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Determination of Plant Spacing and Time of Planting in the Production of Edamame Soybeans for Optimal Yield and Seed Isoflavone Content in Tennessee

Debra Jean Strouse Carpenter
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Debra Jean Strouse Carpenter entitled "Determination of Plant Spacing and Time of Planting in the Production of Edamame Soybeans for Optimal Yield and Seed Isoflavone Content in Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Vincent R. Pantalone, Major Professor

We have read this thesis and recommend its acceptance:

Fred Allen, Dennis Deyton, Carl Sam

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Linda Painter

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A Thesis presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Debra Jean Strouse Carpenter

May 2007

Dedication

I dedicate this thesis to my patient and devoted husband *Don Carpenter* and to our beloved children *Lois Rose and Rebekah Jean Carpenter*, whose love and encouragement gave me the strength to do this; to my wise and loving parents *John and Lois Strouse*, whose guidance and faith made me who I am today, and to my dear friends *Sarah Shinpock, Kay Houser, and Amanda Dalton*, who cheered me on and helped me in more ways than I can mention.

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Abstract

The objectives of this study are three-fold: to determine the within row plant spacing and time of planting that will produce optimal yields and seed isoflavone content, to explore the feasibility of incorporating edamame soybeans [*Glycine max* (L.) Merrill] in a double-cropping system with strawberries [*Fragaria X ananassa*], and to study the potential as an edamame soybean of newly identified line *TN03-349*. Line *TN03-349* was planted into raised, plasticulture, irrigated strawberry beds at the University of Tennessee's East Tennessee Research and Education Center. Five within-row spacings were used (0.08m, 0.15m, 0.30m, 0.60m, and 1.20m) in 2004 and 2005. A second raised bed, irrigated plasticulture experiment was conducted at the Plateau Research and Education Center in Crossville, TN, using four soybean lines (*Gardensoy-43, TN00-60, USG 5601T,* and *TN03-349*) and two planting dates (May 24 and June 14, 2005). A final field experiment at the same location used the same four soybean lines, four within-row spacings (0.04m, 0.08m, 0.15m, and 0.30m), and three planting dates (May 24, June 14, and July 6) in 2005 and 2006. All experimental plantings were harvested at both the R6 (green) and the R8 (dry) stages. Analysis of R6 and R8 data, averaged over two years (2004 and 2005), from the East Tennessee location showed that total pod yield was highest up to plant spacing 0.30m (at a mean of 10,450 kg ha^{-1}) and dropped off at the 0.60m (8370 kg ha^{-1}) and 1.20m (5985 kg ha^{-1}) spacings. Similarly, shelled seed yield responded to spacing treatments with 3419 kg ha^{-1} at 0.30m and decreased to 1880 kg ha^{-1} at the 1.20m spacing. While seed numbers (per 100 pods) from R6 harvest did not change, seed size did, increasing as space between plants increased with means of 371mg $seed^{-1}$ for 0.08m and 916mg $seed^{-1}$ for 1.20m. Seed isoflavone content was not significantly affected by spacing treatment. Analysis of R6 data from the Plateau strawberry bed experiment showed that genotype significantly affected total plot yield ($p<0.01$) and seed size ($p<0.05$). While line *Gardensoy-43* had the lowest shelled seed yield, it had the largest seeds (1070mg $seed^{-1}$). The other lines

did not differ in shelled seed yield, but *TN00-60* and *TN03-349* differed from *USG 5601T* (smallest at 260mg seed^{-1}) in seed size. Planting date did not significantly affect yield or seed size. Analysis of R8 data at Crossville, TN showed line *TN03-349* to have significantly higher total yield than all others at 876 kg ha^{-1} and *Gardensoy-43* (lowest yield at 274 kg ha^{-1}) differed from *TN00-60* (549 kg ha^{-1}) and *USG 5601T* (497 kg ha^{-1}) where $p<0.0001$. Three isoflavones were significant for genotype: daidzin ($p<0.05$), malonyl daidzin ($p<0.001$), and malonyl glycitin ($p<0.0001$). Total seed isoflavone and oil content were not significantly affected by genotype. Seventeen of eighteen measured amino acids were affected by genotype (p-values varied). Results from the field experiment revealed that genotypic effects were significant ($p<0.01$) to all measurements across all three planting dates for the R6 development stage. The edamame lines *Gardensoy-43* and *TN03-349* consistently produced larger seeds and heavier pod weights than the others. Planting date also had a significant effect on seed size ($p<0.05$) with June giving the largest (mean seed size, 360mg seed^{-1}) and May the smallest (mean seed size, 350mg seed^{-1}) size. Spacing treatment significantly affected seed size ($p<0.05$), total pod yield ($p<0.01$), and shelled seed yield ($p<0.01$). The widest spacing treatment, 0.30m, gave the largest seed size (0.36g) while the closest spacing treatment, 0.04m, yielded the lowest seed size (0.35g). Total pod and shelled seed yields only differed at the widest spacing (0.30m) and were significantly lower than all the other treatments, which did not differ from each other. Analysis of R8 harvested soybeans for 2005 and 2006 revealed that the 2005 experimental plantings produced higher yields than those in 2006 ($p<0.0001$), which were reduced by deer damage. Genotype played a significant part in R8 seed yield ($p<0.0001$). Line *TN00-60* produced the highest weight (4374 kg ha^{-1}) and differed significantly from the other three lines, which were not different from each other (*Gardensoy-43*, 2941 kg ha^{-1}; *TN03-349*, 3373 kg ha^{-1}; *USG 5601T*, 2970 kg ha^{-1}). Spacing had no significant effect.

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PART I

Introduction and Literature Review

Soybean [*Glycine max* (L.) Merrill] is a versatile crop, widely adapted and grown world-wide. The major producers of the world's supply are the United States, Brazil, China, Argentina, and India. In the United States, approximately 30 million hectares are devoted to commercial (commodity) soybean production annually and U.S. farmers produce approximately 91 million metric tons each year. About one third of the total annual crop is exported, with China as the largest consumer (in 2003, 9.3 million metric tons), followed by Japan (3.7 million metric tons), the European Union and Mexico (3.3 million metric tons), and Taiwan (1.4 million metric tons) (USDA, 2003). The remaining two thirds are put to use in a myriad of ways. Soybean seed is generally composed of approximately 40% protein, 20% oil, and 35% carbohydrates. It is the high protein and oil content which make soybean so valuable. The oil is extracted to use in baking and frying, and is found in many prepared food products such as mayonnaise, margarine, peanut butter, salad dressings, bakery products, and others. Soybean oil also has industrial applications. Products such as inks, paints, crayons, epoxys, pesticides, and detergents frequently contain soybean oil. Soybean oil also serves as a lubricant in some industrial processes and as an additive to diesel fuel. Soybean meal is primarily used for livestock feed and is an important source of protein for non-ruminants such as poultry and swine. Soybean is also consumed directly by humans in the form of tofu, miso, textured soy protein, and as whole bean. It is the latter use that is of interest in this study.

Soybean seeds harvested and consumed whole at the green stage (corresponding to development stage R6, Fehr and Caviness, 1977) are known by the Japanese term, edamame (eh-dah-mah-may). Edamame has been consumed in Asia for centuries, where soy in its various forms is a dietary staple. Soybean varieties grown for use as edamame have a larger seed size than those grown for oil and meal. Edamame beans have been selected for flavor as well, with preference for a sweet and nutty, not “beany”, flavor. In recent years, the demand for edamame in the U.S. has risen significantly due to two very different factors. The first is the growing Asian population in this country, and the second is the result of research findings indicating that edamame (and soy foods in general) contain compounds with beneficial health effects for humans.

The compounds of interest are isoflavones, also called phytoestrogens. They are a sub-class of a larger group of flavonoids which are nutraceuticals (biologically active non-nutrients). Compared to most flavonoids, isoflavones are available only from a few sources in the plant kingdom. Soybeans are one of only a few known food plants to contain nutritionally relevant amounts of isoflavones, and this is a driving force behind the rising interest in edamame in the U.S.

Consumption of soyfoods (including edamame) in the U.S. has risen dramatically in the last five years, due primarily to the numerous studies which have highlighted the health benefits of soy protein and isoflavones. These studies prompted the October, 1999, FDA soy health claim which states, "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease."

The chemical structure of isoflavones is amazingly similar to that of mammalian estrogens (Figure 1.1). Because of the structural similarities, isoflavones are capable of binding to estrogen receptors and affect estrogen regulated gene products (Markiewicz et al., 1993, and Mayr et al., 1992). The primary isoflavones found in soybeans are genistein (4'5,7-trihydroxysoflavone) and daidzein (4',7-dihydroxyisoflavone), along with their respective β-glucosides (glucose is attached at the 7 position of the A ring), genistin and daidzin. Generally, a larger amount of genist(e)in is present in soybeans and soyfoods than daidz(e)in. Small amounts of a third isoflavone, glycitein (7,4'-dihydroxy-6- methoxyisoflavone), and its glycoside, glycitin, also exist in soybeans. In addition to these six isomers, each of the isoflavone glycosides can have an acetlyl or a malonyl group attached at carbon 6 of the A ring (Figure 1.2). Genist(e)in, in particular, binds with almost the same affinity as the endrogenous estrogen, 17-β- estradiol, to estrogen receptor beta (ERb)

Genistein: R_1=OH, R_2=H

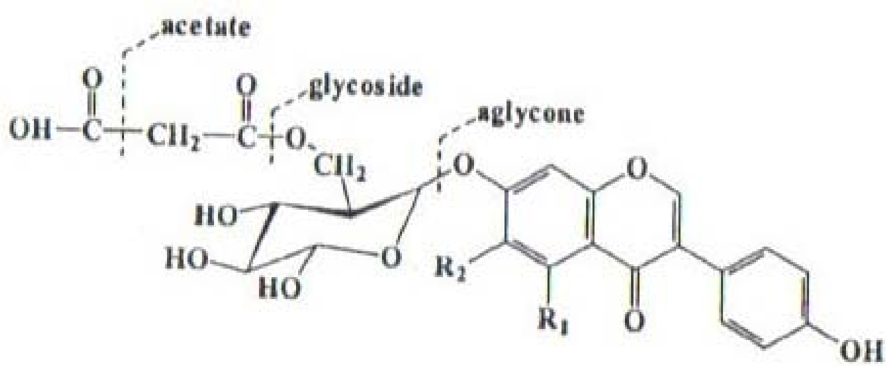
Daidzein: R_1=R_2=H

Glycitein: R_1=H, R_2=OMe

Endogenous estrogen:

17-β-Estradiol

Figure 1.1: Comparison of isoflavone structure with that of 17-β-Estradiol (Wu et al., 2004).



