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RESPONSES OF SOYBEAN ANCESTRAL LINES AND CULTIVARS TO SIMULATED ACID RAIN

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

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CHAPTER I

INTRODUCTION

Acid precipitation has become a growing concern nationally and internationally, especially because it can occur great distances from the source of the acid forming pollutants. The acidity in such precipitation results when nitrogen and sulfur oxides are emitted into the atmosphere and react with atmospheric moisture to form acidic solutions (10). Natural rain water is approximately pH 5.6. As a result of increased pollutant levels, precipitation in the Eastern United States has an average annual pH of 4.4, with individual rainfalls as low as pH 2.1 (10,15). In the Northeastern United States, half of the summer rain events are at pH 4.0 or below and as low as 3.0 (24). Analysis of prevailing winds indicates that in general, much of the acidity originates over industrial areas in the Midwest (7). Predictions have been made that with the increasing use of coal, precipitation will probably be at least as acidic in the future as it is at present (23).

It is important to understand the impact of acid precipitation on growth and yield of crops grown under agronomic conditions in the field. This research project was proposed to assist in evaluating the effect of acid precipitation on soybean [*Glycine max* (L. Merr.)]. The project described in this thesis is a combination of greenhouse (controlled environment) and field

experiments. Controlled environment studies are useful indicators of potential effects and may suggest subtle changes not measurable in a less controlled field situation, as well as reducing the number of variables in the experiment (20).

Foliar symptoms, due to simulated acid rain (hereafter referred to as SAR), occur more easily with plants grown in greenhouse environments than plants exposed to SAR in the field. However, greenhouse data may overestimate effects on growth and yield (25). A greenhouse study, as a source of reduced environmental variation, was conducted to complement the field trials at two of Tennessee's Agricultural Experiment Stations (Knoxville and Milan).

The objective of this research project was to ascertain the affects of simulated acid rain (SAR) on seed yield as well as physiological and morphological traits of soybean ancestral lines and cultivars derived from them.

The null hypotheses tested by these experiments were:

1: Simulated acid rain has no effect on soybean growth and yield.

2: Soil fertility level has no influence on the effect of simulated acid rain on soybeans.

3: There are no differences in sensitivities of the different lines of soybean to simulated acid rain.

CHAPTER II

LITERATURE REVIEW

I. YIELD RESPONSES

Visible foliar injury due to simulated acid precipitation has been documented for several species of crops, but it has not generally been related to effects on yield (23). There is little information on the physiological changes that produce these foliar lesions or what is occurring within plants that are exposed to low pH solutions and do not form lesions (13).

Lee et al. (23) observed varied patterns of yield response of 28 crops to SAR:

> Marketable yield production was decreased for radish, beet, carrot, mustard greens, and broccoli; marketable yield was stimulated for tomato, green pepper, strawberry, alfalfa, orchardgrass, and timothy; yield for potato was ambiguously affected by SAR; and other crops had no significant differences in yield as a result of SAR.
>
> Although foliar injury was not generally related to effects on yield, foliar injury of swiss chard, mustard greens, and spinach were harsh enough to reduce marketability. Variation between species and the portion of the plant to be utilized may determine the

probability that a yield will be affected by acid precipitation (23).

Rain fall acidity may also indirectly influence plant growth by its effect on soils and on plants grown with sub-optimal soil conditions (3). Long term effects of acidic precipitation on poorly managed soils may negatively affect crop productivity through leaching of soil nutrients (20).

Foliar injury is not necessarily related to seed yield or quality. If the photosynthetic area of the leaves is adequate to promote continued growth, foliar injury may be insignificant and only cosmetic. Many times plants can be subjected to severe foliar damage (such as hail or insects) in an early stage of development and still recover from the damage with no reduction in seed yields (29).

"The economic impact of acid rain depends on the part of the plant to be utilized, and how that portion is affected. In soybeans, seed yield is the final product after a growing season of exposure to many environmental factors (acid precipitation included) interacting with several hundred traits of the plant. For acid precipitation to affect yields, it must have a large impact on a few major traits which affect yield, or have small cumulative effects on many minor traits which affect yield" (2). Obtaining a high seed yield of good quality is the main objective of soybean production. Many different genetic traits and environmental factors may affect seed yield.

Brun and Setter (5) indicated that plant growth and yield "may be considered products of photosynthetic rate, integrated over time, and partitioning of the resulting photosynthate between physiological or morphological yield components". Ferenbaugh (15) determined that SAR increased the rate of photosynthesis in snap beans (Phaseolus vulgaris L.). The respiration rate also increased slightly.

Evans and Lewin (12) reported a reduction in pod number per plant in soybean (cv. Amsoy 71) at SAR treatments of pH 3.1 and below. This reduction may have resulted from an alteration in the sequential steps required during flowering, pollination, pod set, and pod development. SAR seemed to have a negative effect on at least one of these processes (12). The same report indicated that seed mass per plant increased by 11% at pH 3.1 compared to pH 5.7, while seed mass per plant decreased by 11% at pH 2.5 compared to pH 5.7. The mean mass per seed of plants exposed to pH 3.1 was greater than that of plants exposed to either pH 5.7 or 2.5. The larger seed yield of plants exposed to pH 3.1 was due to this greater mass per seed since the number of seeds per plant decreased significantly as acidity increased (12).

Troiano et al. (27) found that the size of both pods and seeds, as well as the number of seeds produced per pod, were significantly affected by SAR. The smallest seeds (determined by mass per 100 seeds) were produced at pH 3.4, but this was offset by an increase in the number of pods and seeds per plant. This project showed that the effect of SAR was related to ozone

concentrations in the atmosphere; when ozone was filtered from the air, acidic precipitation stimulated growth (27).

Irving and Miller (20) found similar results, and concluded that acidic precipitation could contribute to the nutritional requirements of soybeans and other plants by providing sulfur and nitrogen in low concentrations. Foliar applications of fertilizers containing nitrogen, phosphorus, potassium, and sulfur during the pod-filling stage have also been shown to result in higher soybean yields (16). This is attributed to replenishing nutrients that are depleted during reproductive growth.

Evans and Thompson (14) exposed soybean plants to SAR of pH 4.1, 3.3, and 2.7, which resulted in decreased yields of 10.6, 16.8, and 23.9%, respectively. The decrease in seed mass per plant was attributed to a decrease in the number of pods per plant, because the number of seeds per pod and the mass of the individual seeds did not vary significantly among the different pH treatments.

II. PLANT GROWTH AND PHYSIOLOGY

Evans and Lewin (12) reported that SAR of pH 3.2 and 2.5 decreased the dry mass of soybean stems and leaves. Some plants have little or no injury from contact with acid rain. One possible explanation is the ability of the tissue of some plant species to buffer the acid before any significant physical or physiological damage can occur. Results of testing by Craker and Bernstein (8) indicated that soybean leaf tissue was the least

susceptible (of six plant species) to acidic solutions. Buffering was illustrated by immersing plant tissue into different acidic solutions and recording the pH over a four-hour time period. The buffering phenomenon could be the result of leachates interacting with the acid solution, or of an internal disruption and release of cell contents neutralizing the hydrogen ion concentration of the acid rain (8). Evans et al. (13) also recognized that the chemistry of leaf surfaces and the cells within the leaf could be altered by acid solutions. Nutrient leaching from plant foliage due to acid precipitation may inhibit plant growth.

Leaves with a smooth waxy surface that makes wetting difficult are less susceptible to leaching. Leaves which are relatively large, flat surfaced, pubescent, and easily wetted are more readily leached (28). Soybean cultivars vary widely in pubescence and stomatal distribution.

Leaves from healthy and vigorously growing plants, with adequate nutrient supplies, are less susceptible to leaching than are leaves which are nutritionally deficient (28). Soil conditions could therefore be a factor indirectly influencing foliar leaching.

A decrease in the leaf area available to soybeans may not be detrimental to yield. Hodgkinson (18) demonstrated that partial defoliation (in alfalfa) promoted an increased photosynthetic rate in the remaining leaves. Brun (4) noted that there has not been a significant correlation between the photosynthetic rates of different soybean cultivars and their yields. He hypothesized

that this could be because photosynthetic rates are usually measured on young vegetative plants; photosynthetic rate at this time has very little effect on the final yield. Also photosynthesis is only one of the processes contributing to yield (4). Specific leaf weight (SLW = leaf dry weight per unit area) has been shown to be positively correlated with photosynthetic rate in alfalfa (26).

The growth and yield response of some plants to acidic precipitation is positive while others show a negative response. The plants which exhibit a positive reaction appear to utilize the rain as a fertilizer. Negative effects appear to be the result of nutrient leaching and foliar lesions. An interaction between positive and negative effects results in the net response to acidic precipitation (21).

III. STOMATAL FREQUENCY

Though stomates are primarily responsible for gas exchange and transpiration, they also allow water exchange in leaves (11). This is important relative to the formation of lesions and their proximity to stomata. Stomatal frequency in soybeans is quite varied. Ciha and Brun (6) examined 43 varieties of soybeans and found that stomatal frequency on the upper layer of the leaves ranged from 81 to 174 mm^{-2}. The lower layer of soybean leaves had between 242 and 345 stomates mm^{-2}. This variation in stomate numbers may result in a large variation in transpiration rates (6). Another possibility may be that there are differences among

soybean cultivars in stomatal sensitivity to environmental factors. The variation in stomatal frequency in combination with the variation in stomatal sensitivity may be important in explaining increased nutrient leaching and foliar lesions due to SAR (11).

IV. GENETIC BACKGROUND OF NORTH AMERICAN SOYBEAN CULTIVARS

The current soybean germplasm collection, maintained by the Plant Sciences Research Division of the USDA, contains about 4,000 accessions (ancestral lines), including 200 named varieties (19). This collection is divided into two groups based on the maturity classification of the accessions. The Northern Soybean Germplasm Collection, maintained at Urbana, Illinois, contains accessions in maturity groups OO-IV. Accessions in maturity groups V-VIII constitute the Southern Soybean Germplasm Collection, maintained at Stoneville, Mississippi (17).

Although a large number of soybean varieties have been developed from each of the two collections, most have their origin in a small number of ancestral lines. According to Delannay et al. (9), the genetic makeup of the northern soybean cultivars developed and released in the United States from 1971-1981 is traceable to ten accessions contained in the northern collection. One such accession is 'Mandarin' which accounts for more than 30% of the genes contained in those cultivars. More than 80% of the southern cultivars released during that same time period have their genetic origin in seven accessions. In this case, the

accessions CNS and S-100 contribute more than 50% of the genes in the southern gene pool. A highly used introductory line, CNS was present in the pedigrees of 20 of the 22 southern cultivars released in the 1971-1981 time span (9). Allen and Bhardwaj (1) presented detailed lineage diagrams of selected soybean cultivars which have been developed from these northern and southern cultivars.

Johnston and Shriner (22) studied the response of three wheat cultivars to SAR and indicated that the two most closely related cultivars (Abe and Arthur 71) responded similarly while the cultivar Oasis which was less closely related responded differently with respect to foliar growth.

Irving (20) suggests that it may be important to consider that crop cultivar recommendations are based on productivities obtained under ambient conditions of precipitation acidity; therefore, crops currently being grown may have been selected indirectly (through natural selection) for their adaptations to rainfall acidity and the presence of other pollutants.

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CHAPTER III

THE EFFECT OF SIMULATED ACID RAIN ON SOYBEAN ANCESTRAL LINES AND CULTIVARS

I. ABSTRACT

During 1985, 1986, and 1987, field and greenhouse experiments were conducted on four ancestral lines and two cultivars from each of the northern and southern gene pools. The ancestral lines have contributed approximately 80% of the genes to their respective gene pools. Representatives from the northern germplasm collection included Mandarin, Manchu, AK Harrow, Richland, 'Amsoy 71', and 'Williams 82'. The southern germplasm collection was represented by CNS, S 100, PI 54610, Tokyo, 'Lee 74', and 'Essex'. Spray-to-wet applications of simulated acid rain (SAR) were applied at three acidity levels (pH 2.8, 3.2, and 4.3). Field experiments were grown under optimum and sub-optimum soil conditions (pH and fertility) at two diverse locations to determine if soybean response to SAR was affected by edaphic factors. In general, SAR had no effect on seed yield. In cases where yield was affected, there were more positive than negative effects. There was no consistent trend with respect to optimum vs sub-optimum soil conditions. Seed weight was affected by SAR for some lines but was not necessarily related to yield. In general, photosynthesis, transpiration, and stomatal frequency were not affected by SAR. Early stages of plant growth (leaf and

stem weight) in the greenhouse was affected by SAR, but measurements taken on the same characteristics during later growth stages were not significant. These results indicate that soybean cultivars in general will not be detrimentally affected by acid rain based on the response of their ancestral lines and evaluations of selected cultivars derived from them.

II. INTRODUCTION

Varied yield responses to simulated acid rain (SAR) have been observed for soybean. Yield increases have been associated with a foliar fertilizer effect contributed by S and N during the critical pod-filling stage (10,12). Evans et al. (6,7) and Banwart (2) observed a decrease in yield for the soybean cultivar 'Amsoy 71'. Additional researchers found no response to SAR for yield of soybean (9,10).

Banwart (2) observed that very few growth parameters of soybean were influenced by SAR of pH 3.0. Additionally he reported that photosynthetic rate was not affected at this pH; however, it was reported that SAR lowered the pH of the upper 2 cm of soil in a highly buffered midwestern soil.

Ciha and Brun (3) reported that stomatal frequency of soybean cultivars was influenced by the environmental factors of light and temperature, and that genotypes varied widely. They also suggested that stomata are one of the regulating factors in photosynthesis and transpiration; they indicated that soybean cultivars may also differ in their sensitivities to environmental

stress. Ferenbaugh (8) observed increased photosynthetic rates at low SAR pH (2.0) for Phaseolus vulgaris plants.

Delannay et al. (5) reported on the narrowness of the soybean genetic base and the loss of genetic diversity among cultivars. Their report indicated that the North American soybean gene pool had its origin in only 50 plant introductions. This lack of diversity has been of concern to breeders and geneticists due to crop vulnerability; comparisons have been made to the narrow genetic base of corn which led to the leaf blight epiphytotic in 1970. Allen et al. (1) suggested that this narrow genetic base which is present in modern cultivars may be used in assessing the impact of acid precipitation on soybeans without having to test all the currently available varieties. If genes were present in the ancestral lines which predisposed susceptibility or lack of response to SAR, these characteristics are likely to be present in their progeny.

The objectives established for this research project were to: 1) study the effect of simulated acid rain on soybean production, 2) compare sensitivities among ancestral lines and cultivars developed from them, 3) compare the responses between optimum and sub-optimum soil conditions, and 4) evaluate several factors that may have an effect on physiological processes and responses.

III. MATERIALS AND METHODS

Field and greenhouse experiments were conducted on twelve lines of soybean; four ancestral lines and two cultivars were chosen

from each of the northern and southern gene pools. The ancestral lines Mandarin, Manchu, AK Harrow, and Richland, and the cultivars 'Amsoy 71' and 'Williams 82' were representative of the northern gene pool. The ancestral lines CNS, S 100, PI 54610, and Tokyo, and the cultivars 'Lee 74' and 'Essex' represented the southern gene pool. Simulated acid rain (SAR) of three acidity levels (pH 2.8, 3.2, and 4.3) was formulated reflecting the average rainfall composition as reported by Cogbill and Likens (4) (Appendix A). Analyses of variance were conducted using the GLM procedure of the Statistical Analysis System (SAS) (11); means were separated using Duncan's multiple range test.

Field Experiments

Field experiments were conducted for two years (1985 and 1986) at two locations (Milan and Knoxville) on optimum and sub-optimum (lower pH and available nutrients) soils. The soil at the Knoxville Plant Science Field Laboratory was a Statler sandy loam variant (Humic Hapludult) with optimum plots at pH 5.9 and sub-optimum plots at pH 5.0. At the Milan Experiment Station, soil was a Grenada silt loam (Glossic Fragiudalf) with optimum plots at pH 5.9 and sub-optimum plots at pH 4.9. The field layout was a split-split plot (in three replications) where soil fertility served as the main plot, SAR pH was the sub-plot, and soybean line was the sub-sub plot. Variety plots consisted of three rows 3.05 m in length with 91 cm row spacing. SAR of three pH levels (4.3,

3.2, and 2.8) was applied as spray-until-run-off treatments three times per week in Knoxville and two times per week in Milan. SAR spray applications began at the V2 stage of vegetative growth and continued through senescence. The simulated rainfall was applied with a tractor mounted spraying system using a full cone stainless steel nozzle delivery approximately 11 liters per minute at 6.9 X 10^3 Pa and traveling 1950 rpm; the rain resembled a moderate intensity rain event.

At maturity, 2.4 m of the center row of each plot (ends were trimmed to eliminate border effects) was harvested for seed yield with a plot thresher. Yield and 100 seed weight were measured and adjusted to 13% moisture.

Additional measurements, taken for seven of the lines at the Knoxville location in 1985, included leaf number, leaf area, leaf dry weight, stem dry weight, and specific leaf weight. In addition, transpiration rate and stomatal conductivity were recorded, on two separate dates (July 12, 1985 and August 22, 1985) for the same seven lines using the LI-COR Steady State Porometer (Model LI 1600). Leaf area was measured using the LI-COR Area Meter (Model LI-3000). Specific leaf weight (SLW), was calculated as the ratio of leaf dry weight per unit of leaf area (g cm^{-2}).

At Knoxville in 1986, stomatal frequency, for each of the twelve lines, was obtained from impressions of the upper leaf surface on the center leaflet of the fourth fully expanded trifoliolate from the plant apex. The plants were between the R5

(beginning seed) and R7 (beginning maturity) stage of reproductive growth. Leaf impressions were taken from two plants of each entry within each SAR pH and soil fertility level for the first and second replications. Leaf impressions were taken from only one plant of each entry within each pH and soil fertility level for the third replication.

These leaf impressions were made by spraying plastic spray (Appendix B) on the leaf surface and allowing it to dry for approximately two minutes. Clear tape was then placed over the dried spray and peeled off; the tape was then applied to a microscope slide. Stomatal counts were made in six randomly chosen (0.12 mm^2) microscopic fields (40X magnification) per replication.

Greenhouse Experiments

A greenhouse experiment was conducted in 1986 and in 1987. Procedures were similar for both experiments. In both experiments 12 to 18 seeds were planted in 20 cm diameter black plastic pots containing approximately 5.7 liters of 'Promix BX'. After germination, seedlings were thinned to 8 plants per pot. The same twelve soybean lines used for the field experiments were used for the greenhouse experiments. The pots were arranged in a split-plot design with four replications. Rainfall pH was the main plot and variety was the sub-plot. SAR treatments of three pH levels (4.3, 3.2, and 2.8) as well as a control of deionized water

(pH 5.2) were applied three times per week for one minute beginning at the V2 stage of vegetative growth. Plants were fertilized at two week intervals with 5 g l^{-1} of soluble 20-8.8-16.3 fertilizer. Spider mites were controlled with 'Kelthane' and 'Tedion dithio' insecticidal bombs. Rain simulant was applied with a '1/2 HH 30 W' full cone nozzle at 35 X 10^3 Pa (8.3 liters per minute) for one minute while plants rotated on a table beneath the nozzle.

Greenhouse Experiment # 1 1986:

Twenty-one days following the first SAR treatment, three of the plants were harvested (excluding the roots) from each pot. Thirty-four days following the first SAR treatment, two additional plants were harvested from each pot. Leaf number, leaf area, leaf dry weight, stem dry weight, and SLW were recorded for each of these harvest dates. Transpiration rates and stomatal conductivity were measured on one plant per pot when the plants reached the R1 (beginning flower) stage of reproductive growth.

Greenhouse Experiment # 2 1987:

Fourteen days following the first SAR treatment, two of the plants were harvested (excluding the roots) from each pot. Twenty-eight days following the first SAR treatment, two additional plants were harvested from each pot. Leaf number, leaf area, leaf dry weight, stem dry weight, and SLW

were recorded for each of these harvest dates. Photosynthetic rates were taken on two dates approximately 14 days apart (38 and 52 days following the first spray date). The photosynthetic measurements were taken on the center leaflet of a fully expanded trifoliolate, one to four nodes above the unifoliate, using an infrared gas analyzer (Analytical Development Corporation).

IV. RESULTS AND DISCUSSION

Field Experiments

Soybean Seed Yield:

Analysis of variance for soybean seed yield was conducted across years and locations (Table 1). Simulated acid rain (SAR) pH had no significant ($P \leq .05$) effect on soybean yield when considered across years, locations, soil conditions, and entries (Table 1). Yield means for each SAR pH level ranged from 1682 kg ha^{-1} for pH 4.3 to 1701 kg ha^{-1} for pH 2.8; these means are presented in Table 2.

There were significant differences ($P \leq 0.01$) between optimum and sub-optimum soil conditions (Table 1). Optimum soil conditions resulted in a 19.7% yield increase over the sub-optimum conditions (1846 and 1543 kg ha^{-1} respectively, Table 3). There was not a significant interaction ($P \leq 0.05$) between soil

Table 1. Analysis of variance for seed yield combined across years, locations, soil fertility levels, SAR treatments, and soybean lines.

Source of Variation	df	MS
Year (Y)	1	219539556 **
Location (L)	1	133871034 **
Y x L	1	7534491 **
Error A	8	762512 **
Soil (S)	1	19906445 **
S X Y	1	1156476
S*L	1	5617616 **
S X Y X L	1	189137
Error B	8	243227
pH	2	35279
pH X Y	2	462625
pH X L	2	475528
pH X Y X L	2	318428
pH X S	2	53967
pH X S X Y	2	86678
pH X S X L	2	727838 *
pH X S X L X Y	2	84323
Error C	32	153240
Entry (E)	11	12343038 **
E X Y	11	3864116 **
E X L	11	438357 **
E X Y X L	11	1915398 **
E X S	11	221613
E X S X Y	11	198912
E X S X L	11	133629
E X S X Y X L	11	202003
E X pH	22	235308
E X pH X Y	22	279028 *
E X pH X L	22	165488
E X pH X Y X L	22	242285
E X pH X S	22	140454
E X pH X S X Y	22	148919
E X pH X S X L	22	179495
E X pH X Y X L X S	22	131179
Error D	528	165414

*, ** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

Table 2. Mean seed yield at each simulated acid rain (SAR) pH level averaged across years, locations, soil fertility levels, and soybean lines.

