SCREENING SOYBEAN LINES FOR HEAT-TOLERANT POLLEN

by

LEVI P. WALKER

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Major Professor
William T. Schapaugh, Ph.D.

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Abstract

Heat and drought stress are common problems for crops grown in Kansas. Rarely do these problems occur separately, more often than not they occur in tandem if not simultaneously. The interaction of heat stress and pollen germination was investigated in order to determine if a physiological screen was a feasible method of determining heat tolerance in soybean [*Glycine max* (L.) Merr]. Ten soybean lines (Group A) from the 2006 Northern Region Uniform Soybean Tests were analyzed over two years in four locations consisting of irrigated and dryland field environments, with an additional twenty lines (Group B) analyzed in the second year. Pollen was collected from plants and incubated at either 28^{o}, 34^{o}, or 38^{o} C to determine pollen germination for optimal and stress-inducing temperatures. A three-way interaction of entry x incubation temperature x environment was observed, as well as significant differences among entries, incubation temperatures and environments. Average pollen germination for soybean entries ranged from 25% to 38% across three incubation temperatures and four environments in Kansas during 2006 – 07. The average environment effect for pollen germination ranged from 29% (dryland, 2006) to 34% (irrigated, 2007), while the average incubation temperature effect on pollen germination ranged from 25% (38^{o}C) to 44% (28^{o}C). This experiment has shown that increasing incubation temperatures significantly decreases pollen germination in vitro. It has also shown that soybean genotypes differ in pollen germination and that an in vitro screen can be used to characterize these differences. Further studies are needed to establish the relationship between pollen germination, seed set and seed yield in soybean. Work also needs to be completed to determine the proper sample size to adequately characterize differences in pollen germination so that performance differences among genotypes can be used as selection criteria in a plant breeding program.

Table of Contents

List of Tables

Acknowledgements

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CHAPTER 1 - LITERATURE REVIEW

Heat and drought stress are common problems for crops grown in Kansas. Rarely are we graced with these problems separately, more often than not they occur in tandem if not simultaneously. One area of crop development that is affected by heat stress is the reproductive cycle; pollination and fruit set tend to succumb to heat stress, thereby lowering crop yields (Warrag and Hall, 1984). This action is compounded by reproductive abortion rates in *Glycine max* (L.) Merr., which range from 36% - 81% (Egli, 2005). This study investigates the effects of heat stress on fertilization in soybean by employing a screening method to differentiate heat tolerant and heat susceptible lines in a breeding program. The hypothesis of this study is that the pollen of some genotypes is more heat-tolerant than others, resulting in increased seed set, and that this characteristic may be heritable.

Effect of Temperature

In other legumes, such as *Arachis hypogaea* L. (peanut) and *Vigna unguiculata* subsp. unguiculata (cowpea), heat can also cause problems with reproductive processes. Prasad et al. (1999) studied the ability of groundnut to produce viable pollen during short periods of high temperatures. The study showed that high daytime temperatures can reduce the number of flowers, pegs/pods, and that high night and day temperatures will reduce the amount of pollen produced and the pollen viability. Part of the negative effect on pollen was due to reduced anther dehiscence caused by the high temperatures. They felt the duration of time the flowers were subjected to high levels of heat was more important than any one temperature itself (Prasad et al., 1999).

In another study on peanut by Prasad et al. (2001), the floral stages of pre-and post-anthesis were examined to determine the critical juncture of development vs. temperature. The results of this study corroborate Prasad et al., 1999; that longer periods at high temperatures are more detrimental to fruit set than short bursts of heat. They did find that two stages were significantly affected by the heat treatments: anthesis and three to four days before anthesis. High temperatures applied during this time reduced pollen viability. Prasad et al. (2001) took the information obtained from the previous two studies and used it to screen peanut lines for heat tolerance. They analyzed the effects of high temperatures applied during flowering and microsporogenesis and found that different processes were independently controlled by heat tolerance traits. The refined screening procedure, measuring the heat tolerance of peanuts during flowering and microsporogenesis, consisted of measuring the amount of fruit-set obtained during these events (Prasad et al., 2002).

A soybean yield study by Gibson and Mullen (1996) examined the effects of high temperature during the day and night periods (35/30^{o} C). They were expressly interested in the effect of high night temperatures. They found no interaction between day/night temperature regimens, but each environment did produce an effect. Plants exposed to a daytime temperature of 35^{o}C during flowering, pod set, and seed fill experienced reduced seed numbers and seed fill, up to 27% reduction in yield, along with a decrease in photosynthesis. Plants exposed to a nighttime temperature of 30^{o}C during early reproductive growth produced an increase in the number of seeds and pods, but this positive effect was offset by smaller seed sizes. Overall, yield did not change significantly during the high night temperatures. This suggested that 30^{o}C may still be in the optimum range, while exposure approaching 35^{o}C would begin reducing yield levels.