Daidzein: R_1=R_2=H

Glycitein: R_1=H, R_2=OMe

Genistein: R_1=OH, R_2=H

Figure 1.2: Representation of chemical structure of isoflavones. (Wu et al., 2004).

(Kuiper et al., 1998). Isoflavones may also be tissue selective, exerting pronounced estrogenic effects on some tissues (Walker et al., 2001), but not in others (Upmalis et al., 2000).

Because of these characteristics, several studies have attempted to determine if soy foods can reduce or reverse the effects of menopause. Potter et al. (1998) studied the influence of soy isoflavones and soy protein on blood lipids and bone density in post-menopausal women. Three groups of women consumed three different levels of soy isoflavones (in the form of isolated soy protein). Group one consumed protein from a source other than soy, group two consumed 55 mg isoflavones per day, and group three consumed 90 mg isoflavones daily. Non-HDL cholesterol in groups two and three was reduced compared to the first group. Significant increases in both bone mineral content and bone density were measured in group three. The researchers concluded that soy protein consumption by post-menopausal women may decrease the risk factors associated with cardiovascular disease through reduction of non-HDL cholesterol serum levels and that consumption of higher amounts of soy isoflavones (90mg/day) protected against spinal bone loss.

In a double blind experiment, Alekel et al. (2000) used 69 peri-menopausal subjects randomly assigned to one of three treatments, isoflavone-rich soy (SP1+, n=24), isoflavone-poor soy (SP1-, n=24), or whey protein (n=21). Measurements of lumbar spine bone mineral density (BMD) and bone mineral content (BMC) were taken pre-treatment for a baseline and post-treatment using dual-energy X-ray absorptiometry. The percentage change in lumbar spine BMD and BMC between baseline and post-treatment did not differ significantly from zero in the soy protein groups, but loss occurred in the whey protein control group (~1.5%). Regression analysis revealed that the SP1+ treatment had a positive effect on change in BMD (+5.6%) and BMC (+10.1%). Single degree of freedom contrasts using analysis of variance with BMD or BMC as the outcome revealed that it was the isoflavones, not the soy protein, that were responsible for the positive effect. The conclusion reached was that soy isoflavones attenuated bone loss from the lumbar spine in peri-menopausal women.

Nagata et al. (1998) studied the relationship between dietary intake of soy products and total serum cholesterol concentration. In 1992, 1242 men and 3596 women participated in an annual health check-up program in Takayama City, Japan. Soy intake, plus the intake of other various foods and nutrients, was assessed by a semi-quantitative food frequency questionnaire. Blood samples from fasting participants were taken to measure total serum cholesterol concentration. The trend observed was significant with a decrease of total serum cholesterol concentration associated with an increase in consumption of soy products in men after controlling for age, smoking status, and intake of total energy, protein, and fat. The negative trend was also observed in women after controlling for age, menopausal status, BMI, and intake of total energy and vitamin C. Results were not changed by an additional adjustment for physical activity, caffeine intake, and consumption of cholesterol, carbohydrates, fiber, and vitamin E. The researchers concluded that soy products might be useful in human cholesterol homeostasis.

Meta-analysis of 38 soy protein studies utilizing over 730 research volunteers (34 studies used only adults while 4 included only children) was conducted by Anderson et al. (1995). All 38 studies were controlled, clinical experiments published in peer-reviewed journals. Twenty of these studies used isolated soy protein, 15 used textured soy protein, and 3 a combination of the two. Average soy protein intake was 47g per day (range 17 – 124 g/day), and 15 studies used fewer than 31 g/day. In 14 studies, the test diets were comparable to a typical western diet while 21 studies used diets low in fat and cholesterol. In 19 studies, the test diets and control diets were comparable with respect to total fat, saturated fat, and cholesterol intake, and weight maintenance. Results of the analysis showed statistically significant reduction in serum cholesterol (9.3%), serum LDL cholesterol (12.9%), and serum triglycerides (10.5%) with the soy protein treatment. A non-statistically significant increase in HDL cholesterol also occurred (2.4%). Changes in serum cholesterol were highly correlated with pre-study serum LDL cholesterol levels. Volunteers with normal levels before the studies had LDL decreases of 7.7%, while those with severe hyper cholesterolemia had LDL decreases of 24% with the soy protein treatment. Adults and children did not differ in response. The results of this meta-analysis prompted the FDA to issue its health claim for soy protein in 1999.

While isoflavones are capable of mimicking estrogen, they are also capable of inhibiting estrogen, probably by tying up available sites and therefore creating anti-estrogenic activity. While estrogen replacement therapy aids in reducing many negative factors associated with menopause, it has been linked with an increase in hormone-dependent cancers. One published study (Horn-Ross et al., 2003) quantified the intake of specific phytoestrogenic compounds and related them to cancer risk. The top two quartiles of total isoflavones were associated with a 41% reduced risk of endometrial cancer, compared to the lowest quartiles of consumption. The reduced risk was associated with higher consumption levels of genistein and daidzein. Those researchers concluded that soy-based foods may have beneficial effects in some women, possibly greater in post-menopausal and obese women.

The estrogen-like structure of isoflavones and their affinity for endogenous estrogen sites are thought to be only partially responsible for their physiological effects. Genistein, in particular, has been found to inhibit the growth of a wide range of both hormone-dependent and independent cancer cells in vitro, probably resulting from genistein's ability to influence signal transduction (Weber et al., 1999). In vitro, genistein inhibits the activity of several enzymes and cellular factors responsible for the control of cell growth.

Pollard and Wolter (2000) conducted a study using Lobund-Wistar rats, which produce higher than normal levels of testosterone. Testosterone is known to promote the development of prostate cancer and about 30% of male Lobund-Wistar rats will develop prostate tumors. Approximately 200 male rats were divided into two groups, and were fed two different diets for a period of time ranging from 2 to 24 months. Group one received a natural diet containing soy meal while group two received the same diet but the soy protein was replaced with casein (milk protein). After 24 months, 3% of the group one rats and 30% of the rats in group two developed prostate cancer. These results suggest that the soy meal contained a factor with an agonist effect.

Zhou et al. (1999) transplanted human prostate cancer cells into 48 male SCID (immuno-deficient) mice and divided them into six groups based on protein source (casein or soy) and three levels of soy isoflavone concentration (0%, 0.2%, 1.0%). The soy protein source contained 2 mg g^{-1} isoflavones and the soy isoflavone concentrate contained 170 mg g^{-1} aglycone isoflavone equivalents (79.2 mg genistein, 70.4 mg daidzein, and 20.4 mg glycitein). Tumor size was reduced in the five groups consuming soy protein and/or soy isoflavone concentrate in the following manner:

- soy protein alone……………………………………………………………11%
- 0.2% soy isoflavone concentrate alone……………………………….19%
- soy protein + 0.2% isoflavone concentrate…………………………..28%
- 1.0% soy isoflavone concentrate alone……………………………….30%
- soy protein + 1.0% isoflavone concentrate…………………………..40%

The data strongly suggests that consumption of soy foods may inhibit the growth of experimental prostate cancer. Soy products appear to have both a direct and an indirect effect on cancer cell growth by inhibiting cell growth and influencing tumor neovasculature.

Data gathered from studies by Wei et al. (1995) suggest that the antioxidant properties of genistein may be responsible for its anticarcinogenic effect. This study investigated the antioxidant and anti-promotional effects of soybean isoflavones, using HL-60 cells and the mouse skin tumorigenesis model. Amounts of hydrogen peroxide (H_2O_2), a powerful oxidant, were measured in 12-0-tetradecanoylphorbol-13-acetate (TPA) activated HL-60 cells and superoxide anion generation by xanthine/xanthine oxidase. Results of the TPA test were: Genistein has the strongest antioxidant effect of the tested isoflavones, followed by daidzein. Results of the oxidase test were: Genistein, apigenin, and prunectin are equally capable of inhibiting O_2 generation by xanthine/xanthine oxidase. Daidzein exhibited only moderate effects. These results suggest that the structure of isoflavones are related to their antioxidant properties. Also, mice [*Mus musculu])* were fed 250 ppm genistein for 30 days and then sacrificed. Tests showed increased activity of antioxidant enzymes in the skin and small intestine. In another mouse study (Wei et al., 1995), low levels of genistein prolonged tumor latency significantly and lowered tumor multiplicity by approximately 50%.

As a result of these and other studies, the health benefits of soy consumption are being recognized by the general public and frozen edamame imports from China and Taiwan increased 20 fold between the 1980's and 2000. Demand is projected to continue to increase in the coming years. Currently, nearly all edamame consumed in the U.S. is imported. U.S. growers are not meeting the demand for fresh and frozen edamame but interest in commercial production of edible soybeans is rising. Although soybeans for human consumption have been cultivated in Asia for thousands of years, not enough research has been done on adaptation of edamame production to modern U.S. agriculture. This is a "new" crop in this country and growers need more information to efficiently and cost effectively produce edamame for the American market. American farmers are increasingly looking to diversify and expand their operations, and vegetable soybean production offers an opportunity. Southern tobacco [*Nicotiana tobaccum*] producers in particular, facing the end of the tobacco quota system and the removal of price supports, may need to look at an alternative crop. Also, the use of edamame in a double-cropping system with spring crops such as strawberries [*Fragaria X ananassa*] is possible due to the relatively short growing season needed when soybeans are harvested green.

Given the increasing evidence of soy's beneficial health effects and the rise in popularity of the edamame soybean, U.S. universities have begun to conduct field trials and agronomic studies on Asian and other varieties of edamame.