SAR pH	Mean Yield	Standard Error
	---------- ($kg\ ha^{-1}$)	---------
2.8	1701 a [s]	56
3.2	1701 a	52
4.3	1682 a	55

[s] Means followed by the same letter are not significantly different ($P \leq .05$) from other means.

Table 3. Mean seed yield by simulated acid rain (SAR) pH within optimum and sub-optimum soil fertility levels averaged across years, locations, and soybean lines.

SOIL CONDITIONS	SAR pH	SEED YIELD	STANDARD ERROR
		------- kg ha^{-1} -------	
OPTIMUM	2.8	1868 a [s]	86
	3.2	1842 a	79
	4.3	1829 a	86
SUB-OPTIMUM	2.8	1534 b	68
	3.2	1560 b	65
	4.3	1534 b	67

[s] Means followed by the same letter are not significantly different ($P \leq .05$).

condition and SAR pH indicating that yield response to SAR was not influenced by soil condition (Table 1).

Analysis of variance indicated, as was expected, significant differences ($P \leq .01$) in yield among entries (Table 1). Soybean seed yield means ranged from 1204 kg ha^{-1} for Richland to 2513 kg ha^{-1} for Essex when averaged across SAR pH levels, soil fertilities, years, and locations (Table C-1). There was not a significant entry X pH interaction for yield indicating that in general there was no difference in the relative rankings of entries with respect to different SAR pH levels (Table 1). There was also no significant entry X soil fertility interaction for yield indicating that in general there was no difference in the relative rankings of entries with respect to soil fertility (Table 1). In addition there was no significance for the entry X pH X soil fertility interaction; thus entries did not respond differently in relation to each other with respect to SAR pH level in combination with soil fertility (Table 1).

Mean yields for each of the soybean lines and cultivars at each SAR pH level are shown in Table 4. Duncan's Multiple Range test revealed that Mandarin and Richland, two of the northern ancestral lines had a positive response to increased acidity of the SAR (Table 4). Yields for Mandarin and Richland increased by approximately 15% when acidity of the SAR treatment increased from pH 4.3 to pH 2.8. No significant responses were observed for the other two northern ancestral lines, Manchu and AK Harrow, nor for either of the northern cultivars, Amsoy 71 or Williams 82. No

Table 4. Mean seed yields of northern and southern ancestral lines and cultivars at each simulated acid rain (SAR) level, averaged across years, locations, and soil fertility levels.

	SAR pH	Mean Yield	Standard Error
Northern Ancestral Lines		------ kg ha^{-1} ------	
	2.8	1434 a [s]	132
MANDARIN	3.2	1304 ab	87
	4.3	1245 b	98
	2.8	1455 a	114
MANCHU	3.2	1318 a	121
	4.3	1399 a	135
	2.8	1296 a	112
AK HARROW	3.2	1361 a	100
	4.3	1219 a	101
	2.8	1302 a	116
RICHLAND	3.2	1176 ab	100
	4.3	1132 b	119
Northern Cultivars			
	2.8	1525 a	138
AMSOY 71	3.2	1575 a	149
	4.3	1575 a	130
	2.8	2078 a	217
WILLIAMS 82	3.2	2188 a	194
	4.3	2180 a	185

Table 4 (continued).

	SAR pH	Mean Yield	Standard Error
Southern Ancestral Lines		------- kg ha^{-1} ------	
	2.8	1288 a [s]	148
CNS	3.2	1376 a	137
	4.3	1412 a	208
	2.8	1764 a	170
S 100	3.2	1982 a	196
	4.3	1792 a	175
	2.8	1571 a	199
PI 54610	3.2	1703 a	201
	4.3	1705 a	204
	2.8	2008 a	291
TOKYO	3.2	1726 a	169
	4.3	2068 a	266
Southern Cultivars			
	2.8	2065 a	168
LEE 74	3.2	2149 a	183
	4.3	2093 a	180
	2.8	2623 a	251
ESSEX	3.2	2554 a	228
	4.3	2361 a	231

[s] Means followed by the same letter are not significantly different ($P \leq .05$) for that entry.

significant responses were observed for the southern ancestral lines or cultivars with respect to SAR pH when analyses of variance were conducted for each entry across years, locations and levels of soil fertility (Table 4).

There were significant differences ($P \leq 0.01$) for yield between the two years, and between the two locations; there was also a significant year X location interaction (Table 1).

The mean yield in 1985 across entries and locations was 2199 kg ha^{-1}; this was 85% higher than the mean yield of 1191 kg ha^{-1} in 1986 (Table C-2). The average yield at Knoxville was 61% higher than the average yield at Milan across entries and years (2089 versus 1301 kg ha^{-1} respectively; Table C-2). The overall mean yield across all entries, SAR pH treatments, soil fertility levels, locations, and years was 1695 kg ha^{-1}.

As indicated by the combined analysis of variance, the yield response to the pH of SAR treatment had a significant interaction with soil fertility and location (Table 1). At the Milan location in 1985, plots treated with SAR of pH 3.2 and 2.8 had significantly higher ($p \leq .05$) yields than plots treated with pH 4.3, when tested across levels of soil fertility (Table C-2). Yields increased from 1805 to 1964 kg ha^{-1} or 8.8% when acidity increased from pH 4.3 to pH 2.8. When rain pH increased from 4.3 to pH 3.2 yield increased by 6.8% (1805 to 1927 kg ha^{-1}). The significant response at Milan in 1985 is a reflection of the significant response to SAR treatments under optimum soil

fertility where the yield at pH 2.8 was 16% higher than the yield at pH 4.3 (Table 5).

The analysis of variance also indicated statistical significance ($P \leq .05$) for entry X year, entry X location, and entry X year X location interactions (Table 1). In addition, there was a significant ($P \leq .05$) entry X pH X year interaction indicating that the entries did not respond the same to SAR pH levels from one year to the next.

When analyses of variance were conducted by entry for each year, location, and level of soil fertility, there were several significant responses observed. There are eight sets of year - location - soil fertility treatment combinations (Figures 1 through 4) for each of the twelve soybean lines. Northern ancestral lines and cultivars will be considered first:

Northern Ancestral Lines:

Mandarin responded positively to increased acidity of SAR in two out of the eight combinations (Figures 1 and 4) and lacked any significant response in the other six. When considered across years and locations, no significant yield response was observed for the entry Mandarin (Table C-3, Appendix). Manchu had one positive response and one negative response (Figures 3 and 1) out of the eight combinations, but when analyzed across years and locations, Manchu showed a positive response to SAR under sub-optimum soil fertility (Table C-3). The responses for AK Harrow were ambiguous in two out of the eight combinations (Figures 2 and 3); both of these figures show higher yields at pH 3.2 than for

Table 5. Mean seed yield at each simulated acid rain (SAR) pH for each location, year, and soil fertility averaged across soybean lines.

LOCATION	YEAR	SOIL CONDITION	SAR pH	MEAN YIELD	STANDARD ERROR
				---- kg ha^{-1} ----	
			2.8	2846 ¶	157
		OPTIMUM a[§]	3.2	2619	147
			4.3	2882	154
Knoxville	**1985**				
			2.8	2219	120
		SUB-OPTIMUM b	3.2	2206	116
			4.3	2219	112
			2.8	1767	106
		OPTIMUM a	3.2	1882	86
			4.3	1926	93
Knoxville	**1986**				
			2.8	1437	86
		SUB-OPTIMUM b	3.2	1592	93
			4.3	1461	76
			2.8	2094 a	146
		OPTIMUM a	3.2	2071 a	143
			4.3	1809 b	142
Milan	**1985**				
			2.8	1833	124
		SUB-OPTIMUM b	3.2	1782	106
			4.3	1800	128
			2.8	764	43
		OPTIMUM a	3.2	794	47
			4.3	700	38
Milan	**1986**				
			2.8	645	27
		SUB-OPTIMUM b	3.2	661	28
			4.3	656	31

[§] Soil conditions followed by the same letters are not significantly different ($P \leq .05$) within a year and location
¶ Means followed by the same letters or no letters are not significantly different ($P \leq .05$). Letters are only for comparison within a year, location, and soil condition.

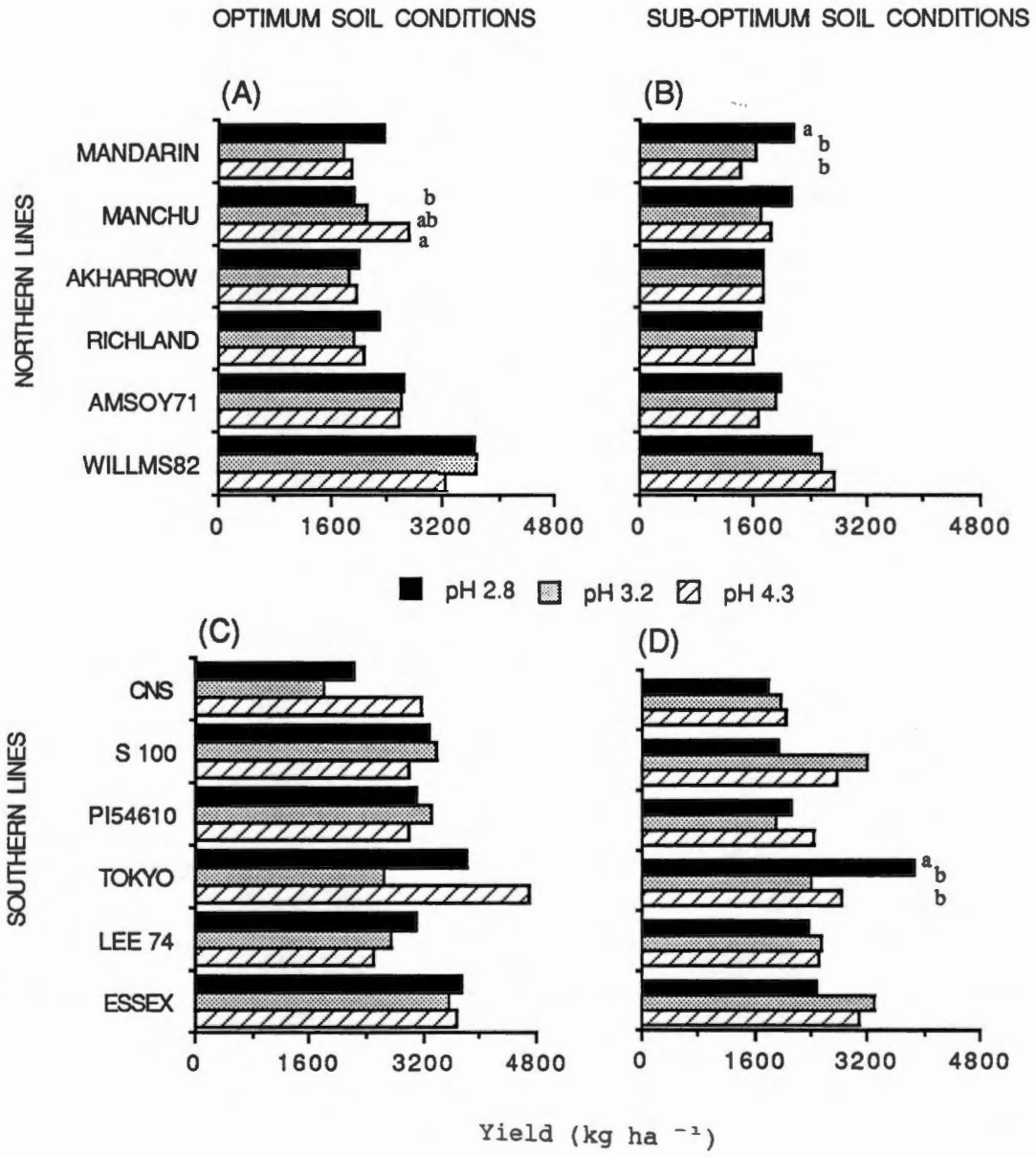

Figure 1. Mean yields for northern (A and B) and southern (C and D) soybean ancestral lines and cultivars grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Knoxville, 1985). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for yield (see Appendix Table C-1).

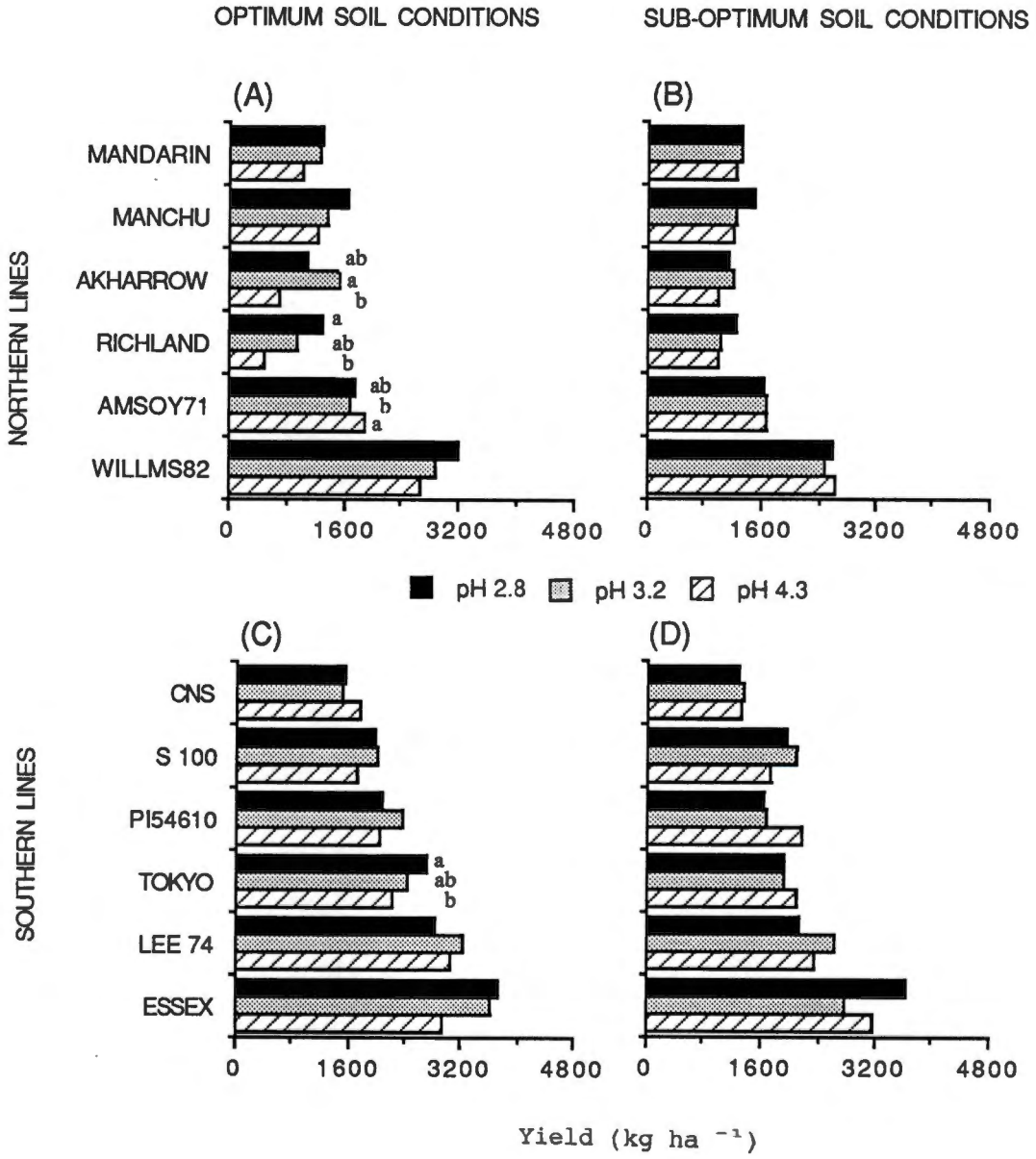

Figure 2. Mean yields for northern (A and B) and southern (C and D) soybean ancestral lines and cultivars grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Milan, 1985). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for yield (see Appendix Table C-1).

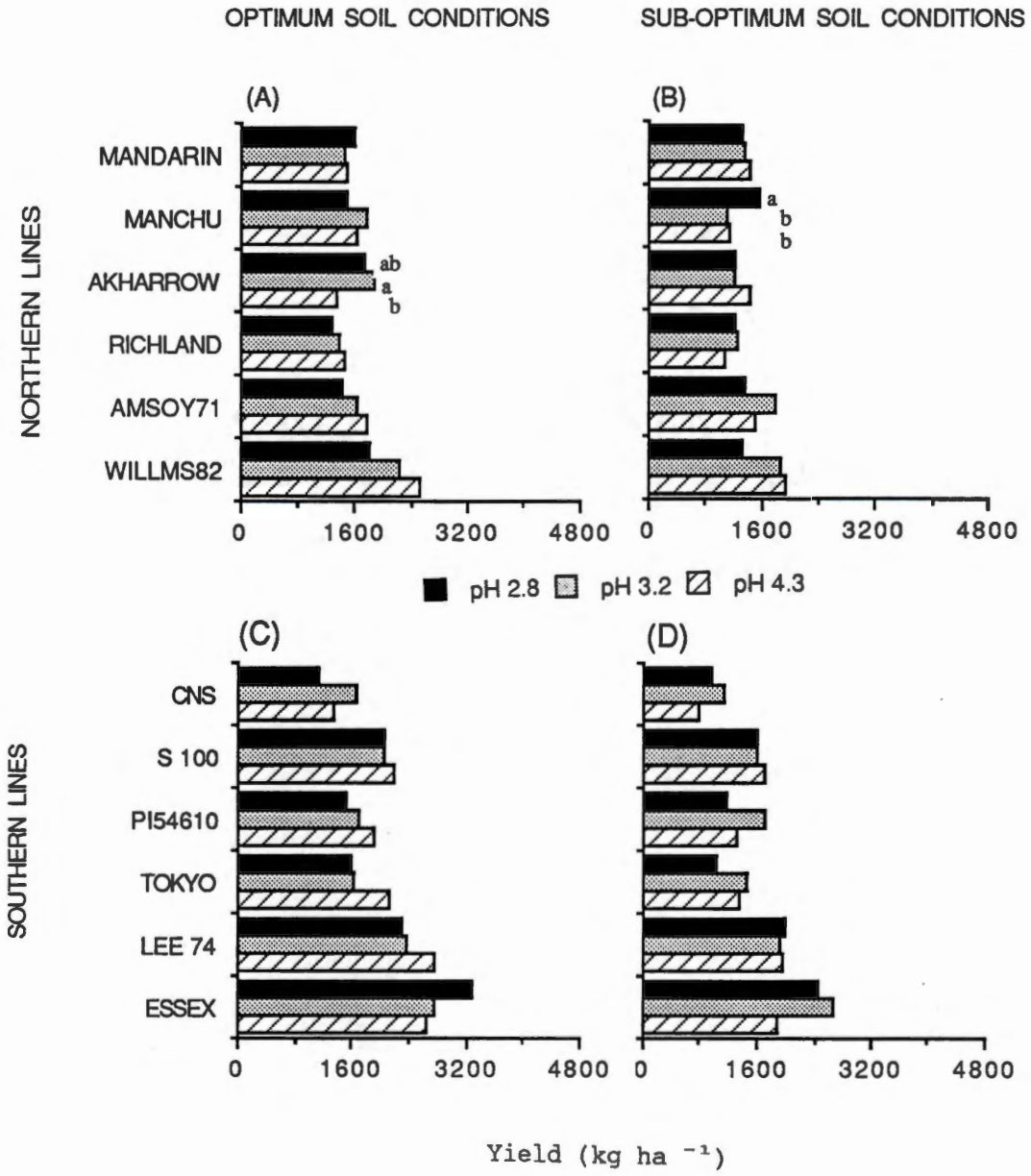

Figure 3. Mean yields for northern (A and B) and southern (C and D) soybean ancestral lines and cultivars grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Knoxville, 1986). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for yield (see Appendix Table C-1).

