Greenhouse and Field Evaluation of Alamo and Two New Genotypes of Switchgrass for Biomass Production

by

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Abstract

Due to its high yields and wide geographic range, switchgrass (*Panicum virgatum*) was chosen by the Department of Energy as a model herbaceous perennial bioenergy feedstock. Alamo is the highest yielding variety, and the “benchmark” recommended for the Deep South. Within the species, and even within this variety, large amounts of genetic variability exist, allowing for the development of cultivars with higher yields, and composition better suited for the needs of either biochemical or thermochemical conversion methods, through selective breeding. Links between the physiological measurements during seedling growth and yield of the mature plant in the field are unknown, but could expedite breeding progress if they were available. Therefore, the objective of this study was to compare Alamo with two new genetic lines of switchgrass, GA-992 and GA-993, in a field study and a study of seedling growth in a greenhouse, to examine whether seedling growth in a greenhouse was indicative of yield and other differences in the field experiment. The field experiment compared yield, cell wall, C, and N composition, and morphological characteristics of the three genetic lines over a 4-year period. Growth of seedlings from each line was measured weekly over a seven-week period in the greenhouse experiment which was conducted twice in 2008. Root measurements and partitioning of C and N in the roots and shoots of the seedlings were also measured. Data from the greenhouse experiment revealed complex interactions, with little or no difference among genetic lines for most variables measured. Height and weight of the two new lines were superior to that of Alamo on certain harvest dates, but this pattern was not consistent over time, and was not detected in the field study where there was mostly no difference in biomass yield among experimental entries. It is concluded that differences among the three genetic lines evaluated in this research were small or not detectable, and results in the field experiment could not be predicted from results in the greenhouse experiment.

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This thesis is dedicated to all of these people but most especially to my grandfather, Glenn Gass.

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Historical Perspective on Bioenergy

Since the 1970's during the oil embargo there has been interest in developing a cheap and domestic source of renewable transportation fuel. The Department of Energy (DOE) became involved in programs supporting relevant research in 1976, and in 1977 began field studies co-funded by the United States Department of Agriculture (USDA) (Wright, 1992). Oak Ridge National Laboratory (ORNL) was asked to provide advice and support to the program and by 1982 the DOE had fully transferred management of their Short Rotation Woody Crops Program to ORNL. In 1984 the Herbaceous Energy Crops Program (HECP) began, funded by the DOE. The goal of this program was to gain the necessary information for using herbaceous biomass as a viable source of feedstock for fuel production, and to do so in a way that would minimize adverse effects on the environment (Berger et al., 1984).

Herbaceous crops were desirable because they would likely be relatively easy to incorporate into preexisting agricultural practices, and because they could serve as an alternative crop for a depressed farm economy. Also, herbaceous crops seemed more suitable to the conversion methods of the time: enzymatic hydrolysis and fermentation to alcohol, and anaerobic digestion (Young, 1986).

The program focused initially on screening a wide variety of species to identify potential candidates for commercialization (McLaughlin and Kszos, 2005). These crops must be suited to grow on marginal land as defined by criteria such as having a high potential for erosion, being excessively wet or dry, possessing poor soil quality, and with nutrient or rooting constraints. Other desired traits in these crops included being a native species, a perennial and established by seed, as well as having the ability to enhance soil and wildlife (McLaughlin and Kszos, 2005). Six universities and one private company were selected by the HECP to participate in an initial herbaceous screening process (Cushman et al., 1985) and each one chosen was required to screen at least two potential feedstock candidates.

Although affected by severe droughts, Parrish et al. reported in 1990 that in the four years after establishment, switchgrass(*Panicum virgatum*) outperformed all other species being tested. Although annual species had better yields in years with higher rainfall, perennials outperformed them in lower rainfall years (Bransby et al., 1990). Switchgrass showed little response to differing amounts of precipitation and after the first establishment year showed high, consistent yields. Six of the seven intuitions included switchgrass in their recommendations for further study (Wright, 1992). Due to higher production costs and high variability, annuals were dropped from the study. Bransby's discovery of the high yield potential of the switchgrass variety Alamo was a major factor in selecting switchgrass as the HECP's model species for herbaceous energy crop production (Sladden et al., 1991).

Conversion Technologies

There are two broad methods for converting biomass into liquid fuels, biochemical and thermochemical. While an economical feedstock with consistently high yields is the factor most important to all conversion methods, conversion efficiency of each method is affected by feedstock composition.

Biochemical conversion is the production of ethanol either by direct fermentation of sugars or by the enzymatic hydrolysis of starch or lingo-cellulosic material to sugars, and subsequent fermentation (Carpita, 1996). Therefore, in biochemical conversion, the composition of the feedstock is critically important. The soluble sugars and cellulose portion of the feedstock are most readily converted while hemicellulose, and much more so, lignin, require more pretreatment beforehand to avoid adversely affecting conversion efficiency (Chang and Holtzapple, 2000). Feedstocks high in available cellulose and low in lignin are best for biochemical conversion (Himmel et al., 1997).

Both feedstock composition and maturity can affect the conversion to ethanol. While desired carbohydrate contents increase as switchgrass matures, their extraction becomes more challenging and an increase in pretreatment severity may be needed to compensate and could possibly lead to lower yields of hemicellulosic sugars (Sanderson et al., 2006).

In order for cellulosic ethanol to become economically viable, improvements must be made to pretreatment processes, and to the feedstocks themselves for more effective release of fermentable sugars. In other words, both plant breeding or genetic modifications to improve the feedstock composition along with improvements to the effectiveness of the pretreatment process would lower the cost of generating fermentable sugars (Himmel, 2007; Sticklen, 2006).

Another broad method of bioenergy production is thermochemical conversion which includes direct combustion, gasification, and pyrolysis. For these methods the cell wall structure of plants does make a small difference, but the physical properties of the biomass are more important. Specifically, these processes generally benefit from the ability to deliver the feedstock in smaller particles which create more surface area to allow greater access of heat to the biomass, and therefore, more efficient conversion (Kumar, 2009). In this regard, the physical structure of switchgrass makes size reduction easier.

While the non-combustible portion (ash) is only a small fraction of the feedstock, it is extremely important: biomass low in ash and minerals such, as N, P, K, and Si are best for thermochemical methods. In particular, ash causes slagging and plugging of downstream equipment that leads to the need for expensive remediation (Kumar, 2009). In addition, when gasified, most of the preexisting nitrogen contained in the feedstock results in formation of NOx (Kumar, 2009), which is a harmful emission.

As the field continues to develop, a better understanding of the interaction between the feedstock and processing performance will help guide the selection of improved crops that are tailor-made to provide feedstocks for specific thermochemical or biological conversion technologies in a low-cost, efficient, and sustainable manner (Koonin, 2006; Ragauskas et al., 2006). Switchgrass, the selected model herbaceous feedstock, continues to show strong potential as one such crop. With its high energy density and low alkali content, it is a relatively clean fuel attractive as a thermochemical feedstock (McLaughlin et al., 1999). Current genetic research and breeding will continue to improve the yield and other properties of switchgrass for more efficient conversion (Sanderson et al., 2006).

An absence of operational commercial biorefineries in the continental United States leads to uncertainty in how best to optimize feedstocks for particular conversion processes. However, research on the production of switchgrass and other feedstocks on rain-fed marginal land on a large scale appears to be an achievable goal (Sarath, 2008), and should be pursued in parallel with research on conversion technologies.

Switchgrass as an Energy Crop

Selected as the model bioenergy crop by, switchgrass exhibits numerous beneficial characteristics to meet that selection criteria listed earlier. It is a native C4 perennial grass that grows throughout much of North America in a wide variety of environments (Sanderson et al., 1996). Throughout its wide geographical range, it has been shown to produce consistently high yields (Fike et al., 2006) and has evolved to tolerate erodible soil with low nutrient or water requirements which avoids competition with food crop production that requires highly productive arable land (McLaughlin et al., 1994).

Other agronomic traits also played a role in the selection of switchgrass. Because it is able to be chopped or bailed, it can be harvested by preexisting farm practices which use hay- and silage-making equipment (Turhollow, 1994). In addition, switchgrass is planted by seed which is less expensive to establish than some other potential feedstocks such as Miscanthus, which is vegetatively propagated by rhizomes, and energycane that is established by billets (Heaton et al., 2004). As for hay crops, switchgrass can be dried by the sun in the field which provides a distinct advantage over other potential biomass crops that have been evaluated for southeastern production such as napiergrass, energycane, and giant reed (Knoll et al., 2012). In addition, it is relatively low in ash and nitrogen concentrations compared to some other alternative crops, making it more preferable for direct thermochemical conversion.

Switchgrass grown for biomass provides environmental benefits as well. It can provide a nesting habitat for migratory birds and cover for other native avian and animal species (Roth et al., 2005). The deep roots of switchgrass allow not only for better drought tolerance, but can also play a role in capturing nutrients associated with non-point pollution (Ma et al., 2000). Switchgrass produces large amounts of biomass above ground for use as a bioenergy feedstock, but also produces substantial root biomass that sequesters large amounts of atmospheric carbon (Ma et al., 2000). Gains in root biomass are anticipated to continue to increase with long term production (McLaughlin et al., 1994).

Geographic Distribution, Morphology, and Crop Improvement

Switchgrass can naturally tolerate a wide variety of habitats including open prairies, open woods and even brackish marshes. It has a range from the eastern seaboard west into Wyoming, North Dakota and New Mexico and from Nova Scotia and Ontario in the north into Central America in the south (Hitchcock, 1971). Both morphologically and genetically, switchgrass is divided into two distinct ecotypes, upland and lowland (Hultquist et al., 1996). The lowland ecotype is tall and vigorous, has a bunch-type growth habit and is adapted to wetter conditions. The upland ecotype, on the other hand, is shorter, rhizomatous, thinner stemmed, and adapted to drier conditions (Sladden and Bransby, 1992). The lowland ecotypes are tetraploid, while the upland ecotypes are octoploid (Lemus et al., 2002). Switchgrass is largely self-incompatible, and breeding does not occur across ecotypes (McLaughlin and Kszos, 2005). However, because of its wide range and varied environments, there are large variations in populations due to genetic factors such as genetic drift and mutation, along with environmental factors such as latitude, altitude, soil type, and climate variation, which have resulted in significant variation even within ecotype (Casler et al., 2007).

Switchgrass has only been studied as a potential crop for the last fifty years (Parrish and Fike, 2005) and for most of that time only as a possible forage crop: very little selective breeding has occurred for improved total biomass yield and composition with the objective to use it for the production of bioenergy. Breeding aimed on its improvement as a forage crop involved mainly upland ecotypes while new biofuel feedstock breeding research, especially the research in the Southeast, has shown that lowland switchgrass is the better suited ecotype and should be the basis for genetic improvement focus (Cassida et al., 2005).

One of the greatest issues in producing switchgrass as an energy crop has been developing protocols for the establishment of strong stands (McLaughlin and Kszos, 2005). Competition from fast growing weeds has been a problem in the first critical season and research within the HECP included studies to improve seed germination, evaluate herbicide treatments for weed control, and alter seedling vigor through breeding. A large amount of the energy captured by switchgrass in the first two years is allocated by the plant to the development of a strong root system and full yield is typically not achieved until the third year after planting (McLaughlin and Kszos, 2005).

