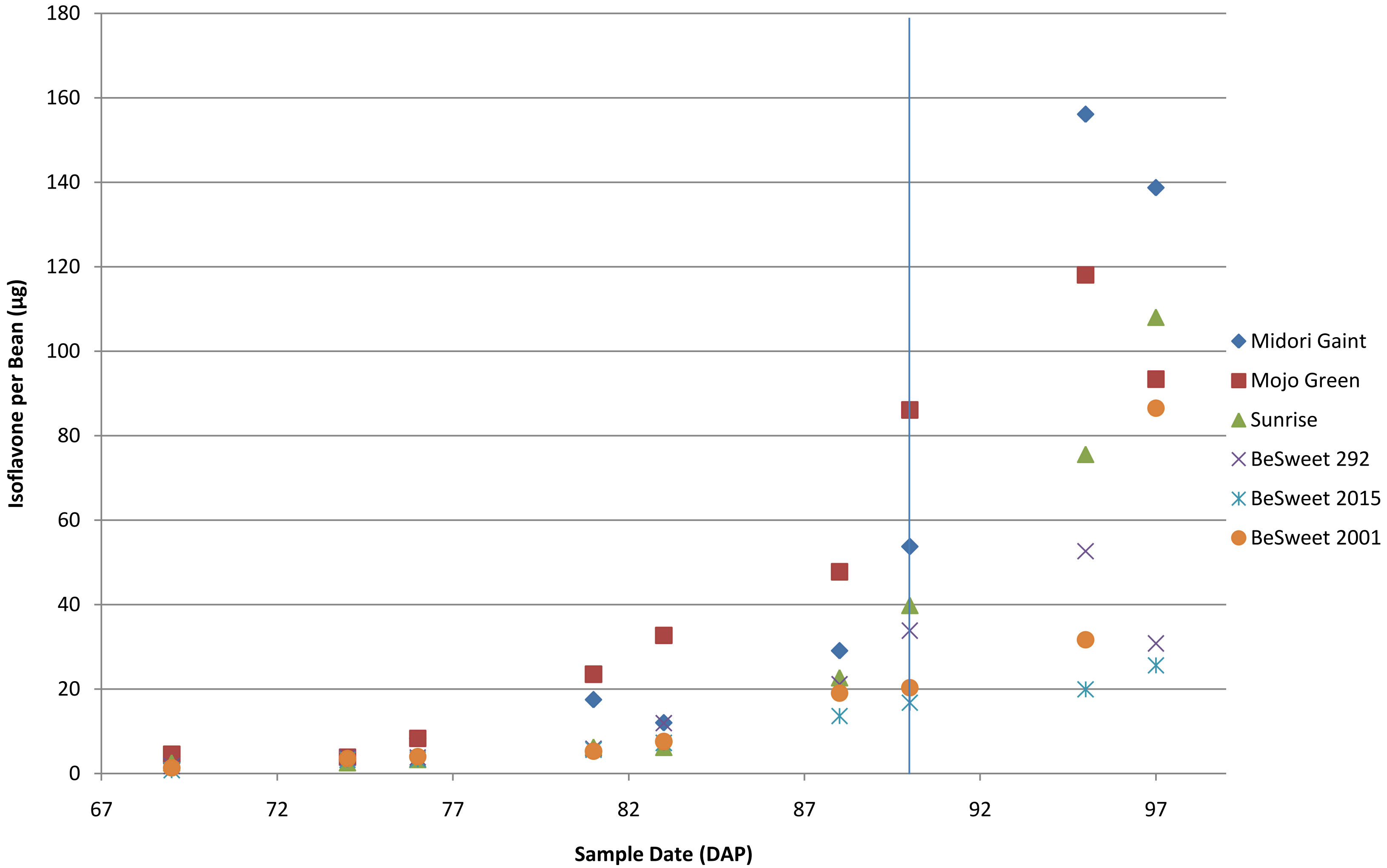


Figure 5.10. The total isoflavone concentration (µg per bean) is the sum of genistin, daidzin, glycitin, malonyl genistin, daidzein, glycitein, and genistein. Total isoflavones were measured across nine sample dates ranging from 69 to 97 days after planting. The edamame cultivar evaluation trial was grown in Painter, VA during 2009. Each isoflavone mean was determined by two sample means. The data point is the sum of the seven isoflavone means. The vertical line represents when the beans were ready for harvest.



References

Abusteit, E.O. 1993. Weed competition in (*Glycine Max* L.). J. Agronomy and Crop Sci. 171:96-101.

AOAC International. 1995. Official methods of analysis of AOAC International. Arlington Va: AOAC International.

Achouri, A., J.I. Boye, and D. Belanger. 2005. Soybean isoflavones: efficacy of extraction conditions and effect of food type on extractability. Food Res Intl. 38:1199-1204.

Beaver, J.S., and R.R. Johnson. 1981. Response of determinate and indeterminate soybeans to varying cultural practices in the northern USA. Agron. J. 73:833-838.

Bennett, J.O., O. Yu, L.G. Heatherly, H.B. Krishnan. 2004. Accumulation of genistein and daidzein, soybean isoflavones implicated in promoting human health, is significantly elevated by irrigation. J. Agr. Food Chem. 52:7574-7579.

Berger, M., C.A. Rasolohery, R. Cazalis, J. Dayde. 2008. Isoflavone accumulation kinetics in soybean seed cotyledons and hypocotyls: distinct pathways and genetic controls. Crop Sci. 48:700-708.

Bernick, K. 2009. Edamame takes root in U.S. Corn and Soybean Dig. Penton Media Inc., Overland Park, KS, p. 2.

Burden, B.J., D.M. Norris. 1992. Role of the isoflavonoid coumestrol in the constitutive antixenosic properties of “Davis” soybeans against an oligophagous insect, the Mexican bean beetle. J Chem. Ecol. 18:1069-1081.

Caldwell, C.R., S.J. Britz, R.M. Mirecki. 2005. Effects of temperature, elevated carbon dioxide, and drought during seed development on the isoflavone content of dwarf soybean

[*Glycine max* (L.) Merrill] grown in controlled environments. J Agr. Food Chem. 53:1125-1129.

Champolivier, L., A. Merrien. 1996. Effects of water stress applied at different growth stages to *Brassica napus* L. var. *olefifera* on yield, yield components and seed quality. European J. Agron. 5:153-160.

Chung, H., S. Hogan, L. Zhang, K. Rainey, K. Zhou. 2008. Characterization and comparison of antioxidant properties and bioactive components of Virginia soybeans. J. Agr. Food Chem. 56:11515-11519.

Cober, E.R., D.W. Stewart, H.D. Voldeng. 2001. Photoperiod and temperature responses in early-maturing near-isogenic soybean lines. Crop Sci. 41:721-707.

Cox, W.J., E. Shields, J.H. Cherney. 2008. Planting date and seed treatment effects on soybean in the northeastern United States. Agron J. 100:1662-1665.

Cui, Z., A.T. James, S. Miyazaki, R.F. Wilson, and T.E. Carter Jr. 2005. Breeding specialty soybeans for traditional and new soyfoods, p. 264-322. In: K. Liu (ed.). Soybeans as functional foods and ingredients. AOCS Press, Champaign, Ill.

Dhaubhadel, S., M. Gijzen, P. Moy, M. Farhangkhoee. 2007. Transcriptome analysis reveals a critical role of CHS7 and CHS8 genes for isoflavonoid synthesis in soybean seeds. Plant Physiol. 143:326-338.