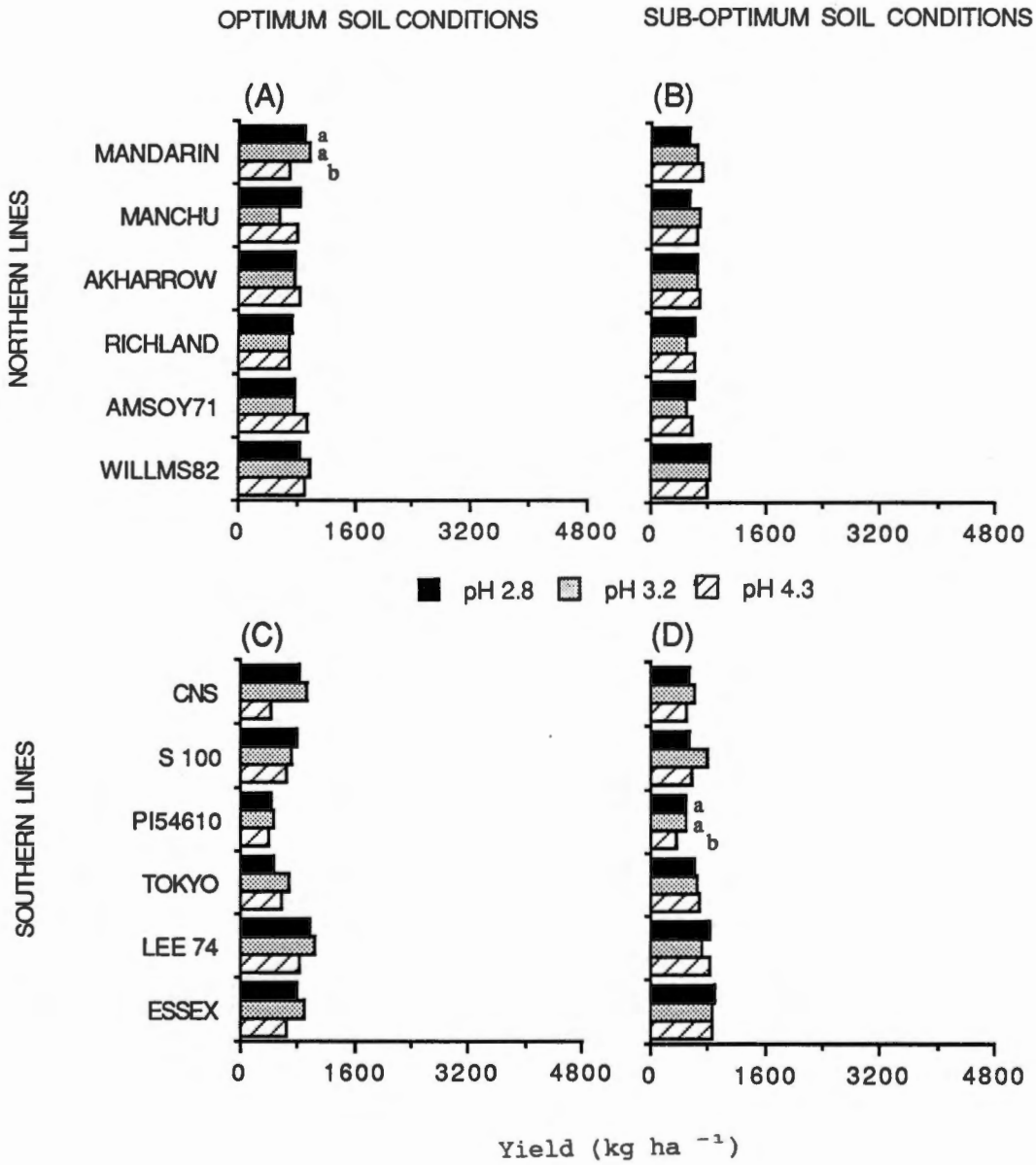

Figure 4. Mean yields for northern (A and B) and southern (C and D) soybean ancestral lines and cultivars grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Milan, 1986). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for yield (see Appendix Table C-1).

pH 4.3, but pH 2.8 was not significantly different from pH 3.2 or 4.3. Richland showed one positive response to increased acidity of SAR (Figure 2); however when analyzed across years and locations, no differences were found.

Northern Cultivars:

Amsoy 71 was ambiguously affected by the SAR treatments for one out of the eight combinations (Figure 2); there was a lower yield for pH 3.2 than for pH 4.3 but pH 3.2 was not different from pH 2.8 and pH 2.8 was not different from pH 4.3. Amsoy 71 did not respond to SAR in any of the other seven combinations. There were no significant yield responses to SAR for the cultivar Williams 82 for any of the treatment combinations. This lack of yield response to SAR is a reflection of the response of the northern ancestral lines. When SAR did cause an effect on yield of the northern lines, it tended to be beneficial rather than detrimental.

Southern Ancestral Lines:

CNS and S 100, the primary contributors of genes to the southern gene pool, had no significant yield responses to SAR for any of the eight combinations. The ancestral line PI 54610 had a positive response to SAR for only one out of eight combinations (Figure 4). Tokyo responded positively to SAR acidity for two out of the eight combinations (Figures 1 and 2).

Southern Cultivars:

Neither of the cultivars, Lee 74 or Essex, exhibited either a positive or negative yield response to SAR for any of the eight

treatment combinations (Figures 1 through 4). This is a reflection of the response of the southern ancestral lines. Although the cultivar Essex did not exhibit a response to SAR for these individual year and location analyses, it did respond positively to SAR under optimum soil fertility when analyzed across years and locations (Table C-3). As with the northern lines, there were very few significant yield responses to SAR among the southern lines and when there were, they were positive rather than negative.

Soybean Seed Weight:

Analysis of variance for seed weight was conducted across years and locations (Table 6). Eleven of the entries were included in this analysis of variance (the ancestral line S 100 was omitted due to a lack of data). The SAR pH had no significant ($P \leq .05$) effect on soybean seed weight when considered across years, locations, soil fertility, and soybean lines (Table 6). Seed weight means for each SAR pH level are presented in Table 7; these means are not significantly different when averaged across years, locations, levels of soil fertility, and soybean lines.

There was a significant difference for seed weight between optimum and sub-optimum soil fertility (Table 6). Optimum soil fertility had an average 100 seed weight of 16.2 g as compared to 15.7 g under sub-optimum soil fertility (Table 8). There was not a significant ($P \leq .05$) interaction between soil fertility and SAR

Table 6. Analysis of variance for 100 seed weight combined across years, locations, soil fertility levels, simulated acid rain (SAR) pHs, and soybean lines.

Source of Variation	df	MS
Year (Y)	1	148.6279 **
Location (L)	1	55.9136 **
Y x L	1	0.0023
Error A	8	1.0143
Soil (S)	1	69.1946 **
S X Y	1	9.6755
S*L	1	24.5671 *
S X Y X L	1	0.0810
Error B	8	2.8105
pH	2	1.0375
pH X Y	2	3.5447
pH X L	2	3.5784
pH X Y X L	2	1.5495
pH X S	2	1.3788
pH X S X Y	2	0.1746
pH X S X L	2	0.0441
pH X S X L X Y	2	1.3759
Error C	32	1.6951
Entry (E)	10	585.4122 **
E X Y	10	127.1242 **
E X L	10	40.4215 **
E X Y X L	10	33.7065 **
E X S	10	1.8906
E X S X Y	10	3.5054 **
E X S X L	10	2.9075 *
E X S X Y X L	10	4.1966 **
E X pH	20	0.8029
E X pH X Y	20	1.6350
E X pH X L	20	2.0258
E X pH X Y X L	20	2.2622
E X pH X S	20	1.4563
E X pH X S X Y	20	1.0662
E X pH X S X L	20	2.1849
E X pH X Y X L X S	20	0.9555
Error D	480	1.4345

*, ** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

Table 7. Means for 100 seed weights at each simulated acid rain (SAR) pH level averaged across years, locations, soil fertility levels, and soybean lines.

SAR pH	Mean Seed Weight	Standard Error
	---------- g ----------	
2.8	15.94 a [s]	0.20
3.2	16.00 a	0.19
4.3	15.89 a	0.20

[s] Means followed by the same letter are not significantly different ($P \leq .05$) from other means.

Table 8. Means for 100 seed weight for each simulated acid rain (SAR) pH within optimum and sub-optimum soil conditions, averaged across years, locations, and soybean lines.

SOIL CONDITIONS	SAR pH	SEED WEIGHT	STANDARD ERROR
		------ g 100^{-1} ------	
	2.8	16.14 a	0.29
OPTIMUM	3.2	16.30 a	0.29
	4.3	16.23 a	0.30
	2.8	15.74 b	0.27
SUB-OPTIMUM	3.2	15.70 b	0.26
	4.3	15.54 b	0.27

[s] Means followed by the same letter are not significantly different ($P \leq .05$).

pH indicating that seed weight response to SAR was not influenced by soil fertility (Table 6).

Analysis of variance indicated as was expected, significant ($P \leq .05$) differences in seed weight among entries (Table 6). Soybean 100 seed weights ranged from 14 g for Essex to 24 g for Tokyo; these means (averaged across years, locations, soil fertility, and SAR pH levels) are shown in Table C-4.

There was no statistical significance ($P \leq .05$) for the entry X pH interaction; seed weight did not respond differently for the different soybean lines with respect to SAR pH (Table 6). There also was not a significant entry X soil fertility interaction for seed weight indicating that in general there was no difference in the relative rankings of the entries with respect to soil fertility (Table 6). In addition there was no significance for the entry X pH X soil fertility interaction; thus entries did not respond differently in relation to each other with respect to SAR pH level in combination with soil fertility (Table 6).

There were genotype X environment interactions as indicated by the statistical significance for entry X soil fertility X year, entry X soil fertility X location, and entry X soil fertility X year X location (Table 6).

When analyses of variance were conducted for each entry within year, location, and soil fertility there were some significant responses observed; there were eight sets of year - location - soil fertility treatment combinations for the twelve soybean lines (Figures 5 through 8).

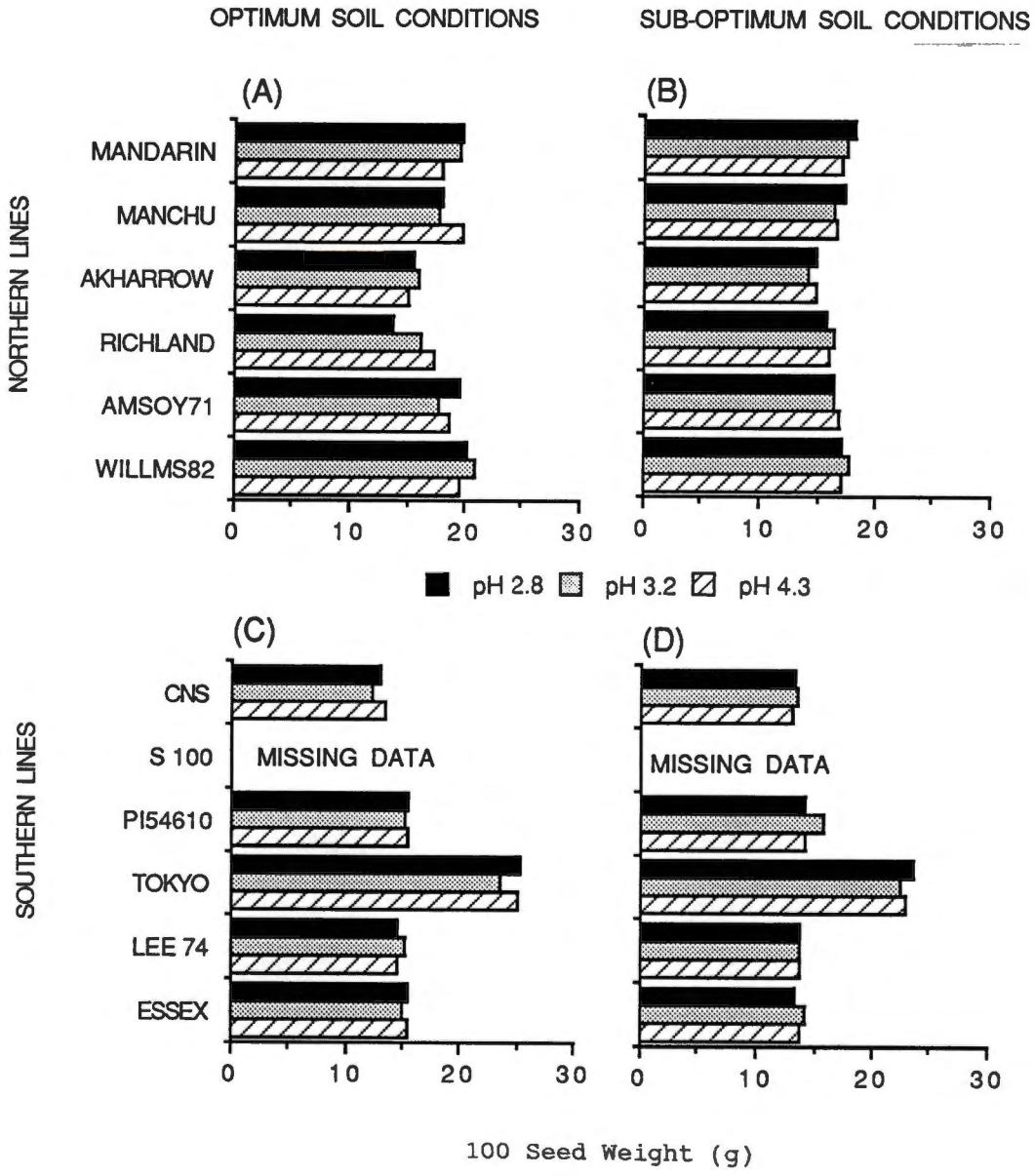

Figure 5. Mean seed (100) weights for northern (A and B) and southern (C and D) ancestral lines and cultivars grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Knoxville, 1985). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for seed weight (see Appendix C-4).

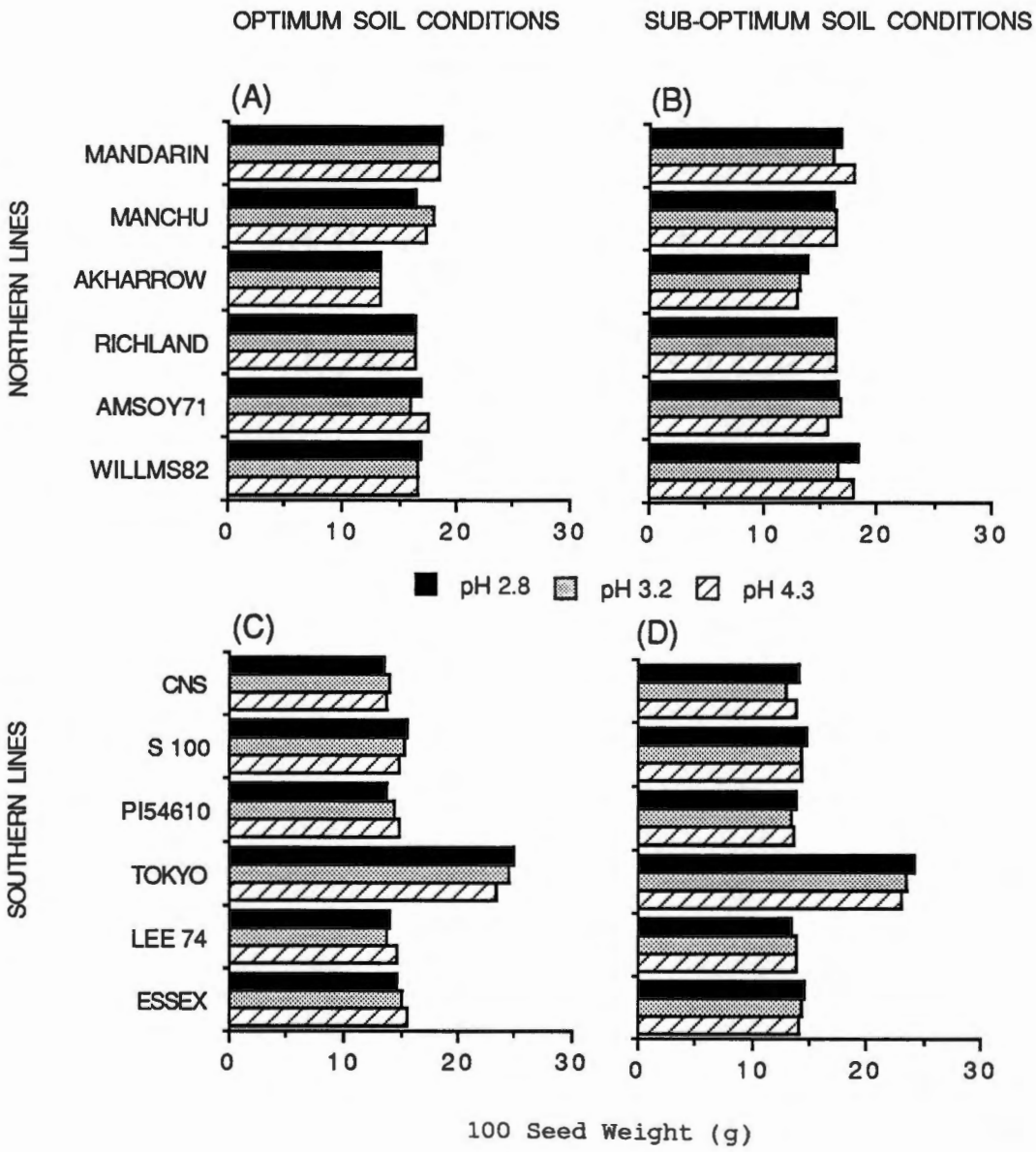

Figure 6. Mean seed (100) weights for northern (A and B) and southern (C and D) ancestral lines and cultivars grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Milan, 1985). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for seed weight (see Appendix Table C-4).

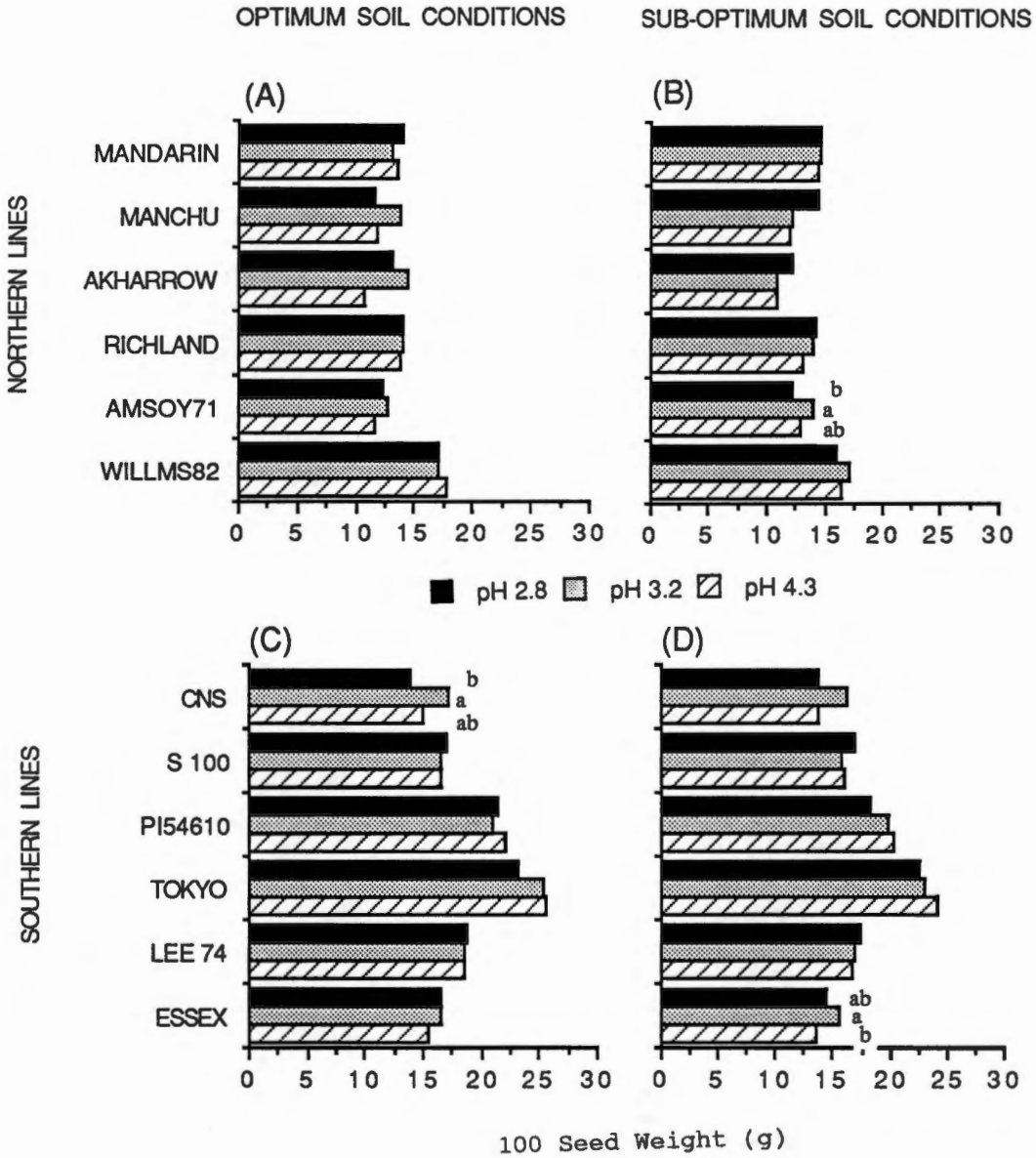

Figure 7. Mean seed (100) weights for northern (A and B) and southern (C and D) ancestral lines and cultivars grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Knoxville, 1986). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for seed weight (see Appendix Table C-4).