The varieties “Alamo” and “Kanlow” have been determined by long term studies in field research plots to be the best commercial varieties (McLaughlin and Kszos, 2005) with high yielding Alamo recommended for the deep south (Sanderson et al., 2006; Sladden et al., 1991).

With the yet unused adaptations of the many isolated populations and the natural variability within each population there are many sources of genetic material available for varietal improvement (Bouton, 2002). Current genetic research and breeding can harness the diverse factors associated with these differences for better, improved varieties with higher yields, better establishment ability, and traits tailored to specific conversion methods (Sanderson, 2006).

Introduction

Switchgrass has been chosen by The Bioenergy Feedstock Development Program initiated by the United States Department of Energy as a model bioenergy feedstock. A native C4 perennial grass, switchgrass has a wide geographic range and is adapted to a variety of environmental conditions. It can serve as a wildlife habitat and its deep roots can sequester large amounts of carbon.

Among other advantages of switchgrass is primarily its potential for consistently high yields on marginal lands unsuitable for food production, and its ability to be utilized for both biochemical and thermochemical conversion methods. However, even though current yields are impressive switchgrass has potential for even higher yields through selective breeding.

The conversion technologies in which switchgrass biomass may be used as a feedstock can be divided into two categories, biochemical and thermochemical. While the feedstock trait most important to both categories is yield, other technology-specific biomass traits are beneficial as well. In biochemical biomass conversion to ethanol, the cell wall composition of the feedstock is an important factor. Specifically, soluble sugars and cellulose can be more readily converted into ethanol using this process, while hemicellulose and even more so, lignin, require more intensive pretreatment beforehand. Feedstocks that are low in lignin are best for this method. Thermochemical methods, comprised of combustion, gasification, and pyrolysis, are affected less by the cell wall components of a feedstock. However, ash, nitrogen, and other non-combustible minerals create problems such as slagging and production of harmful emissions, so feedstocks which are low in these elements are desirable.

Relatively little selective breeding on switchgrass has been conducted to date and what has been done generally focused on potential of the crop to be used as a forage crop and mainly on the upland ecotypes. There are two main commercial varieties of lowland switchgrass, Alamo and Kanlow, both of which are higher yielding than the upland varieties in studies performed in the eastern United States. Alamo is the highest yielding and the “benchmark” variety for use in the Deep South. Even within varieties a large amount of genetic variability exists allowing for higher yielding strains to be produced from within this germplasm. With emerging demand for improvements such as higher biomass yields, conversion specific composition, and more reliable establishment, interest in the production and evaluation of these strains has renewed breeding efforts.

Selection of improved cultivars should focus on all three of the objectives but the ultimate goal is to improve yield and composition in mature stands. Seedling vigor in both above ground growth and root development would lead to advantages in stand establishment. Links between seedling growth in a greenhouse and that of mature stands in the field are unknown. However, if a consistent relationship existed between these two variables it could increase the rate of progress in genetic improvement which is currently severely constrained by the fact that switchgrass takes three years after planting to reach full production in the field. Therefore, the overall objectives of this study were to compare Alamo with two new genotypes of switchgrass, GA-992 and GA-993 and to determine whether seedling growth in a greenhouse was indicative of yield and other differences in a field experiment.

Materials and Methods

Greenhouse Experiment

The greenhouse study was conducted twice, the first beginning in April of 2008 and the second beginning in August of the same year in Auburn, Alabama. The switchgrass seeds were planted in conetainers filled to approximately 1 cm from the top of the conetainer. The substrate used was soil collected from the E.V. Smith Research Station, a Wickham soil (fine-loam, mixed, thermic Typic Hapludult), and sifted to remove large particles.

Approximately 200 conetainers of Alamo, and two new genotypes, GA-992 and GA-993, were planted with 3 to 5 seeds each and later thinned to one plant per conetainer. They were placed in racks and watered daily to field capacity. The racks of seedlings were rotated periodically. Four weeks after germination, seedlings were randomly selected to fill seven racks with twenty seedlings each of Alamo, GA-992, and GA-993, per rack. Racks were rotated periodically to minimize the effects of locational variations across the greenhouse bench.

Beginning four weeks after planting, and continuing once a week for seven weeks, the above ground biomass of the seedlings from one tray was harvested. Just prior to cutting height, number of leaves, and number of tillers of each plant in the tray were recorded. Each seedling was then cut level with the rim of the conetainer, approximately 1 cm above the soil surface. The harvested material of each individual seedling was separately dried at 60 C for 48 hours, and the dry weight of each seedling was recorded.

During the last harvest period, ten of the remaining un-harvested seedlings from each new genotype as well as Alamo were also randomly selected for root analysis. Roots were carefully washed with tap water to remove the soil, blotted dry, and separated from the rest of the plant. Roots were scanned using a WinRHIZO Root Scanner (Model STD 1600+, Regent Instruments, Inc.) to determine the total root length and the average root diameter. The root and top portions of the plant were dried separately at 60 C in a forced air oven for 72 hours and then analyzed separately for carbon and nitrogen.

The seven weekly measurements of seedling growth were analyzed using PROC GLIMMIX. Means were considered significant at $P<0.05$. Analysis of root composition and root mass data was performed using the GLM procedure. Differences in the percentages of carbon and nitrogen were determined using Tukey's least squared means. Duncan's multiple range test was used in evaluating the differences in total root length and average root diameter.

Field Experiment

A field experiment was conducted for four years, 2006-2009. It was planted in June of 2006, and located at the Plant breeding Unit of the E.V. Smith Research and Extension Center near Shorter, Alabama. The site of the study was on a Wickham soil (fine-loam, mixed, thermic Typic Hapludult). A randomized complete block design was used with the three genotypes, each with four replications, over four years. Each plot was 3.05 m x 9.14 m with 0.76 m row spacing. Weather information was obtained from the AWIS Services, Inc., Auburn Alabama, 'E.V. Smith' monitoring (Alabama Mesonet Weather Data, 2009). A 10-year average was calculated to estimate "normal" monthly rainfall (Table 2) and temperatures (Table 1).

At the time of harvest (Table 8), ten tillers were randomly selected from each plot. They were manually cut 5 cm above the soil surface and saved for later analysis. A 1.07 m x 9.14 m strip of switchgrass was then harvested, giving a total harvested area of 9.75 m^2. The biomass cut from each plot was weighed. Subsamples were taken from the harvested biomass of each plot, weighed immediately. Both the ten-tiller samples and harvested subsamples were dried at 60 degrees Celsius for 72 hours in a forced air oven. The dried biomass subsamples were weighted for dry matter determination and discarded.

Data collected from the tiller samples were length, stem diameter and number of leaves. Tillers were then separated into leaf and stem components, with leaf sheaths counting as stem material. All leaves or stems for each individual plot were combined and weighed. The separated biomass was then milled using a Wiley mill to pass through a 2-mm screen for compositional analysis. Carbon, nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and ash analysis were then performed on samples using the procedure described by Goering and van Soest(1970).

The GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC) was used to conduct the analysis of variance for yield, composition and morphological traits. Results were considered significant at $P<0.05$.

Results

Greenhouse Experiment

The greenhouse experiment comparing Alamo and the two new genetic lines, GA-992 and GA-993 was performed twice, one beginning in April of 2008 and the other in August of 2008. Due to the large difference associated with trial dates, there was a treatment x date interaction (Table 3) and the results of the two different dates are presented separately.

April 2008

During the April seedling growth study some differences between Alamo, GA-992, and GA-993 were observed (Table 4), most notably, the differences in dry weight during several weeks (Figure 1). Differences in dry weight after week one were evident with Alamo having less than GA-993 and a lower yield than both GA-992 and GA-993 after week two. GA-992 again out performed Alamo in week 5. There were no differences in the number of tillers (Figure 3) or number of leaves (Figure 5) among the three genotypes during the seventh and final week of the trial. The only difference in seedling height (Figure 7) was in the second week when GA-992 had significantly higher growth than Alamo.

August 2008

Greater differences were recorded in the August study than in April for all variables that were measured (Table 5). Both number of tillers and number of leaves which showed no differences in April, were different among genotypes in the final week of the August study

(Figures 4&6). GA-993 had a higher number of tillers and leaves than Alamo. The dry weight of all seedling samples taken in August (Figure 2) show a more definitive trend than that of the April study (Figure 1). Specifically, Alamo had significantly less dry weight than both of the new genotypes in three of the weeks, and in each it had less dry weight than at least one of them. After the first and second week, GA-992 had greater dry weight than Alamo. After week three, GA-993 had a higher dry weight than Alamo, and in week four, both new genotypes out performed Alamo. After week five, dry weight of all three were different from one another: GA-992 had a higher dry weight than Alamo and GA-993, and GA-993 out yielded Alamo. GA-992 had greater dry weight than GA-993 and Alamo after week six and seven

In August of 2008 seedling height followed a similar trend as dry weight. GA-992 was taller than Alamo after the first and second weeks. There was no difference in the height after week three. After weeks four, five, and six, height of Alamo was lower than that of both GA-992 and GA-993. In week seven, the final week of the study, Alamo and GA-993 had similar average seedling heights, while and GA-992 was taller than both of them.

Seedling Root Study

Carbon content of shoots from GA-992 and GA-993 did not from one another, but was higher than that of Alamo (Table 6). Nitrogen content of shoots from GA- 992 and GA-993 was not different, but that of Alamo was higher than that of both new genotypes (Figure 9).

The percentage of carbon in the roots was not different among Alamo, GA-992, and GA-993 (Table 6). However, differences among each in percent nitrogen in the roots were detected: as with the shoots, the new genotypes had a lower concentration of nitrogen than did Alamo and no difference between them (Table 6).

The roots of the seedlings were scanned to compare both average root diameter and total root length. GA-992 and Alamo were significantly different in total root length, with GA-992 having a greater total length, while GA-993 did not differ from either (Table 7, Figure 11). There was no difference among entries in root diameter (Figure 12).

Field Experiment

With 4-year average yields of 10732, 8644, and 10348 Mg/ha of dry matter for Alamo, GA-992, and GA-993 respectively, no significant difference was shown in the years studied. Year did have a significant impact on dry matter yields which were higher in 2008 than in 2006 and 2007 (Table 9).

There was also no difference between Alamo, GA-992, and GA-993 in leaf weight, stem weight, ratio of leaves to stems, and the total tiller weight determined from samples collected at the time of harvest in 2007 and 2008 (Table 10). However, in 2008 leaf weight, stem weight, leaf to stem ratio, and total weight, were higher than in 2007 (Table 10). This was likely due to higher rainfall in 2008 (Table 2), and harvesting of biomass produced in 2007 in late winter (Table 9) which would have resulted in considerable loss of yield compared to a fall harvest.

Average tiller length and diameter, and leaf number did not vary among the three genotypes (Table 12). Tiller length and leaf number did not differ between years but tiller diameter was larger in the 2007 harvest. Analysis of compositional data also revealed few differences (Tables 14 and 15). The exceptions were a higher level of hemicellulose in Alamo than in GA-992 (Figure 27), and a higher level of N in the leave of Alamo than in the leaves of GA-993 (Table 15, Figure 29).