Dolde, D., C. Vlahakis, J. Hazebroek. 1999. Tocopherols in breeding lines and effects of planting location, fatty acid composition, and temperature during development. J. Amer. Oil Chem. Soc. 76:349-355.

Duppong, L.M., and H. Hatterman-Valenti. 2005. Yield and quality of vegetable soybean cultivars for production in North Dakota. HortTechnology. 15:896-900.

Eaton, B.J., O.G. Russ, K.C. Feltner. 1976. Competition of velvetleaf, prickly sida, and venice mallow in soybeans. Weed Sci. 24:224-228.

Egli, D.B. 1993. Cultivar maturity and potential yield of soybean. Field Crops Res. 32:147-158.

Egli, D.B., W.P. Bruening. 2000. Potential of early-maturing soybean cultivars in late plantings. Agron J. 92:932-537.

Egli, D.B., D.M. TeKrony. 1995. Soybean seed germination, vigor, and field emergence. Seed Sci. Technol. 23:595-607.

Egli, D.B., P.L. Cornelius. 2009. A regional analysis of the response of soybean yield to planting date. Agron J. 101:330-335.

Eldridge, A.C., W.F. Kwolek. 1983. Soybean isoflavones: effect of environment and variety on composition. J. Agr. Food Chem. 31:394-936.

Elsheikh, E.A.E., S.S.M. Salih, A.A. Elhussein, and E.E. Babiker. 2009. Effects of intercropping, *Bradyrhizobium* inoculation and chicken manure fertilization on the chemical composition and physical characteristics of soybean seed. Food Chem. 112:690-694.

Ernst, M. and T. Woods. 2001. Marketing challenges for emerging crops in Kentucky: Vegetable soybeans. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). 2nd Intl. Veg. Soybean Conf. Wash. State Univ. Pullman, WA.

Fehr, W.R., C.E. Caviness, D.T.Burmood, and J.S. Pennington. 1971. Stages of development descriptions for soybeans, *Glycine Max* (L.) Merrill. Crop Sci. 11:929-931.

Graham, T.L. 1991. Flavonoid and isoflavonoid distribution in developing soybean seedling tissues and in seed and root exudates. Plant Physiol. 95:594-603.

Grieshop, C.M. and G.C. Fahey. 2001. Comparison of quality characteristics of soybeans from Brazil, China, and the United States. J. Agr. Food Chem. 49:2669-2673.

Hartwig, E.E. and T.C. Kline. 1991. Yield and composition of soybean seed from parents with different protein, similar yield. Crop Sci. 31:290-292.

Heatherly, L.G. 1998. Early soybean production system (ESPS), p. 103-117. In: L.G. Heatherly and H.E. Hodges(eds.). Soybean production in the mid-South. CRC Pres, Boca Raton, FL.

Hoeck, J.A., W.R. Fehr, P.A. Murphy, G.A. Welke. 2000. Influence of genotype and environment on isoflavone contents of soybean. Crop Sci. 40:48-51.

Hymowitz, T., F.I. Collins, J. Panczner, and W.M. Walker. 1972. Relationship between the content of oil, protein, and sugar in soybean seed. Agron. J. 64:613-616.

Johnson, B.J. 1971. Effect of weed competition on sunflowers. Weed Sci. 19:378-380.

Johnson, D. 1999. Market Improving for Edamame. Coop. Ext., Colorado State Unv. 19:1-4.

Johnson, S.L., W.R. Fehr. and B.J.Alt. 2001. Breeding for seed size and composition of vegetable soybeans. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). 2nd Intl. Veg. Soybean Conf. Wash. State Univ., Pullman, WA.

Kelley, K.M. and E.S. Sanchez. 2005. Accessing and understanding consumer awareness of and potential demand for edamame. HortScience. 40:1347-1353.

Kim, J-A., I-M. Chung. 2007. Change in isoflavone concentration of soybean (*Glycine max* L.) seeds at different growth stages. J. Sci. Food Agr. 87:496-503.

Konovsky, J., T.A. Lumpkin, D. McClary. 1994. Edamame: The vegetable soybean. P. 173-181. In: A.D. O’Rourke (ed.). Understanding the Japanese food and agrimarket: A multifaceted opportunity. Binghamton, Hayworth, UK.

Kumar, V., A. Rani, A.K. Dixit, D. Bhatnagar, G.S. Chauhan. 2009. Relative changes in tocopherols, isoflavones, total phenolic content, and antioxidative activity in soybean seeds at different reproductive stages. J. Agr. Food Chem. 57:2705-2710.

Kumudini, S., L.J. Grabau, T.W. Pfeiffer, C.C. Steele. 2005. Management and production of value-added soybean cultivars in south central USA. Agron J. 97:904-909.

Lai, G., S.H. Lai, and S. Shanmugasundaram. (n.d.). Suggested cultural practices for vegetable soybeans. ARVDC, Shanhua, Taiwan.

Lee, J., M. Renita, R.J. Fioritto, S.K. St. Martin, S.j. Schwartz, Y. Vonovotz. 2004. Isoflavone characterization and antioxidant activity of Ohio soybeans. J. Agr. Food Chem. 52:2647-2651.

Lee, S.J., J.K. Ahn, S.H. Kim, J.T. Kim, S.J. Han, M.Y. Jung, I.M. Chung. 2003. Variation in isoflavone of soybean cultivars with location and storage duration. J. Agr. Food Chem. 51:3382-3389.

Lin, C-C. 2001. Frozen edamame: global market conditions. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). 2nd Intl. Veg. Soybean Conf. Wash. State Univ., Pullman.

Lozovaya, V.V., A.V. Lygin, A.V. Ulanov, R.L. Nelson, J. Dayde, J.M. Widholm. 2005. Effect of temperature and soil moisture status during seed development on soybean seed isoflavone concentration and composition. Crop Sci. 45:1934-1940.

Major, D.J., D.R. Johnson, V.D. Luedders. 1975a. Evaluation of eleven thermal unit methods for predicting soybean development. Crop Sci. 15:172-174.

Major, D.J., D.R. Johnson, J.W. Tanner, I.C. Anderson. 1975b. Effects of daylength and temperature on soybean development. Crop Sci. 15:174:179.

Malencic, D., M. Popovic, J. Miladinovic. 2007. Phenolic content and antioxidant properties of soybean (*Glycine max* L. Merr.) seeds. Molecules. 12:576-581.

Marwede, V., A. Schierholt, C. Mollers, H. Becker. 2004. Genotype × environment interactions and heritability of tocopherol contents in canola. Crop Sci. 44:728-731.

Masuda, R. 2001. Enhancement of sweet components in vegetable soybeans seeds: starch degradation during cooking enhances flavor of immature seeds. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). 2^{nd} Intl. Veg. Soybean Conf. Wash. State Univ., Pullman, WA. p. 105-108

Mathew, J.P., S.J. Herbert, S. Zhang, A.A.F. Rautenkranz, G.V. Litchfield. 2000. Differential response of soybean yield components to the timing of light enrichment. Agron. J. 92:1156-1161.

Mbuvi, S.W., and J.B. Litchfield. 1995. Green soybeans as a vegetable: comparing green soybeans with green peas and lima beans; and maximized harvest time determinations using mathematical modeling. J. Veg. Crop Prod. 1(1):99-121.

Mebrahtu, T. and T.E. Devine. 2008. Combining ability analysis for selected green pod yield components of vegetable soybean genotypes (Glycine Max). N.Z. J. Crop Hort Sci. 36:97-105.

Messina, M. 2001. An overview of the health effects of soyfoods and soybean isoflavones. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). 2^{nd} Intl. Veg. Soybean Conf. Wash. State Univ., Pullman, WA.