Ahmed et al. (1992) attempted to discern exactly what causes heat injury in cowpea during the reproductive cycle. They looked at heat stress by floral development interactions, any resultant damage, and probable scenarios of male sterility caused by high night temperatures. As might be expected, plants that endured an environment with high night temperatures did not set as many pods as those grown in optimum conditions, although the number of flowers produced in each temperature treatment was similar. The plants grown in supraoptimal conditions produced deformed pollen and lacked endothecial development, which led to poor anther dehiscence and low pollen viability.

Warrag and Hall (1983) performed three heat tolerance experiments in California. The first experiment was conducted in the Imperial Valley and screened determinate cowpea genotypes for heat tolerance during flowering by selecting genotypes which would initiate flowering during the hottest portion of the growing season. Notes were made on the number of pods every two weeks. Out of 58 lines, three lines set significantly higher numbers of pods: Prima, TVu 4552, and PI 204647. The balance of the entries set little to no pods, due in part to non-dehiscent anthers. The second experiment tested Prima and TVu 4552 from experiment 1 and compared them with a widely grown commercial variety of cowpea, California Blackeye No. 5. All three cultivars were grown in growth chambers to test their tolerance to heat stress during the night ($33^{o}/22^{o}$ C, $33^{o}/30^{o}$ C day/night temperatures). Plants grown in high night temperatures ($33^{o}/30^{o}$ C) produced more flowers than the control ($33^{o}/22^{o}$ C) group. Of the three lines tested, two abscised all of their flowers within the first two days after anthesis, while the third line attained a 39% pod set. However, seeds produced by the third line were 20% smaller and 25% fewer than those that were produced in the control (optimal environment) group. The main factor responsible for differences in pod set was anther dehiscence. The two lines that had

vast flower abscission had poor anther dehiscence in the heat treatment as compared to their respective control groups. Viability of the pollen was analyzed by performing a stain with lactophenol-cotton blue. This test revealed that the two lines with vast flower abscission (California Blackeye No. 5 and Prima) produced pollen that was deformed and small, while the line that performed well in the heat treatment (TVu 4552) produced healthy, desirable pollen. A test to further implicate pollen performance was conducted by hand-pollinating plants subject to heat treatments with pollen from control groups, which resulted in increased pod set. This implies that the ovules were still viable at higher temperatures, and are more resistant to the effects of heat than anthers. A third experiment looked at high daytime temperature (36^{o} C) effect on pollen performance, and was similar to experiment two in all other aspects. This experiment resulted in reduced pollen performance during a photoperiod of 13 hr 20 min which further confirmed the data produced by experiment two. California Buckeye No. 5 and Prima had at least 30% lower pollen viability and about 50% less anther dehiscence than did TVu 4552.

Another cause of heat-induced sterility could be due to the stomium, which is near the partition between the anther thecae (pollen sacs). In cultivars whose pollen doesn't withstand heat well, the stomium never ruptures. This could be influenced by pollen grains which are smaller than normal, possibly due to low water content (Warrag and Hall, 1984). Female sterility can be ruled out by analyzing the esterase activity of the stigmas. According to their research, the most sensitive timeframe, in regards to heat stress during reproductive development, was nine to seven days before anthesis.

A possible explanation for reduced fertility during heat stress is the reduction of proline in pollen grains. Research performed by Hong-Qi and Croes (1982) examined proline activity in *Lilium longiflorum* (cv. Arai 5). They stated that when plants are stressed, levels of free proline

rise, possibly indicating a defense mechanism against abiotic stress. They found that proline accumulation deters germination in non-stressed pollen, but greatly aids germination in heat-stressed pollen. They found that proline also aids in the respiration of heat-stressed pollen grains. They were not sure what mechanism allowed proline to aid/sustain pollen during adverse conditions, but felt that it involved the osmotic potential of the pollen cells, inducing a quasi-dormant state. However, this response was non-specific to proline; they were able to duplicate the same results with sucrose.

Mutters et al. (1989) cited a lack of proline content in the pollen grains as one cause of heat-induced male sterility. They examined the proline content of leaves and reproductive tissues in cowpea and whether proline accumulation could account for heat sensitivity. They found that proline concentration in anthers was higher in heat sensitive cultivars than heat tolerant cultivars and that transport of proline from the anthers to pollen in cowpea occurs during the plants' most vulnerable reproductive time, six days before anthesis. They concluded that lower levels of proline in pollen grains reduce the level of fertility, due to possible dysfunction of the pollen. It was noted that a proline threshold exists in maize for determining fertility, but it was not known if a threshold existed for cowpea.

Pressman et al. (2002) studied the effects of heat stress in *Solanum lycopersicum* L. (garden tomato) on the metabolism of carbohydrates in the anther walls and pollen grains and found that the starch accumulation in anther walls is dynamic. An initial buildup of starch gave way before anthers were fully developed, and then disappeared. Starch concentration in pollen grains was also dynamic, and followed a wave pattern; starch levels, which were initially low, climaxed three days before anthesis and crashed immediately before anthesis occurred. Pressman et al. (2002) believed this change in starch concentrations, coupled with exposure to

heat stress, was responsible for reduced pollen viability and depressed pollen concentration. It was suggested that heat-induced stress causes a reduction in soluble sugars and starch accumulation in mature pollen grains. Wallwork et al. (1998) in barley found similar results, namely that heat stress reduced the formation of starch from sucrose in pollen grains.