Conclusions

Greenhouse Experiment

Conclusions that were drawn from the greenhouse experiment are as follows:

- complex interactions were observed between treatments (genotypes) and time:
- for most harvest dates there was no significant difference among treatments, and this was partly due to high variation among seedlings, and therefore high experimental error, and
- in all cases where treatment differences were evident, results from GA-992 and/or GA-993 were superior to those for Alamo.

Field Experiment

Results from the field experiment allowed the following conclusions to be drawn:

- no differences were observed among treatments:
- yield differences were observed across years, and appeared to be partially related to rainfall and a late harvest of biomass produced in 2007; and
- there appeared to be no relationship between results from the greenhouse experiment and results from the field experiment.

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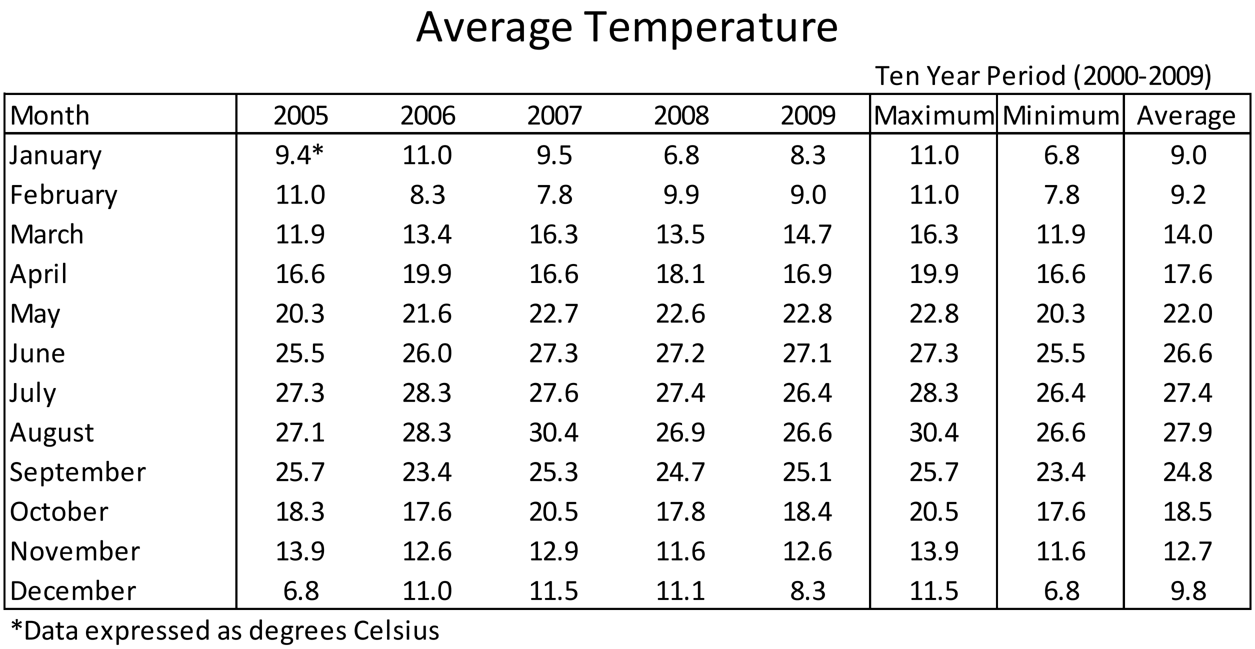
Appendix

Table 1. Average temperature per month during study and 10-year averages.



Average Temperature

						Ten Year Period (2000-2009)		
Month	2005	2006	2007	2008	2009	Maximum	Minimum	Average
January	9.4*	11.0	9.5	6.8	8.3	11.0	6.8	9.0
February	11.0	8.3	7.8	9.9	9.0	11.0	7.8	9.2
March	11.9	13.4	16.3	13.5	14.7	16.3	11.9	14.0
April	16.6	19.9	16.6	18.1	16.9	19.9	16.6	17.6
May	20.3	21.6	22.7	22.6	22.8	22.8	20.3	22.0
June	25.5	26.0	27.3	27.2	27.1	27.3	25.5	26.6
July	27.3	28.3	27.6	27.4	26.4	28.3	26.4	27.4
August	27.1	28.3	30.4	26.9	26.6	30.4	26.6	27.9
September	25.7	23.4	25.3	24.7	25.1	25.7	23.4	24.8
October	18.3	17.6	20.5	17.8	18.4	20.5	17.6	18.5
November	13.9	12.6	12.9	11.6	12.6	13.9	11.6	12.7
December	6.8	11.0	11.5	11.1	8.3	11.5	6.8	9.8

*Data expressed as degrees Celsius

Table 2. Total monthly rainfall for years studied and 10-year averages.

Total Rainfall

						Ten Year Period (2000-2009)		
Month	2005	2006	2007	2008	2009	Maximum	Minimum	Average
January	54.1†	112.3	150.4	110.7	52.1	150.4	52.1	95.9
February	112.8	115.6	75.2	101.9	105.2	115.6	75.2	102.1
March	254.5	79.5	78.0	77.5	243.6	254.5	77.5	146.6
April	184.9	44.2	51.1	100.6	109.2	184.9	44.2	98.0
May	32.3	78.5	11.9	64.0	262.4	262.4	11.9	89.8
June	39.1	18.3	29.2	50.3	99.6	99.6	18.3	47.3
July	215.4	93.2	173.2	126.2	75.2	215.4	75.2	136.7
August	86.6	99.3	82.8	252.0	191.8	252.0	82.8	142.5
September	36.6	103.1	55.9	18.5	148.1	148.1	18.5	72.4
October	45.0	110.7	75.4	83.1	163.6	163.6	45.0	95.6
November	85.9	150.1	54.6	92.7	154.2	154.2	54.6	107.5
December	51.8	62.2	94.5	82.3	276.4	276.4	51.8	113.4
Total	1198.9	1067.1	932.2	1159.8	1881.1	2276.9	607.1	1247.8
*Grownning Season	639.8	547.4	479.6	694.7	1049.8	1325.9	295.9	682.2

*Total growing season rainfall (April to October)

†Data expressed in millimeters

Table 3. Analysis of variance for weekly growth of seedlings in the greenhouse, April & August 2008.

Greenhouse Growth

Effect	Degrees of freedom	*F* statistic	Probability of >*F*
Grams Dry Matter			
Date	1	2089.3	<0.001
Genotype	2	157.4	<0.001
Genotype x Date	2	9.0	0.0001
Day	6	1061.5	0.0000
Date x Day	6	92.6	<0.001
Genotype x Day	12	2.6	0.0023
Genotype x Date x Day	12	5.5	<0.001
Height			
Date	1	2751.8	<0.001
Genotype	2	116.2	<0.001
Genotype x Date	2	12.7	<0.001
Day	6	887.3	0.0000
Date x Day	6	69.7	<0.001
Genotype x Day	12	2.7	0.0014
Genotype x Date x Day	12	4.1	<0.001
Number of Leaves			
Date	1	777.0	<0.001
Genotype	2	12.3	<0.001
Genotype x Date	2	10.7	<0.001
Day	6	169.8	<0.001
Date x Day	6	69.1	<0.001
Genotype x Day	12	1.3	0.2391
Genotype x Date x Day	12	2.2	0.0088
Number of Tillers			
Date	1	285.1	<0.001
Genotype	2	14.4	<0.001
Genotype x Date	2	2.3	0.1008
Day	6	135.9	<0.001
Date x Day	6	45.3	<0.001
Genotype x Day	12	2.5	0.0029
Genotype x Date x Day	12	1.2	0.2616

Table 4. Means of weekly growth measurements from the greenhouse experiment, beginning April 2008.

Seedling Growth April 2008							
Week	1	2	3	4	5	6	7
Dry Weight(grams)							
Alamo	0.04a*	0.08a	0.13†	0.40	0.56a	0.78	1.04
GA_992	0.06ab	0.18b	0.21	0.50	0.73b	0.95	1.15
GA_993	0.09b	0.16b	0.18	0.41	0.71ab	0.93	1.17
Number of Tillers							
Alamo	1.0	1.0	1.0	2.4	2.1	2.5	2.2
GA_992	1.0	1.0	1.0	2.8	2.7	2.7	2.7
GA_993	1.0	1.0	1.1	2.5	2.8	2.6	2.3
Number of Leaves							
Alamo	4.1	5.0	5.1	7.2	8.3	9.6	9.6
GA_992	4.9	5.1	5.4	8.2	9.3	10.7	11.5
GA_993	4.9	5.2	5.3	7.9	9.7	10.1	10.1
Height(cm)							
Alamo	5.5	9.1a	14.1	27.7	33.9	43.6	43.4
GA_992	8.7	13.8b	17.3	29.0	39.3	44.9	50.7
GA_993	7.2	11.3ab	17.1	29.1	40.0	43.8	50.9

*Weekly columns with different letters(a,b) are different at $P<0.05$.

†Weeks with no letters showed no difference in that category.

Table 5. Means of weekly growth measurements from the greenhouse experiment, beginning August 2008.

Seedling Growth August 2008							
Week	1	2	3	4	5	6	7
Dry Weight(grams)							
Alamo	0.01a*	0.03a	0.06a	0.11a	0.10a	0.19a	0.29a
GA_992	0.04b	0.08b	0.09ab	0.19b	0.34b	0.42b	0.57b
GA_993	0.02	0.06ab	0.12b	0.22b	0.22c	0.27a	0.39a
Number of Tillers							
Alamo	1.0†	1.0	1.0	1.1	1.0	1.5	1.5a
GA_992	1.0	1.0	1.1	1.3	1.0	2.0	1.8ab
GA_993	1.0	1.0	1.2	1.6	1.2	1.8	2.1b
Number of Leaves							
Alamo	3.7	4.2	4.9	5.0	4.8	5.3	6.1a
GA_992	4.3	4.6	5.0	4.5	4.0	4.7	6.4ab
GA_993	4.4	4.6	4.9	5.0	4.9	5.6	7.6b
Height(cm)							
Alamo	2.3a	3.8a	6.4	8.5a	7.87a	11.55a	19.9a
GA_992	5.3b	6.8b	8.0	11.8b	17.2b	21.7b	29.8b
GA_993	3.5ab	6.0ab	8.3	13.7b	12.5b	16.8b	19.6a

*Weekly columns with different letters(a,b) are different at $P<0.05$.

†Weeks with no letters showed no difference in that category.

Figure 1. Changes in seedling dry weight of different genotypes with time for the greenhouse experiment starting in April 2008.