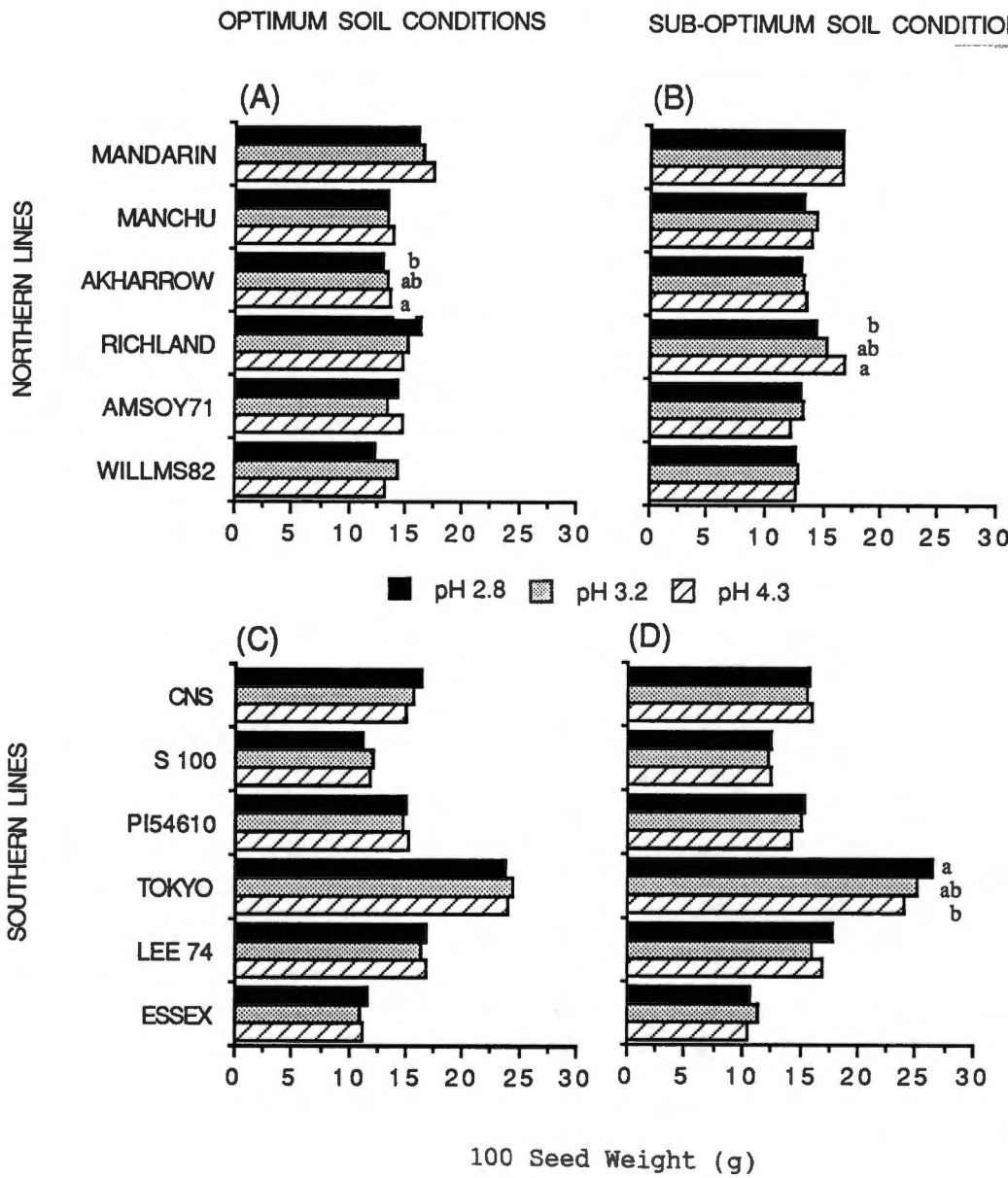

Figure 8. Mean seed (100) weights for northern (A and B) and southern (C and D) ancestral lines and cultivars grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Milan, 1986). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for seed weight (see Appendix Table C-4).

Northern Ancestral Lines:

The seed weights of two predominant ancestral lines, Mandarin and Manchu, were not significantly affected by SAR for any of the above eight combinations.

AK Harrow showed a negative response to SAR (for one out of the eight combinations) under optimum soil fertility at the Milan location in 1986 (Figure 8). There was a 5.8% decrease in seed weight when SAR acidity increased from pH 4.3 to pH 2.8 (13.7 g and 12.9 g respectively). This negative response, however, was not reflected in seed yield, as was shown in Figure 4.

The seed weight of Richland, another northern ancestral line, was decreased by SAR under sub-optimum soil fertility at Milan in 1986 (Figure 8). Seed weight decreased from 17 g to 14 g, a 17.6% decrease, when SAR acidity increased from pH 4.3 to pH 2.8. Again, this negative response for seed weight was not reflected in seed yield (Figure 4).

Northern Cultivars:

Seed weight for Amsoy 71 responded ambiguously to SAR (for one out of the eight treatment combinations) under sub-optimum soil fertility for the Knoxville location in 1986. The seed weight for pH 2.8 was lower than for pH 3.2, but was not significantly different from pH 4.3 (Figure 7). This response was not reflected in seed yield (Figure 3). Williams 82 showed no significant response to SAR for seed weight for any of the eight combinations (Figures 5 through 8). These two cultivars are similar to their

related ancestral lines with their lack of response to SAR for seed weight.

Southern Ancestral Lines:

The seed weight of CNS, the primary contributor of genes to the southern gene pool, showed an ambiguous positive response to SAR in one of the eight combinations. The average seed weight at pH 3.2 was significantly ($P \leq .05$) higher than at pH 2.8, but the means at pH 2.8 and 4.2 were not different under optimum soil fertility at Knoxville in 1986 (Figure 7). There was no reflection of this response in seed yield (Figure 3).

The soybean ancestral lines, S 100 and PI 54610, showed no significant response to SAR for seed weight for any of the eight combinations (Figures 5 through 8).

Seed weight for Tokyo was increased by increased acidity of SAR for one out of the eight treatment combinations (Figure 8). There was a 10.8% increase in seed weight from pH 4.3 (23.9 g 100^{-1}) to pH 2.8 (26.5 g 100^{-1}).

Southern Cultivars:

Lee 74 had no statistically significant ($P \leq .05$) responses to SAR for seed weight for any of the eight combinations (Figures 5 through 8).

There was an ambiguous response, for seed weight to SAR for the southern cultivar Essex in Knoxville 1986 (Figure 7), but no significant responses for any of the other seven combinations.

Although there were some negative seed weight responses to SAR, no individual entry responded to more then one of the eight

treatment combinations and the reduced seed weights were not reflected in seed yields. All significant responses of seed weight to SAR occurred in 1986 at both locations under both optimum and sub-optimum soil fertility (Figures 7 and 8).

Soybean Stomatal Frequency:

When analysis of variance was conducted for stomatal frequency for Knoxville in 1986, there were no significant differences ($P \leq .05$) due to SAR pH (Table 9). The mean stomatal frequencies are shown at each pH in Table 10.

There were significant differences ($P \leq .01$) among entries for stomatal frequency (Table 9). The entry means ranged from 127 mm^{-2} for Richland to 237 mm^{-2} for CNS (Table 11). There was no statistical significance for the entry X SAR pH interaction, indicating that entries did not differ in their stomatal frequency with respect to the SAR treatment (Table 9).

Analysis of variance also indicated a significant difference ($P \leq .05$) for stomatal frequency between optimum and sub-optimum soil fertility (Table 9). The mean stomatal frequency across entries and SAR treatments was higher under sub-optimum soil fertility (189 mm^{-2}) than for optimum soil fertility (182 mm^{-2}). There were no significant entry X soil fertility, pH X soil fertility, or entry X soil fertility X pH interactions for stomatal frequency (Table 9).

Table 9. Analysis of variance for stomatal frequency of twelve soybean lines when grown on two levels of soil fertility and subjected to three levels of simulated acid rain (SAR) pH (Knoxville, 1986)

Source of Variation	df	Mean Square
Rep (R)	2	3.473
Soil (S)	1	35.930 *
Error A	2	1.070
pH	2	19.634
S X pH	2	5.386
Error B	8	7.522
Entry (E)	11	313.150 **
E X S	11	8.861
E X pH	22	12.801
E X S X pH	22	14.512
Error C	132	16.903

*, ** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

Table 10. Mean stomatal frequency at each simulated acid rain (SAR) pH level, averaged across soil fertility levels and soybean lines for Knoxville, 1986.

SAR pH	Mean Stomatal Frequency	Standard Error
	----------mm^{-2}----------	
2.8	181 a [s]	5.2
3.2	190 a	5.8
4.3	184 a	5.0

[s] Means followed by the same letter are not significantly different ($P \leq .05$) from other means.

Table 11. Mean stomatal frequency (adaxial leaf surface) for northern and southern ancestral lines and cultivars averaged across soil fertility level and simulated acid rain (SAR) pH.

	Mean Stomatal Frequency	Standard Error
Northern Ancestral Lines	---------- mm^{-2} ----------	
MANDARIN	157.33 f [s]	8.67
MANCHU	184.42 de	4.94
AK HARROW	190.89 de	7.29
RICHLAND	127.13 g	9.15
Northern Cultivars		
AMSOY 71	224.76 ab	10.48
WILLIAMS 82	218.98 abc	6.17
Southern Ancestral Lines		
CNS	237.19 a	8.37
S 100	175.92 ef	8.27
PI 54610	180.16 def	8.09
TOKYO	205.01 bcd	5.27
Southern Cultivars		
LEE 74	196.45 cde	7.30
ESSEX	129.75 g	5.47

[s] Entry means followed by the same letter are not significantly different ($P \leq .05$) from the other entry means.

Soybean Leaf Areas:

Analysis of variance was conducted for total leaf area per plant for Knoxville in 1985 for five of the soybean lines, AK Harrow, CNS, Essex, Mandarin, and Williams 82 (Table 12). No statistical significance ($P \leq .05$) was indicated for pH in this model demonstrating that leaf areas did not differ in response to the different SAR treatments. Also, there were not significant entry X pH or entry X soil fertility X pH interactions. There were significant ($P \leq .05$) differences among the five entries for leaf area (Table 12).

Soybean Transpiration Rates and Stomatal Conductances:

Analyses of variance were conducted for transpiration rates and stomatal conductivity which were measured on two separate sampling dates for five of the soybean lines at Knoxville in 1985 (Tables 13 and 14). There was no statistical significance for pH for transpiration rate or stomatal conductance on either of the two sampling dates, indicating that transpiration rates and stomatal conductivity were not affected by SAR pH when considered across entries and soil fertility (Tables 13 and 14).

There were no significant differences among entries for transpiration rate on the first sampling date (Table 13). In addition there were no entry X soil fertility, entry X pH , or entry X soil fertility X pH interactions; thus, soil fertility did not make a particular entry more responsive to SAR pH (Table 13). On the other hand, there were significant differences among the

Table 12. Analysis of variance for leaf area per plant of twelve soybean lines when grown on two levels of soil fertility and subjected to three levels of simulated acid rain (SAR), Knoxville, 1985.

Source of Variation	df	Mean Square
Rep (R)	2	15383
Soil (S)	1	493474
Error A	2	88312
pH	2	64253
S X pH	2	113341
Error B	8	33158
Entry (E) ¶	4	4326598 **
E X S	4	372603
E X pH	8	20931
E X S X pH	8	125471
Error C	47	159194

*, ** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

¶ Entries used in this ANOVA included: AK Harrow, CNS, Essex, Mandarin, and Williams 82.

Table 13. Analysis of variance for transpiration rates (TR) and stomatal conductance (SC) across soil fertility level, SAR pH, and soybean line for Knoxville 1985 (1st sampling date).

Source of Variation	df	Mean Square TR	Mean Square SC
Rep (R)	2	97.68 **	0.22478 *
Soil (S)	1	0.44	0.00627
Error A	2	0.53	0.00843
pH	2	1.33	0.01143
S X pH	2	0.43	0.01354
Error B	8	1.95	0.01341
Entry (E) ¶	6	1.71	0.02622 *
E X S	6	0.99	0.01340
E X pH	12	0.78	0.01055
E X S X pH	12	1.13	0.01278
Error C	53	0.92	0.01112

*, ** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

¶ Entries used in this ANOVA included: AK Harrow, CNS, Essex, Mandarin, PI 54610, S 100, and Williams 82.

Table 14. Analysis of variance for transpiration rates (TR) and stomatal conductance (SC) across soil fertility level, SAR pH, and soybean line for Knoxville 1985 (2nd sampling date).

Source of Variation	df	Mean Square TR	Mean Square SC
Rep (R)	5	14.1964 *	0.0224
Soil (S)	1	169.9212	0.3285
Error A	5	23.8604	0.0577
pH	2	14.7424	0.0348
S X pH	2	9.0283	0.0256
Error B	20	8.3062	0.0207
Entry (E) ¶	4	237.7997 **	0.6025 **
E X S	4	38.1074 **	0.1000 **
E X pH	8	11.8772 *	0.0298 *
E X S X pH	8	16.3727 **	0.0413 **
Error C	118	4.9112	0.0126

*, ** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

¶ Entries used in this ANOVA included: AK Harrow, CNS, Essex, Mandarin, and Williams 82.

five entries for transpiration rate at the second sampling date (Table 14). Also, there were entry X pH, entry X soil fertility, and entry X soil fertility X pH interactions (Table 14).

When analyses of variance and mean separations were conducted separately for each entry, there were significant differences in transpiration rates for some entries under optimum and/or sub-optimum soil fertility for the second sampling date (Figure 9). The northern ancestral line, Mandarin, responded ambiguously to SAR under optimum soil fertility and positively under sub-optimum soil fertility. The northern ancestral line, AK Harrow, and northern cultivar, Williams 82, showed no significant response to SAR under either optimum or sub-optimum soil fertility. The southern ancestral line, CNS, had higher rates of transpiration at SAR pH levels of 2.8 and 3.2 compared to 4.3 under optimum soil fertility but they were equal under sub-optimum soil fertility. The southern cultivar, Essex, had similar transpiration rates at the different SAR pH levels under optimum soil fertility but it had lower rates at 2.8 and 3.2 compared to 4.3 under sub-optimum soil fertility on the second sampling date (Figure 9).

Analyses of variance indicated significant differences among entries for stomatal conductance for both the first and second sampling dates (Tables 13 and 14). There were no significant interactions for the first sampling date (Table 13), but there were significant entry X pH, entry X soil fertility, and entry X soil fertility X pH interactions for the second sampling date for stomatal conductance (Table 14).

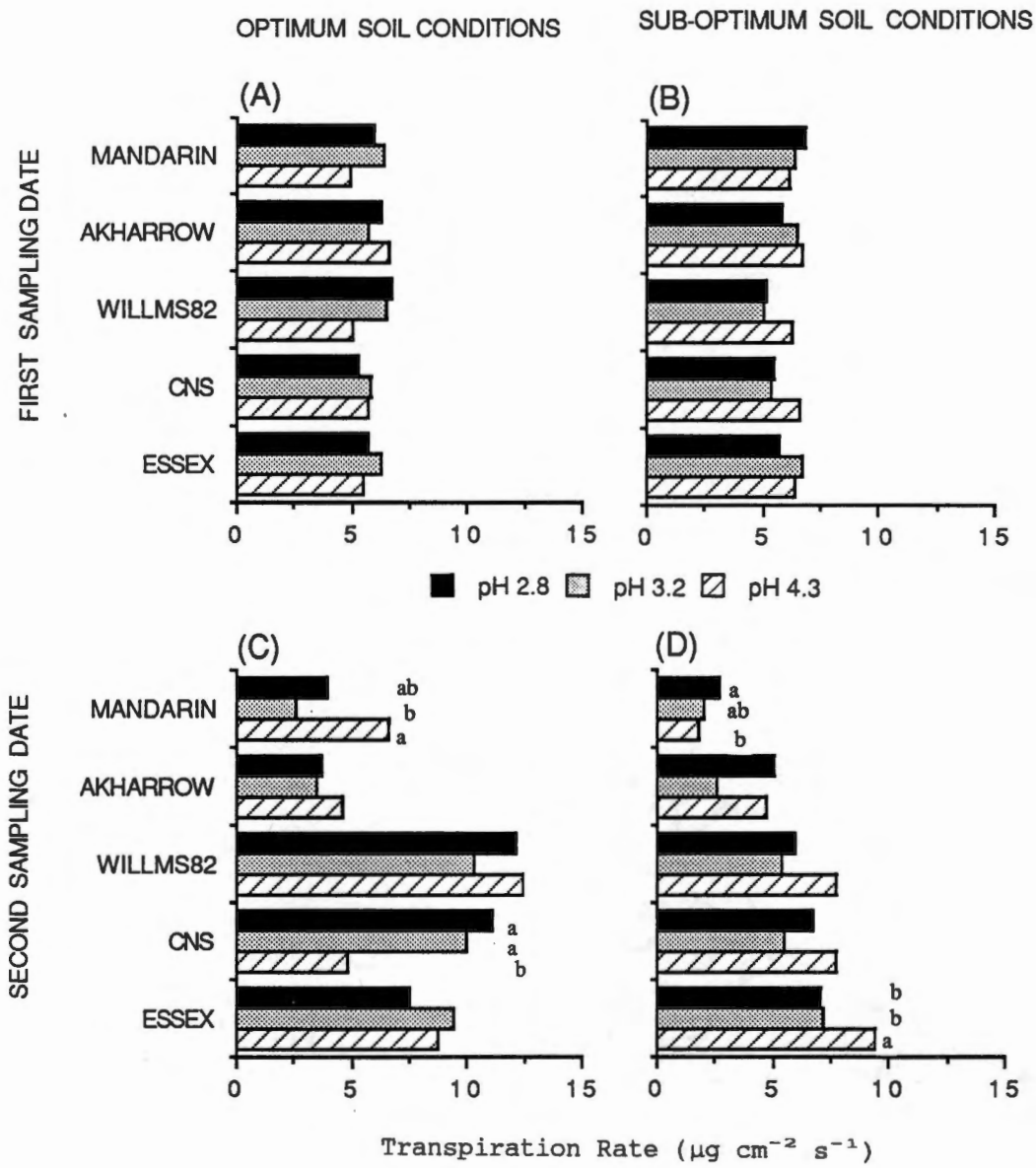

Figure 9. Mean transpiration rates, for five soybean lines, at two separate sampling dates 7/12/85 (A and C) and 8/12/85 (B and D) grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Knoxville, 1985). Bars followed by the same letters or no letters are not significantly different ($P \leq 0.05$) for that entry. There are significant differences among entries at the second sampling date.

When analyses of variance and mean separations were conducted for each entry, there were responses for some entries for stomatal conductance (Figure 10). The northern ancestral line, Mandarin, responded ambiguously to SAR under optimum soil fertility and lacked any significant response under sub-optimum soil fertility. Another northern ancestral line, AK Harrow, and northern cultivar Williams 82, showed no differences in stomatal conductance under either soil fertility. The southern ancestral line, CNS, had higher stomatal conductance at SAR pH levels 2.8 and 3.2 compared to 4.3 under optimum soil fertility, but the rates were not different under sub-optimum soil fertility. The southern cultivar Essex, lacked response under optimum soil conditions but the rates were lower at the two higher SAR acidity levels under sub-optimum soil conditions.

Greenhouse Experiments

First Greenhouse Experiment (1986):

Based on analyses of variance, there were no significant differences among the SAR pH levels for leaf number, leaf area, specific leaf weight (SLW), leaf weight , stem weight, or total plant weight (Tables 15 and 16). There were significant differences ($P \leq 0.01$) among entries for these measurements, but these differences were not associated with the SAR treatment as indicated by a lack of significance for the entry X pH interaction.

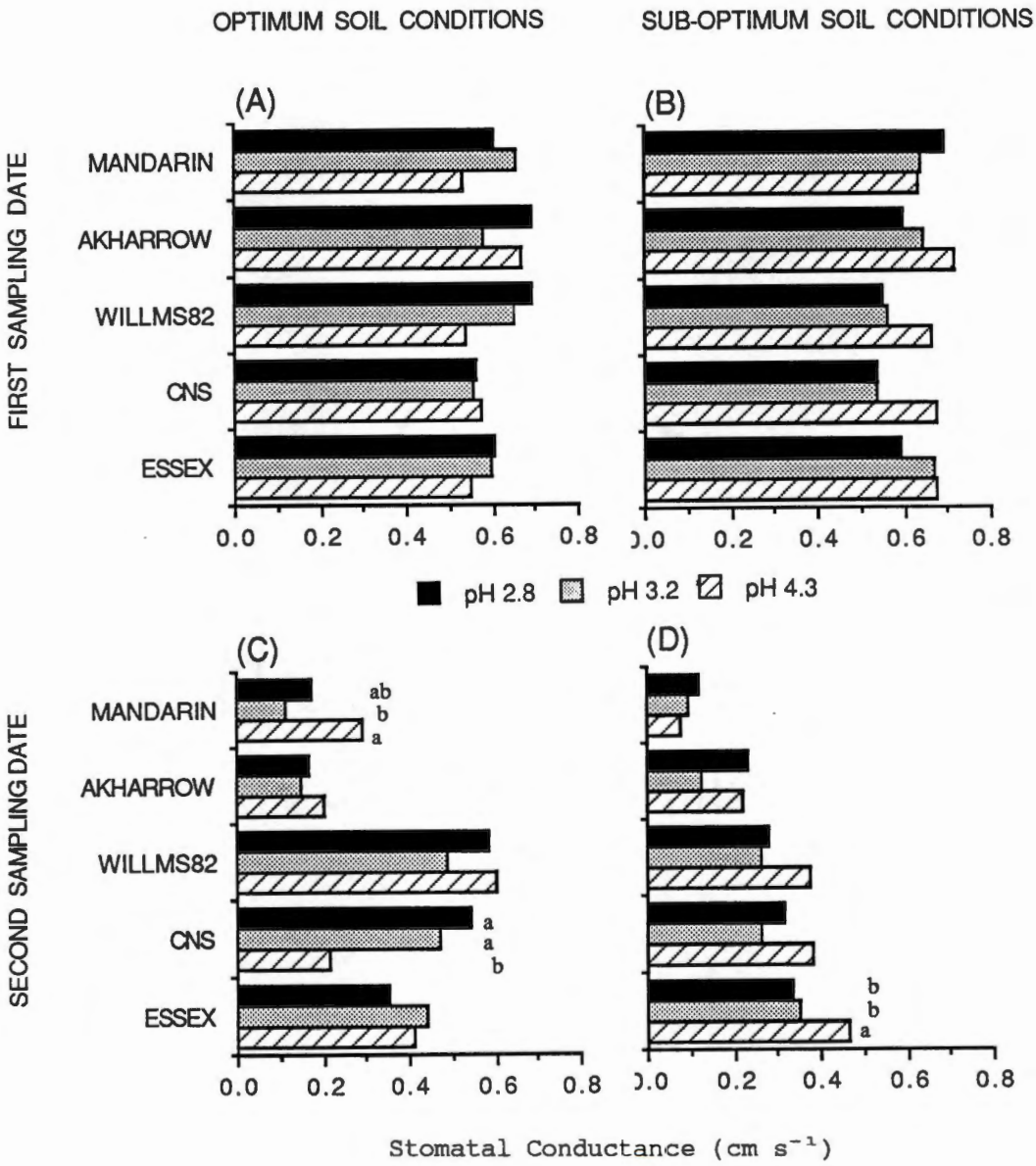

Figure 10. Mean stomatal conductance, for five soybean lines, at two separate sampling dates 7/12/85 (A and C) and 8/12/85 (B and D) grown on optimum (A and C) and sub-optimum (B and D) soil fertility and treated with three levels of SAR (Knoxville, 1985). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries.