Effect of Drought

Lack of adequate water can spell certain disaster for a crop. Desclaux and Roumet (1996) investigated the timing of drought stress on Maturity Group 1 soybean in southern France while comparing the responses of determinant and indeterminant cultivars. Drought stress was applied during the vegetative, flowering, pod lengthening, and pod filling stages. They observed that drought stress significantly shortened the duration of each stage of development. This lead to a reduced number of nodes, a shorter flowering period, and was especially demonstrated during pod maturation. This acceleration of development naturally resulted in smaller seed size due to a shortened pod filling period. The implication of having a shorter flowering period is that heat stress in likely present, and could also lower yields via production of deformed and/or sterile pollen. Interestingly, there was a difference in how growth habit interacts with drought stress. The determinant cultivar favored partitioning of assimilate to the branches, whereas the indeterminant cultivar partitioned 80% of assimilate to the main stem of the plant.

Kokubun et al. (2001) indirectly looked at how drought stress prior to anthesis interacts with formation of reproductive structures in soybean. Reduced levels of photosynthesis and assimilate production in leaves were observed during the stress period, but made a near complete recovery when watered after the allotted stress time. They theorized that photosynthesis and assimilate production are not the main cause of floral abortion during short-term drought stress. To test this idea, they hand pollinated flowers from well-watered pots with those from drought-

stressed pots. Pistils from well-watered plants achieved moderate fertilization rate (10%-60%) with pollen derived from drought-stressed plants, but pistils from drought-stressed plants did not achieve as much success when crossed with pollen from well-watered plants. They hypothesized that the difference in water potential of the pistil and pollen created an incompatibility that could not be overcome, or that the act of emasculation created water loss and stressed the water potential of the pistils even more.

CHAPTER 2 - MATERIALS AND METHODS

In the first year of the experiment (2006), ten entries (Group A) were selected from the 2006 Uniform Test III for evaluation. In 2007, 20 additional early Group III to mid-Group IV soybean lines (Group B) were selected for evaluation from the Kansas State University Soybean Breeding program (Table 2.1). Key differences between 2006 and 2007 included randomly assigning incubation temperatures for pollen sampling dates and the inability to sample all reps each day. Another notable difference between the 2006 and 2007 growing seasons was the timing of heat stress in the field. In 2006 average maximum temperatures ranged from 31^{o} C to 33.8^{o} C in the period of June – August. The same period in 2007 had average maximum temperatures ranging from 28^{o} C to 34.3 ^{o} C, with the majority of the heat stress occurring in August (Table 2.2).

Entries evaluated ranged from early Group III to mid-Group IV in maturity. Two locations were selected to perform the experiment; an irrigated field consisting of fine-silty, mixed, superactive, mesic, Cumuli Hapludolls (Kahola silt loam) and a dryland field consisting of coarse-silty, mixed, superactive, mesic, Fluventic Hapludolls (Eudora silt loam), both located near Manhattan, Kansas. A randomized complete block design was utilized, consisting of three replicates. On 5/24/2006 and 6/21/2006, entries were planted in four-row plots, 3.6 m long with a row spacing of 0.762 m, at a rate of six seeds per 0.3 m to a depth of 0.025 m in the irrigated, dry land plots, respectively. The plants were allowed to grow with minimal intervention, which consisted of manually removing weeds.

From beginning flowering, or R1 (Fehr et al., 1971), until beginning pod, or R3, 30 floral buds were randomly sampled from each plot between 0700 h and 0800 h. These flowers were placed in a Petri dish, which was then stored in a cooler containing a single layer of ice on the

bottom to prevent blooming of the flowers. The samples were then transported to the lab for pollen extraction, arriving no later than 0830 h. The Petri dishes were placed upon the bench top and allowed to acclimate for 30 min. Pollen extraction consisted of jointly removing the pistil and stamen structures from the flower with tweezers, and gently rapping the configuration against the edge of the bench top in such a manner as to allow the dislodged pollen to fall onto a glass microscope slide; the slide was then placed back into the corresponding Petri dish. Pollen extraction was completed by 01100 h. Each plot was sampled three times for pollen extraction at each incubation temperature.

After all of the samples had been emasculated, pollen was then dusted from the slide into a chamber cell culture slide using a 000 paintbrush. The samples were incubated for 30 min at either 28^{o}, 34^{o}, or 38^{o} C in batches of 10 entries and then removed to be photographed under 4x magnification with an Olympus DP70 digital camera mounted on top of an Olympus BX51 microscope. Plating and incubation were completed by 1300 h. The growth media consisted of 15 g sucrose ($C_{12}H_{22}0_{11}$), 0.03 g calcium nitrate [$Ca(NO_3)_2*4H_2O$], and 0.01 g boric acid (H_3BO_3) dissolved in 100 mL ddH_2O (Koti et al., 2004). This mixture was stirred and heated on a hot plate before adding 0.6 g agar. After the agar had completely dissolved into solution, the media was removed from heat and allowed to cool to 40^{o}C before pouring into the chamber cell culture slides in a laminar flow hood. After solidification, the chamber cell culture slides were then refrigerated until needed. Prior to use, the growth media was placed in an incubator set at that day's incubation temperature to equilibrate for pollen germination. At the conclusion of flowering, the digital photographs were analyzed to determine percent germination for each sample by counting germinated and un-germinated pollen grains. Pollen grains were considered germinated if the pollen tube was 1.5 x the diameter of the pollen grain it grew from.