Miles, C.A. and C. Chen. 2000. Washington State University edamame trials. Wash. St. Univ. Coop. Ext., Vancouver, WA.

Mohamed, A., M.S.S. Rao and T. Mebrahtu. 2001. Nutritional and health benefits of vegetable soybean: beyond protein and oil. p. 131–134. In: T.A. Lumpkin and S. Shanmugasundaram (Compilers), 2nd Int. Vegetable Soybean Conf., Washington State Univ., Pullman, WA.

Molyneux, P. 2004. The use of the stable free radical Diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci. Technol. 26:211-219.

Moller, I.M. 2006. Reactive oxygen species (ROS) and plant respiration, Essay 11.5. In L. Taiz, and E. Zeiger (eds.). Plant Physiol.-online. Sinauer, Sunderland, MA.

Montri, D.N., K.M. Kelley, and E.S. Sanchez. 2006. Consumer interest in fresh, in-shell edamame and acceptance of edamame based patties. HortScience 41:1616-1622.

Murphy, P.A., T.Song, G.Buseman, K. Barua. 1997. Isoflavones in soy-based infant formulas. J. Agr. Food Chem. 45:4635-4638.

Naeve, S. 2005. Just for growers-MN soybean production: growth and development. 04/03/2010. http://www.soybeans.umn.edu/crop/growth/index.htm

Patel, R.P., B.J. Boersma, J.H. Crawford, N. Hogg, M. Kirk, B. Kalyanaraman, D.A. Parks, D. Barnes, and V. Darley-Usmar. 2001. Antioxidant mechanisms of isoflavones in lipid systems: paradoxical effects of peroxyl radical scavenging. Free Radical Biol. Med. 31: 1570-1581.

Pendersen, P., J.G. Lauer. 2003. Soybean agronomic response to management systems in the upper Midwest. Agron J. 95:1146-1151.

Pendersen, P., J.G. Lauer. 2004. Response of soybean yield components to management system and planting date. Agron J. 96:1372-1381.

Phillips, S.B., G.L. Mullins, S.J. Donohue. 2002. Changes in snap bean yield, nutrient composition, and soil chemical characteristics when using broiler litter as fertilizer source. J Plant Nutr. 25:1607-1620.

Piper, E.L., K.J. Boote. 1999. Temperature and cultivar effects on soybean seed oil and protein concentrations. J. Amer. Oil Chemist Soc. 76:1233-1241.

Prabhakaran, M.P., L.S. Hui, C.O. Perera. 2006. Evaluation of the composition and concentration of isoflavones in soy based supplements, health products, and infant formulas. Food Res. Intl. 39:730-738.

Rao, M.S.S., A.S. Bhagsari, A.I. Mohamed. 2002. Fresh green seed yield and seed nutritional traits of vegetable soybean genotypes. Crop Sci. 42:1950-1958.

Ray, C.L., E.R. Shipe, W.C. Bridges. 2008. Planting date influence on soybean agronomic traits and seed composition in modified fatty acid breeding lines. Crop Sci. 48:181-188.

Renquist, A.R., J.B. Reid. 1998. Quality of processing tomato (*Lycoperscion esculentum*) fruit from four bloom dates in relation to optimal harvest timing. N.Z. J. Crop Hort Sci. 26:161-168.

Rice-Evans, C.A., N.J. Miller, G. Paganga. 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2:152-159.

Riedl K.M., J.H. Lee, M. Renita, S.K. St.Martin, S.J. Schwartz, Y. Vodovotz. 2007. Isoflavone profiles, phenol content, and antioxidant activity of soybean seeds as influenced by cultivar and growing location in Ohio. J Sci. Food Agr. 87:1197-1206.

Robinson, A.P., S.P. Conley, J.J. Volenec, J.B. Santini. 2009. Analysis of high yielding early-planted soybean in Indiana. Agron J. 101:131-139.

Sakthivelu, G., M.K. Akitha Devi, P. Giridhar, T. Rajasekaran, G.A. Ravishankar., M.T. Nikolova, G.B. Angelov, R.M. Todorova, G.P. Kosturkova. 2008. Isoflavone composition, phenol content, and antioxidant activity of soybean seeds from India and Bulgaria. J. Agr. Food Chem. 56:2090-2095.

Sanchez, E., K. Kelley, and L. Butler. 2005. Edamame production as influenced by seedling emergence and plant population. HortTechnology 15:672-676.

Sciarappa, W.J., L.K. Hunsberger, D. Shen, Q.-L. Wu, J. Simon, and S. Hulme. 2007. Evaluation of edamame cultivars in New Jersey and Maryland. In: J. Janick and A. Whipkey (eds.). Trends in new crops and new uses. ASHS Press, Alexandria, VA.

Shanmugasundaram, S., M.R. Yan, and R.Y. Yang. 2001. Association between protein, oil, and sugar in vegetable soybean. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). 2^{nd} Intl. Veg. Soybean Conf. Wash. State Univ., Pullman.

Showalter, R.K. 1969. Detachment force for harvesting snap beans. In: Proc of the FL State Hort Soc. 82:115-118.

Shurtleff, W., and T.A. Lumpkin. 2001. Chronology of green vegetable soybeans and vegetable-type soybean. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). 2^{nd} Intl. Veg. Soybean Conf. Wash. State Univ., Pullman, WA.

Sikka, K.C., A.K. Gupta, R. Singh, and D.P. Gupta. Comparative nutritive value, amino acid content, chemical composition, and digestibility in vitro of vegetable- and grain-type soybeans. J. Agr. Food Chem. 26:312-316.

Smith, T.J., H.M. Camper, M.T. Carter, G.D. Jones, M.W. Alexander. 1961. Soybean production in Virginia as affected by variety and planting dates. VA Agr. Expt. Sta. Bul. 526.

Soyatech. 2010. Soy foods: the US market 2009. Soyatech, Southwest Harbor, ME.

Swinny, E., K.G. Ryan. 2005. Red clover *Trifolium pretense* L. phytoestrogens: UV-B radiation increases isoflavone yield, and postharvest drying methods change the glucoside conjugate profiles. J. Agr. Food Chem. 53:8273-8278.

Tsou, S.C.S., and T.L. Hong. 1991. Research on vegetable soybean quality in Taiwan. In: S. Shanmugasundaram (ed.). Vegetable soybean: research needs for production and quality improvement. AVRDC, Shanhua, Taiwan, p. 107-107.

Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun. K. Okubo, K. Kitamura. 1995. Factors affecting isoflavone content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. J. Agr. Food Chem. 43:1184-1192.

U.S. Dept. of Agr. 2007. USDA-Iowa State University database on the isoflavone content of foods. Release 1.4. U.S. Dept. Agr., Beltsville, MD.

U.S. Depr. Of Agr. 2008. USDA database for the isoflavone content of selected foods. Release 2.0. U.S. Dept. Agr., Beltsville, MD.

U.S. Dept of Agric. 2009. Agriculture statistics for 2008. U.S. Dept. Agr, Washington, D.C.

Virginia Cooperative Extension. 2000. Agronomy handbook. VA Coop. Ext. Publ. 424-100.

Wang, H-J., P.A. Murphy. 1994. Isoflavone content in commercial soybean foods. J. Agr. Food Chem. 42:1666-1673.

Wang, H-J., P.A. Murphy. 1996. Mass balance study of isoflavones during soybean processing. J. Agr. Food Chem. 44:2377-2383.