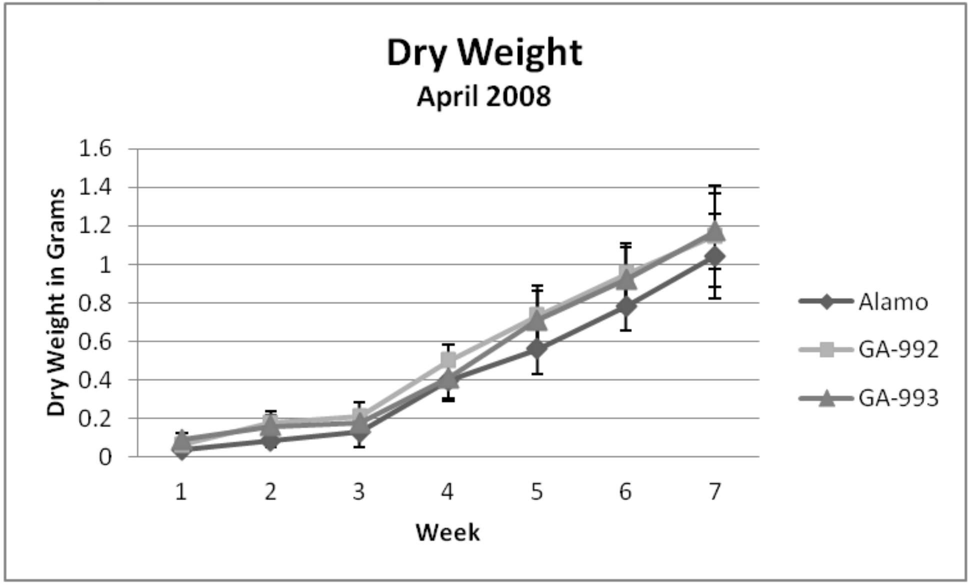


Figure 2. Changes in seedling dry weight of different genotypes with time for the greenhouse experiment starting in August 2008.

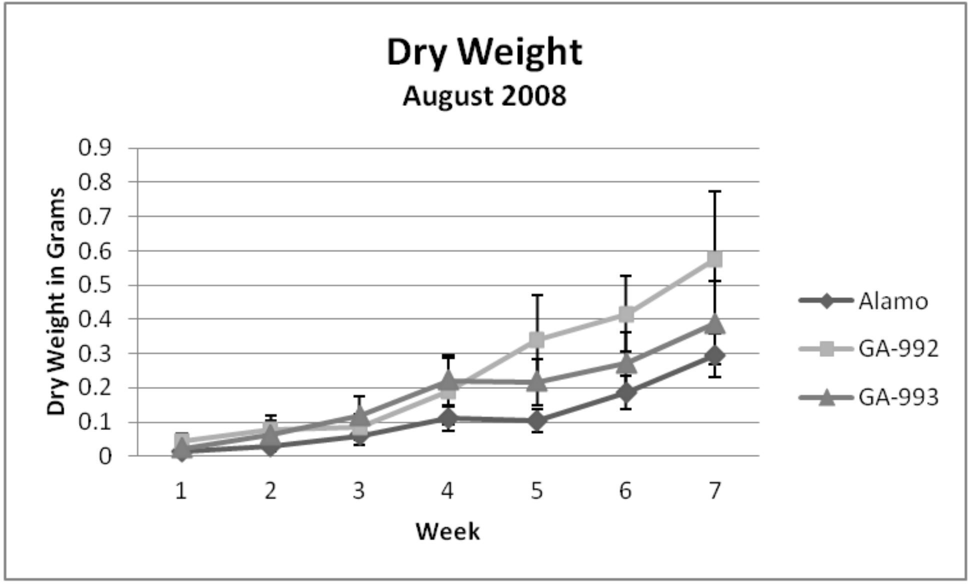


Figure 3. Changes in the tiller number of different genotypes with time for the greenhouse experiment starting in April 2008.

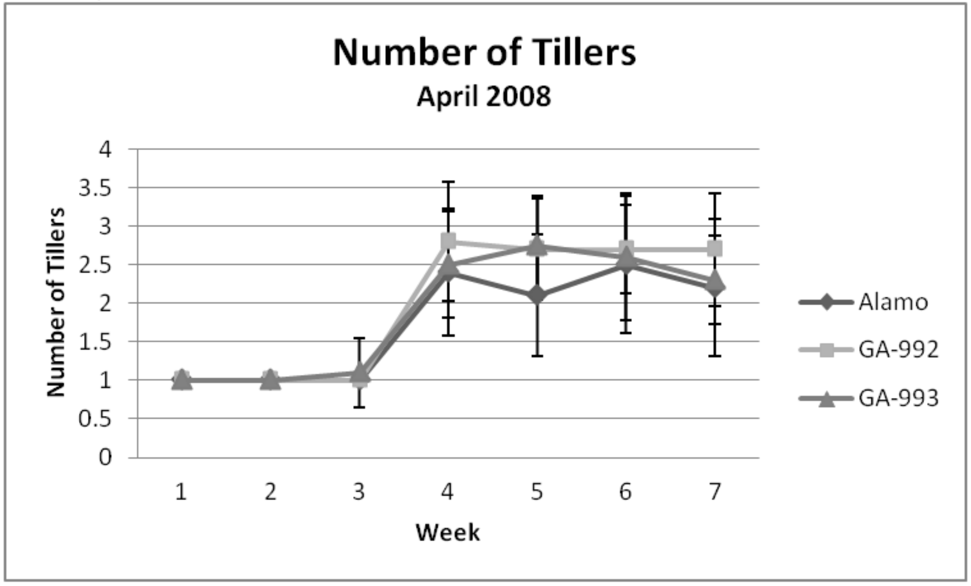


Figure 4. Changes in the tiller number of different genotypes with time for the greenhouse experiment starting in August 2008.

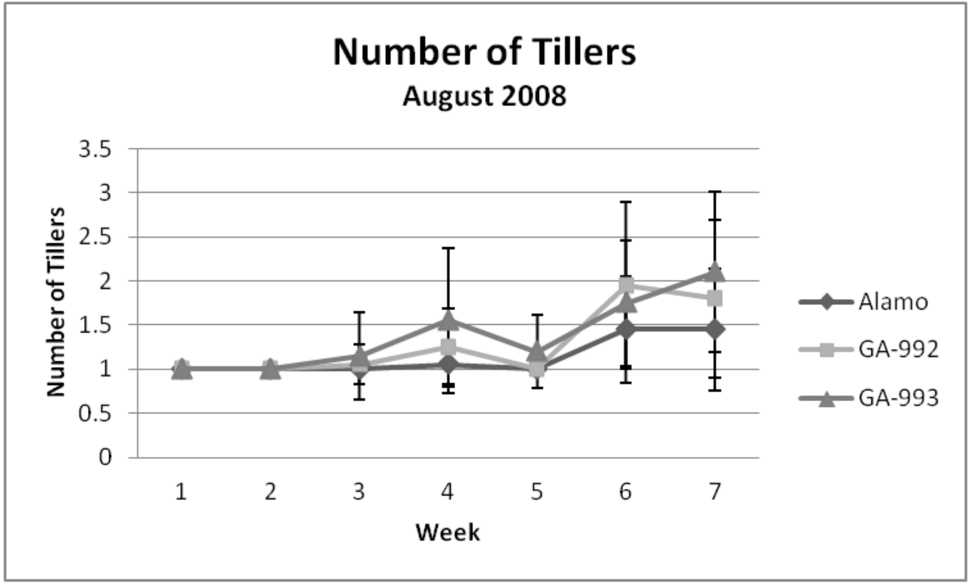


Figure 5. Changes in the leaf number of different genotypes with time for the greenhouse experiment starting in April 2008.

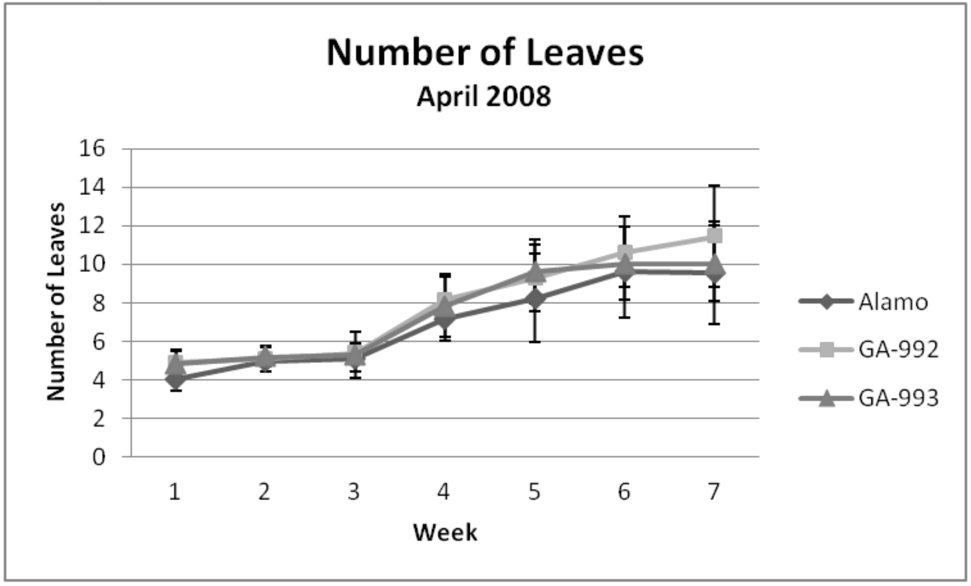


Figure 6. Changes in the leaf number of different genotypes with time for the greenhouse experiment starting in August 2008.

Figure 7. Changes in seedling height of different genotypes with time for the greenhouse experiment starting in April 2008.

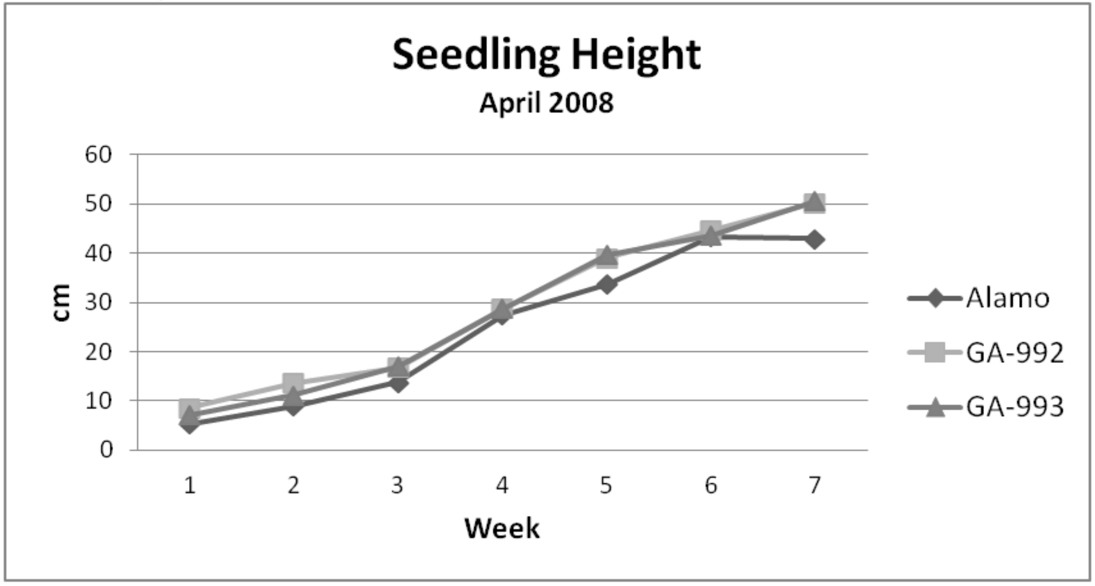


Figure 8. Changes in seedling height of different genotypes with time for the greenhouse experiment starting in August 2008.