Table 15. Summary of analyses of variance for leaf number, leaf area, and specific leaf weight (SLW) per plant for twelve soybean lines at two separate sampling dates, and transpiration rate (TR), and stomatal conductance (SC) at a third date in the first greenhouse experiment.

	Sampling date							
	3/14/86			3/27/86			3/20/86	
	LEAF							
Source of Variation	no.	Area	SLW	no.	Area	SLW	TR	SC
Rep (R)	NS	NS	NS	NS	NS	NS	*	NS
pH	NS	NS	NS	*	NS	NS	NS	NS
Error A								
Entry (E)	**	**	**	**	**	**	**	**
E X pH	NS	NS	NS	NS	NS	NS	NS	NS
Error B								

*,** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

Table 16. Summary of analyses of variance for leaf weight, stem weight, and total plant weight per plant for twelve soybean lines at two separate sampling dates in the first greenhouse experiment.

	Sampling date					
	3/14/86			3/27/86		
	WEIGHT					
Source of Variation	leaf	stem	total	leaf	stem	total
Rep (R)	NS	NS	NS	NS	NS	NS
pH	NS	NS	NS	NS	NS	NS
Error A						
Entry (E)	**	**	**	**	**	**
E X pH	NS	NS	NS	NS	NS	NS
Error B						

*,** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

Analyses of variance of leaf number for the second sampling date revealed a significant difference among SAR pH levels (Table 15). The mean leaf number at each SAR pH level averaged across all entries are shown in Table 17. The mean leaf number at each of the SAR pH levels was significantly ($P < 0.05$) higher than that of the control (pH 5.2). The leaf number increased by 32% when SAR pH decreased from pH 5.2 (control) to pH 2.8.

Analyses of variance were conducted for each entry and harvest date. The means and the statistical differences for leaf number of each entry at each harvest date are graphed in Figure 11. There were no significant responses to SAR for leaf number for any of the northern lines (Figure 11). There were significant differences for some southern entries among SAR pH treatments at the second harvest date. The southern ancestral line, S100, exhibited a positive response to SAR (Figure 11). The leaf number increased from 25 to 34 (36%), when acidity increased from pH 5.2 to pH 2.8. CNS and PI 54610 responded ambiguously to SAR treatment. Tokyo, Lee74, and Essex showed no significant response to SAR for leaf number.

Leaf area, leaf weight, stem weight, total above ground plant weight, SLW, transpiration rate, and stomatal conductance exhibited no significant ($P \leq .05$) response to SAR pH (Tables 15 and 16). There were significant differences among soybean lines, but this was not associated with the SAR treatments as illustrated by the lack of significance for the entry X pH interaction. Means

Table 17. Mean leaf number for each simulated acid rain (SAR) pH level at the second sampling date for the first greenhouse experiment, 1986.

SAR pH	Mean Leaf Number	Standard Error
2.8	26 a [s]	1.4
3.2	23 a	1.3
4.3	24 a	1.2
Control [¥]	20 b	1.2

[s] Means followed by the same letter are not significantly different ($P \leq .05$) from other means.
[¥] pH of 5.2.

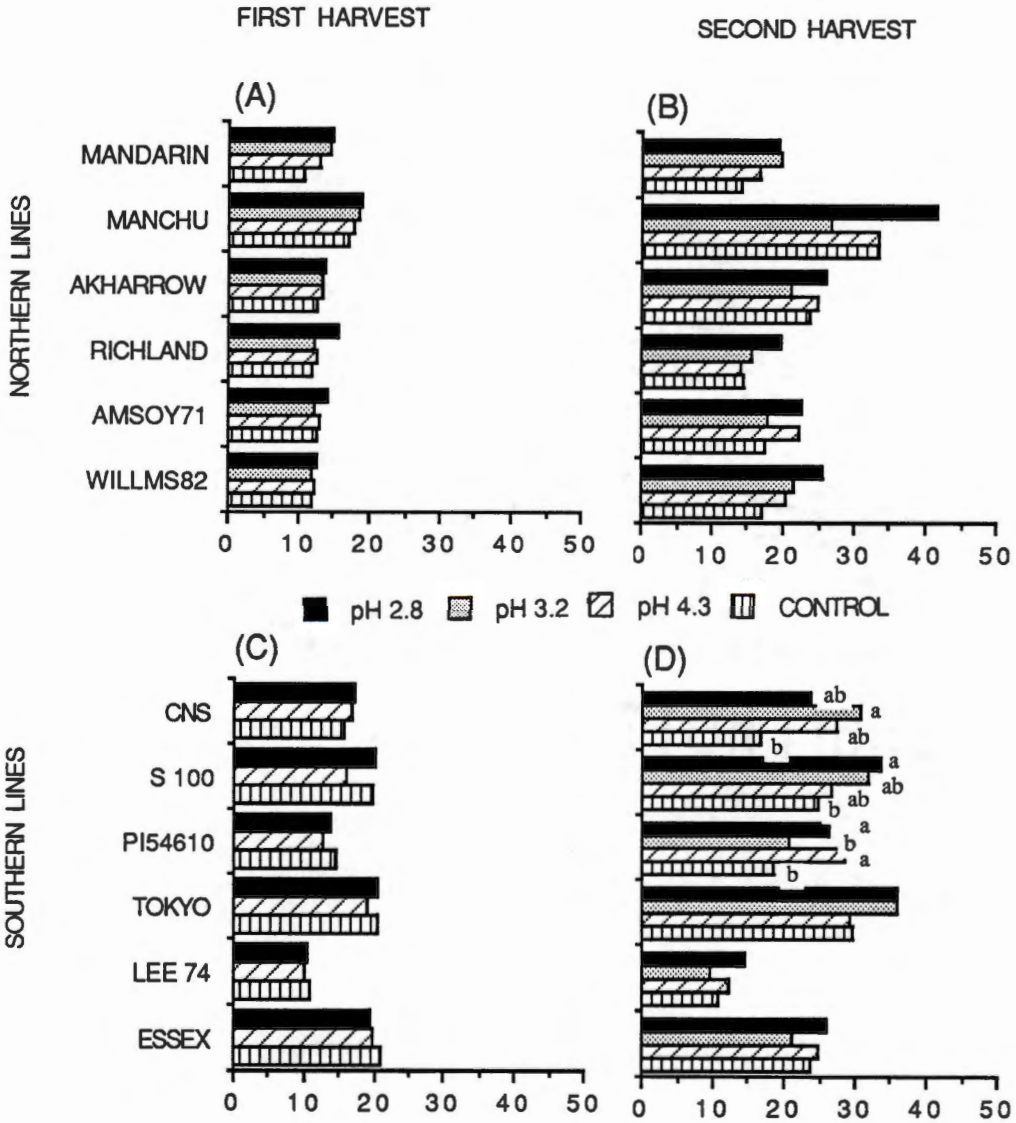

Figure 11. Mean leaf number for northern (A and B) and southern (C and D) ancestral lines and cultivars at the first harvest date (3/14/86, A and C) and second harvest date (3/27/86, B and D) when treated with four levels of SAR (first greenhouse experiment, 1986). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for leaf number.

for individual measurements for each entry and SAR pH are shown in the Appendix Tables D-1 through D-7.

Second Greenhouse Experiment (1987):

For the second greenhouse experiment and first sampling date, there were no significant ($P \leq .05$) differences due to SAR pH for the traits: leaf number, leaf area, SLW, or photosynthetic rate. Analyses of variance did detect significant differences, due to SAR pH, for the plant growth measurements leaf, stem, and total plant weights across all entries (Tables 18 and 19). There were significant differences among entries, but these differences were not associated with the SAR treatments as indicated by the lack of statistical significance for the entry X pH interaction. Means for individual measurements for each entry and sampling date are presented in Appendix Tables E-1 through E-8.

Means for above ground plant dry weight measurements for each pH are exhibited in Table 20. Plant growth was reduced by the SAR pH, as reflected in both leaf and stem dry weight. Plant weights for all three SAR treatments differed significantly ($P \leq .05$) from those for the control of pH 5.2 at the first sampling date (Table 20). Total above ground dry weight decreased by 20% when SAR acidity increased from pH 5.2 to pH 2.8. Leaf dry weight decreased by 21% when SAR acidity increased from pH 5.2 to pH 2.8. Stem weight also decreased by 18% for the same increase in acidity for the first sampling date (Table 20).

Table 18. Summary of analyses of variance for leaf number, leaf area, and specific leaf weight (SLW) per plant for twelve soybean lines at two separate sampling dates, and transpiration rate (TR), and stomatal conductance (SC) at a third date in the second greenhouse experiment.

	Sampling date							
	2/27/87			3/13/87			4/10/87	
	LEAF							
Source of Variation	no.	Area	SLW	no.	Area	SLW	TR	SC
Rep (R)	NS	NS	NS	NS	NS	NS	NS	NS
pH	NS	NS	NS	NS	NS	NS	*	**
Error A								
Entry (E)	**	**	**	**	**	**	NS	NS
E X pH	NS	NS	NS	NS	NS	NS	NS	NS
Error B								

*,** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

Table 19. Summary of analyses of variance for leaf weight, stem weight, and total plant weight per plant and photosynthetic rate (PN) for twelve soybean lines at two separate sampling dates in the second greenhouse experiment.

	Sampling date							
	2/27/87			3/13/87			3/23	4/8
	WEIGHT							
Source of Variation	leaf	stem	total	leaf	stem	total	PN	PN
Rep (R)	NS	NS	NS	NS	NS	NS	NS	NS
pH	**	*	**	*	*	*	NS	NS
Error A								
Entry (E)	**	**	**	**	**	**	NS	NS
E X pH	NS	NS	NS	NS	NS	NS	NS	NS
Error B								

*,** Denote statistical significance at the 0.05 and 0.01 probability levels, respectively. All effects are assumed to be random.

Table 20. Means for leaf weight, stem weight, and total plant weight per plant for each SAR pH level across all soybean lines at two separate sampling dates for the second greenhouse experiment, 1987.

SAR pH	Mean Leaf Weight	Mean Stem Weight	Mean Total Weight
	---------- g ----------		
	First Sampling Date (2/27/87)		
2.8	0.287 b [s]	0.229 b	0.516 b
3.2	0.283 b	0.225 b	0.508 b
4.3	0.258 b	0.205 b	0.464 b
Control [x]	0.361 a	0.281 a	0.643 a
	Second Sampling Date (3/13/87)		
2.8	0.743 a [s]	0.707 ab	1.451 a
3.2	0.796 a	0.726 a	1.523 a
4.3	0.606 b	0.597 b	1.203 b
Control [x]	0.817 a	0.771 a	1.589 a

[s] Means followed by the same letter are not significantly different ($P \leq .05$) from other means.
[x] pH = 5.2.

For the second greenhouse experiment and second sampling date, there were no significant ($P \leq .05$) differences due to SAR pH for the traits: leaf number, leaf area, SLW, or photosynthetic rate (Table 20). However, analyses of variance did reveal significant differences due to SAR pH for the plant growth measurements: leaf, stem, and total dry weights and for the physiological measurements: transpiration rate and stomatal conductivity. Means for plant growth measurements at each SAR pH level at the second sampling date are shown in Table 20.

Although there were significant differences for above ground plant weight among the treatments there was not a biological trend, and it appeared that the plants had overcome their negative response to the SAR treatments observed for the first sampling date.

When analyses of variance were conducted on these growth parameters for each entry at each harvest date, several of the entries showed a significant response to SAR.

Leaf Weight:

The northern ancestral lines, Mandarin and Manchu, showed no significant response to SAR for leaf weight at the first sampling date (Figure 12). Another ancestral line, AK Harrow responded with decreased weight for the SAR treatments when compared with the control. The leaf weight decreased 49% (from 0.37 g to 0.19 g) when acidity increased from pH 5.2 (control) to pH 2.8 (Appendix Table E-3). Richland responded ambiguously to SAR treatments for leaf weight (Figure 12). The two northern

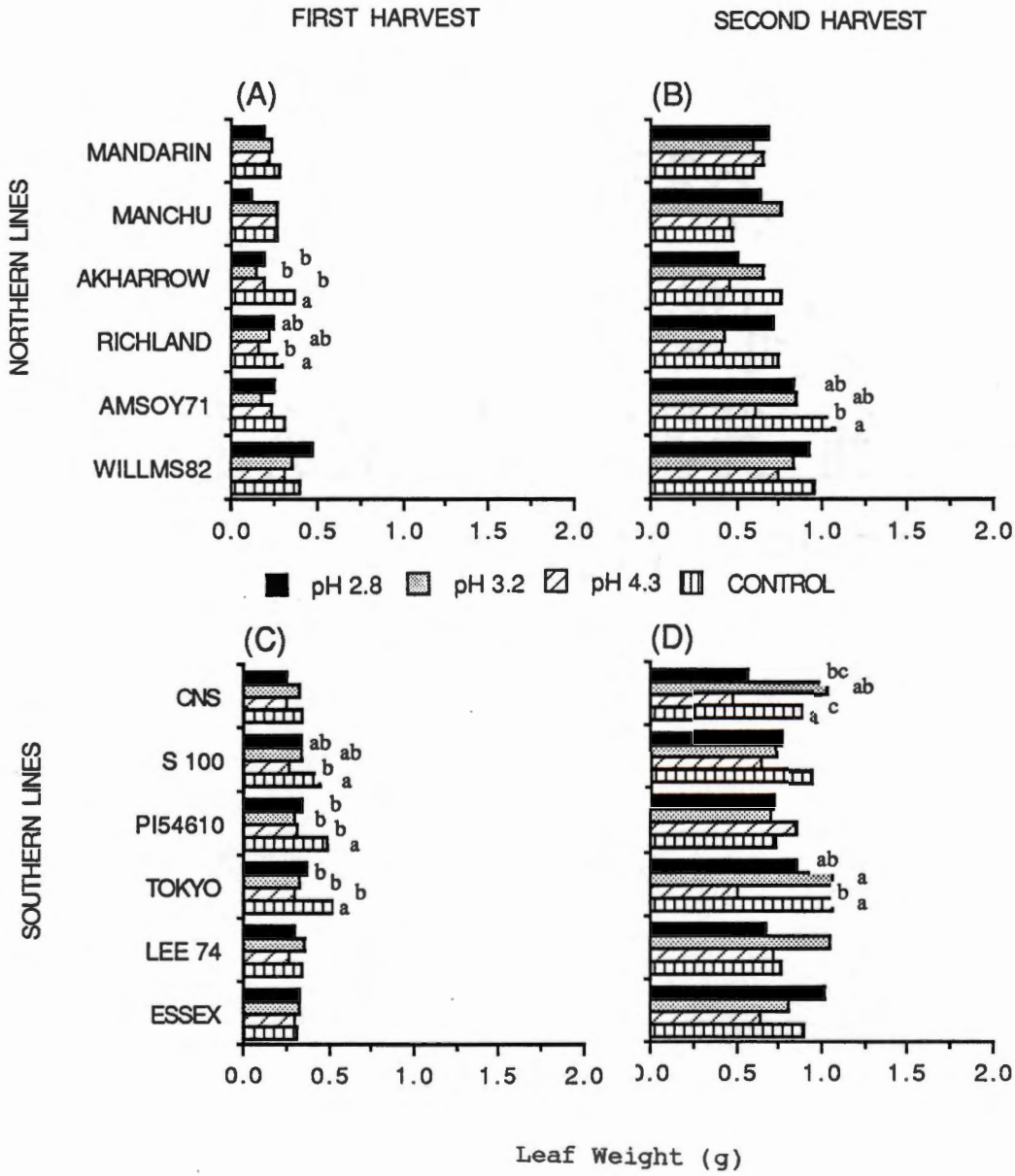

Figure 12. Mean leaf weight for northern (A and B) and southern (C and D) ancestral lines and cultivars at the first harvest date (2/27/87, A and C) and second harvest date (3/13/87, B and D) and treated with four levels of SAR (second greenhouse experiment, 1987). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for leaf weight.

cultivars, Amsoy 71 and Williams 82 did not respond significantly ($P \leq 0.05$) to SAR treatments for leaf weight at the first sampling date. Amsoy 71 responded ambiguously to SAR for the second sampling date and the other northern lines showed no significant response (Figure 12).

The predominant southern ancestral line, CNS, did not respond significantly to SAR for leaf weight (Figure 12). S 100 responded ambiguously to SAR. The other two ancestral lines, PI 54610 and Tokyo, both responded negatively to SAR with reduced leaf weight for the plants treated with SAR as compared to the control. Leaf weight for PI 54610 decreased by 31% (from 0.49 g to 0.34 g) when acidity increased from pH 5.2 to pH 2.8 and leaf weight for Tokyo decreased by 25% (from 0.53 g to 0.40 g) for the same increase in acidity (Appendix Table E-3). The two southern cultivars, Lee 74 and Essex, did not show a significant response to SAR for leaf weight at the first sampling date (Figure 12). Two southern ancestral lines, CNS and Tokyo, responded ambiguously to SAR for leaf weight at the second harvest date while the other soybean lines showed no significant response (Figure 12).

Stem weight:

The northern ancestral lines Mandarin, Manchu, and AK Harrow had lower stem weights due to SAR at the first harvest date (Figure 13). Stem weight for Mandarin decreased by 39% (from 0.23 g to 0.14 g) when acidity increased from pH 5.2 to pH 2.8 and stem weight for Manchu decreased by 55% (from 0.22 g to 0.10 g) for the same increase in acidity (Appendix Table E-4). AK Harrow had a

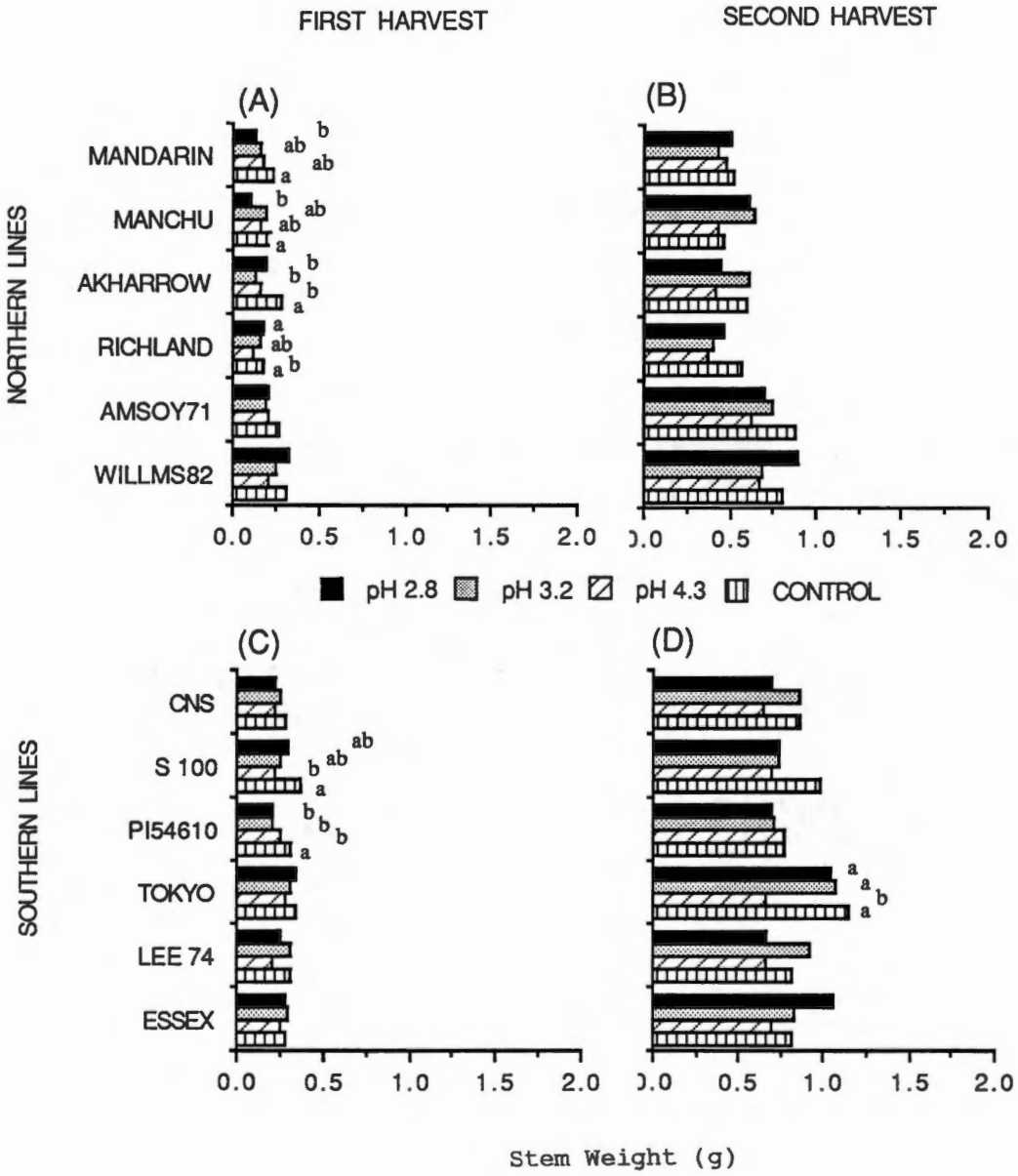

Figure 13. Mean stem weight for northern (A and B) and southern (C and D) ancestral lines and cultivars at the first harvest date (2/27/87, A and C) and second harvest date (3/13/87, B and D) when treated with four levels of SAR (second greenhouse experiment, 1987). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for stem weight.

decreased stem weight at the first harvest date of 32% (from 0.28 g to 0.19 g) and Richland responded ambiguously to SAR for stem weight at the first harvest date (Figure 13). The two northern cultivars, Amsoy 71 and Williams 82, did not respond significantly to SAR for stem weight at the first harvest. None of the northern soybean lines showed a significant response to SAR for stem weight at the second harvest date (Figure 13).