Whent, M., J. Hao, M. Slavin, M. Zhou, J. Song, W. Kenworthy, L. Yu. 2009. Effects of genotype, environment, and their interaction on chemical composition and antioxidant properties of low-linolenic soybeans grown in Maryland. J. Agr. Food Chem. 57:10163-10174.

Wszelaki, A.L., J.F. Delwiche, S.D. Walker, R.E. Liggett, S.A. Miller, M.D. Kleinhenz. 2005. Consumer liking and descriptive analysis of six varieties of organically grown edamame-type soybean. Food Quality and Preference. 16:651-658.

Xu, B.J., and S.K.C. Chang. 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. J. Food Sci. 72:s159-s166.

Yin, X., and T.J. Vyn. 2005. Relationship of isoflavones, oil, and protein in seed with yield of soybean. Agron. J. 97:1314-1321.

Young, G., T. Mebrahtu, J. Johnson. 2000. Acceptability of green soybeans as a vegetable entity. Plant Foods Human Nutr. 55:323-333.

Zhang, L.X., and S. Kyei-Boahen. 2007. Growth and yield of vegetable soybean (Edamame) in Mississippi. HortTechnology 17:26-31.

Zhou, K., J.J. Laux, L. Yu. 2004. Comparison of swiss red winter wheat grain and fractions for their antioxidant properties. J. Agr. Food Chem. 52:1118-1123.

Appendix A-1

2008 Temperature and Rainfall Data

Painter, VA

January					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	55	36	45.5		
2	49	28	38.5	0.02	
3	34	24	29		
4	39	16	27.5		
5	48	26	37		
6	59	39	49		
7	68	42	55		
8	69	53	61		
9	70	56	63		
10	60	36	48		
11	70	44	57	0.1	
12	67	47	57		
13	47	34	40.5	0.03	
14	47	39	43		
15	42	27	34.5		
16	41	31	36		
17	43	29	36	0.56	
18	51	36	43.5	0.7	
19	50	30	40	0.11	
20	33	24	28.5	0.2	
21	30	17	23.5		
22	44	16	30		
23	46	32	39	0.4	
24	43	29	36	0.06	
25	32	24	28		
26	42	20	31		
27	41	32	36.5		
28	41	29	35		
29	53	26	39.5		
30	57	40	48.5	0.01	
31	46	28	37		
				2.19	0

February					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	59	34	46.5	0.51	
2	58	32	45	0.3	
3	58	33	45.5		
4	58	41	49.5		
5	70	48	59	0.03	
6	75	63	69		
7	73	53	63		
8	53	35	44		
9	57	33	45		
10	59	41	50		
11	56	22	39		
12	43	22	32.5	0.02	
13	63	42	52.5	1.33	
14	43	29	36	0.34	1
15	57	27	42		
16	57	36	46.5		
17	54	27	40.5		
18	65	51	58	0.41	
19	67	38	52.5		
20	49	26	37.5		
21	46	25	35.5	0.06	
22	38	28	33	0.44	
23	40	37	38.5	0.05	
24	42	29	35.5		
25	49	33	41	0.04	
26	56	32	44		
27	60	39	49.5		
28	42	29	35.5		
29	49	33	41	0.04	
				3.57	1

2008 Temperature and Rainfall Data

Painter, VA

March					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	56	41	48.5		
2	49	32	40.5		
3	61	32	46.5		
4	66	52	59		
5	63	53	58	1.02	
6	60	34	47		
7	58	42	50	0.42	
8	64	57	60.5	0.56	
9	62	33	47.5		
10	47	28	37.5		
11	54	35	44.5		
12	57	43	50		
13	57	29	43		
14	71	50	60.5		
15	69	48	58.5		
16	59	44	51.5	0.33	
17	51	35	43		
18	52	35	43.5		
19	73	47	60		
20	72	46	59		
21	55	41	48		
22	61	40	50.5		
23	53	34	43.5	0.09	
24	51	29	40		
25	51	31	41		
26	68	43	55.5		
27	73	50	61.5		
28	79	54	66.5		
29	79	39	59	0.01	
30	48	32	40		
31	63	42	52.5		
				2.43	0

April					
Temperature					
Day	Max.	Min.	Mean	Rain	Snow
1	72	55	63.5		
2	71	48	59.5	0.15	
3	52	35	43.5		
4	68	47	57.5	0.92	
5	69	58	63.5	1.73	
6	58	46	52	0.61	
7	47	43	45	0.01	
8	53	44	48.5		
9	53	44	48.5	0.07	
10	71	49	60	0.02	
11	71	52	61.5		
12	76	64	70		
13	74	53	63.5		
14	58	43	50.5	0.04	
15	55	35	45		
16	64	31	47.5		
17	68	33	50.5		
18	82	42	62		
19	82	53	67.5		
20	75	57	66		
21	66	55	60.5	0.51	
22	69	58	63.5	0.15	
23	71	58	64.5		
24	77	50	63.5		
25	79	54	66.5		
26	81	60	70.5		
27	81	57	69		
28	70	54	62	0.32	
29	64	51	57.5	0.48	
30	62	50	56		
				5.01	0

2008 Temperature and Rainfall Data

Painter, VA

May

	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	65	43	54		
2	78	60	69		
3	80	64	72		
4	80	67	73.5		
5	75	50	62.5		
6	76	47	61.5		
7	80	57	68.5		
8	80	66	73	0.5	
9	76	62	69	1.12	
10	62	49	55.5	0.11	
11	61	44	52.5		
12	60	48	54	1.6	
13	66	47	56.5	0.13	
14	74	42	58		
15	77	54	65.5		
16	77	61	69	1.03	
17	77	54	65.5		
18	76	59	67.5		
19	72	55	63.5		
20	75	53	64	0.35	
21	68	47	57.5		
22	71	49	60		
23	72	47	59.5		
24	71	56	63.5		
25	75	51	63		
26	81	55	68		
27	83	67	75		
28	84	58	71	0.86	
29	72	44	58		
30	78	51	64.5		
31	84	67	75.5		
				5.7	0

June

	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	86	66	76	1.67	
2	84	63	73.5	0.22	
3	78	56	67		
4	88	66	77	0.51	
5	87	67	77	1.29	
6	77	64	70.5		
7	95	69	82		
8	96	73	84.5		
9	95	74	84.5		
10	97	73	85		
11	91	73	82		
12	89	62	75.5		
13	87	65	76		
14	88	68	78		
15	83	70	76.5	0.27	
16	89	65	77		
17	85	64	74.5	0.89	
18	81	56	68.5		
19	80	58	69	0.6	
20	83	60	71.5	0.06	
21	84	69	76.5		
22	83	65	74		
23	84	70	77	0.04	
24	82	64	73		
25	84	63	73.5		
26	93	69	81		
27	93	77	85		
28	93	74	83.5		
29	93	77	85		
30	91	71	81	0.59	
				6.14	0