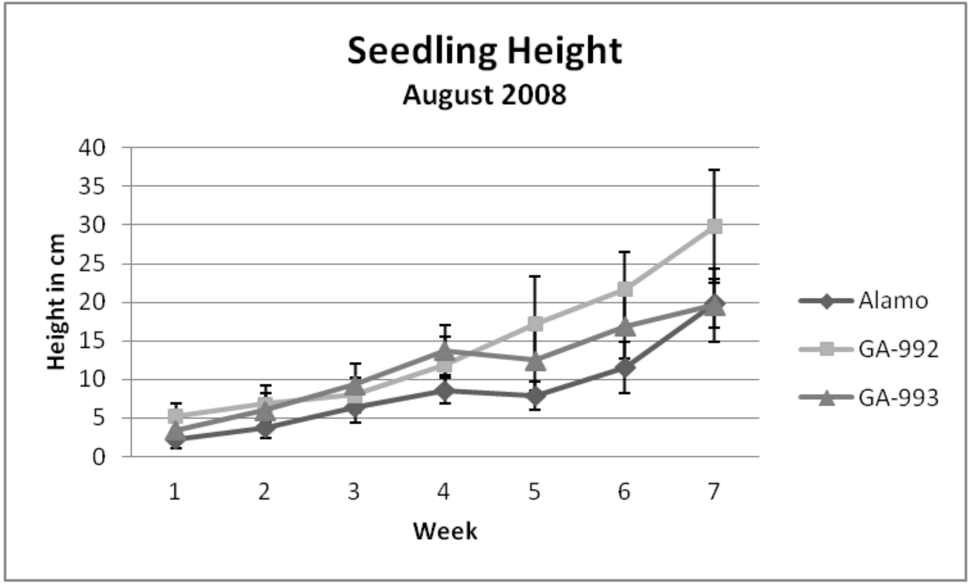


Table 6. Percentage of nitrogen and carbon in roots and shoot of samples from the greenhouse experiment.

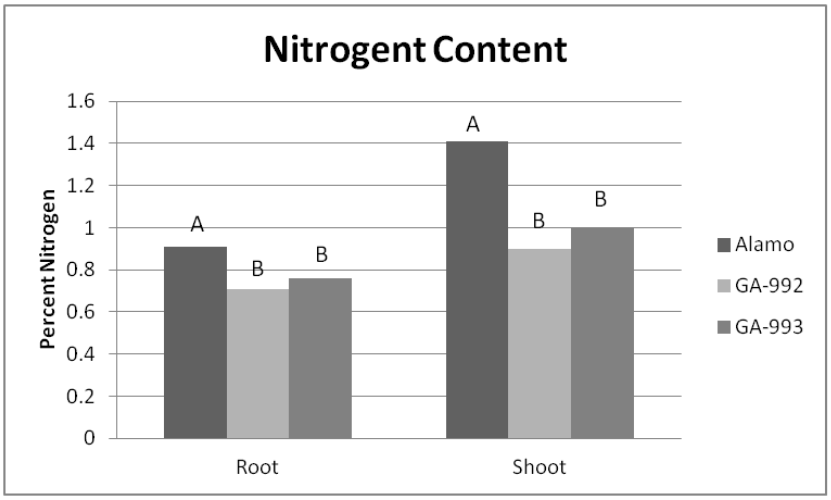
	Shoot		Root	
	Nitrogen	Carbon	Nitrogen	Carbon
Alamo	1.41*(0.29)†a‡	45.19(0.55)a	0.91(0.13)a	43.87(0.93)a
GA-992	0.90(0.30)b	45.17(0.30)b	0.71(0.14)b	43.78(1.20)a
GA-993	1.00(0.20)b	44.33(0.67)b	0.76(0.10)b	44.27(0.84)a

*Values based on the average percent dry matter of ten samples.

†Standard deviations (SD) in parentheses.

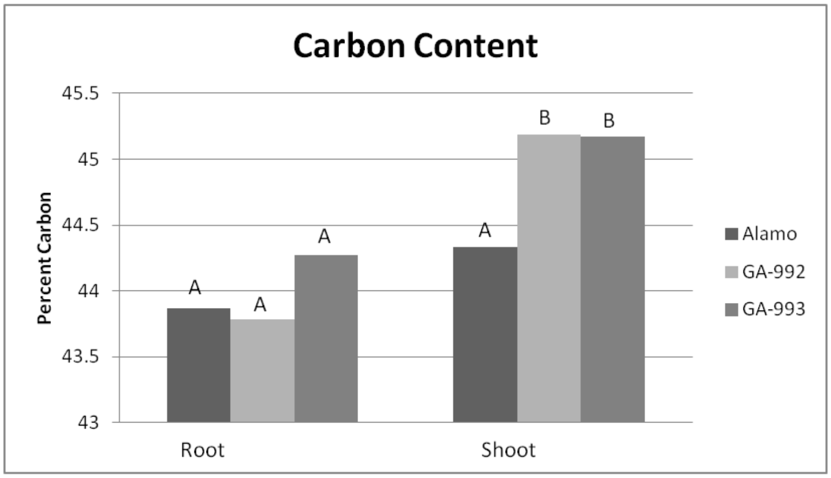
‡Columns with the same letter do not differ (P>0.05).

Figure 9. Percent nitrogen in roots and shoots from the greenhouse experiment on a dry matter basis.



Root or shoot components with the same letter do not differ (P>0.05).

Figure 10. Percent carbon in roots and shoots from the greenhouse experiment on a dry matter basis.



Root or shoot components with the same letter do not differ (P>0.05).

Table 7. Root measurement of samples from the greenhouse experiment.

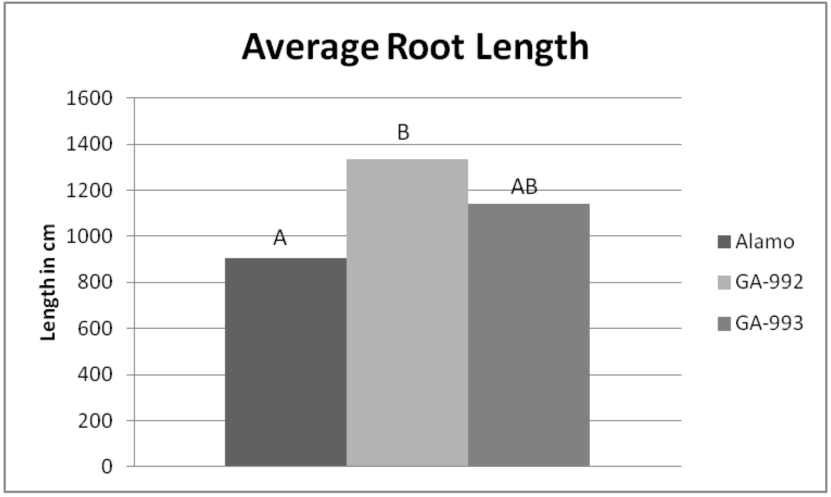
	Root Length(cm)	Root Diameter(mm)
Alamo	902.5*(312.4)†a‡	0.404(0.065)a
GA-992	1336.0(320.3)b	0.352(0.043)a
GA-993	1138.3(407.7)ab	0.349(0.060)a

*Values based on the average percent dry matter of ten samples.

†Standard deviations (SD) in parentheses.

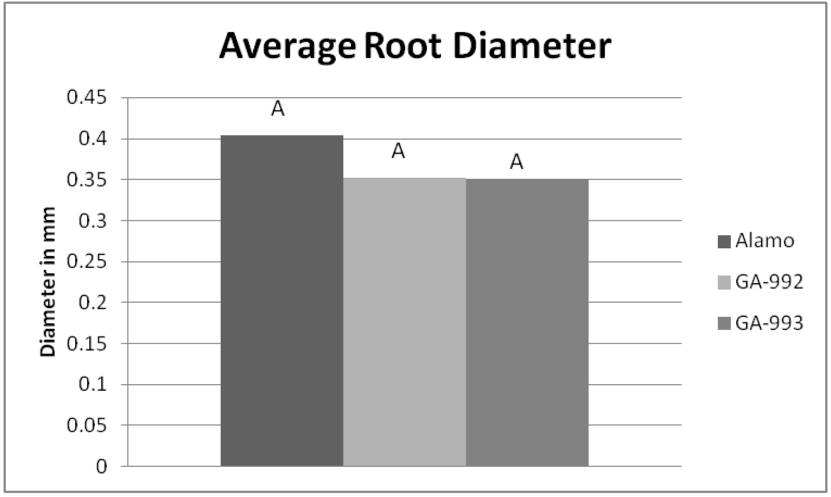
‡Columns with the same letter do not differ (P>0.05).

Figure 11. Average root length of samples from the greenhouse experiment.



Values with the same letter do not differ (P<0.05).

Figure 12. Average root diameter of samples from the greenhouse experiment.



Values with the same letter do not differ (P<0.05).

Table 8. Harvest dates and biomass yields from the field experiment planted on 6/12/2006.

Yield and Harvest Dates - Switchgrass Field Experiment

Alamo, GA-992 and GA-993 - Planted 6/12/06

Date of Harvest	Year	Alamo	GA_992	GA_993
11/2/2006	2006	7009*a†	5481a	7682a
2/29/2008	2007	9245a	9083a	8416a
10/10/2008	2008	15645b	10331b	16418b
11/9/2009	2009	11028ab	9681ab	8876ab
	Mean	10732	8644	10348

*Yields expressed as kg dry matter per hectare.

†Means with the same letter do not differ at $P<0.05$.

Figure 13. Total biomass yield (kg/ha) from the field experiment, 2006-2009.

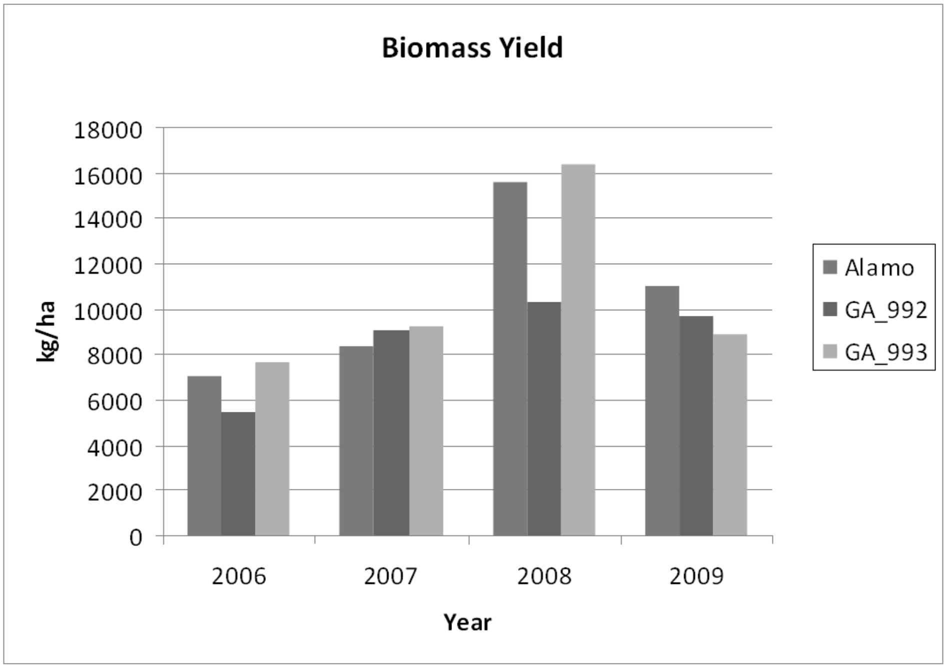


Table 9. Analysis of variance for weight of tiller components of samples from the field experiment, 2007 and 2008.