In 2007 yield components were measured for each plot after hand-harvesting one meter of plant row from an outer row, bundling the plants, and allowing them to dry. The number of pods per plant were counted and then threshed with an Almaco belt thresher. The seed was then collected and cleaned over a screen to eliminate debris. Cleaned seed was then weighed for total seed weight and seed size was obtained by weighing 100 seeds. Average seeds per pod was calculated by dividing total seed weight by total pods per 1 m of plant-row. Plot yield was measured by harvesting the inner-two plot rows (3.6 m per row) with a Massey Ferguson XP combine.

Pod set was measured at the fifth node of six plants from the two inner rows of each plot. Consistent identification of the plants and fifth node was accomplished by placing a metal clip just below the node. Flower count notes were conducted by counting new floral buds at the fifth node, beginning at R1 and conducted every other day during the work week, or three days a week until R3. Attempts to permanently identify counted floral buds with physical demarcation of the bud were unsuccessful. Pods at the fifth node were counted after plants had reached R8. Pod set was calculated by dividing the total number of pods at the fifth node from the total number of floral buds observed at the fifth node.

Data Analysis

Pollen data were analyzed using the Mixed procedure in SAS 9.1.3. The model considered the entry x incubation temperature interaction as a fixed effect within each environment, and the plot and plot x entry interaction as random. Student's *t*-tests were used to test the null hypothesis of the Least Squares Means of each entry at each temperature for each environment. Differences of least squares means were used to differentiate comparisons of entry means of pollen germination rates at each temperature per environment ($P = 0.05$).

Agronomic data (seed size, yield and its components) were also analyzed using the Mixed procedure. The models for the agronomic data considered environment and entry as fixed effects. Student's *t*-tests were again used to test the null hypothesis of the Least Squares Means of each entry for each agronomic trait measured. Differences of least squares means were utilized to separate comparisons of entry means for the agronomic traits per environment. Proc Correlation was used to characterize the relationship between yield, its components, and pollen data.

Figures and Tables

Table 2.1 Soybean germplasm used to screen for heat-tolerant pollen.

Entry List		
Group A	Group B	
2006 - 2007	2007	
HS4-3143	IA3023	KS4103sp
K03-2897	K05-4602	KS4202
LD00-2817W	K05-4624	KS4302sp
LD00-3309	K06-6017	KS4303sp
LG03-3853	K06-6081	KS4402sp
LS03-4993	K06-6219	KS4602N
LS93-0375	K06-6325	KS4607
Macon	K06-6536	KS4694
MD03-5469	K06-6597	KS4702sp
MD03-5872	K06-6643	

Table 2.2 Weather during the 2006, 2007 growing season and 20 year climatic data for Manhattan, Kansas.

Year	Month	Average Air Temperature		Total Precipitation	Average Relative Humidity	Solar Radiation
		Maximum	Minimum			
		°C		mm	%	MJ/m2
2006	April	22.8	7.3	2.34	62.3	17.2
	May	25.2	11.9	2.36	64.2	17.9
	June	31.0	16.8	1.23	59.3	21.1
	July	33.8	20.6	3.04	60.3	19.8
	August	32.5	19.5	9.13	56.2	16.0
	September	24.2	10.6	1.74	68.4	14.7
	October	19.0	5.7	2.05	67.8	10.2
	November	13.8	-0.4	0.06	67.0	8.3
2007	April	17.7	4.3	2.61	64.4	13.2
	May	25.0	14.4	12.08	74.5	14.5
	June	28.4	18.2	3.08	73.6	17.5
	July	31.6	19.4	3.38	75.9	19.0
	August	34.3	21.8	1.77	73.3	17.4
	September	28.1	13.8	1.51	70.9	13.6
	October	22.2	8.2	3.24	69.4	11.6
	November	13.8	-2.0	0.04	62.7	8.6
Avg.†		24.7	11.8	2.76	65.9	16.9

†Twenty year average of climate for Manhattan, Kansas.