2008 Temperature and Rainfall Data

Painter, VA

July						August					
	Temperature						Temperature				
Day	Max.	Min.	Mean	Rain	Snow	Day	Max.	Min.	Mean	Rain	Snow
1	86	69	77.5			1	90	72	81		
2	84	63	73.5	1.66		2	91	74	75.5		
3	88	67	77.5			3	89	68	72		
4	88	74	81			4	87	66	74		
5	82	70	76	0.11		5	88	69	73		
6	81	69	75	0.03		6	89	73	73.5		
7	85	68	76.5			7	89	68	73.5		
8	88	74	81			8	86	65	72	0.47	
9	81	70	75.5	0.35		9	82	62	76		
10	86	73	79.5			10	85	63	74		
11	85	64	74.5			11	82	64	74	0.88	
12	84	64	74			12	81	66	76		
13	83	63	73			13	82	65	80.5		
14	85	70	77.5	0.01		14	83	61	77.5		
15	83	68	75.5			15	85	67	68		
16	85	63	74			16	83	65	68.5	0.39	
17	90	62	76			17	86	62	72		
18	90	64	77			18	87	65	71		
19	87	71	79			19	90	71	79		
20	88	76	82			20	89	66	78.5		
21	92	70	81			21	78	58	69.5		
22	93	75	84	0.02		22	79	58	72.5		
23	90	71	80.5			23	81	63	76.5		
24	83	69	76	1.43		24	82	60	71		
25	84	63	73.5			25	90	68	79		
26	83	62	72.5			26	88	69	78.5	0.03	
27	86	69	77.5			27	75	64	69.5		
28	87	67	77	0.05		28	75	70	72.5	0.38	
29	86	66	76			29	82	71	76.5	0.09	
30	91	72	81.5	0.7		30	85	71	78		
31	91	78	84.5			31	85	68	76.5		
				4.36	0					2.24	0

2008 Temperature and Rainfall Data

Painter, VA

September						October					
	Temperature						Temperature				
Day	Max.	Min.	Mean	Rain	Snow	Day	Max.	Min.	Mean	Rain	Snow
1	86	57	71.5			1	72	61	66.5	0.15	
2	87	58	72.5			2	67	57	62	0.06	
3	92	65	78.5			3	72	47	59.5		
4	92	67	79.5			4	75	50	62.5		
5	88	66	77			5	78	55	66.5		
6	80	69	74.5	1.32		6	77	54	65.5		
7	82	70	76	0.02		7	70	48	59		
8	84	63	73.5			8	69	43	56		
9	85	70	77.5			9	79	63	71	0.02	
10	75	68	71.5	0.14		10	78	65	71.5		
11	77	65	71	0.14		11	73	56	64.5		
12	80	66	73			12	73	54	63.5		
13	88	74	81			13	74	49	61.5		
14	91	75	83			14	76	52	64		
15	89	78	83.5			15	80	58	69		
16	84	67	75.5			16	84	59	71.5		
17	74	59	66.5			17	84	51	67.5	0.21	
18	79	53	66			18	59	51	55	0.47	
19	77	61	69			19	59	49	54		
20	73	57	65			20	60	34	47		
21	76	56	66			21	70	39	54.5		
22	76	60	68			22	67	48	57.5		
23	72	62	67			23	58	40	49		
24	72	55	63.5			24	61	41	51		
25	67	62	64.5	1.65		25	70	59	64.5	0.11	
26	77	66	71.5	0.35		26	68	52	60	0.2	
27	75	69	72	1.76		27	64	47	55.5		
28	77	67	72	0.02		28	55	42	48.5	0.63	
29	77	66	71.5			29	54	43	48.5		
30	75	56	65.5	0.43		30	51	41	46		
						31	62	32	47		
				5.83	0					1.85	0

2008 Temperature and Rainfall Data

Painter, VA

November						December					
	Temperature						Temperature				
Day	Max.	Min.	Mean	Rain	Snow	Day	Max.	Min.	Mean	Rain	Snow
1	70	42	56			1	58	47	52.5	0.21	
2	68	44	56			2	52	38	45		
3	65	49	57			3	47	27	37		
4	62	58	60	0.46		4	54	35	44.5		
5	62	58	60	0.86		5	54	38	46		
6	60	55	57.5	0.26		6	47	25	36		
7	65	59	62	0.02		7	45	35	40	0.01	
8	65	54	59.5	0.34		8	37	26	31.5		
9	61	42	51.5			9	57	29	43		
10	61	38	49.5			10	69	54	61.5	0.06	
11	52	33	42.5			11	66	50	58	2.38	
12	54	37	45.5			12	64	42	53	1.25	
13	64	41	52.5	0.82		13	43	33	38		
14	60	53	56.5	0.1		14	52	25	38.5		
15	70	59	64.5	0.27		15	67	44	55.5		
16	67	46	56.5	0.42		16	62	42	52	0.03	
17	51	32	41.5			17	52	41	46.5	0.06	
18	46	37	41.5			18	51	42	46.5	0.01	
19	40	31	35.5			19	59	42	50.5	0.09	
20	50	33	41.5			20	63	41	52		
21	46	34	40			21	50	35	42.5	0.65	
22	38	29	33.5	0.01		22	41	24	32.5		
23	43	22	32.5			23	34	22	28		
24	53	28	40.5			24	62	27	44.5		
25	50	42	46	0.62		25	65	50	57.5	0.02	
26	49	37	43			26	50	30	40		
27	51	28	39.5			27	60	44	52	0.02	
28	58	33	45.5			28	73	50	61.5		
29	54	34	44			29	72	45	58.5		
30	56	38	47	0.83		30	53	37	45		
						31	55	37	46		
				5.01	0					4.79	0

Appendix A-2

2009 Temperature and Rainfall Data

Painter, VA

January					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	37	25	31		
2	39	21	30	0.24	
3	44	34	39		
4	45	26	35.5		
5	52	42	47	0.01	
6	46	36	41	0.29	
7	63	41	52	0.18	
8	60	41	50.5	0.15	
9	43	30	36.5		
10	55	28	41.5	0.01	
11	57	39	48	0.16	
12	40	28	34		
13	41	25	33	0.05	
14	39	27	33	0.11	
15	38	27	32.5		
16	28	18	23		
17	23	8	15.5	0.02	
18	40	20	30		
19	39	29	34	0.1	
20	36	23	29.5		
21	32	23	27.5		
22	42	19	30.5		
23	54	27	40.5		
24	50	39	44.5		
25	40	20	30		
26	36	28	32		
27	34	31	32.5	0.11	
28	57	31	44	0.21	
29	60	28	44	0.44	
30	46	23	34.5		
31	39	25	32		
				2.08	0

February					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	58	25	41.5		
2	60	44	52		
3	54	34	44		
4	37	27	32		
5	31	19	25		
6	42	12	27		
7	55	26	40.5		
8	71	49	60		
9	65	28	46.5		
10	68	34	51		
11	68	47	57.5		
12	68	54	61		
13	59	37	48		
14	49	26	37.5		
15	46	32	39		
16	41	29	35		
17	40	29	34.5		
18	44	24	34	0.13	
19	57	42	49.5	0.14	
20	48	26	37		
21	44	23	33.5		
22	51	35	43	0.07	
23	39	29	34		
24	38	24	31		
25	46	19	32.5		
26	56	30	43		
27	66	44	55		
28	61	37	49	0.12	
29			####		
				0.46	0

2009 Temperature and Rainfall Data

Painter, VA

March					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	39	35	37	0.81	
2	36	24	30	0.83	2.5
3	26	18	22		
4	35	14	24.5		
5	48	21	34.5		
6	67	40	53.5		
7	72	53	62.5		
8	75	56	65.5		
9	75	61	68		
10	66	37	51.5		
11	74	40	57		
12	74	42	58		
13	45	33	39	0.15	
14	42	35	38.5	0.28	
15	46	38	42	0.66	
16	49	43	46	0.25	
17	50	43	46.5	0.4	
18	58	30	44		
19	66	46	56		
20	54	42	48	0.13	
21	49	28	38.5		
22	56	27	41.5		
23	56	37	46.5		
24	46	28	37		
25	48	26	37		
26	56	43	49.5	0.03	
27	59	47	53	0.19	
28	64	46	55	1.06	
29	73	48	60.5	0.16	
30	70	46	58		
31	59	38	48.5		
				4.95	2.5