Effect		Degrees of freedom	*F* statistic	Probability of >*F*
Leaf Weight				
	Year	1	65.8	0.0000
	Genotype	2	0.7	0.5175
	Genotype x Year	2	0.1	0.9308
Stem Weight				
	Year	1	17.1	0.0025
	Genotype	2	0.2	0.8103
	Genotype x Year	2	0.2	0.8440
Leaf:Stem Ratio				
	Year	1	52.2	0.0001
	Genotype	2	2.0	0.2056
	Genotype x Year	2	0.3	0.7628
Total Weight				
	Year	1	24.0	0.0009
	Genotype	2	0.1	0.8757
	Genotype x Year	2	0.1	0.8861

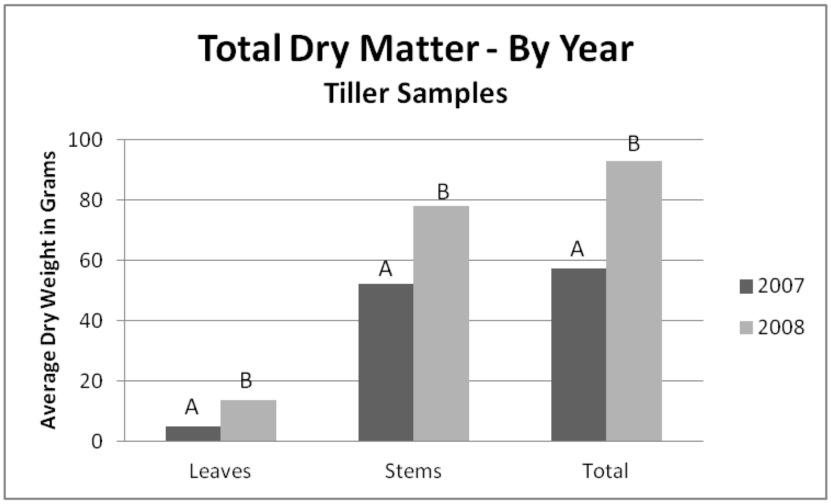
Table 10. Means and standard deviations of the weights of tiller components of samples from the field experiment, 2007 and 2008.

Data Based on the Total Dry Matter of the Leaves and Stems of Tillers Collected				
	Leaves	Stems	Total	Leaf:Stem Ratio
2007				
Alamo	5.15*	56.82	61.97	0.091
	(1.51)†	(14.83)	(15.96)	(0.019)
GA-992	4.60	51.67	56.27	0.091
	(0.53)	(9.28)	(9.17)	(0.020)
GA-993	4.39	48.16	53.65	0.114
	(2.61)	(7.81)	(8.68)	(0.011)
2008				
Alamo	15.00	77.50	92.50	0.193
	(4.08)	(14.27)	(17.82)	(0.031)
GA-992	13.75	80.50	94.25	0.172
	(3.77)	(20.34)	(23.23)	(0.037)
GA-993	12.60	76.25	92.00	0.216
	(7.54)	(21.79)	(23.42)	(0.058)

*All Values Expressed as Grams Dry Matter

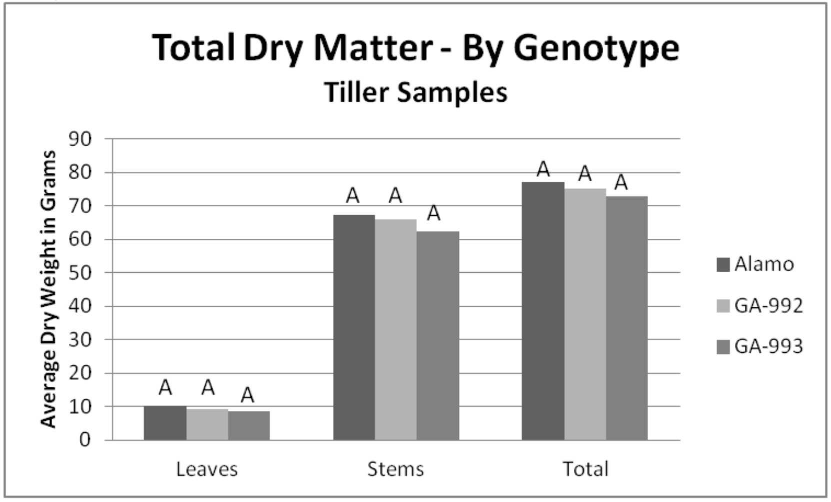
†Standard deviation (SD) in parentheses

Figure 14. Dry weight of whole, leaf, and stem components of tiller samples from the field experiment by year, 2007-2008.



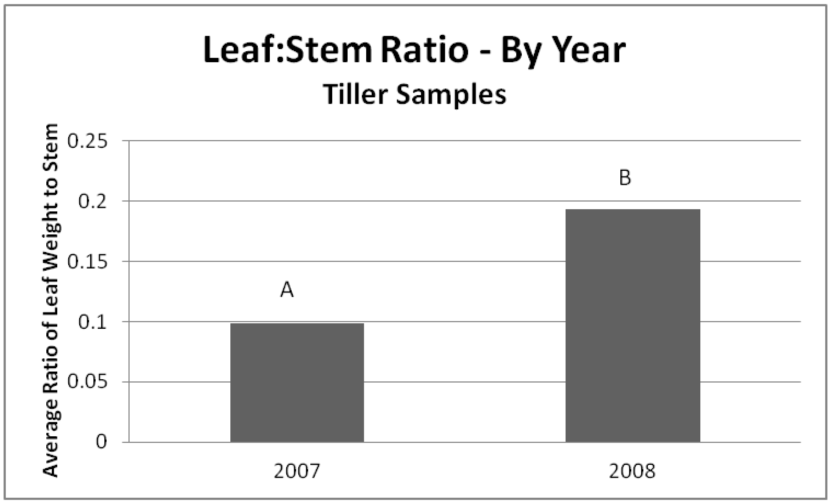
Values with the same letter do not differ (P<0.05).

Figure 15. Dry weight of whole, leaf, and stem components of tiller samples from the field experiment by genotype, 2007-2008.



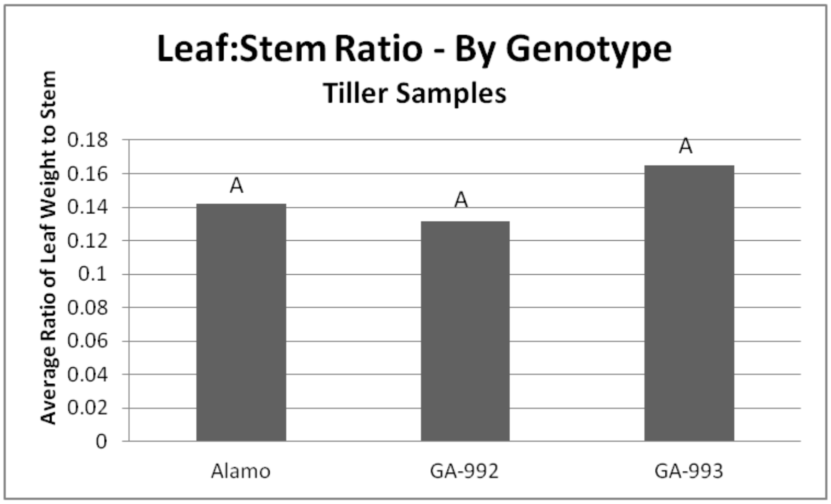
Values with the same letter do not differ (P<0.05).

Figure 16. Leaf to stem weight ratio on a dry matter basis from the field experiment by year, 2007-2008.



Values with the same letter do not differ (P<0.05).

Figure 17. Leaf to stem weight ratio on a dry matter basis from the field experiment by genotype, 2007-2008.



Values with the same letter do not differ (P<0.05).

Table 11. Analysis of variance for tiller length, diameter, and leaf number of samples from the field experiment, 2007 and 2008.

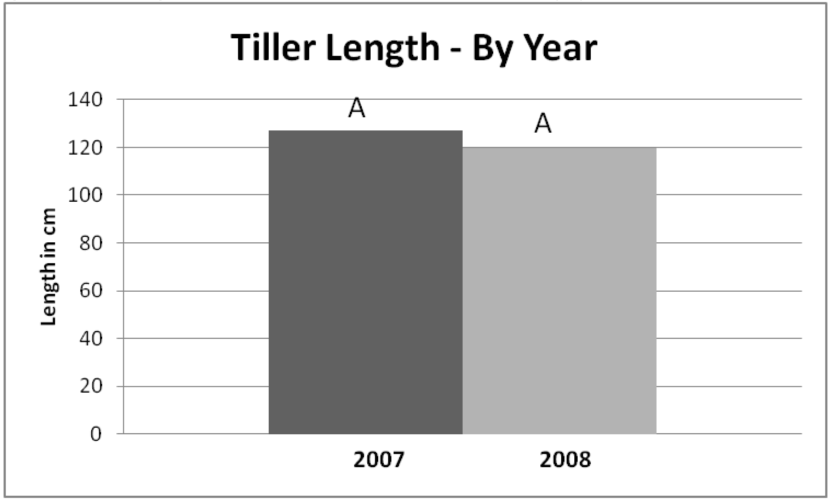
Effect	Degrees of freedom	*F* Statistic	Probability of >*F*
Tiller Length(cm)			
Year	1	2.4	0.1725
Genotype	2	0.1	0.8649
Genotype x Year	2	0.9	0.4361
Diameter(mm)			
Year	1	12.2	0.0068
Genotype	2	1.5	0.2729
Genotype x Year	2	0.6	0.5935
Number of Leaves			
Year	1	0.4	0.5879
Genotype	2	0.3	0.7687
Genotype x Year	2	0.1	0.8971

Table 12. Means and standard deviations for tiller length, diameter, and leaf number of samples from the field experiment, 2007 and 2008.