Different studies have evaluated optimal seeding rate, environmental and genotypic effects on isoflavone levels, isoflavone content of RoundUp Ready® versus conventional soybeans, and retention of soy isoflavones in edamame after processing. Most of these studies have been conducted by land-grant public universities and are published in the form of Agricultural Extension reports and publications and not as refereed journal articles. Thus, the current proposed study provides an opportunity to add to the scientific literature base.

At the University of Missouri Greenley Research Center, yield tests were conducted in 2001 on several Asian edamame varieties. The variety '*Sapporo Midori*' was used in a pod per plant optimal yield test. Given the fact that edamame seed is generally expensive (up to $48 per kg.), the influence of plant population on marketable pod yield is an important factor. Soybeans were planted in 0.762 m wide rows in 3.03 m x 9.09 m plots on June 20, 2001. The experiment was conducted as a randomized complete block design with five replications. The plot was maintained as a weed-free area for the experiment. Starter fertilizer was applied at planting with a side-dress application applied at the R1 development stage. Edamame soybeans were harvested by hand at the R6 stage. Pods were sorted by the number of beans per pod, frozen, counted, and then weighed. Pod numbers per plant were 25, 19, and 16 for 40,000, 70,000, and 100,000 plants per acre, respectively. Pod yield was maximized at 40,000 plants per acre. Total bean yield was highest for plant population at 100,000 plants per acre, but the difference between yields for populations of 40,000 and 100,000 plants per acre was not statistically significant.

According to the 2003 New Crop Opportunities Research Report published by the University of Kentucky, Department of Agronomy, a study conducted to determine the optimal seeding rate for novel soybean cultivars revealed a low variability in mean yields due to the seeding rate treatments. In that study, two high protein, two tofu, and two natto soybean varieties were planted at two different locations in 2002 and 2003. All experiments were planted as a randomized complete block with three replications. The treatments consisted of the six cultivars being tested and a range of seven seeding rates. The seeding rates were: 2, 3, 4, 5(standard), 6, 7, and 8 viable seeds per 0.30m of row. Plot sizes were six, 4.55m wide rows, 6.06m in length. Plantings were done May 17 and May 23, 2002 and June 2, 2003. Stand counts were taken from the center two rows of each plot and totals extrapolated. Seeding rates did impact plant stand. There was a linear increase in plant stand corresponding to a linear increase in seeding rate. Statistically, the seeding rate did not have a significant impact on yield. However, dry growing conditions in 2002 probably limited yield potential and the researchers concluded that further tests under more optimal growing conditions would be necessary to determine whether seeding rates of novel soybean cultivars does or does not impact yield.

Hoeck et al. (2000) of Iowa State University studied the effects of genotype and environment on isoflavone content of soybean. Nine isoflavones were measured in six cultivars grown in eight Iowa locations over a two year period. The mean contents of total isoflavones and six of the nine individual isoflavones were significantly higher in 1996 than in 1995. There were significant differences in total and individual isoflavone content among the planting locations in one or both years. The genotype, genotype x year, genotype x location, and genotype x year x location interactions were significant for both total and individual isoflavone contents. However, the differences between the cultivars with the highest and lowest total and individual isoflavone contents were relatively consistent across the different locations. The significant year x location interactions showed the impact of variable climatic conditions on isoflavone concentration.

Isoflavones, like many phytochemicals, are substances called phytoalexins formed by the plant tissue in response to physiological stimuli (infectious agents or their products) that accumulate to levels that inhibit the growth of micro-organisms (Dakora and Phillips, 1996). Isoflavones possess antifungal, antimicrobial, antioxidant, and other properties that enhance survivability of the soybean plant. Because of this, soybean isoflavone concentrations greatly increase when the plant is stressed, such as when moisture is limited, and are influenced by the environmental conditions under which the soybean is grown (Eldridge and Kwolek, 1983 and Wang and Murphy, 1994). Tsukamoto et al. (1995) observed that total isoflavone content was significantly lower for cultivars planted in April and May in Kyushu, Japan than for those planted in June and July. In a temperature-controlled growth chamber experiment, Tsukamoto et al. (1995) found that isoflavone concentrations were significantly greater when seed developed under low temperature than when development occurred at high temperature. Unlike many phytoalexins, however, isoflavones are always present in significant amounts in soybeans because one of their primary functions is to stimulate nodulation genes in the soil bacteria called *Bradyrhizobium japonica*. Rhizobia induce the formulation of nodules on legume roots (Rolfe, 1988). The rhizobia inside these nodules are able to reduce atmospheric nitrogen to ammonia which the plant then uses as a nitrogen source for growth.

Isoflavones are relatively stable compounds mostly unaffected by heat or freezing. In a study conducted by Simonne et al. (2000), the effects of processing on edamame isoflavone content were evaluated. The plants were hand harvested from the field at the R6 developmental stage and transported to a cool room (15°C). On the same day, the pods were hand-picked from the plants and hand-shelled to minimize damage. The shelled soybeans were divided into several 100g portions for various treatments (untreated control, blanching, boiling, freezing, and freeze-drying). Each treatment was completed in duplicate. Beans were blanched before freezing and freeze-drying. Significant loss of measured isoflavones occurred in all treatments except control due to leaching during the blanching or boiling process. Only 4% loss actually occurred as a result of the freeze-drying and less still in the frozen samples.

Maturity stage can affect isoflavone concentrations. Simonne et al. (2000) conducted an experiment that examined the effects of development stage on the isoflavone content of varieties developed for production in the southeast region of the U.S. It was determined that interaction between genotypes and stage of maturity was significant. The experimental lines showed a decrease in ratios of malonyldaidzin and glucosides from R6 to R8 whereas the commercial cultivar '*Hutcheson*' had no change. The experimental lines contained higher total isoflavones at the R6 stage but the opposite was observed with the *Hutcheson* soybeans. Thus, breeders may be able to capitalize on utilizing specific genetic backgrounds to enhance isoflavone concentrations at desired growth stages as new knowledge of soybean genotypes becomes available.

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PART II

Raised, Irrigated Bed Plasticulture of Edamame Soybean

Abstract

Recent studies have begun to reveal the benefits of soy consumption and demand for edamame soybeans [*Glycine max* (L.) Merrill] has risen rapidly in the U.S. in the last five years. Most of the edamame consumed in this country is currently imported, but interest in production of edamame soybean has risen as well. In order to provide U.S. growers with the most complete information possible, more research must be conducted on production methods. The objectives of this study are three-fold, to: (a) determine the within row plant spacing and time of planting that will produce optimal yields and seed isoflavone content, (b) explore the feasibility of incorporating edamame soybeans in a double-cropping system with strawberries [*Fragaria X ananassa*], and (c) study the potential as an edamame soybean of newly identified line '*TN03-349*'. Line *TN03-349* was planted into raised, irrigated plasticulture beds at the University of Tennessee's East Tennessee Research and Education Center (Knoxville, TN). Five within-row spacings were used (0.08m, 0.15m, 0.30m, 0.60m, and 1.20m) in 2004 and 2005. A second raised bed, irrigated plasticulture experiment was conducted at the Plateau Research and Education Center (Crossville, TN) using four soybean lines (*'Gardensoy-43', 'TN00-60', 'USG 5601T',* and *TN03-349*) and two planting dates (May 24 and June 14, 2005). All experimental plantings were harvested at both the R6 (green) and the R8 (dry) stages. Analysis of R6 and R8 data from the Knoxville location showed that total pod yield was highest up to plant spacing 0.30m (at a mean of 10,450 kg ha^{-1}) and dropped off at 0.60m (8370 kg ha^{-1}) and 1.20m (5985 kg ha^{-1}) spacing. While seed numbers (per 100 pods) from R6 harvest did not change, seed size did, increasing as space between plants increased with means of 371mg $seed^{-1}$ for 0.08m and 916mg $seed^{-1}$ for 1.20m. Seed isoflavone content was not significantly affected by spacing treatment. Analysis of R6 data from the Crossville location showed that genotype significantly affected total plot yield ($p<0.01$) and seed size ($p<0.05$). While line *Gardensoy-43* had the lowest shelled seed yield, it had the largest seeds (1070mg $seed^{-1}$). The

other lines did not differ in shelled seed yield, but *TN00-60* and *TN03-349* differed from *USG 5601T* (smallest at 260mg $seed^{-1}$) in seed size. Planting date did not significantly affect yield or seed size. Analysis of R8 data showed line *TN03-349* to have significantly higher total yield than all others at 876 kg ha^{-1} and *Gardensoy-43* (lowest yield at 274 kg ha^{-1}) differed from *TN00-60* (549 kg ha^{-1}) and *USG 5601T* (497 kg ha^{-1}), where $p<0.0001$. Three isoflavones were significant for genotype: daidzin ($p<0.05$), malonyl daidzin ($p<0.001$), and malonyl glycitin ($p<0.0001$). Total seed isoflavone and oil content were not significantly affected by genotype. Seventeen of eighteen measured amino acids were affected by genotype (p-values varied).

Part II of this thesis is a lightly revised version of a paper by the same name that will be submitted to the journal *Horticultural Technology* in 2007 by Debra J.S. Carpenter, Vincent R. Pantalone, Fred L. Allen, Dennis E. Deyton, and Carl E. Sams.

My primary contributions to this paper include (1) organization, development, and management in all aspects of this project, (2) collection of data from Knoxville and Plateau experimental plantings, (3) chemical analysis of seed for isoflavone and amino acid content, (4) statistical analysis of data, and (5) all of the writing with the exception of some minor revisions.

Introduction

Soybean [*Glycine max* (L.) Merrill] is a versatile crop, widely adapted and grown world-wide. The major producers of the world's supply are the United States, Brazil, China, Argentina, and India. In the United States, approximately 30 million hectares are devoted to commercial (commodity) soybean production annually and U.S. farmers produce approximately 91 million metric tons each year. About one third of the total annual crop is exported, with China as the largest consumer (in 2003, 9.3 million metric tons), followed by Japan (3.7 million metric tons), the European Union and Mexico (3.3 million metric tons), and Taiwan (1.4 million metric tons) (USDA, 2003). The remaining two thirds are put to use in a myriad of ways. Soybean seed is generally composed of approximately 40% protein, 20% oil, and 35% carbohydrates. It is the high protein and oil content which make soybean so valuable. The oil is extracted to use in baking and frying, and is found in many prepared food products such as mayonnaise, margarine, peanut butter, salad dressings, bakery products, and others. Soybean oil also has industrial applications. Products such as inks, paints, crayons, epoxys, pesticides, and detergents frequently contain soybean oil. Soybean oil also serves as a lubricant in some industrial processes and as an additive to diesel fuel. Soybean meal is primarily used for livestock feed and is an important source of protein for non-ruminants such as poultry and swine. Soybean is also consumed directly by humans in the form of tofu, miso, textured soy protein, and as whole bean. It is the latter use that is of interest in this study.