April					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	60	42	51		
2	65	53	59		
3	75	55	65	0.12	
4	73	51	62		
5	68	45	56.5		
6	64	53	58.5	0.65	
7	63	42	52.5	0.07	
8	56	40	48		
9	63	38	50.5		
10	75	49	62		
11	77	52	64.5	0.21	
12	52	40	46		
13	52	32	42		
14	65	45	55	0.69	
15	60	45	52.5	0.06	
16	57	39	48	0.11	
17	68	33	50.5		
18	74	45	59.5		
19	76	53	64.5		
20	62	50	56	0.14	
21	70	53	61.5	0.03	
22	68	51	59.5	0.25	
23	62	44	53		
24	68	41	54.5		
25	89	58	73.5		
26	89	69	79		
27	89	64	76.5		
28	87	66	76.5		
29	83	51	67		
30	68	50	59		
				2.33	0

2009 Temperature and Rainfall Data

Painter, VA

May						June					
	Temperature						Temperature				
Day	Max.	Min.	Mean	Rain	Snow	Day	Max.	Min.	Mean	Rain	Snow
1	80	55	67.5			1	82	60	71		
2	80	66	73			2	90	60	75		
3	80	55	67.5	0.41		3	89	65	77		
4	67	53	60	0.32		4	80	61	70.5	1.78	
5	67	56	61.5	0.29		5	80	61	70.5	1.42	
6	65	55	60	0.37		6	73	60	66.5	0.41	
7	78	59	68.5	0.32		7	75	58	66.5	0.01	
8	78	59	68.5	0.05		8	84	62	73		
9	86	68	77	0.03		9	86	70	78	0.01	
10	86	64	75	0.27		10	85	64	74.5	0.17	
11	75	53	64	0.03		11	84	69	76.5		
12	69	49	59	0.01		12	84	72	78		
13	69	47	58			13	85	69	77	1.23	
14	73	57	65			14	80	66	73		
15	78	66	72			15	80	61	70.5	0.01	
16	79	65	72			16	74	62	68		
17	77	55	66	0.65		17	72	64	68		
18	60	50	55	0.11		18	78	65	71.5	0.49	
19	62	45	53.5			19	82	66	74	0.86	
20	68	39	53.5			20	88	70	79	0.03	
21	74	44	59			21	85	71	78	0.85	
22	80	55	67.5			22	82	69	75.5		
23	80	62	71			23	82	64	73	0.05	
24	78	66	72			24	83	68	75.5		
25	78	70	74			25	86	71	78.5		
26	78	64	71			26	89	67	78		
27	77	61	69			27	85	74	79.5		
28	81	64	72.5			28	85	65	75		
29	83	66	74.5	0.64		29	85	67	76	0.34	
30	83	66	74.5	0.12		30	88	67	77.5		
31	82	67	74.5								
				3.62	0					7.66	0

2009 Temperature and Rainfall Data

Painter, VA

July					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	88	68	78		
2	88	68	78	0.2	
3	84	68	76		
4	83	69	76		
5	83	67	75	0.19	
6	80	66	73	0.12	
7	85	62	73.5		
8	85	68	76.5		
9	82	62	72		
10	75	59	67		
11	79	56	67.5		
12	88	73	80.5	0.03	
13	80	70	75	0.83	
14	82	71	76.5		
15	82	59	70.5		
16	86	73	79.5		
17	86	75	80.5	0.01	
18	84	74	79	0.05	
19	84	64	74		
20	79	67	73		
21	83	69	76		
22	84	67	75.5		
23	83	71	77		
24	86	73	79.5		
25	89	69	79		
26	90	75	82.5		
27	89	67	78	3.03	
28	85	70	77.5	0.28	
29	87	73	80	0.11	
30	86	73	79.5	0.06	
31	91	76	83.5	0.02	
				4.93	

August					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	91	70	80.5	0.1	
2	85	73	74	0.21	
3	87	74	82.5		
4	86	72	83.5		
5	90	75	84.5		
6	89	70	81.5	0.17	
7	81	64	81.5	0.05	
8	84	64	76	0.02	
9	92	73	76		
10	93	74	75		
11	93	76	77		
12	92	71	79.5	0.37	
13	92	71	85	1.29	
14	80	72	82.5		
15	81	71	84		
16	86	64	82		
17	88	66	78.5		
18	90	69	77		
19	92	78	78		
20	92	73	79	0.3	
21	91	77	81.5		
22	87	77	79	0.04	
23	84	73	79	0.35	
24	84	70	77	0.95	
25	87	69	78	0.01	
26	89	69	79		
27	90	73	81.5		
28	86	72	79		
29	86	72	79	0.14	
30	86	76	81		
31	85	64	74.5	0.1	
				4.1	0

2009 Temperature and Rainfall Data

Painter, VA

September					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	75	59	67		
2	76	59	67.5		
3	75	63	69		
4	82	67	74.5		
5	84	59	71.5		
6	84	59	71.5		
7	80	68	74	0.75	
8	72	68	70	3.06	
9	76	62	69	0.15	
10	70	64	67	0.16	
11	67	62	64.5	1.26	
12	75	57	66		
13	77	65	71	0.02	
14	80	61	70.5		
15	83	64	73.5		
16	82	66	74		
17	74	67	70.5		
18	76	57	66.5		
19	77	59	68		
20	73	49	61		
21	77	55	66		
22	80	66	73		
23	82	70	76	0.02	
24	86	72	79		
25	85	65	75	0.02	
26	71	61	66		
27	79	68	73.5	0.12	
28	81	56	68.5		
29	80	57	68.5		
30	71	59	65		
				5.56	

October					
	Temperature				
Day	Max.	Min.	Mean	Rain	Snow
1	70	51	60.5	0.01	
2	75	50	62.5		
3	79	66	72.5	0.01	
4	79	54	66.5		
5	75	54	64.5		
6	71	51	61		
7	78	64	71	0.06	
8	78	56	67		
9	82	53	67.5		
10	82	67	74.5		
11	68	48	58		
12	66	45	55.5		
13	73	53	63		
14	71	48	59.5		
15	58	49	53.5	1.11	
16	59	51	55	0.17	
17	56	48	52	0.53	
18	52	45	48.5	0.13	
19	55	40	47.5	0.01	
20	67	37	52		
21	74	45	59.5		
22	74	51	62.5		
23	76	55	65.5		
24	76	66	71		
25	74	56	65	0.32	
26	64	42	53		
27	66	58	62	0.35	
28	71	58	64.5	0.56	
29	70	54	62		
30	66	48	57		
31	77	59	68		
				3.26	