Average Measurements of Tillers Collected from Each Genotype (2007-2008)			
	Tiller Length(cm)	Diameter(mm)	Number of Leaves
2007			
Alamo	124.98	4.66	7.58
	(15.66)*	(0.87)	(0.93)
GA-992	130.18	4.84	7.30
	(22.02)	(0.78)	(1.09)
GA-993	125.50	4.45	7.43
	(14.50)	(0.76)	(0.98)
2008			
Alamo	124.98	4.34	7.40
	(18.34)	(0.65)	(1.24)
GA-992	116.00	4.11	7.30
	(18.50)	(0.58)	(1.47)
GA-993	118.45	3.83	7.23
	(18.53)	(0.83)	(1.67)

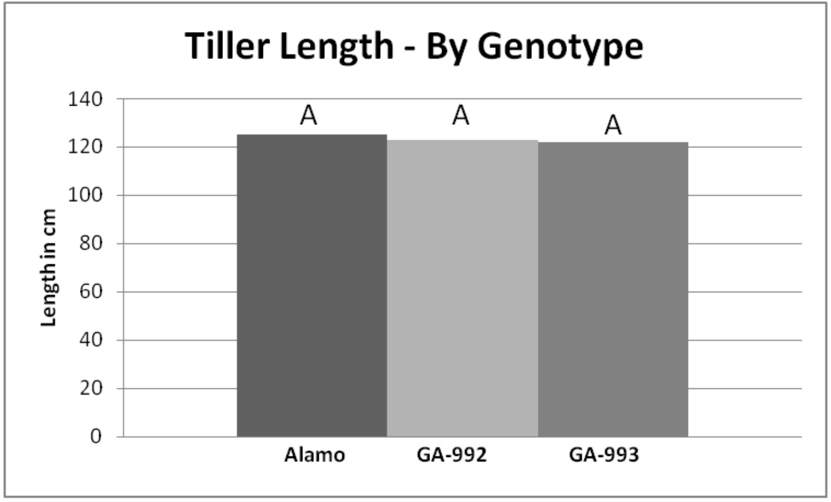
*Standard deviation (SD) in parentheses

Figure 18. Average tiller length from the field experiment by year, 2007 and 2008.



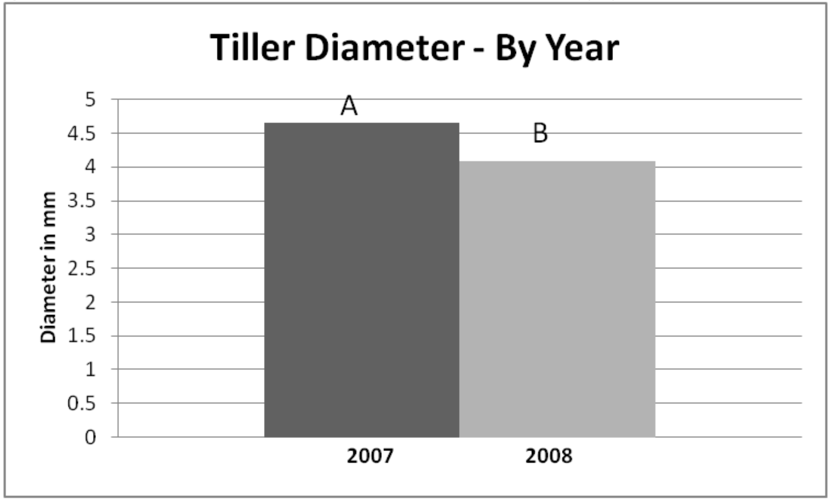
Values with the same letter do not differ (P<0.05).

Figure 19. Average tiller length from the field experiment by genotype, 2007 and 2008.



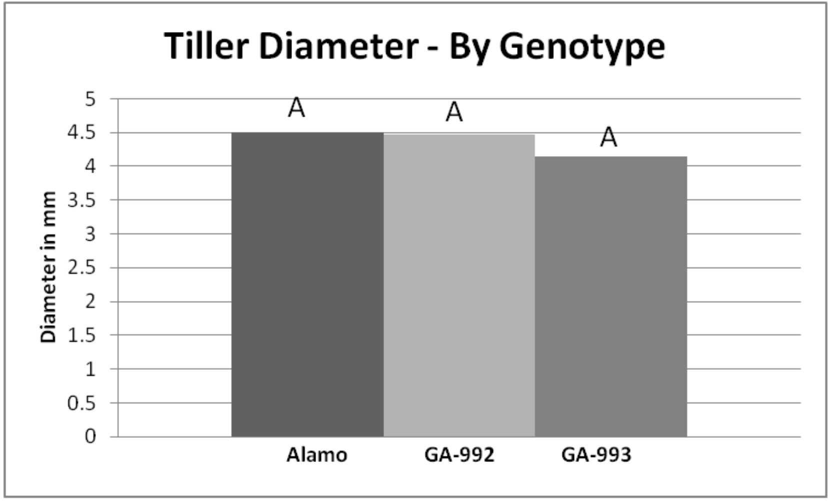
Values with the same letter do not differ (P<0.05).

Figure 20. Average tiller diameter from the field experiment by year, 2007 and 2008.



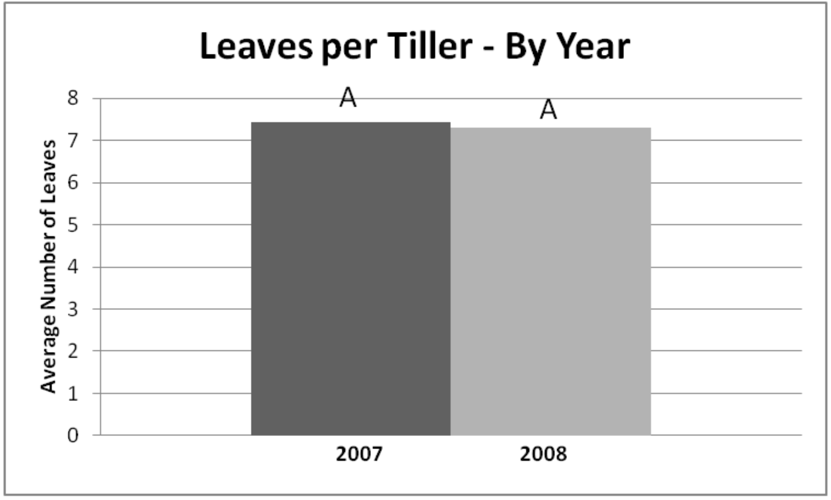
Values with the same letter do not differ (P<0.05).

Figure 21. Average tiller diameter from the field experiment by genotype, 2007 and 2008.



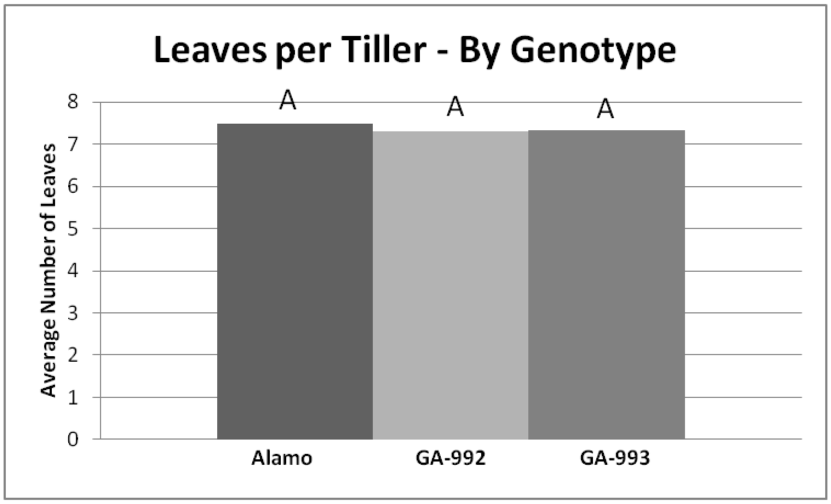
Values with the same letter do not differ (P<0.05).

Figure 22. Average number of leaves per tiller from the field experiment by year, 2007 and 2008.



Values with the same letter do not differ (P<0.05).

Figure 23. Average number of leaves per tiller from the field experiment by genotype, 2007 and 2008.



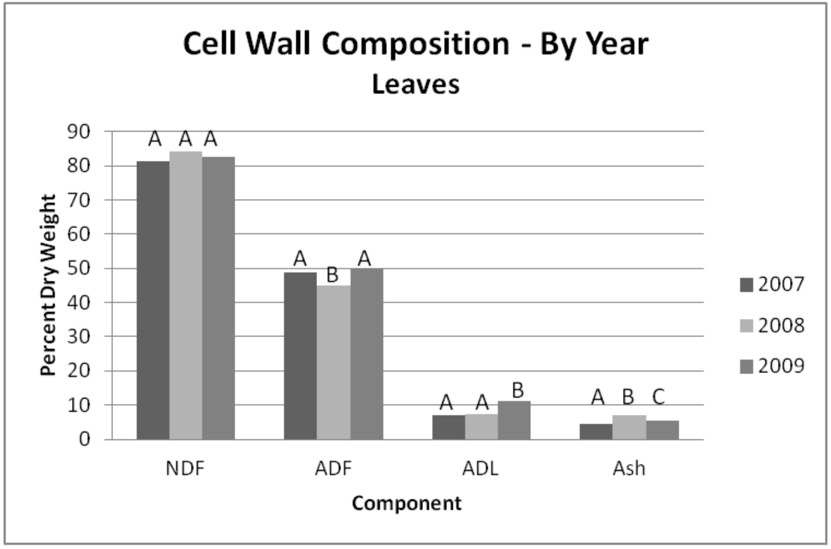
Values with the same letter do not differ (P<0.05).

Table 13. Analysis of variance for leaf cell wall composition of samples from the field experiment, 2007-2009.

Leaf Composition

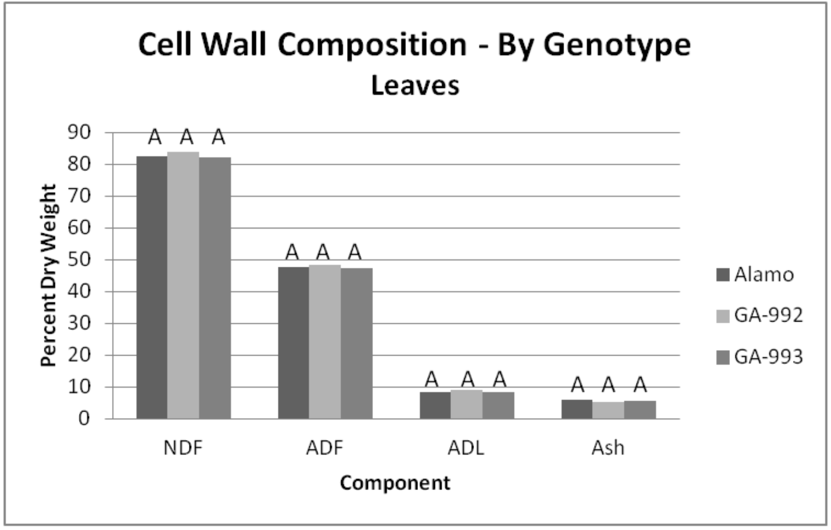
Effect	Degrees of freedom	*F* statistic	Probability of >*F*
Neutral Detergent Fiber			
Genotype	2	0.632	0.5533
Year	2	1.743	0.2349
Genotype x Year	4	0.788	0.5612
Acid Detergent Fiber			
Genotype	2	0.890	0.4502
Year	2	19.933	<0.001
Genotype x Year	4	0.825	0.5265
Acid Detergent Lignin			
Genotype	2	0.999	0.4125
Year	2	36.909	<0.001
Genotype x Year	4	1.566	0.2500
Ash			
Genotype	2	2.628	0.1218
Year	2	28.771	<0.001
Genotype x Year	4	0.352	0.8385

Figure 24. Leaf cell wall composition from the field experiment by year, 2007-2009.