CHAPTER 3 - RESULTS AND DISCUSSION

Pollen Germination

GROUP A

In the analysis of the data, three main effects (entry, temperature, and environment) and four interaction (entry x temperature, entry x environment, temperature x environment, and entry x temperature x environment) sources of variation were significant (Table 3.1). Average pollen germination for soybean entries ranged from 25% (LD00-3309) - 38% (LS03-4993) across three incubation temperatures and four environments in Kansas during 2006 – 07 (Table 3.2). The average environment effect for pollen germination ranged from 29% (dryland, 2006) - 34% (irrigated, 2007) (Table 3.3), while the average incubation temperature effect on pollen germination ranged from 25% (38^{o} C) - 44% (28^{o} C) (Table 3.4). One possible explanation for the significant temperature x environment interaction could be the timing of heat stress during the growing season. In 2006, the average maximum air temperature during June and July were 31.0 ^{o}C and 33.8 ^{o}C, respectively; in 2007 the same period registered maximum air temperatures of 28.4 ^{o}C and 31.6 ^{o}C, respectively (Table 2.2). Overall, an increase in temperature decreased the pollen germination in entries, with no significant difference in germination between 34^{o} and 38^{o} C. As with Prasad et al. (2001), pollen germination at 38^{o} C are close to half that obtained at 28^{o} C. Average pollen germination of entries among incubation temperatures ranged from 18% (K03-2897, 38^{o}C) to 62% (HS4-3143, 28^{o}C) (Table 3.5). The highest consistent performer across incubation temperatures was MD03-5469, which had the second highest pollen germination rate among entries for both incubation temperatures 34^{o} and 38^{o} C. Entry HS4-3143 had the highest pollen germination for incubation temperature 28^{o} C, and was statistically the same as entry MD03-5469 at 38^{o} C. Interestingly, entries LD00-2817W and LD00-3309 each expressed pollen germinations that were essentially the same across temperatures and environments. These results generally agree with Abdul-Baki and Stommel (1995), in that exposure to heat stress significantly reduces the rate of pollen germination.

Considering the entry x environment interaction, average pollen germination for entries from the four environments ranged from 19% to 51% (Table 3.6). Of the four environments

sampled, three of them had statistically significant (Pr < 0.0314 or less) entry x temperature interactions, while the two-way interaction in the fourth environment (dryland, 2007) was not significant (Table 3.7). These entry x temperature responses across environments contributed to a significant entry x temperature x environment interaction. Significant differences among entries across temperatures and environments for pollen germination are noted in Table 3.8. Entry HS4-3143 was the best performer in the irrigated environment with an average pollen germination rate of 57% at 38^{o} C. In 2007, entry LG03-3853 was the top germinating entry in the dryland environment, and was also the highest germinating entry at 34^{o} and 38^{o} C in the irrigated environment. Some of the entries performed better in one environment than another. Entry MD03-5469 had the lowest pollen germination rates at all temperatures in the 2006 dryland environment, but was one of the top germinating entries at 28^{o} and 34^{o} C in the 2006 irrigated environment. In 2007 a similar trend happened with another entry, MD03-5872. It ranked the lowest in the dryland environment, but performance increased in the irrigated environment at 28^{o} and 34^{o} C.

GROUP B

Of the 20 entries added in 2007, only 19 were analyzed due to late flowering of entry K05-3457. Significant main effects for the entries added in 2007 include entry, environment, incubation temperature, and the entry x environment interaction (Table 3.9). Average pollen germination across environments and incubation temperatures for these entries ranged from 26% (KS4702sp) - 51% (KS4302sp) (Table 3.10). Environments were significantly different (Pr < 0.0007) and average soybean pollen germination across environments ranged from 33% (dryland, 2007) - 39% (irrigated, 2007) (Table 3.11). All incubation temperatures were significantly different (Pr < 0.0001) from one another; soybean pollen germination ranged from 20% (38^{o} C) - 55% (28^{o} C) across the incubation temperatures (Table 3.12). Group B did not have a three-way interaction, but did express an entry x environment interaction. Significant differences among entry x environment interactions for each of the two environments are noted in Table 3.13. Some entries performed better in one environment than the other. Entry KS4103sp was the best performer in the 2007 dryland environment, but was not significantly different from most of the poorer performing entries in the 2007 irrigated environment. The best

overall performance of an entry in both environments belongs to KS4302sp, which posted pollen germination which were not significantly different from the top entries in each category, having the highest pollen germination rate in the irrigated field (62%) and the second highest rate in the dryland field (48%).

Agronomic Measurements

Yield

In Group A, environment and entry effects were highly significant (<0.0001), as well as the environment x entry interaction (<0.0001) (Table 3.14). Average seed yield for entries across the four environments ranged from 2004 kg ha^{-1} (MD03-5872) to 2831 kg ha^{-1} (K03-2897) (Table 3.15). Mean yield for the environments ranged from 1950 kg ha^{-1} (dryland, 2006) – 2757 kg ha^{-1} (irrigated, 2007) (Table3.16). Table 3.17 delineates seed yield's environment x entry interaction, with LD00-2817W and MD03-5469 producing 1439 kg ha^{-1} (dryland, 2006) and 3510 kgha^{-1} (irrigated, 2007), respectively. Entry HS4-3143 produced the highest average seed yield in each environment, while MD03-5872 produced the lowest average seed yield in the same environments. Entry KS4303sp was one of several which produced more seed in one environment over the other. In this case, KS4303sp produced 770 kg ha-1 less in the dryland environment than it did in the irrigated environment.