For the southern ancestral lines, CNS and Tokyo failed to respond, S 100 responded ambiguously, and PI 54610 had a reduced stem weight due to SAR at the first harvest (Figure 13). PI 54610 had a 34% decrease in stem weight, from 0.32 g to 0.21 g, as acidity increased from pH 5.2 to pH 2.8 (Appendix Table E-4). The stem weights of the two southern cultivars, Lee 74 and Essex, were not significantly ($P \leq 0.05$) affected by SAR at the first or second harvest dates (Figure 13).

Total Above Ground Plant Weight:

The measurements for total dry weight are reflective of both leaf and stem dry weights. There were negative responses to SAR for the northern soybean lines Mandarin, Manchu, and AK Harrow in the first sampling date and a lack of significant response to SAR for total weight for any of the northern lines at the second sampling date (Figure 14).

Total plant weight was reduced by SAR for the southern soybean lines S 100, PI 54610, and Tokyo for the first harvest date (Figure 14). Total plant weight responded ambiguously to SAR for CNS and Tokyo at the second harvest date and failed to show a

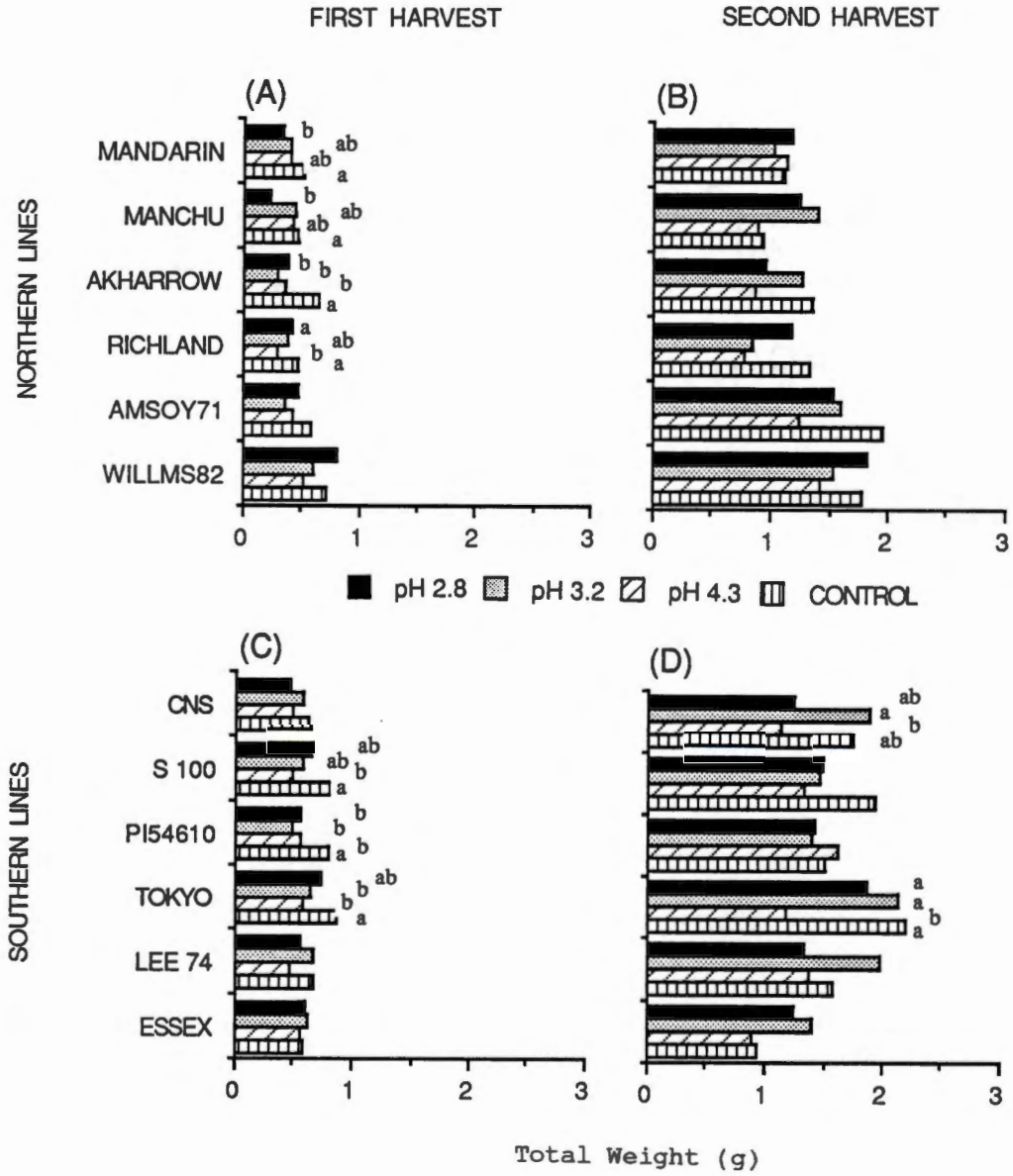

Figure 14. Mean total weight for northern (A and B) and southern (C and D) ancestral lines and cultivars at the first harvest date (2/27/87, A and C) and second harvest date (3/13/87, B and D) when treated with four levels of SAR (second greenhouse experiment, 1987). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for total weight.

response for other southern lines (Figure 14). It appears that acid precipitation may negatively affect young plants, but that they are able to overcome any detrimental effects as they continue to grow.

Transpiration Rate and Stomatal Conductance:

Analyses of variance indicated significant affects of SAR on transpiration rate and stomatal conductance for the second greenhouse experiment (Table 18). Stomatal conductance is an indication of the capacity for diffusion of water through the stomata and an indirect measure of stomatal opening which relates to the regulation of transpiration.

Means for transpiration rate and stomatal conductivity at each SAR pH level averaged across entries at the second harvest date are shown in Table 21. There does not appear to be a consistent trend with respect to SAR pH. The responses for both transpiration rate and stomatal conductance to SAR are ambiguous.

When analyses of variance were conducted for each entry, there were several significant responses to SAR (Figure 15). The northern ancestral line, Mandarin, responded ambiguously to SAR for both transpiration rate and stomatal conductance (Figure 15). Manchu, AK Harrow, and Amsoy 71 lacked significant response for either measurement. Williams 82 did not respond significantly for transpiration rate, but had an ambiguous response for stomatal conductance (Figure 15).

The southern lines CNS, Tokyo, Lee 74, and Essex, lacked significant response to SAR for either measurement (Figure 15).

Table 21. Mean transpiration rate and stomatal conductance for each SAR pH level across all soybean lines for the second greenhouse experiment.

SAR pH	Transpiration Rate	Stomatal Conductance
	-- $\mu g\ cm^{-2}\ s^{-1}$ --	-- $cm\ s^{-1}$ --
2.8	0.4095 ab [s]	0.023 a
3.2	0.3147 bc	0.015 b
4.3	0.2433 c	0.011 b
Control	0.4621 a	0.023 a

[s] Means followed by the same letter are not significantly different ($P \leq .05$) from other means.

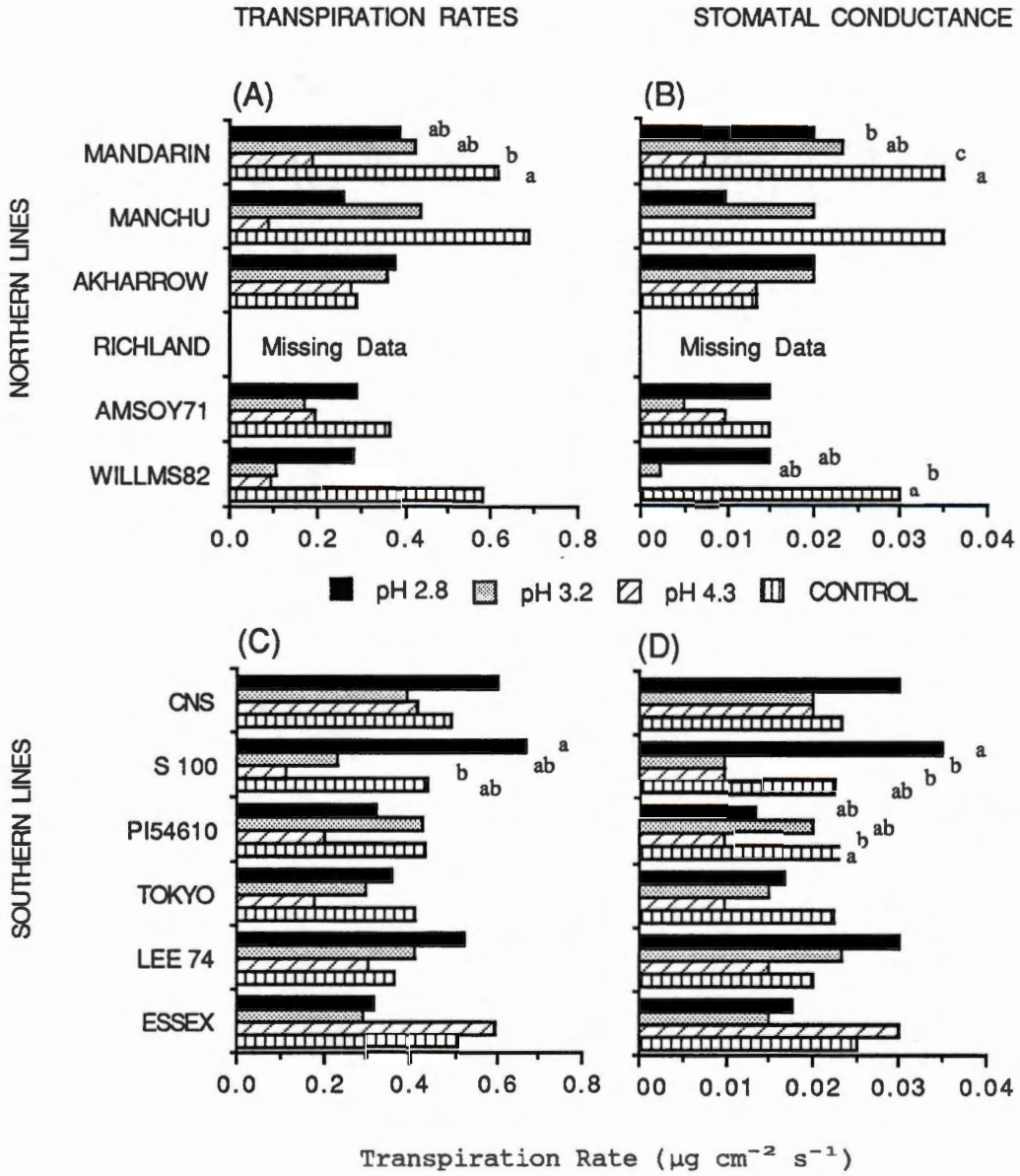

Figure 15. Mean transpiration rates for northern (A) and southern (C) ancestral lines and cultivars, and stomatal conductivity for northern (B) and southern (D) ancestral lines and cultivars treated with four levels of SAR (second greenhouse experiment, 1987). Bars followed by the same letters or no letters are not significantly different ($P \leq .05$) for that entry. There are significant differences among entries for transpiration rate and stomatal conductivity.

S 100 responded ambiguously to SAR for both transpiration rate and stomatal conductance. PI 54610 did not respond to SAR for transpiration rate, while it responded ambiguously for stomatal conductance.

While there were responses for transpiration rate and stomatal conductivity, there was no consistent trend with respect to pH or entries; therefore the significant responses were likely random.

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CHAPTER IV

SUMMARY AND CONCLUSIONS

Field and greenhouse experiments were conducted to determine the affects of simulated acid rain (SAR) on yield and growth of twelve lines of soybean. The twelve lines consisted of four ancestral lines and two cultivars from each of the northern and southern gene pools. The ancestral lines Mandarin, Manchu, AK Harrow, and Richland, and the cultivars Amsoy 71 and Williams 82 represented the northern gene pool. The ancestral lines CNS, S 100, PI 54610, and Tokyo and the cultivars Lee 74 and Essex were representative of the southern gene pool. The objective of the experiments was to evaluate the impact of acidic precipitation on these lines, which contained approximately 80% of the genes present in the current gene pool, and to make inferences about soybean response to acid precipitation in general. Three levels of simulated acid rain (SAR) were utilized (pH 2.8, 3.2, and 4.3). This simulant was formulated to reflect the chemical composition of actual rainfall of the Northeastern United States.

Field experiments were conducted on optimum (recommended soil pH and fertility) and sub-optimum soil fertility in order to determine if response to acid precipitation was affected by soil management practices. There was no trend to indicate that soybeans were more sensitive to SAR in either optimum or sub-optimum soils when yield, 100-seed weight, leaf area, plant

weight, transpiration rate, stomatal conductivity, and stomatal frequency were evaluated. In most instances when there was a response to SAR, the affect was positive, indicating the possibility of a foliar fertilizer effect. There was only one instance where yield decreased as a result of an increased acidity, but this was true for only one year/location combination. Entries did not respond the same from one year or location to the next indicating that responses were random.

When there were responses to SAR, the pattern was not consistent from one year to the next or for more than one location or experiment. Overall, the trend was for a lack of yield response of soybean to SAR and in the cases where there were responses, the effect was generally beneficial rather than detrimental.

Results obtained from the second greenhouse experiment indicated that plant growth (plant weight) was inhibited in early stages but the effect did not persist so that the plants recovered and grew normally at later stages of growth.

Based on the response of the ancestral lines (which contribute approximately 80% of the genes to the gene pool) and cultivars developed from them, it appears that, in general, soybean yield and growth will not be detrimentally affected by SAR.

APPENDICES

APPENDIX A

ACID RAIN MIXTURES

SOLUTIONS:			
	$(NH_4)_2SO_4$	125.5 g/L H_2O	Mwt.=132.14
	$Ca(NO_3)_2 \cdot 4H_2O$	236.1 g/L H_2O	Mwt.=236.15
	$CaCl_2 \cdot 2H_2O$	139.6 g/L H_2O	Mwt.=147.02
	Na_2SO_4	56.8 g/L H_2O	Mwt.=142.05
	K_2SO_4	43.5 g/L H_2O	Mwt.=174.27
	$MgSO_4 \cdot 7H_2O$	98.5 g/L H_2O	Mwt.=246.48

Stock Rain Solution: To a 1 L volumetric flask, add 10 ml each of:

$(NH_4)_2SO_4$
Na_2SO_4
K_2SO_4
$MgSO_4 \cdot 7H_2O$

Add approximately 900 ml deionized H_2O followed by 10 ml each of:

$Ca(NO_3)_2 \cdot 4H_2O$
$CaCl_2 \cdot 2H_2O$

Take to 1 L total volume with deionized H_2O and mix.

Stock Acid Solution: 1 M HNO_3 + 1 M H_2SO_4

To a 1 L volumetric flask containing about 200 ml deionized H_2O, add:

63 ml conc. HNO_3 (~70%)
and 56 ml conc. H_2SO_4 (~96%).

Take to 1 L total volume with deionized H_2O and mix.

SIMULATED ACID RAIN MIXTURES FOR THE THREE pH LEVELS:

pH	STOCK ACID SOLUTION	STOCK RAIN SOLUTION
2.8	26.4 ml	50 ml
3.2	10.5 ml	150 ml
4.3	835 µl	50 ml

Mix the stock acid and stock rain solutions in the above proportions to make 50 L of SAR by taking to 50 L total volume with deionized H_2O and mixing.

This recipe reflects average rainfall composition as reported by: Cogbill, C.V. and G.E. Likens. 1974. Acid precipitation in the northeastern United States. Water Resour. Res. 10:1133-1137.

APPENDIX B

PLASTIC SPRAY FOR STOMATAL FREQUENCY

PLASTIC SPRAY AND IGNITION SEALER:

Acra-Seal Part No. M4-06

Radiator Specialty Company, Charlotte,
North Carolina 28234.

APPENDIX C

TABLES OF MEANS FOR THE FIELD EXPERIMENT

Table C-1. Mean seed yield for northern and southern ancestral lines and cultivars across years, locations, soil conditions, and simulated acid rain (SAR) pHs.

	Mean Yield		Standard Error
	--------- kg ha^{-1} ---------		
Northern Ancestral Lines			
MANDARIN	1328	ef [s]	62
MANCHU	1391	e	70
AK HARROW	1292	ef	60
RICHLAND	1203	f	64
Northern Cultivars			
AMSOY 71	1558	d	79
WILLIAMS 82	2149	b	113
Southern Ancestral Lines			
CNS	1358	e	95
S 100	1846	c	103
PI 54610	1660	d	114
TOKYO	1934	c	113
Southern Cultivars			
LEE 74	2102	b	101
ESSEX	2513	a	135

[s] Entry means followed by the same letter are not significantly different ($P \leq .05$) from the other entry means.

Table C-2. Mean seed yields by simulated acid rain (SAR) pH level at each location in each year averaged across soil conditions and soybean lines.

LOCATION	YEAR	SAR pH	MEAN YIELD
			-- kg ha^{-1} --
Knoxville	**1985**	2.8	2533 [s]
		3.2	2413
		4.3	2551
Knoxville	**1986**	2.8	1602
		3.2	1737
		4.3	1694
Milan	**1985**	2.8	1964 a
		3.2	1927 a
		4.3	1805 b
Milan	**1986**	2.8	704
		3.2	728
		4.3	678

[s] Means followed by the same letters or no letters are not significantly different ($P \leq .05$). Letters are only for comparison within a location and year.

Table C-3. Mean seed yields for northern and southern ancestral lines and cultivars by simulated acid rain (SAR) pH level under optimum and sub-optimum soil conditions averaged across years and locations.

	SAR pH	OPTIMUM SOIL	SUB-OPT SOIL
Northern Ancestral Lines		-------- kg ha^{-1} --------	
	2.8	1536 s	1332 s
MANDARIN	3.2	1372	1235
	4.3	1281	1208
	2.8	1472	1438 a
MANCHU	3.2	1450	1187 b
	4.3	1598	1201 b
	2.8	1396 ab	1195
AK HARROW	3.2	1522 a	1200
	4.3	1216 b	1221
	2.8	1411	1193
RICHLAND	3.2	1245	1107
	4.3	1184	1080
Northern Cultivars			
	2.8	1656	1395
AMSOY 71	3.2	1684	1465
	4.3	1790	1359
	2.8	2371	1785
WILLIAMS 82	3.2	2443	1934
	4.3	2340	2021

Table C-3 (continued).

		SAR pH	OPTIMUM SOIL	SUB-OPT SOIL
Southern Ancestral Lines			------- kg ha^{-1} -------	
		2.8	1429 [s]	1146 [s]
	CNS	3.2	1476	1275
		4.3	1668	1157
		2.8	1772	1421
	S 100	3.2	1773	1667
		4.3	1787	1487
		2.8	1776	1367
	PI 54610	3.2	1966	1441
		4.3	1833	1577
		2.8	2152	1864
	TOKYO	3.2	1848	1604
		4.3	2393	1742
Southern Cultivars				
		2.8	2304	1826
	LEE 74	3.2	2352	1947
		4.3	2283	1903
		2.8	2888 a	2358
	ESSEX	3.2	2703 ab	2406
		4.3	2479 b	2244

[s] Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are only for comparison within an entry and soil condition.

Table C-4. Mean seed weight for northern and southern ancestral lines and cultivars averaged across years, locations, soil conditions, and simulated acid rain (SAR) pHs.

	Mean Seed Weight		Standard Error
	---------- g ----------		
Northern Ancestral Lines			
MANDARIN	16.70	b [s]	0.24
MANCHU	15.22	f	0.28
AK HARROW	13.44	i	0.18
RICHLAND	15.42	ef	0.19
Northern Cultivars			
AMSOY 71	15.09	f	0.29
WILLIAMS 82	16.47	bc	0.29
Southern Ancestral Lines			
CNS	14.38	g	0.18
S 100	14.47	g	0.27
PI 54610	16.09	cd	0.33
TOKYO	24.06	a	0.20
Southern Cultivars			
LEE 74	15.70	de	0.22
ESSEX	13.87	h	0.23

[s] Entry means followed by the same letter are not significantly different ($P \leq .05$).

APPENDIX D

TREATMENT MEANS FOR THE FIRST GREENHOUSE EXPERIMENT

Table D-1. Mean leaf number per plant for northern and southern ancestral lines and cultivars for the first greenhouse experiment for the first and second harvest dates.

		SAR pH	Leaf Number FIRST HARVEST	Leaf Number SECOND HARVEST
Northern Ancestral Lines			------- no. --------	
		2.8	14 a ¶	19 a ¶
d ˢ	MANDARIN	3.2	14 a	19 a
		4.3	13 a	16 a
		Control¥	10 a	14 a
		2.8	18 a	41 a
a	MANCHU	3.2	18 a	26 a
		4.3	17 a	33 a
		Control¥	17 a	33 a
		2.8	13 a	26 a
bc	AK HARROW	3.2	13 a	21 a
		4.3	13 a	24 a
		Control¥	12 a	23 a
		2.8	15 a	19 a
d	RICHLAND	3.2	12 a	15 a
		4.3	12 a	13 a
		Control¥	11 a	14 a
Northern Cultivars				
		2.8	14 a	22 a
cd	AMSOY 71	3.2	12 a	17 a
		4.3	13 a	22 a
		Control¥	12 a	17 a
		2.8	12 a	25 a
cd	WILLIAMS 82	3.2	11 a	21 a
		4.3	12 a	20 a
		Control¥	11 a	17 a

Table D-1 (continued).