2009 Temperature and Rainfall Data

Painter, VA

November						December					
	Temperature						Temperature				
Day	Max.	Min.	Mean	Rain	Snow	Day	Max.	Min.	Mean	Rain	Snow
1	73	53	63	0.1		1	55	40	47.5	0.53	
2	58	51	54.5	0.02		2	58	38	48	0.1	
3	63	43	53			3	66	57	61.5	0.85	
4	63	40	51.5			4	60	39	49.5		
5	59	43	51			5	49	41	45	0.56	
6	55	45	50			6	41	35	38	0.13	
7	58	30	44			7	50	28	39		
8	69	43	56			8	46	33	39.5		
9	69	43	56			9	69	41	55	2.48	
10	69	53	61			10	68	45	56.5		
11	65	51	58	1.1		11	46	31	38.5		
12	54	51	52.5	3.63		12	44	25	34.5		
13	58	52	55	2.3		13	57	30	43.5	0.16	
14	57	54	55.5	0.07		14	53	36	44.5	0.11	
15	65	54	59.5			15	53	41	47		
16	71	48	59.5			16	48	37	42.5		
17	63	44	53.5			17	38	31	34.5		
18	62	47	54.5			18	37	23	30		
19	63	56	59.5	0.04		19	44	30	37	1.33	
20	63	55	59	0.07		20	36	29	32.5	0.03	0.5
21	57	39	48			21	41	32	36.5		
22	59	39	49			22	42	29	35.5		
23	56	50	53	0.12		23	42	26	34		
24	57	55	56	0.08		24	39	26	32.5		
25	59	53	56	0.06		25	52	27	39.5		
26	60	45	52.5	0.01		26	58	52	55	1.44	
27	54	41	47.5	0.73		27	58	41	49.5		
28	52	41	46.5			28	46	37	41.5		
29	64	36	50			29	41	31	36		
30	62	53	57.5	0.02		30	34	23	28.5	0.04	
						31	42	27	34.5	0.38	
				8.35						8.14	0

Appendix B

Appendix B-1. Analysis of variance table for an edamame cultivar evaluation trial conducted in Painter, VA during 2008 and 2009.

Source	DF	Sum of Squares	Mean Square	F Ratio	P-Value
Cultivar	4	95.74063	23.93516	4.484486	0.0066
Year	1	27.225	27.225	5.100871	0.0322
Cultivar x Year	4	50.13313	12.53328	2.348233	0.0797
Block	3	29.1385	9.712833	1.819795	0.1674
Error	27	144.1078	5.337324		
Total	39	346.345			

Appendix B-2. Analysis of variance table for the 100 seed weight of five edamame cultivars grown in Painter, VA during 2008 and 2009.

Source	DF	Sum of Squares	Mean Square	F Ratio	P-Value
Cultivar	4	1493.504	373.376	19.84206	<0.0001
Year	1	463.0802	463.0802	24.60915	<0.0001
Cultivar x Year	4	388.5335	97.13338	5.161892	0.0032
Block	3	14.0628	4.6876	0.24911	0.8612
Error	27	508.0697	18.8174		
Total	39	2867.25			

2008	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	956.737	239.1843	41.9211	<.0001
Block	3	34.1655	11.3885	1.996	0.1684
Error	12	68.467	5.706		
Total	19	1059.37			

2009	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	925.3	231.325	8.8208	0.0015
Block	3	104.8	34.93333	1.3321	0.3099
Error	12	314.7	26.225		
Total	19	1344.8			

Appendix B-3. Analysis of variance table for the percent marketable pods of five edamame cultivars grown in Painter, VA during 2008 and 2009.

Source	DF	Sum of Squares	Mean Square	F Ratio	P-Value
Cultivar	4	0.095208	0.023802	4.327432	0.0078
Year	1	0.123118	0.123118	22.38404	<0.0001
Cultivar x Year	4	0.01129	0.002823	0.513158	0.7318
Block	3	0.015483	0.005161	0.938319	0.4358
Error	27	0.148507	0.0055		
Total	39	0.393445			

Appendix B-4. Analysis of variance table for the harvest efficiency of five edamame cultivars grown in Painter, VA during 2008 and 2009. Due to the year x cultivar interaction, there is an ANOVA table below for each year. The percentage data in used in this analysis was transformed using the arcsine transformation.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Year	1	0.09800537	0.098005	16.7851	0.0003
Cultivar	4	0.04320224	0.010801	1.8498	0.1484
Year x Cultivar	4	0.10587362	0.026468	4.5332	0.0062
Block	3	0.04142178	0.013807	2.3647	0.0932
Error	27	0.15764863	0.005839		
Total	39	0.44615164			

2008	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	0.03575584	0.008939	1.0114	0.4397
Block	3	0.0132031	0.004401	0.498	0.6906
Error	12	0.10605871	0.008838		
Total	19	0.15501765			

2009	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	0.11332001	0.02833	7.7629	0.0025
Block	3	0.03601576	0.012005	3.2896	0.0581
Error	12	0.04379284	0.003649		
Total	19	0.19312861			

Appendix B-5. Analysis of variance tables for average plant height of five edamame cultivars grown in 2008 and 2009 in Painter, VA. There is an ANOVA table for each year below the whole model table because of the interactions between year and cultivar.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	198.271	49.5677	12.6678	<.0001
Year	1	4.84416	4.84416	1.238	0.2757
Cultivar*Year	4	60.1168	15.0292	3.8409	0.0135
Block	3	7.44998	2.48333	0.6347	0.5991
Error	27	105.648	3.9129		
Total	39	376.33			

2008	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	131.065	32.7663	10.3473	0.0007
Block	3	10.0985	3.36615	1.063	0.401
Error	12	37.9997	3.1666		
Total	19	179.163			

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	127.323	31.8306	6.2301	0.006
Block	3	3.68978	1.22993	0.2407	0.8663
Error	12	61.3103	5.1092		
Total	19	192.323			

Appendix B-6. Analysis of variance table for yield of a planting date study with three planting dates and two cultivars. The planting date study was conducted in Painter, VA during 2008 and 2009.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	1	13.878252	13.87825	3.4551	0.072
Planting date	2	21.931254	10.96563	2.73	0.08
Year	1	15.402002	15.402	3.8344	0.0587
Cultivar*Planting date	2	0.159254	0.079627	0.0198	0.9804
Cultivar*Year	1	0.003502	0.003502	0.0009	0.9766
Planting date*Year	2	32.912629	16.45631	4.0969	0.0257
Cultivar*Planting date*Year	2	5.929629	2.964815	0.7381	0.4857
Block	3	28.333173	9.444391	2.3513	0.0902
Error	33	132.55265	4.01675		
Total	47	251.10235			

Appendix B-7. Analysis of variance table for the percent marketable pods of a planting date study with two cultivars and three planting dates conducted in Painter, VA during 2008 and 2009

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	1	271.7008	271.7008	6.6912	0.0143
Planting date	2	1806.935	903.4675	22.2498	<0.0001
Cultivar*Planting date	2	118.0317	59.01585	1.4534	0.0546
Year	1	161.3333	161.3333	3.9732	0.2484
Cultivar*Year	1	124.1633	124.1633	3.0578	0.0896
Planting date*Year	2	865.5617	432.7809	10.6582	0.0003
Cultivar*Planting date*Year	2	277.3217	138.6609	3.4148	0.0449
Block	3	261.9008	87.30027	2.15	0.1127
Error	33	1339.984	40.606		
Total	47	5226.933			

Appendix B-8. Analysis of variance table for percent protein concentration in five edamame cultivars grown during 2008 and 2009 in Painter, VA. The data used in this analysis was transformed using the arcsine transformation. Due to the interactions between cultivar and year an analysis of variance table for each year is also below.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	0.000523	0.000131	3.7719	0.0403
Year	1	0.000389	0.000389	11.2312	0.0073
Cultivar x Year	4	0.001433	0.000358	10.3361	0.0014
Error	10	0.000347	0.000035		
Total	19	0.002691			

2008	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	0.000796	0.000199	6.193	0.0356
Error	5	0.000161	0.000032		
C. Total	9	0.000956			

2009	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	0.00116	0.00029	7.7976	0.0224
Error	5	0.000186	0.000037		
C. Total	9	0.001346			

Appendix B-9. Analysis of variance table for lipid concentrations of five edamame cultivars grown in Painter, VA during 2008 and 2009. Due to the significant interactions between cultivar and year, there are two ANOVA tables below for each year. The percentage data in these tables were transformed using the arcsine transformation