In Group B, the main effects and the environment x entry interaction were again significant (Table 3.18). Average yield for entries across environments ranged from 1015 kg ha^{-1} (K06-6219) to 2878 kg ha^{-1} (KS4607) (Table 3.19). The dryland field (2007) produced a yield of 2334 kg ha^{-1} while the irrigated field produced 2482 kg ha^{-1} (Table 3.20). Entry K06-6219 produced the lowest yield in both the dryland (799 kg ha^{-1}) and irrigated (1237 kg ha^{-1}) fields, while K06-6597 was the high yielder in the irrigated environment (3143 kg ha^{-1}) and KS4607 was the high yielder in the dryland environment (3174 kg ha^{-1}) (Table 3.21).

Yield Components

In Group A, the number of pods produced at the fifth node was significantly affected by environment, entry, and the interaction between environment and entry (Table 3.22). The average number of pods at the fifth node was statistically similar for both environments of each

year (Table 3.23), and half of the entries produced statistically similar numbers of pods at the fifth node (Table 3.24). Rankings and comparisons of Group A entry performance are detailed in Table 3.25. Three entries, HS4-3143, LG03-3853, and Macon, managed to be among the top entries for pods at the fifth node in three of the four environments. Entry MD03-5872 managed to be the lowest producer in three of the four environments, and also one of the largest producers in the fourth environment (irrigated 2006).

Pod set had one main effect, environment, which was on the brink of significance ($Pr < 0.0507$) (Table 3.26). If it would be judged significant, the 2006 irrigated field would have a significantly higher pod set at the fifth node than the 2007 dryland and irrigated environments, and similar rate to the 2006 dryland environment (Table 3.27).

In Group B, the environment x entry interaction affected pod production at the fifth node (Table 3.28). Rankings and comparisons of Group B entries are detailed in Table 3.29. Entry IA3023 was in the top tier of pod production at the fifth node in both the irrigated and dryland fields. Again, there were some entries that performed better in one environment than the other. Entry KS4303sp illustrates this point well. In the irrigated environment it produced on average 3.6 pods at the fifth node, while in the dryland field it produced 0.9 pods at the fifth node. Three entries, K06-6219, KS4302sp, and KS4607, were seemingly unaffected by environment, producing the same results in both the irrigated and dryland fields.

A correlation analysis of pollen germination rate, yield components, and yield for entries (Group A) grown in either two or four Kansas environments was performed to characterize the relationships between the factors. The correlations tended to small and non-significant (Table 3.30). Only two correlations were statistically significant from 0. The correlation between pollen germination averaged over all temperatures was positively correlated with the pollen germination at 28^{o} C ($r = 0.70$). The correlation between the pollen germination at 34^{o} C was inversely correlated with pollen germination at 38^{o} C ($r = -0.53$).

Conclusions

Overall, trends in pollen germination for Groups A and B genotypes were similar: as incubation temperature increased, pollen germination decreased. These results agree with those obtained by Salem et al., (2007) in that pollen germination in soybean decreased as the incubation temperature increased past the optimal.

Significant differences in pollen germination were noted among entries in Group A and Group B at each of the three incubation temperatures. These results prove the first part of the hypothesis, which stated that some of the soybean genotypes would be more tolerant of heat stress than others. However, significant genotype by environment interactions were noted among both groups of entries for pollen germination. Among the Group A entries, the Entry*Temp*Env source of variation was also significant. These interactions were generally smaller than the genotype by environment interactions observed for seed yield. The reason for these large genotype by environment, and genotype by incubation temperature interactions for pollen germination are not clear. Pollen germination for all of the genotypes was not evaluated for all of the temperatures and in all environments in one sampling period. The sampling process to evaluate the pollen response to temperature was extended throughout the entire flowering period. For example, pollen that developed during a period of cool, wet weather may have responded differently to the treatments than pollen that developed during a hot, dry period. Perhaps differences in the micro-climate that occurred throughout this period contributed to these interactions.

This study was unable to confirm the second part of the hypothesis, which stated that higher levels of heat-tolerance would result in increased seed set. No differences were detected among the entries for pod-set at the fifth node. The modest sample size of the experimental unit to characterize this trait may have been insufficient to provide the necessary precision to detect differences among entries. Significant differences in seed yield were noted among the entries. The average number of pods at the fifth node differed among the entries, but, the entry by environment interaction was significant for pod number for both Group A and Group B entries. The contribution of the entry by environment and entry by treatment interactions for pollen germination and pod number, and the limited variability measure in pod set among the entries likely contributed to inability to detect significant correlations between pollen germination, pod number, pod-set and seed yield.

This experiment has shown that increasing incubation temperatures significantly decreases pollen germination in vitro. It has also shown that soybean genotypes differ in pollen germination and that an in vitro screen can be used to characterize these differences. Further studies are needed to establish the relationship between pollen germination, seed set and seed yield in soybean. Work also needs to be completed to determine the proper sample size to

adequately characterize differences in pollen germination so that performance differences among genotypes can be used as selection criteria in a plant breeding program.