Soybean seeds harvested and consumed whole at the green stage (corresponding to development stage R6, Fehr and Caviness, 1977) are known by the Japanese term, edamame (eh-dah-mah-may). Edamame has been consumed in Asia for centuries, where soy in its various forms is a dietary staple. Soybean varieties grown for use as edamame have a larger seed size than those grown for oil and meal. Edamame beans have been selected for flavor as well, with preference for a sweet and nutty, not “beany”, flavor. In recent years, the demand for edamame in the U.S. has risen significantly due to two very different factors. The first is the growing Asian population in this country, and the second is the result of research findings indicating that edamame (and soy foods in general) contain compounds with beneficial health effects for humans.

The compounds of interest are isoflavones, also called phytoestrogens. They are a sub-class of a larger group of flavonoids which are nutraceuticals (biologically active non-nutrients). Compared to most flavonoids, isoflavones are available only from a few sources in the plant kingdom. Soybeans are one of only a few known food plants to contain nutritionally relevant amounts of isoflavones, and this is a driving force behind the rising interest in edamame in the U.S.

Consumption of soyfoods (including edamame) in the U.S. has risen dramatically in the last five years, due primarily to the numerous studies which have highlighted the health benefits of soy protein and isoflavones. These studies prompted the October, 1999, FDA soy health claim which states, “25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.”

Frozen edamame imports from China and Taiwan increased 20 fold between the 1980’s and 2000. Demand is projected to continue to increase in the coming years. Currently, nearly all edamame consumed in the U.S. is imported. U.S. growers are not meeting the demand for fresh and frozen edamame but interest in commercial production of edible soybeans is rising. Although soybeans for human consumption have been cultivated in Asia for thousands of years, not enough research has been done on adaptation of edamame production to modern U.S. agriculture. This is a “new” crop in this country and growers need more information to efficiently and cost effectively produce edamame for the American market. American farmers are increasingly looking to diversify and expand their operations, and vegetable soybean production offers an opportunity. Southern tobacco [*Nicotiana tobaccum*] producers in particular, facing the end of the tobacco quota system and the removal of price supports, may need to look at an alternative crop. Also, the use of edamame in a double-cropping system with spring crops such as strawberries [*Fragaria X ananassa*] is possible due to the relatively short growing season needed when soybeans are harvested green.

Materials and Methods, General:

Description of Cultivars, Lines, and Experimental Field Procedures

Four lines were utilized for these studies: i) '*Gardensoy-43',* a marketed edamame cultivar, ii) a new high yielding line, '*TN00-60',* iii) Tennessee Agricultural Experiment Station cultivar '*USG 5601T'* (Pantalone et al., 2004) which currently serves as a USDA check in the Southern Uniform Maturity Group (MG) V tests, and which contains high levels of isoflavones (Charron et al., 2005), and iv) '*TN03-349',* a promising candidate for edamame production in the mid-south and southeast region. Relative maturities for these lines are 4.6, 4.8, 5.6, and 6.0, respectively.

The experimental field procedures for planting are divided into two categories: i) within-row spacing into raised, irrigated plasticulture beds and ii) time of planting into raised, irrigated plasticulture beds. Two primary attributes were analyzed through these studies: a) agronomic traits and b) chemical composition. Seed protein and oil concentration, and amino acid composition were evaluated at the R8 development stage; isoflavone concentration was evaluated at both the R6 and R8 development stages. Two different harvest methods were used depending on the stage of development and planting location. The soybeans were hand-harvested at the R6 stage and combined at the R8 stage.

Plantings took place at two locations in Tennessee, the East Tennessee Research and Education Center (Knoxville) and the Plateau Research and Education Center (Crossville).

Isoflavone Extraction and Analysis

Approximately 50 seeds were randomly selected from the previously shelled green plot samples. Excess moisture was removed by freeze-drying the 50 seed samples to facilitate more efficient grinding of the samples for extraction. The samples were then ground in a water-cooled Knifetec 1095 Sample Mill (FOSS Tecator, S-26321, Hogana, Sweden) for 20 seconds. This setting produces soybean flour with a uniform particle size. The strawberry bed plots harvested at the R8 stage were processed for extraction by first grinding a random sample of the mature beans from each plot in a water-cooled Knifetec. At least 300 mg of the resulting soybean meal was placed in a plastic weigh boat and then dried at 45°C for one hour. Isoflavone extraction was accomplished using a protocol modified from Griffith and Collison (2001). The procedure is as follows:

1. Weigh 200.0 ± 10.0 mg of ground sample into a glass culture tube (16 x 100 mm).
2. Prepare internal standard. Weigh 10.0 mg apigenin onto weigh paper, transfer to 10-ml volumetric flask. Bring to volume with dimethyl sulfoxide. Add stir bar and mix for 5 minutes. Place 1-ml aliquots of internal standard solution into 2-ml glass vials and store at -20°C.
3. Add 2.0 ml acetonitrile, 1.2 ml high-purity deionized water (at least 18.1 megohms), and 100 µl of internal standard (apigenin, 1.0 mg ml^{-1}) to glass culture tube containing sample. Cap each tube.
4. Place on rotary mixer (Vortex Genie, setting = 3) and extract for 2 hours.
5. Add 0.7 ml high purity water and vortex for 3-5 seconds.
6. Centrifuge for 10 minutes at 2000 *g* and 20°C.
7. Remove 1 ml of supernatant with a 1-ml plastic syringe, put on a nylon 0.45 µm filter, inject into a 2-ml cryovial, and store at -80°C.

Following extraction, HPLC analysis took place. The protocol used for this analysis was also drawn from the method presented by Griffith et al. (2001). The column used for this procedure was a YMC ODS-AM, 250x3 mm with 5 µm packing. Volume of sample injected was 5µl. The solvent flow-rate was set at 8 ml per minute. Column temperature was maintained at 40°C. Solvent A was 0.1% acetic acid in water and solvent B was 0.1% acetic acid in acetonitrile. Gradient elution was from 12% to 24% solvent B in a linear gradient over 34 minutes. The gradient began immediately upon sample injection. The column was then washed with 90% B for 3 minutes and equilibrated for 10 minutes between runs at 12% B. Total sample to sample time for this method was 51 minutes. Detection was by UV absorbance at 260 nm. Peak areas were integrated for quantification.

Determination of Seed Protein and Oil Content, and Amino Acid Composition

Approximately 20 g of the dry-combined soybean seeds was ground in a water-cooled Knifetec 1095 Sample Mill (FOSS Tecator, S-26321, Hogana, Sweden) for 20 seconds. The near infrared (NIR) instrument (NIR 6500, FOSS North America) required a warm up period of 2 hours once the lamp was turned on, after which auto diagnostics were run for instrument response, wavelength accuracy and NIR repeatability. A dehumidifier was used during the analysis to reduce room humidity to 40%, and room temperature was maintained at approximately 20ºC. The ground soybean samples were then scanned to obtain the predicted concentrations of all 18 amino acids, plus protein and oil concentration, using Winisi II 1.5 software.

Data Collection and Analyses

Plot yields for the edamame soybeans were determined by weighing the pods picked from each plot. Also, 100 pods were randomly selected from each plot, shelled, and the beans weighed. Shelled seed yields per plot were then extrapolated from the 100 bean sample. Seed size comparison was facilitated by weighing a random selection of 100 seeds of the shelled beans from each plot. Isoflavone content was measured by HPLC analysis. Seed protein, oil concentration (g kg^{-1}), and amino acid composition were measured using a FOSS 6500 NIR spectrometer.

The statistical methods that were utilized for comparison of plot yields, seed size, isoflavone content, oil content, and amino acid composition are analysis of variance using the MIXED procedure, and regression analysis. Data were analyzed by SAS v9.1.3 (SAS Institute, 2005).

East Tennessee Research and Education Center Study
Materials and Methods:

At the Knoxville location, soybean line *TN03-349* was planted following strawberries into four raised, irrigated plasticulture beds in 2004 and 2005. Five spacing intervals were utilized, 0.08m, 0.15m, 0.30m, 0.60m, and 1.20m, in 40 randomized plots (Figure 2.1). The plants were hand-sown in double rows approximately 0.45 m apart, three to a hill, and hand-thinned to one plant per hill, post emergence. One side of each double row was harvested by hand at the green stage (R6), and one side was harvested with a combine at maturity (R8). There were four replicates, each consisting of a 32.4m length of strawberry bed planted using the five spacing treatments, each in a 6.0m section, with a 0.60m alley between each spacing treatment. Each spacing treatment x harvest stage (R6 or R8) was designated as a plot, for a total of ten plots per replication. At harvest, all plots were end-trimmed to 4.9m by hand and harvested by hand cutting the whole plant at the base.

Data collected were total yield per plot (both R6 and R8 stages), seed size (R6 stage only), isoflavone content, protein and oil content (R8 stage only), and amino acid composition (R8 stage only).