		SAR pH	Leaf Number FIRST HARVEST	Leaf Number SECOND HARVEST
Southern Ancestral Lines			------- no.	----------
		2.8	17 a ¶	23 ab ¶
b [s]	CNS	3.2	16 a	30 a
		4.3	16 a	27 ab
		Control[¥]	15 a	16 b
		2.8	20 a	34 a
a	S 100	3.2	19 a	32 ab
		4.3	16 a	26 ab
		Control[¥]	19 a	24 b
		2.8	13 a	26 a
bc	PI 54610	3.2	15 a	20 b
		4.3	12 a	28 a
		Control[¥]	14 a	18 b
		2.8	20 a	36 a
e	TOKYO	3.2	20 a	35 a
		4.3	19 a	29 a
		Control[¥]	20 a	29 a
Southern Cultivars				
		2.8	10 a	14 a
e	LEE 74	3.2	9 a	9 a
		4.3	9 a	12 a
		Control[¥]	10 a	10 a
		2.8	19 a	32 a
a	ESSEX	3.2	19 a	33 a
		4.3	19 a	34 a
		Control[¥]	20 a	23 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for leaf number. Letters are for comparisons among the entries in both northern and southern gene pools.

[¥] pH = 5.2.

[¶] Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are to be used only for comparisons within entries.

Table D-2. Mean leaf areas per plant for northern and southern ancestral lines and cultivars for the first greenhouse experiment for the first and second harvest dates.

		SAR pH	Leaf Area FIRST HARVEST	Leaf Area SECOND HARVEST
Northern Ancestral Lines			----------cm^2----------	
		2.8	258.5 a ¶	409.1 a ¶
d ⁵	MANDARIN	3.2	296.8 a	458.0 a
		4.3	269.2 a	363.3 a
		Control[¥]	174.6 a	320.5 a
		2.8	445.1 a	1019.3 a
ab	MANCHU	3.2	446.5 a	621.5 a
		4.3	481.9 a	976.0 a
		Control[¥]	684.7 a	876.6 a
		2.8	241.6 a	494.5 a
d	AK HARROW	3.2	243.1 a	448.5 a
		4.3	259.9 a	576.8 a
		Control[¥]	189.8 a	564.6 a
		2.8	297.0 a	362.8 a
d	RICHLAND	3.2	198.9 a	283.3 a
		4.3	195.8 a	263.7 a
		Control[¥]	197.1 a	310.2 a
Northern Cultivars				
		2.8	245.6 a	429.0 a
d	AMSOY 71	3.2	169.4 a	302.0 a
		4.3	201.2 a	432.2 a
		Control[¥]	186.8 a	295.9 a
		2.8	270.1 a	596.5 a
d	WILLIAMS 82	3.2	251.7 a	541.8 a
		4.3	294.0 a	452.2 a
		Control[¥]	221.7 a	373.3 a

Table D-2 (continued).

		SAR pH	Leaf Area FIRST HARVEST	Leaf Area SECOND HARVEST
Southern Ancestral Lines			----------cm^2----------	
		2.8	514.8 a ¶	1080.4 a ¶
ab [s]	CNS	3.2	473.4 a	1288.4 a
		4.3	470.6 a	1068.9 a
		Control[¥]	439.8 a	564.9 a
		2.8	466.8 ab	1003.2 a
b	S 100	3.2	460.9 ab	964.8 a
		4.3	335.2 b	757.9 a
		Control[¥]	490.1 a	963.3 a
		2.8	290.0 a	875.80 a
c	PI 54610	3.2	351.4 a	583.60 a
		4.3	267.0 a	925.70 a
		Control[¥]	359.2 a	626.10 a
		2.8	461.5 a	1233.8 a
a	TOKYO	3.2	427.0 a	1150.0 a
		4.3	420.1 a	1046.4 a
		Control[¥]	526.7 a	1150.6 a
Southern Cultivars				
		2.8	115.7 a	209.9 a
e	LEE 74	3.2	100.0 a	106.6 a
		4.3	118.3 a	163.0 a
		Control[¥]	139.5 a	136.2 a
		2.8	477.9 a	975.0 a
ab	ESSEX	3.2	474.3 a	1063.6 a
		4.3	500.6 a	1024.7 a
		Control[¥]	585.4 a	705.1 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for leaf area. Letters are for comparisons among the entries in both northern and southern gene pools.

[¥] pH =5.2.

¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table D-3. Mean leaf weight per plant for northern and southern ancestral lines and cultivars for the first greenhouse experiment for the first and second harvest dates.

		SAR pH	Leaf Weight FIRST HARVEST	Leaf Weight SECOND HARVEST
Northern Ancestral Lines			--------- g	----------
		2.8	0.541 a ¶	1.575 ab ¶
d [s]	MANDARIN	3.2	0.508 a	1.925 a
		4.3	0.450 a	1.362 ab
		Control[*]	0.425 a	1.175 b
		2.8	0.666 a	2.400 a
bc	MANCHU	3.2	0.708 a	1.625 a
		4.3	0.666 a	2.337 a
		Control[*]	0.625 a	2.125 a
		2.8	0.391 a	1.600 a
d	AK HARROW	3.2	0.408 a	1.400 a
		4.3	0.466 a	1.762 a
		Control[*]	0.300 a	1.783 a
		2.8	0.550 a	1.312 a
d	RICHLAND	3.2	0.383 a	1.500 a
		4.3	0.325 a	1.187 a
		Control[*]	0.358 a	1.300 a
Northern Cultivars				
		2.8	0.475 a	1.600 a
d	AMSOY 71	3.2	0.316 a	1.387 a
		4.3	0.333 a	1.350 a
		Control[*]	0.383 a	1.116 a
		2.8	0.458 a	1.825 a
d	WILLIAMS 82	3.2	0.433 a	1.687 a
		4.3	0.500 a	1.466 a
		Control[*]	0.400 a	1.275 a

Table D-3 (continued).

		SAR pH	Leaf Weight FIRST HARVEST	Leaf Weight SECOND HARVEST
Southern Ancestral Lines			---------- g	----------
		2.8	0.716 a ¶	2.433 a ¶
bc [s]	CNS	3.2	0.600 a	2.562 a
		4.3	0.541 a	2.012 a
		Control[*]	0.641 a	1.733 a
		2.8	0.791 a	2.675 a
ab	S 100	3.2	0.741 ab	2.462 a
		4.3	0.533 b	1.912 a
		Control[*]	0.750 ab	2.612 a
		2.8	0.458 a	2.137 a
c	PI 54610	3.2	0.550 a	1.666 a
		4.3	0.391 a	2.412 a
		Control[*]	0.533 a	2.062 a
		2.8	0.783 a	2.850 a
a	TOKYO	3.2	0.650 a	2.650 a
		4.3	0.641 a	2.425 a
		Control[*]	0.858 a	3.212 a
Southern Cultivars				
		2.8	0.200 a	1.037 a
e	LEE 74	3.2	0.183 a	0.962 a
		4.3	0.166 a	0.950 a
		Control[*]	0.233 a	0.925 a
		2.8	0.875 a	2.587 a
a	ESSEX	3.2	0.750 a	2.675 a
		4.3	0.833 a	2.450 a
		Control[*]	0.958 a	2.416 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for leaf weight. Letters are for comparisons among the entries in both northern and southern gene pools.
[*] pH = 5.2.
¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table D-4. Mean stem weight per plant for northern and southern ancestral lines and cultivars for the first greenhouse experiment for first and second harvest dates.

		SAR pH	Stem Weight FIRST HARVEST	Stem Weight SECOND HARVEST
Northern Ancestral Lines			---------- g ----------	
		2.8	0.441 a ¶	1.737 ab ¶
d ˢ	MANDARIN	3.2	0.450 a	2.125 a
		4.3	0.408 a	1.387 b
		Control ¥	0.325 a	1.437 b
		2.8	0.583 a	2.487 a
bc	MANCHU	3.2	0.641 a	1.850 a
		4.3	0.600 a	2.350 a
		Control ¥	0.591 a	2.562 a
		2.8	0.433 a	1.587 a
d	AK HARROW	3.2	0.433 a	1.462 a
		4.3	0.408 a	1.725 a
		Control ¥	0.541 a	1.762 a
		2.8	0.450 a	1.400 a
d	RICHLAND	3.2	0.341 a	1.800 a
		4.3	0.316 a	1.500 a
		Control ¥	0.341 a	1.462 a
Northern Cultivars				
		2.8	0.400 a	1.500 a
d	AMSOY 71	3.2	0.308 a	1.225 a
		4.3	0.283 a	1.400 a
		Control ¥	0.408 a	1.237 a
		2.8	0.466 a	1.787 a
d	WILLIAMS 82	3.2	0.466 a	1.725 a
		4.3	0.491 a	1.575 a
		Control ¥	0.383 a	1.412 a

Table D-4 (continued).

		SAR pH	Stem Weight FIRST HARVEST	Stem Weight SECOND HARVEST
Southern Ancestral Lines			---------- g	----------
		2.8	0.675 a ¶	2.550 a ¶
bc [s]	CNS	3.2	0.608 a	2.562 a
		4.3	0.508 a	2.225 a
		Control[*]	0.625 a	1.800 a
		2.8	0.675 a	2.375 a
b	S 100	3.2	0.666 a	2.475 a
		4.3	0.475 b	1.962 a
		Control[*]	0.650 a	2.725 a
		2.8	0.466 a	2.362 a
c	PI 54610	3.2	0.525 a	1.633 a
		4.3	0.366 a	2.325 a
		Control[*]	0.566 a	1.925 a
		2.8	0.708 a	3.137 a
a	TOKYO	3.2	0.641 a	2.775 a
		4.3	0.633 a	2.725 a
		Control[*]	0.925 a	3.300 a
Southern Cultivars				
		2.8	0.225 a	0.975 a
e	LEE 74	3.2	0.200 a	0.775 a
		4.3	0.200 a	0.812 a
		Control[*]	0.241 a	0.875 a
		2.8	0.716 a	2.300 a
b	ESSEX	3.2	0.725 a	2.800 a
		4.3	0.733 a	2.412 a
		Control[*]	0.916 a	2.225 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for stem weight. Letters are for comparisons among the entries in both northern and southern gene pools.
[*] pH = 5.2.
¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table D-5. Mean total plant weight (leaf + stem weight for each plant) for northern and southern ancestral lines and cultivars for the first greenhouse experiment for the first and second harvest dates.

		SAR pH	Total Plant Weight FIRST HARVEST	Total Plant Weight SECOND HARVEST
Northern Ancestral Lines			---------- g ----------	
		2.8	0.983 a ¶	3.312 ab ¶
e ˢ	MANDARIN	3.2	0.958 a	4.050 a
		4.3	0.858 a	2.750 b
		Control¥	0.750 a	2.612 b
		2.8	1.250 a	4.887 a
cd	MANCHU	3.2	1.350 a	3.475 a
		4.3	1.266 a	4.687 a
		Control¥	1.216 a	4.687 a
		2.8	0.825 a	3.187 a
e	AK HARROW	3.2	0.841 a	2.862 a
		4.3	0.875 a	3.487 a
		Control¥	0.841 a	3.450 a
		2.8	1.000 a	2.712 a
e	RICHLAND	3.2	0.725 a	3.475 a
		4.3	0.641 a	2.687 a
		Control¥	0.700 a	2.762 a
Northern Cultivars				
		2.8	0.875 a	3.100 a
e	AMSOY 71	3.2	0.625 a	2.612 a
		4.3	0.616 a	2.750 a
		Control¥	0.791 a	2.250 a
		2.8	0.925 a	3.612 a
e	WILLIAMS 82	3.2	0.900 a	3.412 a
		4.3	0.991 a	3.133 a
		Control¥	0.783 a	2.687 a

Table D-5 (continued).

		SAR pH	Total Plant Weight FIRST HARVEST	Total Plant Weight SECOND HARVEST
Southern Ancestral Lines			---------- g ----------	
		2.8	1.391 a ¶	4.983 a ¶
cd [s]	CNS	3.2	1.208 a	5.125 a
		4.3	1.050 a	4.237 a
		Control[¥]	1.266 a	3.517 a
		2.8	1.466 a	5.050 a
bc	S 100	3.2	1.408 a	4.937 a
		4.3	1.008 b	3.875 a
		Control[¥]	1.400 a	5.337 a
		2.8	0.925 a	4.500 a
d	PI 54610	3.2	1.075 a	3.300 a
		4.3	0.758 a	4.737 a
		Control[¥]	1.100 a	3.987 a
		2.8	1.491 a	5.987 a
a	TOKYO	3.2	1.291 a	5.425 a
		4.3	1.275 a	5.150 a
		Control[¥]	1.783 a	6.466 a
Southern Cultivars				
		2.8	0.425 a	2.012 a
f	LEE 74	3.2	0.383 a	1.737 a
		4.3	0.366 a	1.762 a
		Control[¥]	0.475 a	1.800 a
		2.8	1.591 a	4.887 a
ab	ESSEX	3.2	1.475 a	5.475 a
		4.3	1.566 a	4.862 a
		Control[¥]	1.875 a	4.983 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for total weight. Letters are for comparisons among the entries in both northern and southern gene pools.

[¥] pH = 5.2.

¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table D-6. Mean specific leaf weight for northern and southern ancestral lines and cultivars for the first greenhouse experiment for the first and second harvest dates.

		SAR pH	Specific Leaf Weight FIRST HARVEST	Specific Leaf Weight SECOND HARVEST
Northern Ancestral Lines			-------- g cm^{-2} --------	
		2.8	0.0021 a ¶	0.0038 a ¶
c ˢ	MANDARIN	3.2	0.0017 a	0.0041 a
		4.3	0.0017 a	0.0041 a
		Control¥	0.0021 a	0.0037 a
		2.8	0.0014 a	0.0024 a
e	MANCHU	3.2	0.0017 a	0.0027 a
		4.3	0.0013 a	0.0023 a
		Control¥	0.0012 a	0.0025 a
		2.8	0.0019 a	0.0036 a
cd	AK HARROW	3.2	0.0016 a	0.0037 a
		4.3	0.0017 a	0.0034 a
		Control¥	0.0015 a	0.0031 a
		2.8	0.0018 a	0.0053 a
b	RICHLAND	3.2	0.0019 a	0.0057 a
		4.3	0.0016 a	0.0056 a
		Control¥	0.0018 a	0.0041 a
Northern Cultivars				
		2.8	0.0018 a	0.0036 ab
c	AMSOY 71	3.2	0.0018 a	0.0049 a
		4.3	0.0016 a	0.0030 b
		Control¥	0.0021 a	0.0044 ab
		2.8	0.0017 a	0.0031 a
cd	WILLIAMS 82	3.2	0.0018 a	0.0038 a
		4.3	0.0017 a	0.0029 a
		Control¥	0.0021 a	0.0035 a

Table D-6 (continued).

		SAR pH	Specific Leaf Weight FIRST HARVEST	Specific Leaf Weight SECOND HARVEST
Southern Ancestral Lines			-------- g cm^{-2} --------	
		2.8	0.0014 a ¶	0.0040 a ¶
	e [s] CNS	3.2	0.0012 a	0.0019 a
		4.3	0.0011 a	0.0018 a
		Control[¥]	0.0014 a	0.0034 a
		2.8	0.0016 a	0.0026 a
	de S 100	3.2	0.0016 a	0.0025 a
		4.3	0.0016 a	0.0028 a
		Control[¥]	0.0015 a	0.0026 a
		2.8	0.0016 a	0.0024 a
	de PI 54610	3.2	0.0015 a	0.0031 a
		4.3	0.0014 a	0.0025 a
		Control[¥]	0.0015 a	0.0033 a
		2.8	0.0016 a	0.0023 b
	e TOKYO	3.2	0.0015 a	0.0023 b
		4.3	0.0015 a	0.0023 b
		Control[¥]	0.0016 a	0.0027 a
Southern Cultivars				
		2.8	0.0016 a	0.0057 b
	a LEE 74	3.2	0.0017 a	0.0096 a
		4.3	0.0014 a	0.0064 ab
		Control[¥]	0.0018 a	0.0072 ab
		2.8	0.0018 a	0.0026 a
	e ESSEX	3.2	0.0015 a	0.0025 a
		4.3	0.0016 a	0.0024 a
		Control[¥]	0.0017 a	0.0027 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for SLW. Letters are for comparisons among the entries in both northern and southern gene pools.
[¥] pH = 5.2.
¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table D-7. Mean transpiration rate and stomatal conductance for northern and southern ancestral lines and cultivars for the first greenhouse experiment.

		SAR pH	TRANS. RATE	STOMATAL CONDUCT.
Northern Ancestral Lines			-- μg cm^{-2} s^{-1} --	-- cm s^{-1} --
	MANDARIN	2.8	3.092 a ¶	0.135 a ¶
		3.2	4.745 a	0.217 a
		4.3	3.368 a	0.155 a
		Control*	4.761 a	0.212 a
	MANCHU	2.8	4.684 a	0.210 a
		3.2	4.566 a	0.207 a
		4.3	4.037 a	0.190 a
		Control*	3.258 a	0.150 a
	AK HARROW	2.8	4.3697 a	0.200 a
		3.2	5.9910 a	0.280 a
		4.3	5.5092 a	0.255 a
		Control*	3.9747 a	0.177 a
	RICHLAND	2.8	2.935 a	0.130 a
		3.2	3.081 a	0.137 a
		4.3	3.300 a	0.150 a
		Control*	3.410 a	0.165 a
Northern Cultivars				
	AMSOY 71	2.8	3.104 a	0.137 a
		3.2	2.943 a	0.135 a
		4.3	2.912 a	0.130 a
		Control*	3.142 a	0.145 a
	WILLIAMS 82	2.8	2.6932 a	0.130 a
		3.2	1.6922 a	0.077 a
		4.3	3.1275 a	0.150 a
		Control*	2.7272 a	0.125 a

Table D-7 (continued).

	SAR pH	TRANS. RATE	STOMATAL CONDUCT.
Southern Ancestral Lines		-- μg cm^{-2} s^{-1} -----	cm s^{-1} --
CNS	2.8	-	-
	3.2	-	-
	4.3	-	-
	Control*	-	-
S 100	2.8	2.0217 a ¶	0.090 ab ¶
	3.2	2.5022 a	0.122 a
	4.3	1.1870 a	0.057 b
	Control*	2.4545 a	0.112 ab
PI 54610	2.8	2.6740 a	0.122 a
	3.2	1.5100 a	0.070 a
	4.3	1.6120 a	0.075 a
	Control*	2.1020 a	0.100 a
TOKYO	2.8	-	-
	3.2	-	-
	4.3	-	-
	Control*	-	-
Southern Cultivars			
LEE 74	2.8	2.5477 a	0.122 a
	3.2	2.8710 a	0.142 a
	4.3	2.3830 a	0.115 a
	Control*	2.3110 a	0.105 a
ESSEX	2.8	2.4247 ab	0.112 ab
	3.2	2.8660 a	0.140 a
	4.3	1.4100 b	0.067 b
	Control*	1.6417 ab	0.072 b

* pH = 5.2.
¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are for comparisons within each entry only.

APPENDIX E

TREATMENT MEANS FOR THE SECOND GREENHOUSE EXPERIMENT

Table E-1. Mean leaf number per plant for northern and southern ancestral lines and cultivars for the second greenhouse experiment for the first and second harvest dates.

			Leaf Number	
		SAR pH	FIRST HARVEST	SECOND HARVEST
Northern Ancestral Lines			-------- no. ---------	
		2.8	7 a ¶	12 a ¶
cd §	MANDARIN	3.2	7 a	12 a
		4.3	6 a	11 a
		Control*	7 a	11 a
		2.8	5 b	14 a
bcd	MANCHU	3.2	7 a	17 a
		4.3	8 a	12 a
		Control*	8 a	15 a
		2.8	6 b	14 a
bcd	AK HARROW	3.2	6 b	14 a
		4.3	7 ab	12 a
		Control*	9 a	16 a
		2.8	7 a	11 a
d	RICHLAND	3.2	7 a	9 a
		4.3	5 a	8 a
		Control*	6 a	14 a
Northern Cultivars				
		2.8	7 a	20 a
bcd	AMSOY 71	3.2	4 a	19 a
		4.3	6 a	13 b
		Control*	7 a	17 ab
		2.8	10 a	16 ab
bcd	WILLIAMS 82	3.2	7 a	13 b
		4.3	7 a	13 b
		Control*	8 a	20 a

Table E-1 (continued).

		SAR pH	Leaf Number FIRST HARVEST	Leaf Number SECOND HARVEST
Southern Ancestral Lines			-------- no.	---------
		2.8	6 a ¶	18 a ¶
ab §	CNS	3.2	9 a	22 a
		4.3	7 a	17 a
		Control*	9 a	21 a
		2.8	8 a	17 ab
ab	S 100	3.2	8 a	19 ab
		4.3	7 b	13 b
		Control*	9 a	23 a
		2.8	9 a	15 a
abc	PI 54610	3.2	7 a	18 a
		4.3	8 a	19 a
		Control*	9 a	17 a
		2.8	9 ab	25 a
a	TOKYO	3.2	9 b	26 a
		4.3	6 c	16 a
		Control*	11 a	25 a
Southern Cultivars				
		2.8	9 a	16 a
abc	LEE 74	3.2	8 a	24 a
		4.3	7 a	16 a
		Control*	8 a	14 a
		2.8	8 a	21 a
ab	ESSEX	3.2	7 a	20 a
		4.3	7 a	15 a
		Control*	8 a	23 a

§ Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for leaf number. Letters are for comparisons among the entries in both northern and southern gene pools.