Figures and Tables

Table 3.1 Combined analysis of variance for pollen germination of ten soybean lines (Group A) in 2006 – 2007 in Kansas.

Effect	df	F	Pr > F
Env†	3	3.04	0.0302
Temp‡	2	74.22	<0.0001
Entry	9	3.35	0.0136
Entry*Env	27	3.27	<0.0001
Entry*Temp	18	4.57	<0.0001
Temp*Env	6	14.49	<0.0001
Entry*Temp*Env	54	7.47	<0.0001

†Env = Environment

‡Temp = Incubation Temperature

Table 3.2 Pollen germination rates of ten soybean lines (Group A) across three incubation temperatures and four environments in Kansas in 2006 and 2007.

Entry	Pollen Germination
	%
LS03-4993	38a†
LG03-3853	37a
HS4-3143	36ab
MD03-5469	34abc
LS93-0375	31abc
MD03-5872	31a-c
MACON	29bcd
LD00-2817W	28cd
K03-2897	27cd
LD00-3309	25d

† means followed by the same letter are not significantly different from each other.

Table 3.3 Average soybean pollen germination rates at Ashland (irrigated) and Manhattan (dryland) Kansas across soybean lines (Group A) during the 2006 – 2007 growing seasons.

Environment	Year	Pollen Germination
Irrigated	2007	34a†
Dryland	2007	33ab
Irrigated	2006	29b
Dryland	2006	30b

† means followed by the same letter are not significantly different from each other

Table 3.4 Soybean pollen germination rates for three incubation temperatures averaged over ten soybean lines (Group A) and four environments in Kansas in 2006 and 2007.

Temp‡	Pollen Germination
°C	%
28	44a†
34	26b
38	25b

† means followed by the same letter are not significantly different from each other.
‡Temp = incubation temperature

Table 3.5 Pollen germination at three incubation temperatures of ten soybean lines (Group A) averaged over four environments evaluated in 2006 – 2007 in Kansas.

	Pollen Germination		
	Incubation Temperature		
Entry	28 °C	34° C	38° C
		%	
HS4-3143	62a†	18c	28ab
K03-2897	46bc	17c	18b
LD00-2817W	32de	19bc	34a
LD00-3309	24e	24bc	27ab
LG03-3853	52ab	37a	22ab
LS03-4993	60a	28abc	25ab
LS93-0375	40cd	26abc	26ab
MACON	36cd	29ab	22b
MD03-5469	42bcd	30ab	29ab
MD03-5872	46bc	27abc	18b

† means within a column followed by the same letter are not significantly different from each other

Table 3.6 Soybean pollen germination average across three temperatures for ten soybean lines (Group A) grown in dryland and irrigated environments during 2006 – 2007 in Kansas.

	Pollen Germination			
	2006		2007	
Entry	Dryland	Irrigated	Dryland	Irrigated
	%			
HS4-3143	30a-d†	38a	39a	36bcd
K03-2897	22cd	34ab	26bcd	25de
LD00-2817W	36abc	18c	38abc	21e
LD00-3309	23cd	19c	25cd	32cde
LG03-3853	44a	26bc	40a	38abc
LS03-4993	30bcd	36ab	39ab	46ab
LS93-0375	27bcd	22c	24d	51a
MACON	19d	20c	37abc	39abc
MD03-5469	28bcd	40a	33a-d	33b-e
MD03-5872	39ab	36ab	24d	23de

† means within a column followed by the same letter are not significantly different from each other

Table 3.7 Results from analyses of variance of pollen germination for the entry x incubation temperature interaction in regards to pollen germination of ten soybean lines (Group A) grown in four different environments in Kansas.

Environment	Effect	df	F	Pr > F
2006 Irrigated	entry*temp	9	3.12	0.0013
2006 Dryland	entry*temp	9	13.93	<0.0001
2007 Irrigated	entry*temp	9	2.09	0.0314
2007 Dryland	entry*temp	9	1.39	0.1937

Table 3.8 Average pollen germination for ten soybean entries (Group A) incubated at 28°, 34°, and 38° C from four different environments in Kansas in 2006 and 2007.