Plateau Research and Education Center Study
Materials and Methods:

A second raised bed experiment was conducted at the Plateau Research and Education Center, Crossville, TN. The four lines described previously (*Gardensoy-43, TN00-60, USG 5601T, and TN03-349*) were used in a time of planting study utilizing two raised, irrigated plasticulture beds. Two planting

1.20m R6	spacing R8
0.075m R6	Spacing R8
0.60m R6	Spacing R8
0.30m R6	Spacing R8
0.15m R6	Spacing R8

REP 1

0.30m R6	spacing R8
0.15m R6	Spacing R8
1.20m R6	Spacing R8
0.075m R6	Spacing R8
0.60m R6	Spacing R8

REP 2

0.60m R6	spacing R8
0.075m R6	Spacing R8
0.30m R6	Spacing R8
0.15m R6	Spacing R8
1.20m R6	Spacing R8

REP 3

1.20m R6	spacing R8
0.30m R6	Spacing R8
0.075m R6	Spacing R8
0.15m R6	Spacing R8
0.60m R6	Spacing R8

REP 4

Figure 2.1: Illustration of plot design for one year including spacing treatment and stage of harvest; raised, irrigated plasticulture bed experiment, East Tennessee Research and Education Center, Knoxville, TN.

dates (at three week intervals) were used in this study, May 24 and June 14. Seeds were hand-planted at 0.30m intervals in double rows approximately 0.45m apart, three seeds to a hill and thinned to one plant per hill post emergence. One side of the double row was harvested green and one side dry. Each plot consisted of planting date treatment x 1 genotype x harvest stage, three replicates each for all three time of planting treatments, therefore 24 plots per planting date treatment. Plot length was 3.63m with a 0.9m alley between each genotype, requiring a total bed length of 53.6m for each planting date treatment (Figure 2.2). Plots were end-trimmed to 3.0m at harvest by hand and harvested by hand-cutting five randomly chosen plants at the base.

Data collected were yield totals for the five plants per plot (both R6 and R8 stages), seed size and isoflavone content (R6 stage only), protein and oil content (R8 stage only), and amino acid composition (R8 stage only).

Results and Discussion

Edamame soybeans are judged by consumers according to bean size and flavor. Producers of edamame must also be concerned with yield if they are to have a profitable operation. These experiments were an effort to determine the effect of plant spacing, time of planting, and genotype on both yield and bean size. Isoflavone, protein, and oil content were also measured on the soybeans line used in this experiment to aid in evaluation for potential edamame production. Because the soybeans in this study were grown under irrigated, plasticulture conditions, the effects of rainfall and weed competition were not considered to be concerns.

Yield data evaluated from the R6 harvest of the Knoxville location experiment for each spacing treatment included pod yield (kg ha^{-1}), shelled seed yield (kg ha^{-1}), seed weight (g) from 100 randomly selected pods/plot,

Rep	Green Plot	Genotype	Dry Plot
1	1201	Gardensoy-43	1301
1	1202	TN00-60	1302
1	1203	TN03-349	1303
1	1204	5601T	1304
2	1205	Gardensoy-43	1305
2	1206	TN00-60	1306
2	1207	TN03-349	1307
2	1208	5601T	1308
3	1209	TN00-60	1309
3	1210	TN03-349	1310
3	1211	5601T	1311
3	1212	Gardensoy-43	1312

Figure 2.2: Illustration of plot design for one planting date; Plateau Research and Education Center, Crossville, TN, raised irrigated plasticulture bed experiment.

number of seeds from 100 pods/plot and seed size (g). Spacing was a statistically significant contributor to overall yield as measured by pod yield and shelled seed yield. As shown in table 2.1, averaged over two years (2004-2005), yields from the denser spacing treatments 0.08m, 0.15m, and 0.30m were significantly greater than pod yields from the treatments 0.60m and 1.20m ($p<0.0001$). While pod yield from the closest spacing (0.08m) measured 11,477 kg ha^{-1}, the widest spacing (1.20m) produced significantly lower pod yield than all other treatments at 5985 kg ha^{-1}. Similarly, shelled seed yields responded with 3416 kg ha^{-1} at the 0.08m spacing, 3265 kg ha^{-1} for 0.15m, 3419 kg ha^{-1} for 0.30m, 2868 kg ha^{-1} for 0.60m, and 1880 kg ha^{-1} for 1.20m. Seed weight per 100 pods was also effected by spacing treatment ($p<0.0001$), although the number of seeds per 100 pods was not significant ($p>0.05$). Seed weight per 100 pods was greatest for spacing treatment 1.20m at 91.6g, and least for treatment 0.08m at 91.6g (data not shown). Treatments 1.20m, 0.60m, and 0.30m yielded values that were statistically the same, while treatments 0.15m and 0.08m differed from the other treatments and each other. These results show that seed size increased as plant spacing increased, although differences were not statistically significant. Thus, producers could optimize plant spacing at 0.30m to produce both larger seed and high yield levels.

As noted at the time of the green (R6) harvest, plant growth characteristics changed as spacing between plants increased. Plants grown under treatment 0.08m had slender, unbranched stems and pods were borne only along the stem. Stem thickness and amount of lateral branching increased as spacing between plants increased. Plants grown 1.20m apart exhibited extremely thick stems and were heavily branched, with pods borne mostly on the lateral branches. Yield data suggests that this ability to branch and spread in response to increasing space between plants allowed yields to remain statistically the same for the spacing treatments 0.08m, 0.15m, and 0.30m. Plants were unable to compensate adequately at the 0.60m and 1.20m spacings and so yields were negatively affected.

Table 2.1: Mean total pod yield and shelled seed yield in kg ha^{-1} of soybean line *TN03-349* at R6 stage of development in response to within-row plant spacing during 2004 and 2005 raised bed, irrigated plasticulture experiment, Knoxville, TN.

	†Pod Yield (kg ha^{-1})			†Shelled Seed Yield (kg ha^{-1})		
Spacing (m)	**2004**	**2005**	**2004-05**	**2004**	**2005**	**2004-05**
0.08	11520a	11392a	11477a	3402a	3430a	3416a
0.15	10811a	10695a	10759ab	3227a	3303a	3265a
0.30	11074a	9740ab	10450ab	3450a	3388a	3419a
0.60	9029b	7665ab	8370bc	2989b	2747b	2868b
1.20	6227c	5784b	5985c	1831c	1930c	1880c

†Means followed by the same letter within a column are not significantly different at the 0.05 level of significance using the Tukey-Kramer method.

Dry harvest (R8) yields, measured in kg ha^{-1}, were also affected by spacing. Yields for spacing treatments 0.08m, 0.15m, and 0.30m did not differ statistically, with the highest (0.08m) producing 2,209 kg ha^{-1}. The 1.20m spacing treatment had the lowest yield at 1,121 kg ha^{-1}, and was not statistically different than the 0.60m spacing (Table 2.2).

Isoflavone analysis of both green (R6) and dry (R8) harvest samples yielded no significant differences for spacing treatments in total seed isoflavone content ($p>0.05$). When individual isoflavones from the two experimental years were analyzed separately, two different isoflavones showed significance for each year (data not shown). For the 2004 R8 harvest, daidzin was significantly different for the spacing treatments ($p<0.05$). In (R8) 2005, genistin was significantly different for the spacing treatments ($p<0.05$). For the R6 soybeans, isoflavone analysis revealed no significant difference ($p>0.05$) for total seed isoflavone content when both years were analyzed together. When data from 2004 and 2005 were analyzed separately, however, total seed isoflavone content differed between spacing treatments in 2004 ($p<0.05$) but not in 2005 ($p>0.05$). No difference was found for any of the isoflavones when data from both years were analyzed together, but differences did appear when each year's data was analyzed separately. In 2004, genistin and malonyl genistin differed according to spacing treatment ($p<0.05$). Treatment 0.30m had the highest levels of both isoflavones. In 2005, only genistin ($p<0.05$) differed according to treatment. Spacing 0.60m yielded the highest levels of genistin. Isoflavone levels have been shown to fluctuate depending on the temperatures during the growing season (Tsukamoto et al., 1995). Therefore, this may explain the different results for different growing seasons. While water availability and weed competition could be virtually eliminated as variables in this experiment, temperature could not.

Analysis of individual amino acid and total protein content for R8 harvested beans did not reveal any differences due to spacing treatments ($p>0.05$) for either 2004 or 2005. Seed oil content differences were also not significant.

Table 2.2: Dry yield response (kg ha^{-1}) at R8 development stage of soybean line *TN03-349* to within-row spacing treatments, grown in irrigated plasticulture raised beds, Knoxville, TN (2004-2005).

Spacing (m)	†Yield 2004 (kg ha^{-1})	†Yield 2005 (kg ha^{-1})	†Yield 2004-05 (kg ha^{-1})
0.08	2763a	1654a	2209a
0.15	2493a	1621a	2057a
0.30	1688b	1652a	1737ab
0.60	1455b	1132a	1293b
1.20	1239b	1003a	1121c

†Means followed by the same letter within a column are not significantly different at the 0.05 level of significance using the Tukey-Kramer method.

Analysis of individual amino acid and total protein content for R8 harvested beans did not reveal any differences due to spacing treatments ($p>0.05$) for either 2004 or 2005. Seed oil content differences were also not significant.

Analysis of yield data from the experimental plantings at the Crossville, TN location revealed that genotype had a measurable effect on R6 total pod and shelled seed yield ($p<0.01$), seed weight per 100 pods ($p<0.0001$), number of seeds per 100 pods ($p<0.0001$), and seed size ($p<0.05$). When shelled seed yield was examined, three of the genotypes were statistically the same (*USG 5601T, TN00-60*, and *TN03-349*) with significantly higher yield than the fourth (*Gardensoy-43*), see Table 2.3. Number of seeds per 100 pod data gave the following results: *TN00-60* had the highest number, and differed significantly from all others. *Gardensoy-43* differed from *TN03-349* (which had the lowest number) but *TN03-349* did not differ from *USG 5601T*. The genotype with the lowest total seed yield (*Gardensoy-43*) had the highest seed weight per 100 pods (163.7g) and differed significantly from all others. Average seed size for each genotype was extrapolated from seed weight and number of seeds data and proved to be significant for genotype at the 0.05 probability level. *Gardensoy-43*, with the largest seed size (1070mg seed^{-1}, see Figure 2.3), did not differ from *TN00-60*, but was significantly different from *TN03-349* and *USG 5601T*, which had the smallest seeds at 260mg seed^{-1}. However, *TN00-60* was not found to differ *from TN03-349* or *USG 5601T*.

While genotypic effects were statistically significant, planting date effects were not except for total pod yield ($p<0.05$). The May plantings yielded higher at 9854 kg ha^{-1}, with June differing statistically at 8187 kg ha^{-1}. Shelled seed yield (kg ha^{-1}) was not affected. There was no significant genotype x planting date interaction ($p>0.05$).