* pH = 5.2.

¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table E-2. Mean leaf area per plant for northern and southern ancestral lines and cultivars for the second greenhouse experiment for the first and second harvest dates.

		SAR pH	Leaf Area FIRST HARVEST	Leaf Area SECOND HARVEST
Northern Ancestral Lines			----------cm^2----------	
		2.8	85.7 a ¶	192.4 a ¶
bc [s]	MANDARIN	3.2	110.4 a	161.8 a
		4.3	91.1 a	173.1 a
		Control[*]	122.0 a	167.0 a
		2.8	63.8 b	186.1 a
bc	MANCHU	3.2	129.1 a	243.8 a
		4.3	125.2 a	148.0 a
		Control[*]	121.9 a	169.0 a
		2.8	93.8 b	154.2 a
ab	AK HARROW	3.2	72.3 b	217.8 a
		4.3	104.5 b	149.3 a
		Control[*]	163.9 a	237.7 a
		2.8	114.7 a	152.1 a
c	RICHLAND	3.2	98.3 ab	122.2 a
		4.3	70.7 b	115.6 a
		Control[*]	87.7 ab	202.7 a
Northern Cultivars				
		2.8	111.5 a	293.0 a
bc	AMSOY 71	3.2	83.3 a	270.6 a
		4.3	103.5 a	204.6 a
		Control[*]	137.6 a	305.5 a
		2.8	226.4 a	323.0 a
a	WILLIAMS 82	3.2	163.4 a	251.2 a
		4.3	139.0 a	224.1 a
		Control[*]	179.6 a	297.8 a

Table E-2 (continued).

		SAR pH	Leaf Area FIRST HARVEST	Leaf Area SECOND HARVEST
Southern Ancestral Lines			----------cm^2----------	
		2.8	109.6 a ¶	204.3 b ¶
a [s]	CNS	3.2	169.2 a	434.6 a
		4.3	136.4 a	213.4 b
		Control[¥]	150.1 a	332.7 ab
		2.8	164.9 ab	274.9 a
a	S 100	3.2	161.3 ab	243.9 a
		4.3	123.5 b	207.6 a
		Control[¥]	193.2 a	325.7 a
		2.8	157.4 a	248.0 a
a	PI 54610	3.2	135.6 a	237.1 a
		4.3	139.0 a	295.2 a
		Control[¥]	193.8 a	267.9 a
		2.8	178.2 ab	294.6 ab
a	TOKYO	3.2	152.5 bc	372.9 a
		4.3	108.4 c	171.3 b
		Control[¥]	225.0 a	346.1 a
Southern Cultivars				
		2.8	154.0 a	230.6 a
a	LEE 74	3.2	168.6 a	380.7 a
		4.3	118.1 a	238.7 a
		Control[¥]	157.0 a	247.1 a
		2.8	165.0 a	388.4 a
a	ESSEX	3.2	149.4 a	293.6 a
		4.3	156.4 a	230.0 a
		Control[¥]	137.2 a	354.5 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for leaf area. Letters are for comparisons among the entries in both northern and southern gene pools.
[¥] pH = 5.2.
¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table E-3. Mean leaf weight per plant for northern and southern ancestral lines and cultivars for the second greenhouse experiment for the first and second harvest dates.

		SAR pH	Leaf Weight FIRST HARVEST	Leaf Weight SECOND HARVEST
Northern Ancestral Lines			---------- g ----------	
		2.8	0.190 a ¶	0.688 a ¶
bcd §	MANDARIN	3.2	0.240 a	0.590 a
		4.3	0.227 a	0.650 a
		Control*	0.283 a	0.602 a
		2.8	0.125 a	0.647 a
cd	MANCHU	3.2	0.270 a	0.765 a
		4.3	0.267 a	0.460 a
		Control*	0.261 a	0.478 a
		2.8	0.196 b	0.505 a
abcd	AK HARROW	3.2	0.150 b	0.653 a
		4.3	0.193 b	0.465 a
		Control*	0.370 a	0.762 a
		2.8	0.256 ab	0.713 a
d	RICHLAND	3.2	0.215 ab	0.440 a
		4.3	0.166 b	0.415 a
		Control*	0.290 a	0.752 a
Northern Cultivars				
		2.8	0.248 a	0.837 ab
cd	AMSOY 71	3.2	0.178 a	0.852 ab
		4.3	0.231 a	0.611 b
		Control*	0.310 a	1.068 a
		2.8	0.468 a	0.928 a
ab	WILLIAMS 82	3.2	0.352 a	0.832 a
		4.3	0.316 a	0.742 a
		Control*	0.400 a	0.961 a

Table E-3 (continued).

		SAR pH	Leaf Weight FIRST HARVEST	Leaf Weight SECOND HARVEST
Southern Ancestral Lines			---------- g	----------
		2.8	0.252 a ¶	0.562 bc ¶
abcd [s]	CNS	3.2	0.325 a	1.023 a
		4.3	0.252 a	0.485 c
		Control*	0.337 a	0.880 ab
		2.8	0.346 ab	0.756 a
abcd	S 100	3.2	0.338 ab	0.730 a
		4.3	0.266 b	0.641 a
		Control*	0.446 a	0.947 a
		2.8	0.338 b	0.720 a
abcd	PI 54610	3.2	0.295 b	0.701 a
		4.3	0.311 b	0.848 a
		Control*	0.487 a	0.736 a
		2.8	0.396 b	0.843 ab
a	TOKYO	3.2	0.331 b	1.063 a
		4.3	0.305 b	0.511 b
		Control*	0.530 a	1.052 a
Southern Cultivars				
		2.8	0.305 a	0.673 a
abcd	LEE 74	3.2	0.353 a	1.048 a
		4.3	0.265 a	0.720 a
		Control*	0.347 a	0.763 a
		2.8	0.321 a	1.021 a
abc	ESSEX	3.2	0.327 a	0.807 a
		4.3	0.301 a	0.647 a
		Control*	0.310 a	0.893 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for leaf weight. Letters are for comparisons among the entries in both northern and southern gene pools.

* pH = 5.2.

¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table E-4. Mean stem weight per plant for northern and southern ancestral lines and cultivars for the second greenhouse experiment for first and second harvest dates.

		SAR pH	Stem Weight FIRST HARVEST	Stem Weight SECOND HARVEST
Northern Ancestral Lines			---------- g ----------	
		2.8	0.135 b ¶	0.500 a ¶
de §	MANDARIN	3.2	0.166 ab	0.433 a
		4.3	0.180 ab	0.480 a
		Control¥	0.233 a	0.518 a
		2.8	0.103 b	0.605 a
de	MANCHU	3.2	0.187 ab	0.640 a
		4.3	0.163 ab	0.436 a
		Control¥	0.220 a	0.461 a
		2.8	0.187 b	0.452 a
cd	AK HARROW	3.2	0.136 b	0.616 a
		4.3	0.163 b	0.411 a
		Control¥	0.275 a	0.596 a
		2.8	0.175 a	0.461 a
e	RICHLAND	3.2	0.161 ab	0.405 a
		4.3	0.121 b	0.370 a
		Control¥	0.181 a	0.570 a
Northern Cultivars				
		2.8	0.212 a	0.697 a
cde	AMSOY 71	3.2	0.190 a	0.740 a
		4.3	0.203 a	0.632 a
		Control¥	0.273 a	0.883 a
		2.8	0.332 a	0.900 a
abc	WILLIAMS 82	3.2	0.246 a	0.692 a
		4.3	0.208 a	0.673 a
		Control¥	0.311 a	0.808 a

Table E-4 (continued).

		SAR pH	Stem Weight FIRST HARVEST	Stem Weight SECOND HARVEST
Southern Ancestral Lines			---------- g ----------	
		2.8	0.221 a ¶	0.691 a ¶
abcd §	CNS	3.2	0.247 a	0.862 a
		4.3	0.228 a	0.640 a
		Control¥	0.281 a	0.855 a
		2.8	0.291 ab	0.730 a
ab	S 100	3.2	0.245 ab	0.741 a
		4.3	0.220 b	0.690 a
		Control¥	0.365 a	0.978 a
		2.8	0.212 b	0.693 a
bcd	PI 54610	3.2	0.202 b	0.700 a
		4.3	0.246 b	0.772 a
		Control¥	0.317 a	0.765 a
		2.8	0.346 a	1.033 a
a	TOKYO	3.2	0.313 a	1.066 a
		4.3	0.280 a	0.662 b
		Control¥	0.335 a	1.146 a
Southern Cultivars				
		2.8	0.252 a	0.661 a
abc	LEE 74	3.2	0.305 a	0.922 a
		4.3	0.205 a	0.655 a
		Control¥	0.317 a	0.806 a
		2.8	0.286 a	1.051 a
ab	ESSEX	3.2	0.292 a	0.831 a
		4.3	0.250 a	0.692 a
		Control¥	0.275 a	0.815 a

§ Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for stem weight. Letters are for comparisons among the entries in both northern and southern gene pools.

¥ pH = 5.2.

¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table E-5. Mean total plant weight (leaf wt. + stem wt. for each plant) for northern and southern ancestral lines and cultivars for the second greenhouse experiment for the first and second harvest dates.

		SAR pH	Total Plant Weight FIRST HARVEST	Total Plant Weight SECOND HARVEST
Northern Ancestral Lines			---------- g ----------	
		2.8	0.325 b ¶	1.188 a ¶
bcd ⁵	MANDARIN	3.2	0.406 ab	1.023 a
		4.3	0.407 ab	1.130 a
		Control¥	0.517 a	1.121 a
		2.8	0.228 b	1.252 a
cd	MANCHU	3.2	0.457 ab	1.405 a
		4.3	0.431 ab	0.896 a
		Control¥	0.481 a	0.940 a
		2.8	0.387 b	0.957 a
abcd	AK HARROW	3.2	0.286 b	1.270 a
		4.3	0.357 b	0.876 a
		Control¥	0.645 a	1.358 a
		2.8	0.431 a	1.175 a
d	RICHLAND	3.2	0.376 ab	0.845 a
		4.3	0.287 b	0.785 a
		Control¥	0.471 a	1.322 a
Northern Cultivars				
		2.8	0.461 a	1.535 a
cd	AMSOY 71	3.2	0.368 a	1.592 a
		4.3	0.435 a	1.243 a
		Control¥	0.583 a	1.951 a
		2.8	0.801 a	1.828 a
a	WILLIAMS 82	3.2	0.598 a	1.525 a
		4.3	0.525 a	1.416 a
		Control¥	0.711 a	1.770 a

Table E-5 (continued).

		SAR pH	Total Plant Weight FIRST HARVEST	Total Plant Weight SECOND HARVEST
Southern Ancestral Lines			---------- g ----------	
		2.8	0.473 a ¶	1.253 ab ¶
abc [s]	CNS	3.2	0.572 a	1.886 a
		4.3	0.481 a	1.125 b
		Control[*]	0.618 a	1.735 ab
		2.8	0.637 ab	1.486 a
ab	S 100	3.2	0.583 ab	1.471 a
		4.3	0.486 b	1.331 a
		Control[*]	0.811 a	1.926 a
		2.8	0.551 b	1.413 a
abc	PI 54610	3.2	0.497 b	1.401 a
		4.3	0.557 b	1.621 a
		Control[*]	0.805 a	1.501 a
		2.8	0.742 ab	1.877 a
a	TOKYO	3.2	0.645 b	2.130 a
		4.3	0.585 b	1.173 b
		Control[*]	0.865 a	2.198 a
Southern Cultivars				
		2.8	0.557 a	1.335 a
abc	LEE 74	3.2	0.658 a	1.971 a
		4.3	0.470 a	1.375 a
		Control[*]	0.665 a	1.570 a
		2.8	0.607 a	2.072 a
a	ESSEX	3.2	0.620 a	1.638 a
		4.3	0.551 a	1.340 a
		Control[*]	0.585 a	1.708 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for total weight. Letters are for comparisons among the entries in both northern and southern gene pools.
[*] pH = 5.2.
¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table E-6. Mean specific leaf weight per plant for northern and southern ancestral lines and cultivars for the second greenhouse experiment for the first and second harvest dates.

		SAR pH	Specific Leaf Weight FIRST HARVEST	Specific Leaf Weight SECOND HARVEST
Northern Ancestral Lines			--------g cm^{-2}----------	
		2.8	0.0022 a ¶	0.0036 a ¶
ab ⁵	MANDARIN	3.2	0.0022 a	0.0037 a
		4.3	0.0025 a	0.0039 a
		Control¥	0.0023 a	0.0035 a
		2.8	0.0019 a	0.0033 a
c	MANCHU	3.2	0.0020 a	0.0032 a
		4.3	0.0021 a	0.0030 a
		Control¥	0.0021 a	0.0028 a
		2.8	0.0021 a	0.0032 a
bc	AK HARROW	3.2	0.0020 a	0.0029 a
		4.3	0.0018 a	0.0030 a
		Control¥	0.0022 a	0.0033 a
		2.8	0.0022 b	0.0056 a
a	RICHLAND	3.2	0.0022 b	0.0038 a
		4.3	0.0023 b	0.0035 a
		Control¥	0.0033 a	0.0039 a
Northern Cultivars				
		2.8	0.0022 a	0.0028 a
c	AMSOY 71	3.0	0.0022 a	0.0031 a
		4.3	0.0023 a	0.0029 a
		Control¥	0.0025 a	0.0038 a
		2.8	0.0020 a	0.0027 a
bc	WILLIAMS 82	3.2	0.0021 a	0.0033 a
		4.3	0.0023 a	0.0034 a
		Control¥	0.0023 a	0.0032 a

Table E-6 (continued).

		SAR pH	Specific Leaf Weight FIRST HARVEST	SECOND HARVEST
Southern Ancestral Lines			----------g cm^{-2}--------	
		2.8	0.0022 a ¶	0.0027 a¶
c [s]	CNS	3.2	0.0019 a	0.0023 a
		4.3	0.0018 a	0.0023 a
		Control[¥]	0.0023 a	0.0027 a
		2.8	0.0020 a	0.0027 b
c	S 100	3.2	0.0021 a	0.0030 ab
		4.3	0.0021 a	0.0032 a
		Control[¥]	0.0023 a	0.0029 ab
		2.8	0.0021 a	0.0029 a
bc	PI 54610	3.2	0.0021 a	0.0029 a
		4.3	0.0023 a	0.0029 a
		Control[¥]	0.0026 a	0.0030 a
		2.8	0.0022 b	0.0028 a
bc	TOKYO	3.2	0.0022 b	0.0028 a
		4.3	0.0028 a	0.0029 a
		Control[¥]	0.0024 b	0.0030 a
Southern Cultivars				
		2.8	0.0019 a	0.0028 a
c	LEE 74	3.2	0.0022 a	0.0027 a
		4.3	0.0021 a	0.0031 a
		Control[¥]	0.0023 a	0.0033 a
		2.8	0.0019 a	0.0026 a
c	ESSEX	3.2	0.0022 a	0.0028 a
		4.3	0.0021 a	0.0031 a
		Control[¥]	0.0023 a	0.0025 a

[s] Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for specific leaf weight. Letters are for comparisons among the entries in both northern and southern gene pools.

[¥] pH = 5.2.

¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

Table E-7. Mean transpiration rates and stomatal conductance for northern and southern ancestral lines and cultivars for the second greenhouse experiment.

	SAR pH	TRANS. RATE	STOMATAL CONDUCT.
Northern Ancestral Lines		-- $\mu g\ cm^{-2}\ s^{-1}$ --	-- $cm\ s^{-1}$ --
MANDARIN	2.8	0.3930 ab ¶	0.020 b ¶
	3.2	0.4273 ab	0.023 ab
	4.3	0.1870 b	0.007 c
	Control*	0.6165 a	0.035 a
MANCHU	2.8	0.2580 a	0.010 a
	3.2	0.4410 a	0.020 a
	4.3	0.0900 a	0.000 a
	Control*	0.6890 a	0.035 a
AK HARROW	2.8	0.3820 a	0.020 a
	3.2	0.3620 a	0.020 a
	4.3	0.2787 a	0.013 a
	Control*	0.2910 a	0.013 a
RICHLAND	2.8	0.0339 a	0.069 a
	3.2	0.0298 a	0.048 ab
	4.3	0.0293 a	0.045 b
	Control*	0.0492 a	0.050 ab
Northern Cultivars			
AMSOY 71	2.8	-	-
	3.2	-	-
	4.3	-	-
	Control*	-	-
WILLIAMS 82	2.8	0.2860 a	0.015 ab
	3.2	0.1040 a	0.002 ab
	4.3	0.0960 a	0.000 b
	Control*	0.5810 a	0.030 a

Table E-7 (continued).

	SAR pH	TRANS. RATE	STOMATAL CONDUCT.
Southern Ancestral Lines		-- μg cm^{-2} s^{-1} --	-- cm s^{-1} --
CNS	2.8	0.6000 a ¶	0.030 a ¶
	3.2	0.3967 a	0.020 a
	4.3	0.4185 a	0.020 a
	Control*	0.4943 a	0.023 a
S 100	2.8	0.6685 a	0.035 a
	3.2	0.2343 ab	0.010 b
	4.3	0.1160 b	0.010 b
	Control*	0.4447 ab	0.022 ab
PI 54610	2.8	0.3220 a	0.013 ab
	3.2	0.4270 a	0.020 ab
	4.3	0.2023 a	0.010 b
	Control*	0.4377 a	0.023 a
TOKYO	2.8	0.3610 a	0.016 a
	3.2	0.3010 a	0.015 a
	4.3	0.1810 a	0.010 a
	Control*	0.4122 a	0.022 a
Southern Cultivars			
LEE 74	2.8	0.5240 a	0.030 a
	3.2	0.4147 a	0.023 a
	4.3	0.3040 a	0.015 a
	Control*	0.3670 a	0.020 a
ESSEX	2.8	0.3172 a	0.017 a
	3.2	0.2910 a	0.015 a
	4.3	0.5970 a	0.030 a
	Control*	0.5100 a	0.025 a

* pH = 5.2.
¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are for comparison within entries only.

Table E-8. Mean photosynthetic rate for northern and southern ancestral lines and cultivars for the second greenhouse experiment at two separate sampling dates.

		SAR pH	Photosynthetic Rate FIRST SAMPLE	Photosynthetic Rate SECOND SAMPLE
Northern Ancestral Lines			---- Mg dm^{-2} hr^{-1} ----	
		2.8	2395 a ¶	2566 a ¶
b ˢ	MANDARIN	3.2	2480 a	1924 a
		4.3	1924 a	2117 a
		Control¥	2502 a	1155 a
		2.8	3336 a	5645 a
a	MANCHU	3.2	4362 a	3079 a
		4.3	513 a	770 a
		Control¥	1283 a	6543 a
		2.8	855 a	1026 a
b	AK HARROW	3.2	257 a	1539 a
		4.3	1368 a	1967 a
		Control¥	1882 a	2053 a
		2.8	-	-
b	RICHLAND	3.2	-	-
		4.3	-	-
		Control¥	-	-
Northern Cultivars				
		2.8	2053 a	-
ab	AMSOY 71	3.2	4875 a	-
		4.3	1539 a	-
		Control¥	2566 a	-
		2.8	642 a	1539 a
b	WILLIAMS 82	3.2	898 a	1283 a
		4.3	257 a	2053 a
		Control¥	1988 a	3849 a

Table E-8 (continued).

		SAR pH	Photosynthetic Rate FIRST HARVEST	Photosynthetic Rate SECOND HARVEST
Southern Ancestral Lines			---- Mg dm^{-2} hr^{-1} ----	
		2.8	2651 a ¶	3677 a ¶
	b ˢ CNS	3.2	1218 a	1796 b
		4.3	2437 a	1667 b
		Control¥	2993 a	2651 ab
		2.8	1155 a	2502 a
	b S 100	3.2	1882 a	1283 a
		4.3	770 a	257 a
		Control¥	2373 a	3271 a
		2.8	1111 a	2395 a
	b PI 54610	3.2	1411 a	2181 a
		4.3	2180 a	2502 a
		Control¥	1368 a	1967 a
		2.8	2758 a	1539 a
	b TOKYO	3.2	1667 ab	2245 a
		4.3	1026 b	2565 a
		Control¥	2309 a	2116 a
Southern Cultivars				
		2.8	385 c	641 a
	b LEE 74	3.2	1454 bc	2480 a
		4.3	2565 ab	3335 a
		Control¥	3592 a	3336 a
		2.8	1026 a	1347 a
	b ESSEX	3.2	1539 a	2565 a
		4.3	2052 a	3335 a
		Control¥	2630 a	1860 a

ˢ Lines or cultivars preceded by the same letter do not differ significantly ($P < .05$) for photosynthetic rate. Letters are for comparisons among the entries in both northern and southern gene pools.

¥ pH = 5.2.

¶ Means followed by the same letter or no letters are not significantly different ($P \leq .05$). Letters are used only for comparisons within entries.

VITA

Deborah Ruth Landau was born in Oak Ridge, Tennessee on June 19, 1957. She attended Glenwood elementary school and Jefferson Junior High School in that city and was graduated from Oak Ridge High School in June 1975. The following September she entered the University of Tennessee at Martin. As part of her curriculum she was a Co-op student at Flowerwood Nursery in Mobile, Alabama for three quarters. In June of 1980 she received a Bachelor of Science degree in Agriculture.

After five years of experience working as a greenhouse manager for Evergreen Garden Center in Kingsport, Tennessee, she entered Graduate School at The University of Tennessee in Knoxville, in September of 1985 to major in Plant and Soil Science. While working towards her Master of Science degree in the area of plant breeding, she worked as a graduate research assistant for the Soybean Breeding Project.

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