Source	DF	Sum of Squares	Men Square	F Ratio	Prob > F
Cultivar	4	36.35749	9.089372	713.4359	<0.0001
Year	1	29.11384	29.11384	2285.181	<0.0001
Cultivar x Year	4	4.686917	1.171729	91.9705	<0.0001
Error	10	0.127403	0.01274		
C. Total	19	70.28565			

2008	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	0.005955	1.49E-03	651.2497	<0.0001
Error	5	1.14E-05	2.29E-06		
C. Total	9	0.005966			

2009	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	0.001814	0.000453	164.7063	<.0001
Error	5	1.38E-05	2.75E-06		
C. Total	9	0.001828			

Appendix B-10. Analysis of variance table for a diphenylpicrylhydrazyl (DPPH) assay of five edamame cultivars grown in Painter, VA during 2008 and 2009. Due to significant interactions between year and cultivar, there is an ANOVA tables below for each year.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Year	1	17.58268	17.58268	5.3839	0.0276
Cultivar	4	211.3214	52.83036	16.1768	<0.0001
Year*Cultivar	4	78.20357	19.55089	5.9865	0.0012
Error	29	94.70829	3.2658		
C. Total	38	396.1833			

2008	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	4	197.8293	49.4573	8.6378	0.001
Error	14	80.16003	5.7257		
C. Total	18	277.9893			

2009	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	4	76.61955	19.1549	19.7497	<.0001
Error	15	14.54826	0.9699		
C. Total	19	91.16781			

Appendix B-11. Analysis of variance tables for an Oxygen Radical Absorbing Capacity (ORAC) Assay performed on five edamame cultivars. The edamame used in this assay was grown in Painter, VA during 2008 and 2009. Due to significant interactions between cultivar and year, there is an ANOVA tables below for each year.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Year	1	82.1205	82.1205	2.1656	0.1567
Cultivar	4	511.951	127.988	3.3751	0.0289
Year*Cultivar	4	631.154	157.788	4.161	0.013
Error	20	758.414	37.921		
Total	29	1983.64			

2008	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	313.547	78.3867	3.801	0.0395
Error	10	206.227	20.6227		
Total	14	519.773			

2009	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	829.558	207.39	3.7558	0.0408
Error	10	552.188	55.219		
Total	14	1381.75			

Appendix B-12 . Analysis of variance tables for five edamame cultivars grown in 2008 and 2009 in Painter, VA. Due to the three way interactions, below is an ANOVA table for each isoflavone for cultivar and year interactions and also an ANOVA table for each isoflavone by cultivar. Total isoflavones is not included in the whole model ANOVA table, only in the individual tables.

Whole Model	DF	Sum of Squares	F Ratio	Prob > F
Cultivar	4	34944.7	765.821	<.0001
Year	1	3013.53	264.168	<.0001
Cultivar x Year	4	7615.05	166.885	<.0001
Isoflavone	5	511124	8961.1	<.0001
Cultivar x Isoflavone	20	219835	963.545	<.0001
Year x Isoflavone	5	5627.33	98.6592	<.0001
Cultivar x Year x Isoflavone	20	60078.5	263.326	<.0001
Error	60	684.46	11.4	
Total	119	842922		

Daidzin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	3466.4	866.601	142.66	<.0001
Year	1	6677.46	6677.46	1099.25	<.0001
Cultivar x Year	4	3194.8	798.7	131.482	<.0001
Error	10	60.746	6.07		
Total	19	13399.4			

Genistin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	500.378	125.095	249.988	<.0001
Year	1	1265.34	1265.34	2528.65	<.0001
Cultivar x Year	4	429.456	107.364	214.556	<.0001
Error	10	5.004	0.5		
Total	19	2200.18			

Malonyl Genistin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	250460	62614.9	1035.56	<.0001
Year	1	6.56	6.56	0.1085	0.7486
Cultivar x Year	4	62786.9	15696.7	259.601	<.0001
Error	10	604.65	60.5		
Total	19	313858			

Appendix B-12. Continued

Genistein	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	4.08109	1.02027	1058.59	<.0001
Year	1	2.55723	2.55723	2653.27	<.0001
Cultivar x Year	4	4.08109	1.02027	1058.59	<.0001
Error	10	0.00964	0.00096		
Total	19	10.729			

Glycitein	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	339.956	84.9889	67.3478	<.0001
Year	1	668.538	668.538	529.769	<.0001
Cultivar x Year	4	1267.82	316.956	251.165	<.0001
Error	10	12.6194	1.262		
Total	19	2288.94			

Daidzein	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	9.26371	2.31593	16.1975	0.0002
Year	1	20.3969	20.3969	142.655	<.0001
Cultivar x Year	4	10.4711	2.61777	18.3086	0.0001
Error	10	1.42981	0.14298		
Total	19	41.5615			

Total Isoflavone Content	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
cultivar	4	209922	52480.6	545.676	<.0001
Year	1	17986.4	17986.4	187.016	<.0001
cultivar*Year	4	45751.4	11437.9	118.927	<.0001
Error	10	961.75	96.2		
C. Total	19	274622			

Appendix B-13. Analysis of variance tables for individual isoflavones concentrations of five edamame cultivars grown in in Painter, VA during 2008.

Daidzin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	6644.23	1661.06	137.544	<.0001
Error	5	60.3827	12.08		
Total	9	6704.61			

Genistin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	913.298	228.324	233.573	<.0001
Error	5	4.88765	0.978		
Total	9	918.185			

Malonyl Genistin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	273632	68408	3221.24	<.0001
Error	5	106.18	21.2		
Total	9	273738			

Daidzein	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	14.4275	3.60688	13.072	0.0074
Error	5	1.37962	0.27592		
Total	9	15.8071			

Glycitein	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	342.77	85.6924	33.9887	0.0008
Error	5	12.606	2.5212		
Total	9	355.376			

Genistein (Not found in 2008)	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	0	0		
Error	5	0	0		
	9	0			

Total Isoflavone Content	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	213693	53423.2	612.774	<.0001
Error	5	435.91	87.2		
Total	9	214129			

Appendix B-14. Analysis of variance tables for individual isoflavone concentrations of five edamame cultivars grown in Painter, VA during 2009.

Daidzin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	16.9713	4.24284	58.4198	0.0002
Error	5	0.36313	0.07263		
Total	9	17.3345			

Genistin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	16.5371	4.13427	177.639	<.0001
Error	5	0.11637	0.02327		
Total	9	16.6534			

Malonyl Genistin	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	39614.6	9903.65	99.3414	<.0001
Error	5	498.465	99.69		
Total	9	40113			

Daidzein	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	5.30729	1.32682	132.179	<.0001
Error	5	0.05019	0.01004		
Total	9	5.35748			

Glycitein	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	1265.01	316.253	117825	<.0001
Error	5	0.0134	0.00268		
Total	9	1265.02			

Genistein	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	8.16217	2.04054	1058.59	<.0001
Error	5	0.00964	0.00193		
Total	9	8.17181			

Total Isoflavone Concentration	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	4	41981.2	10495.3	99.7951	<.0001
Error	5	525.843	105.2		
Total	9	42507.1			

Appendix B-15. Average seed size of six edamame cultivars grown during 2009 in Painter, VA.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	5	5	0.077096	24.6595	<.0001
Sample Date	8	8	0.410857	82.1337	<.0001
Cultivar x Sample Date	40	40	0.03574	1.429	0.1102
Error	54	0.033766	0.000625		
Total	107	0.557459			