Dry (R8) yields were analyzed with consideration to genotypic effects using seed weight per plot (represented by seeds harvested from five randomly chosen plants per plot) converted into kg ha^{-1} and found to have significance at the $p<0.0001$ probability level. Soybean line *TN03-349* had the highest dry seed

Table 2.3: Comparison of R6 development stage means for pod yield and shelled seed yield (kg ha^{-1}) in four genotypes grown under raised bed, irrigated plasticulture conditions at Crossville, TN, 2005.

Genotype	†Pod Yield (kg ha^{-1})	†Shelled Seed Yield (kg ha^{-1})
Gardensoy-43	5592b	2783b
TN03-349	10642a	6057a
USG 5601T	10065a	6711a
TN00-60	9783a	5075a

†Means followed by the same letter within a column are not significantly different at the 0.05 level of significance using the Tukey-Kramer method.

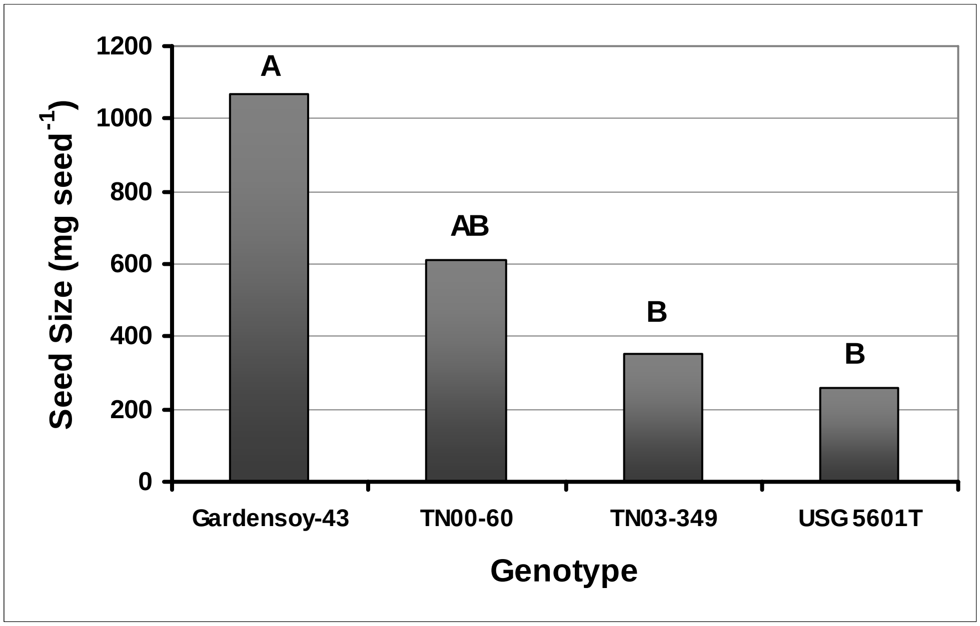

Figure 2.3: Comparison of R6 development stage seed size of four genotypes grown at Crossville, TN, 2005 under raised bed, irrigated plasticulture conditions. Means followed by the same letter within a column are not significantly different at the 0.05 level of significance using the Tukey-Kramer method.

yield (876 kg ha^{-1}) and differed significantly from all others. *TN00-60* and *USG 5601T* were similar, but *Gardensoy-43* was different from all others with the lowest seed yield at 274 kg ha^{-1} (Table 2.4). The R8 harvested seeds were also analyzed for isoflavone, amino acid, total protein, and oil content. Only three isoflavones were found to differ with respect to genotype: daidzin ($p<0.05$), malonyl daidzi ($p<0.001$), and malonyl glycitin ($p<0.0001$). Planting date did affect isoflavone content with June yielding higher levels of most isoflavones. See Table 2.5. Seventeen of the eighteen amino acids measured differed with respect to genotype (p-values varied, see Table 2.6). Total seed isoflavone, total protein, and oil content were not significant ($p>0.05$).

Table 2.4: Dry yield (kg ha^{-1}) at R8 stage of development of four genotypes planted at Crossville, TN, 2005 in an irrigated, raised bed plasticulture system.

Genotype	†R8 Total Yield (kg ha-1)
Gardensoy-43	274c
TN03-349	876a
USG 5601T	497b
TN00-60	549b

†Means followed by the same letter within a column are not significantly different at the 0.05 level of significance using the Tukey-Kramer method.

Figure 2.5: R8 development stage isoflavone content of four soybean genotypes planted on two different dates at Crossville, TN in 2005. Tukey-Kramer method of mean separation used.

Genotype	daidzin	glycitin	genistin	malonyl daidzin	malonyl glycitin	malonyl genistin	daidzein	genistein	Total
					$\mu g\ g^{-1}$				
Gardensoy-43	3.9	1	1.5	39.9	6.4	30.3	0.14	0.15	83.47
TN03-349	3.8	1.3	2.8	66.8	5.4	53.3	0.03	0.06	132.78
USG 5601T	5.7	0.3	2.6	24.8	1.3	14.3	0.14	0.11	48.92
TN00-60	2.9	0.8	1.4	67.7	3.8	35.3	0.17	0.06	113.35
LSD (0.05)	1.8	2.2	3.2	86.3	7.3	54.4	0.09	0.17	149
Date									
May	2.8	0.8	1.1	62.2	1.7	38.9	0.12	0.11	75.18
June	5.3	0.9	3	37.4	1.5	27.7	0.12	0.08	114.09
LSD (0.05)	1.1	0.9	1.3	1.6	1.6	1.6	0.06	0.06	1.63

Table 2.6: Total protein and eighteen individual amino acids measured (g kg^{-1}) in R8 harvested soybeans from four genotypes grown in a raised bed, irrigated plasticulture experiment at Crossville, TN, 2005.

Genotype	Aspartic	Threonine	Serine	Glutamine	Proline	Glycine	Alanine	Cysteine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine	Tryptophan	Total Protein
Gardensoy-43	4.63	2.25	3.50	4.23	2.76	3.56	2.60	0.63	3.07	0.85	1.94	2.46	2.07	1.98	1.57	2.05	3.44	0.45	44.03
TN03-349	4.72	2.31	3.71	3.86	2.83	3.80	2.67	0.60	3.13	0.86	1.93	2.35	2.10	2.02	1.59	1.97	3.59	0.48	44.54
USG5601T	4.73	2.36	3.74	3.93	2.83	3.82	2.68	0.62	3.17	0.87	1.94	2.37	2.14	2.04	1.60	2.03	3.54	0.48	44.90
TN00-60	4.36	2.20	3.38	3.77	2.71	3.51	2.54	0.58	3.04	0.82	1.86	2.34	2.00	1.87	1.51	1.92	3.19	0.43	42.02
LSD (0.05)	0.19	0.07	0.16	0.38	0.07	0.21	0.07	0.05	0.09	0.02	0.07	0.15	0.06	0.09	0.05	0.12	0.19	0.02	1.36

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Part III

Attaining Maximum Yield with Optimal Spacing and Time of Planting for Edamame Soybean

Abstract

The objectives of this study are two-fold: to determine the within row plant spacing and time of planting that will produce optimal yields, and to study the potential as an edamame soybean [*Glycine max* (L.) Merrill] of newly identified line '*TN03-349*'. A field experiment at the Plateau Research and Education Center near Crossville, TN, used four soybean lines ('*Gardensoy-43*', '*TN00-60*', '*USG 5601T*', and *TN03-349*), four within-row spacings (0.04m, 0.08m, 0.15m, and 0.30m), and three planting dates (May 24, June 14, and July 6) in 2005 and 2006. All experimental plantings were harvested at both the R6 (green) and the R8 (dry) developmental stages. Results revealed that genotypic effects were significant ($p<0.01$) to all measurements across all three planting dates for the R6 development stage. The edamame lines *Gardensoy-43* and *TN03-349* consistently produced larger seeds and heavier pod weights than the others. Planting date also had a significant effect on seed size ($p<0.05$) with June giving the largest (mean seed size, 360mg $seed^{-1}$) and May the smallest (mean seed size, 350mg $seed^{-1}$) sizes. Spacing treatment significantly affected seed size ($p<0.05$), total pod yield ($p<0.01$), and shelled seed yield ($p<0.01$). The widest spacing treatment, 0.30m, gave the largest seed size (360mg $seed^{-1}$) while the closest spacing treatment, 0.04m, produced the lowest seed size (350mg $seed^{-1}$). Total pod and shelled seed yields only differed at the widest spacing (0.30m) and were significantly lower than all the other treatments, which did not differ from each other. Analysis of R8 harvested soybeans for 2005 and 2006 revealed that the 2005 experimental plantings produced higher yields than those in 2006 ($p<0.0001$), which were reduced by deer damage. Genotype played a significant part in R8 seed yield ($p<0.0001$). Line *TN00-60* produced the highest yield (4374 kg ha^{-1}) and differed significantly from the other three lines, which were not different from each other (*Gardensoy-43*, 2941 kg ha^{-1}; *TN03-349*, 3373 kg ha^{-1}; *USG 5601T*, 2970 kg ha^{-1}). Spacing had no significant effect on R8 yields.

Part III of this thesis is a lightly revised version of a paper by the same name that will be submitted to the *Agronomy Journal* in 2007 by Debra J.S. Carpenter, Vincent R. Pantalone, Fred L. Allen, Dennis E. Deyton, and Carl E. Sams.

My primary contributions to this paper include (1) organization, development, and management in all aspects of this project, (2) collection of data from experimental plantings, (3) statistical analysis of data, and (4) all of the writing with the exception of some minor revisions.