Cell wall components with the same letter do not differ (P>0.05).

Figure 25. Leaf cell wall composition from the field experiment by genotype, 2007-2009.



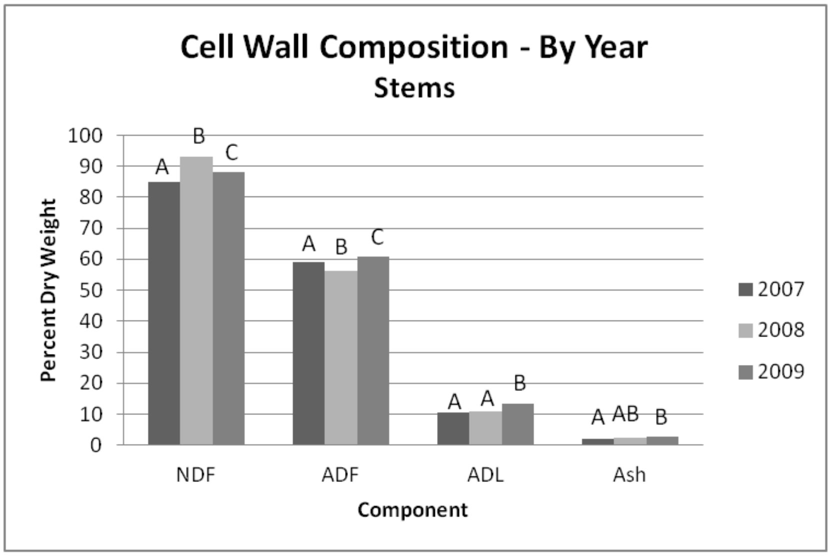
Cell wall components with the same letter do not differ (P>0.05).

Table 14. Analysis of variance of stem cell wall composition of samples from the field experiment, 2007-2009.

Stem Composition

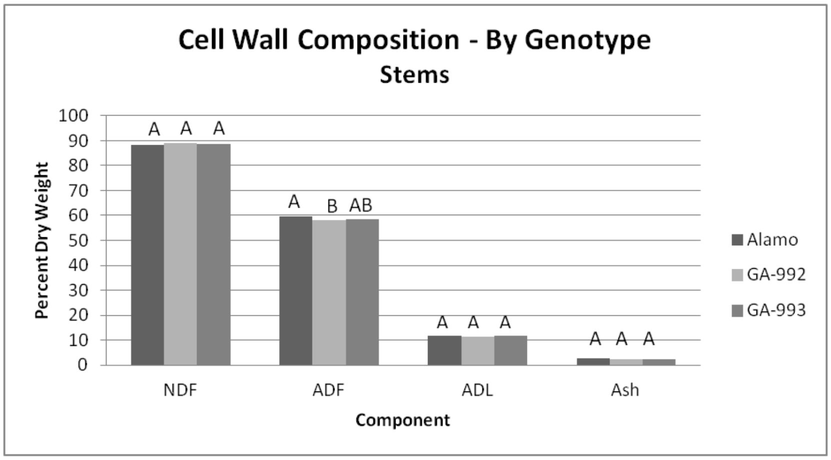
Effect	Degrees of freedom	*F* statistic	Probability of >*F*
Neutral Detergent Fiber			
Genotype	2	0.638	0.5550
Year	2	116.787	<0.001
Genotype x Year	4	0.211	0.9276
Acid Detergent Fiber			
Genotype	2	4.377	0.0434
Year	2	32.121	<0.001
Genotype x Year	4	0.142	0.9626
Acid Detergent Lignin			
Genotype	2	2.282	0.1496
Year	2	55.604	0.0001
Genotype x Year	4	0.177	0.9401
Ash			
Genotype	2	3.023	0.1089
Year	2	5.272	0.0332
Genotype x Year	4	1.951	0.1657

Figure 26. Stem cell wall composition from the field experiment by year 2007-2009.



Cell wall components with the same letter do not differ (P>0.05).

Figure 27. Stem cell wall composition from the field experiment by genotype, 2007-2009.



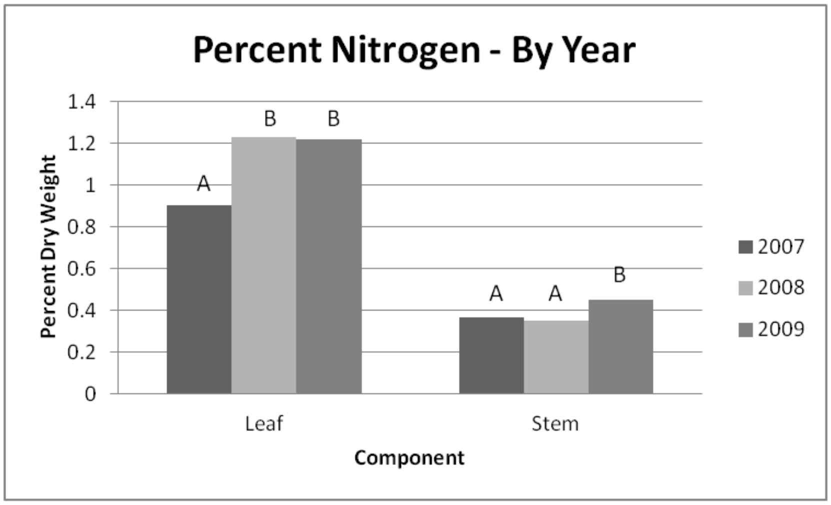
Cell wall components with the same letter do not differ (P>0.05).

Table 15. Analysis of variance of the percentage of nitrogen and carbon in the leaves and stems of samples from the field experiment, 2007-2009.

Percentage of Carbon and Nitrogen in Leaves and Stems

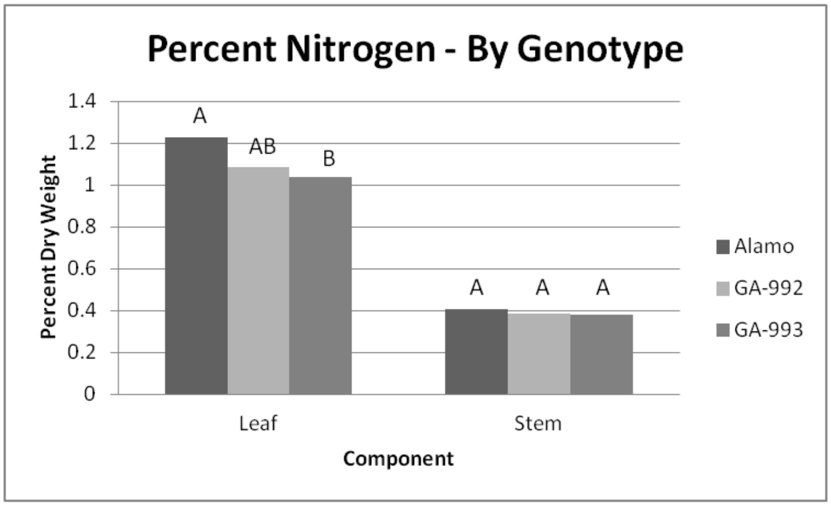
Effect		Degrees of freedom	*F* Statistic	Probability of >*F*
Leaves				
Carbon				
	Genotype	2	0.6	0.5856
	Year	2	11.1	0.0009
	Genotype x Year	4	1.7	0.2042
Nitrogen				
	Genotype	2	5.7	0.0206
	Year	2	15.6	0.0011
	Genotype x Year	4	0.4	0.7959
Stems				
Carbon				
	Genotype	2	0.1	0.8676
	Year	2	8.9	0.0047
	Genotype x Year	4	0.9	0.5284
Nitrogen				
	Genotype	2	1.2	0.3705
	Year	2	36.7	<0.001
	Genotype x Year	4	0.3	0.8612

Figure 28. Percent nitrogen in samples from the field experiment on a dry matter basis by year, 2007-2009.



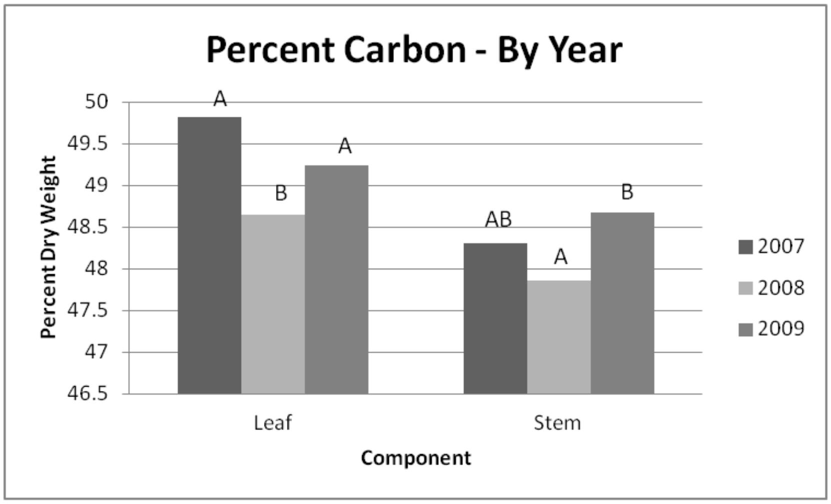
Leaf or stem components with the same letter do not differ (P>0.05).

Figure 29. Percent nitrogen in samples from the field experiment on a dry matter basis by genotype, 2007-2009.



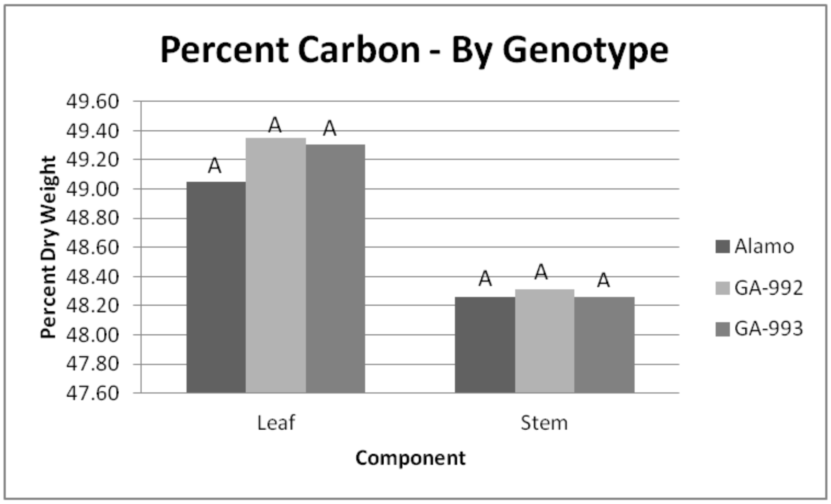
Leaf or stem components with the same letter do not differ (P>0.05).

Figure 30. Percent carbon in samples from the field experiment on a dry matter basis by year, 2007-2009.



Leaf or stem components with the same letter do not differ (P>0.05).

Figure 31. Percent carbon in samples from the field experiment on a dry matter basis by genotype, 2007-2009.



Leaf or stem components with the same letter do not differ (P>0.05).