Introduction

Soybean [*Glycine max* (L.) Merrill] is a versatile crop, widely adapted and grown world-wide. The major producers of the world's supply are the United States, Brazil, China, Argentina, and India. In the United States, approximately 30 million hectares are devoted to commercial (commodity) soybean production annually and U.S. farmers produce approximately 91 million metric tons each year. About one third of the total annual crop is exported, with China as the largest consumer (in 2003, 9.3 million metric tons), followed by Japan (3.7 million metric tons), the European Union and Mexico (3.3 million metric tons), and Taiwan (1.4 million metric tons) (USDA, 2003). The remaining two thirds are put to use in a myriad of ways. Soybean seed is generally composed of approximately 40% protein, 20% oil, and 35% carbohydrates. It is the high protein and oil content which make soybean so valuable. The oil is extracted to use in baking and frying, and is found in many prepared food products such as mayonnaise, margarine, peanut butter, salad dressings, bakery products, and others. Soybean oil also has industrial applications. Products such as inks, paints, crayons, epoxys, pesticides, and detergents frequently contain soybean oil. Soybean oil also serves as a lubricant in some industrial processes and as an additive to diesel fuel. Soybean meal is primarily used for livestock feed and is an important source of protein for non-ruminants such as poultry and swine. Soybean is also consumed directly by humans in the form of tofu, miso, textured soy protein, and as whole bean. It is the latter use that is of interest in this study.

Soybean seeds harvested and consumed whole at the green stage (corresponding to development stage R6, Fehr and Caviness, 1977) are known by the Japanese term, edamame (eh-dah-mah-may). Edamame has been consumed in Asia for centuries, where soy in its various forms is a dietary staple. Soybean varieties grown for use as edamame have a larger seed size than those grown for oil and meal. Edamame beans have been selected for flavor as well, with preference for a sweet and nutty, not “beany”, flavor. In recent years, the demand for edamame in the U.S. has risen significantly due to two very different factors. The first is the growing Asian population in this country, and the second is the result of research findings indicating that edamame (and soy foods in general) contain compounds with beneficial health effects for humans.

The compounds of interest are isoflavones, also called phytoestrogens. They are a sub-class of a larger group of flavonoids which are nutraceuticals (biologically active non-nutrients). Compared to most flavonoids, isoflavones are available only from a few sources in the plant kingdom. Soybeans are one of only a few known food plants to contain nutritionally relevant amounts of isoflavones, and this is a driving force behind the rising interest in edamame in the U.S.

Consumption of soyfoods (including edamame) in the U.S. has risen dramatically in the last five years, due primarily to the numerous studies which have highlighted the health benefits of soy protein and isoflavones. These studies prompted the October, 1999, FDA soy health claim which states, “25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.”

Frozen edamame imports from China and Taiwan increased 20 fold between the 1980’s and 2000 and demand is projected to continue to increase in the coming years. Currently, nearly all edamame consumed in the U.S. is imported. U.S. growers are not meeting the demand for fresh and frozen edamame but interest in commercial production of edible soybeans is rising. Although soybeans for human consumption have been cultivated in Asia for thousands of years, not enough research has been done on adaptation of edamame production to modern U.S. agriculture. This is a "new" crop in this country and growers need more information to efficiently and cost effectively produce edamame for the American market. American farmers are increasingly looking to diversify and expand their operations, and vegetable soybean production offers an opportunity. Southern tobacco [*Nicotiana tobaccum*] producers in particular, facing the end of the tobacco quota system and the removal of price supports, may need to look at an alternative crop.

The field tests conducted for this study were for the purpose of determining the within row plant spacing and the time of planting which together produce optimal yield in a field situation. Potential edamame soybean line *TN03-349* was included in this study to further determine its suitability as an edamame type. Mechanized harvest was utilized for this experiment to simulate a potential commercial production method.

Materials and Methods:

Description of Cultivars, Lines, and Experimental Field Procedures

Four lines were utilized for these studies: i) '*Gardensoy-43',* a marketed edamame cultivar, ii) a new high yielding line, '*TN00-60',* iii) Tennessee Agricultural Experiment Station cultivar '*USG 5601T'* (Pantalone et al., 2004) which currently serves as a USDA check in the Southern Uniform Maturity Group V tests, and which contains high levels of isoflavones (Charron et al., 2005), and iv) '*TN03-349',* a promising candidate for edamame production in the mid-south and southeast region. Relative maturities for these lines are 4.6, 4.8, 5.6, and 6.0, respectively.

Field tests were conducted for both spacing and time of planting studies. These treatments were run simultaneously in two of the three fields used. A field block was planted as a single, full season date and was harvested dry (R8), while the others were picked green (R6).

The dates chosen for the time of planting study in 2005 were May 24, June 14, and July 6. The dates were the same for 2006. The plant spacings used within the rows were 0.04m, 0.08m, 0.15m, and 0.30m.

Each plot in the edamame soybean (harvested at R6 stage of development) experiment consisted of genotype x spacing treatment x time of planting. Plots were 6.36m in length with a 3.63m alley between each genotype. For each planting date, there were four rows, 9.09m apart, of each soybean line planted in three replicates in strips for harvest by genotype. The three replicates per planting date contained the four plant spacing treatments, randomized within each replicate. Harvest was mechanized, using a Pix-all single-row green bean picker which removed the pods from the plants, leaving the plants standing.

The plots harvested at the R8 developmental stage were planted on May 24, the earliest planting date. These plots consisted of genotype x spacing treatment and were 6.36m in length with a 3.63m alley between genotypes. Three replicates each contained the four plant spacing treatments, randomized within each replicate. Four rows per genotype, spaced 0.75m apart and planted in strips, were utilized in this part of the time of planting study. The plots of mature soybeans were harvested with a combine.

Four rows of “filler” soybeans (*Gardensoy-43*) were planted between green-picked plots and dry-combined beans in 2005. These outside four rows were used for practice with the green bean picker to determine the optimal settings (tractor was operated in Low range, first gear, at 1750 rpm in order to achieve the cleanest picking possible).

Plot yields were determined by weighing the total pods picked from each plot. Also, 200 pods were randomly selected from each plot, weighed, shelled, and the beans weighed. Total seed weights per plot have been extrapolated from the 200 pod sample. Seed size comparison was facilitated by weighing a random selection of 200 seeds of the shelled beans from each plot.

The statistical method used for comparison of yields and seed size was analysis of variance using the MIXED procedure. Data were analyzed by SAS v9.1.3 (SAS Institute, 2005).

Results and Discussion

The purpose of this study is to determine the optimal spacing and planting date for edamame type soybeans. The four lines utilized for this experiment included two standard commodity soybean lines for yield and maturity comparison, one known edamame line, and the experimental line, *TN03-349*. Further examination of soybean line *TN03-349* for desirable edamame qualities, such as seed size and taste, was also intended. The commercially available edamame cultivar, *Gardensoy-43*, was included as a reference point for these traits.

The first year of the study, 2005, all experimental plantings were grown under conventional tillage conditions. Germination was uniform and weeds sufficiently controlled to avoid yield-reducing competition. Damage from wildlife grazing was prevented with electric fencing around fields. As a result, harvest was consistent across plots and data was gathered from all three planting dates. The second year of the study, 2006, experimental plantings were grown using no-till methods due to wet field conditions. Planter malfunction resulted in poor germination for the first planting date (May 14) and several plots were lost. The R8 harvested soybeans were also planted on that date and suffered the same difficulty. The electric fencing failed twice, allowing deer into the fields. So much damage occurred that data from the July planting date could not be obtained.

Yield data gathered included total pod weight per plot, pod weight of 200 pods per plot, number of seeds (from those 200 pods), and shelled seed weight (same). Seed size was extrapolated using number of seeds and seed weight data. Yields in kg ha^{-1} were extrapolated from plot weights. The edamame soybean lines (*Gardensoy-43* and *TN03-349*) consistently produced larger seeds than the standard commodity lines (*TN00-60* and *USG 5601T*). Genotype also played a significant role in determining total pod ($p<0.0001$) and shelled seed ($p<0.01$) yields both years. In 2005, the planting date itself was a significant factor in seed size ($p<0.05$) and total pod yield in kg ha^{-1} ($p<0.0001$) with the July planting date having the largest seed size and the lowest yield (mean seed size, 344.5mg $seed^{-1}$, total pod yield, 3024 kg ha^{-1}, and shelled seed yield, 1664 kg ha^{-1}) and May the smallest seed size and the highest yield (mean seed size, 324.4mg $seed^{-1}$, and total pod yield, 4191 kg ha^{-1}). June produced the highest shelled seed yield at 2464 kg ha^{-1} and did not differ from May in total pod yield or from July in seed size. In 2006, only planting dates in May and June were analyzed but findings were not the same for total pod yield. June differed significantly from May ($p<0.0001$) with a mean yield of 3722 kg ha^{-1} for total pod yield and 2435 kg ha^{-1} for shelled seed yield over May's 2987 kg ha^{-1} and 1786 kg ha^{-1}, respectively. However, due to planting difficulties in May, 2006, yields were probably lowered by poor germination. Spacing treatment effects were significant for seed size ($p<0.05$) for planting dates May and June (July data not available), with the widest spacing (0.30m) giving the largest seeds (344.5mg seed^{-1}) and the closest spacing (0.04m) the smallest at 324.4mg seed^{-1}. The number of seeds produced was not significantly affected for any of the planting dates by spacing ($p>0.05$) but spacing did affect total pod ($p<0.01$) and shelled seed ($p<0.05$) yields. Total pod yield was lowest and significantly different at 2309 kg ha^{-1} for spacing treatment 0.30m while yields for the other three spacings did not differ from each other (highest yield was 3912 kg ha^{-1} at 0.04m spacing). Shelled seed yield was also lowest at the 0.30m spacing. Significant interaction ($p<0.0001$) also existed between planting date and genotype for total pod and shelled seed yield data. Interaction between spacing and genotype was also significant at the $p<0.05$ level. Combined analysis of 2005 and 2006 data showed that genotype ($p<0.01$) and spacing ($p<0.05$) were significant factors in determining seed size, total pod yield, and shelled seed yield. Planting date was significant for seed size and shelled seed yield at $p<0.05$. Planting year was also significant for all three (seed size, $p<0.0001$; total pod yield, $p<0.0001$; shelled seed yield, $p<0.05$) with 2005 giving higher total pod and shelled seed yields and 2006 producing larger seed size. See Table 3.1 and Figure 3.1 for summary. The data suggests that seed size, important in edamame soybeans, increased as spacing between plants increased from 0.04m to 0.30m, and that planting in June instead of May does not lower yields significantly.

Analysis of R8 harvested soybeans for 2005 and 2006 revealed that the 2005 experimental plantings produced higher yields than those in 2006 ($p<0.0001$), which were reduced by deer damage and planter malfunction. Genotype played a significant part in R8 seed yield ($p<0.0001$). Line *TN00-60* produced the highest yield at 4374 kg ha^{-1} and was significantly different from the other three genotypes, which did not differ from each other (*Gardensoy-43*, 2941 kg ha^{-1}; *TN03-349*, 3373 kg ha^{-1}; *USG 5601T*, 2970 kg ha^{-1}). Interaction between genotype and planting year was significant ($p<0.0001$, see Figure 3.2).

Table 3.1: Comparison of R6 development stage means for total pod and shelled seed yield (kg ha^{-1}) of four genotypes grown under four different within-row spacing treatments and planted on three different dates at Crossville,TN, 2005 and 2006.

	Total Pod Yield kg ha-1			Shelled Seed Yield kg ha-1		
Genotype	**2005**	**2006**	**2005-06**	**2005**	**2006**	**2005-06**
Gardensoy-43	4432a	2133c	4006a	2397a	1787b	2604a
TN03-349	3757ab	4335a	4347a	2143ab	2557a	2570a
USG 5601T	3259b	3438b	3462b	1926b	2140b	2153b
TN00-60	3670b	3511b	3524b	2269ab	1958b	2072b
Spacing						
0.04	4128a	3912a	4200a	2453a	2441a	2595a
0.08	4105a	3890a	4243a	2411a	2439a	2560a
0.15	3918a	3301a	3905a	2315a	2080ab	2410a
0.30	2966b	2309b	2989b	1555b	1482b	1832b
Date						
May	4191a	2987b	3731a*	2422a	1786b	2243b
June	4123a	3722a	3939a*	2464a	2435a	2457a
July	3024b	NA	NA	1664b	NA	NA

†Means followed by the same letter within a column are not significantly different at the 0.05 level of significance using the Tukey-Kramer method.

NA=not available

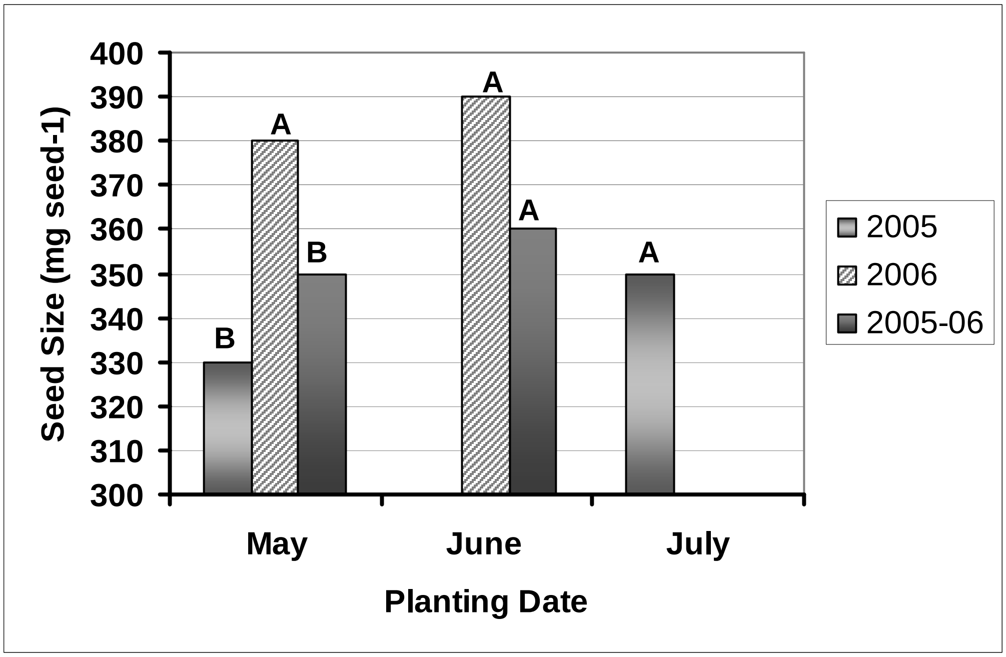

Figure 3.1: Comparison of R6 development stage seed size of soybeans planted at Crossville, TN, in 2005 and 2006 on three different dates. Means followed by the same letter are not significantly different at the 0.05 level of significance using the Tukey-Kramer method.

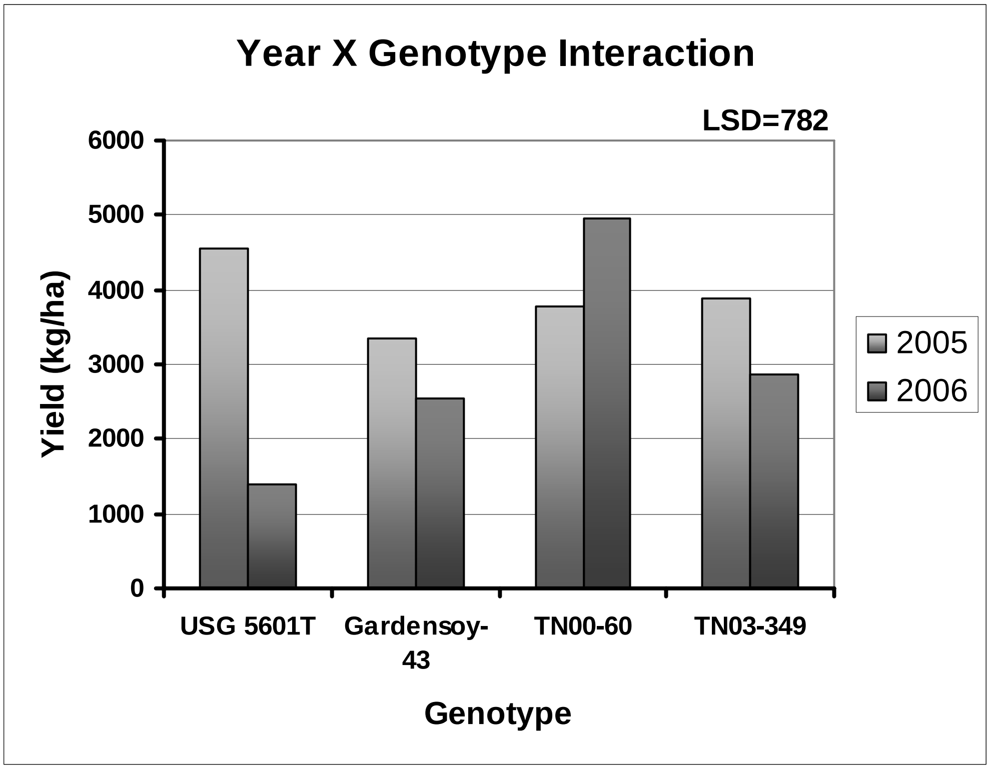

Figure 3.2: Comparison of R8 development stage yield of four soybean genotypes planted in Crossville, TN, in 2005 and 2006. Means followed by the same letter are not significantly different at the 0.05 level of significance using the Tukey-Kramer method.

List of References

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PART IV

Concluding Remarks and Future Research

The experimental plantings at the East Tennessee Research and Education Center in 2004 and 2005 gave results suggesting that within-row spacing can be stretched as far as 0.30m without significant loss in yield. Seed size was also shown to be affected by spacing and tended to increase as plant spacing increased. Total protein and oil content were not significantly affected by plant spacing and seed isoflavone content was not controlled by spacing treatments as much as year differences, probably due to temperature differences in the two growing seasons. Although other studies have investigated this effect, none of the soybean lines used in our experiments have been included in a study of this nature. Perhaps research conducted under field conditions using these genotypes could lend more understanding of this phenomenon and add to present knowledge.

Planting date proved to be a factor in seed size in the Plateau field experiment (the June and July plantings gave larger seed size) and the June planting date produced the highest yields. Thus, use of edamame as a second crop in a double-cropping system should produce both good seed size and yield.

The genotypic effects on yield data and seed size can be dramatic. One of the characteristics that make edamame soybeans different from standard commodity soybeans is seed size. Soybean line *TN03-349* exhibited large seed size, comparable to that of the known edamame cultivar, *Gardensoy-43*. The sweet, nutty flavor of *TN03-349* also makes it a good candidate for use as an edamame soybean. Edamame seed tends to be expensive, mostly due to a tendency to shatter when harvested at the R8 stage. Yields for *TN03-349* were not reduced by excessive shattering, however, *Gardensoy-43* did suffer shattering yield loss from the R6 to R8 stages. It was noted that *TN03-349* grows to be very tall (as much as 0.90m when grown in Tennessee) and lodging occurred at the 0.04m plant spacing treatment because the plants were spindly. This was not a problem at the wider spacing treatments.

Mechanized harvest of R6 stage soybeans using the modified green bean picker was made more efficient when the pods were set higher on the plant.

Gardensoy-43 plants were much shorter than the other genotypes (averaged only 0.46 to 0.53m in height) and set pods very low to the ground which could be missed by the picker. This was more of a problem than overall plant height. The mechanized picker was able to pick even the tallest plants fairly clean. Yields for *Gardensoy-43* were no doubt higher than were reported in the Plateau Research and Education Center field study due to this. *Gardensoy-43* is an early MG IV and perhaps grows taller in its ideal climate.

Differences in results due to spacing treatments between the raised bed and the field experiments are probably due to differences in spacing between rows for these experiments. Spacing between plants became more critical as spacing between rows narrowed.

Loss of data due to planter malfunction and wildlife damage in the second year of the Plateau field study leaves open the need for more research along these lines. Because of this loss, more data might be needed before definitive conclusions can be drawn from the field experiment.

Future research might include a study on height of pod set to facilitate mechanized picking and an investigation into the effects that planting an edamame genotype out of its MG range might have on plant height. Because most edamame lines were developed in early maturity groups, not many exist that ideally grow in the southern U.S. Opportunities then exist to breed new southern adapted edamame varieties.

Vita

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Debra is currently pursuing a career with Purdue University's Cooperative Extension Service as an Extension Educator.