Pollen Germination

	2006						
	Dryland				Irrigated		
	Incubation Temperature				Incubation Temperature		
Entry	28° C	34° C	38° C	%	28° C	34° C	38° C
HS4-3143	87 (1)	4 (6)	0 (9)		29 (6)	10 (6)	76 (1)
K03-2897	50 (3)	16 (5)	0 (9)		50 (3)	6 (9)	47 (5)
LD00-2817W	27 (7)	0 (8)	80 (1)		11 (7)	27 (4)	15 (7)
LD00-3309	0 (10)	0 (8)	68 (2)		10 (8)	44 (2)	5 (10)
LG03-3853	49 (4)	56 (3)	26 (5)		43 (5)	21 (5)	15 (7)
LS03-4993	63 (2)	25 (4)	2 (8)		50 (3)	9 (7)	50 (3)
LS93-0375	31 (6)	4 (6)	45 (3)		10 (8)	34 (3)	22 (6)
MACON	18 (8)	0 (8)	40 (4)		2 (10)	47 (1)	11 (9)
MD03-5469	5 (9)	66 (1)	12 (6)		64 (1)	8 (8)	49 (4)
MD03-5872	48 (5)	66 (1)	5 (7)		56 (2)	2 (10)	52 (2)
LSD (0.05)	19	15	26		17	19	17
	2007						
	Dryland				Irrigated		
	Incubation Temperature				Incubation Temperature		
	Mean of 28°, 34°, and 38° C			%	28° C	34° C	38° C
HS4-3143	40 (1)				64 (3)	31 (4)	13 (8)
K03-2897	27 (7)				38 (9)	23 (6)	12 (9)
LD00-2817W	38 (4)				38 (9)	9 (10)	17 (5)
LD00-3309	25 (8)				54 (6)	25 (6)	17 (5)
LG03-3853	40 (1)				57 (5)	28 (5)	29 (2)
LS03-4993	39 (3)				74 (2)	36 (2)	29 (2)
LS93-0375	24 (9)				77 (1)	49 (1)	28 (4)
MACON	37 (5)				62 (4)	41 (2)	14 (7)
MD03-5469	34 (6)				44 (8)	15 (9)	41 (1)
MD03-5872	24 (9)				47 (7)	17 (8)	5 (10)
LSD (0.05)	NS				25	18	29

† means within a column followed by the same letter are not significantly different from each other.

‡ yield rank, within a column of entries listed.

Table 3.9 Combined analysis of variance for pollen germination of 19 soybean lines (Group B) in 2007 in Kansas.

Effect	df	F	Pr > F
Env	1	11.89	0.0007
Temp†	2	132.73	<0.0001
Entry	18	2.46	0.0012
Entry*Env‡	18	4.31	<0.0001
Entry*Temp	36	0.74	0.8589
Temp*Env	2	0.14	0.8674
Entry*Temp*Env	36	0.64	0.9468

†Temp = incubation temperature

‡Env = environment

Table 3.10 Average pollen germination rates of 19 soybean lines (Group B) across three incubation temperatures and two environments in Kansas in 2007.

Entry	Pollen Germination
	%
KS4302sp	52a†
KS4303sp	44ab
KS4103sp	44ab
K06-6219	41abc
KS4202	41abc
KS4694	42a-d
K05-4602	37b-e
K06-6081	37b-e
K06-6017	36b-e
IA3023	35b-e
K06-6597	34b-e
K06-6325	34b-e
KS4607	33cde
K05-4624	32bcd
KS4402sp	32cde
K06-6536	31de
K06-6643	40e
KS4602N	27e
KS4702sp	27e

†means followed by the same letter are not significantly different from each other

Table 3.11 Average soybean pollen germination at Ashland (irrigated) and Manhattan (dryland) Kansas across ten soybean lines (Group B) and three incubation temperatures during the 2007 growing season.

Environment	Year	Pollen Germination
		%
Irrigated	2007	39a†
Dryland	2007	33b

† means followed by the same letter are not significantly different from each other

Table 3.12 Soybean pollen germination for three incubation temperatures averaged over 19 soybean lines (Group B) and two environments in Kansas in 2007.

Temp‡	Pollen Germination
^{o}C	%
28	55a†
34	33b
38	20c

† means followed by the same letter are not significantly different from each other

‡ Temp = incubation temperature

Table 3.13 Soybean pollen germination rate averaged over three incubation temperatures for 19 soybean lines (Group B) grown in dryland and irrigated environments during 2007 in Kansas.

	Pollen Germination	
	2007	
Entry	Dryland	Irrigated
	%	
IA3023	34bc†	35c-f
K05-4602	29bc	44a-d
K05-4624	35abc	29d-g
K06-6017	29bc	42b-c
K06-6081	29bc	44a-d
K06-6219	32bc	50ab
K06-6325	30bc	37b-f
K06-6536	33bc	28efg
K06-6597	23c	45abc
K06-6643	34bc	25fg
KS4103sp	49a	39b-f
KS4202	25c	57a
KS4302sp	41ab	62a
KS4303sp	30bc	69a
KS4402sp	35abc	28fg
KS4602N	37abc	17g
KS4607	33bc	32c-g
KS4694	31bc	50a
KS4702sp	37abc	16g

† means followed by the same letter are not significantly different from each other.

Table 3.14 Analysis of variance of seed yield (Group A) for soybeans grown in four Kansas environments during 2006 - 2007.

Effect	df	F	Pr > |t|
Env†	3	45.08	<0.0001
Entry	9	9.35	<0.0001
Env*Entry	27	2.98	0.0001

†Env = environment

Table 3.15 Average seed yield for ten soybean entries (Group A) grown in four Kansas Environments during 2006 - 2007.

Entry	Yield
	kg ha^{-1}
K03-2897	2831a†
LD00-3309	2737ab
HS4-3143	2589ab
LS03-4993	2529b
MACON	2523bc
LG03-3853	2508bc
LD00-2817W	2306cd
LS93-0375	2293cd
MD03-5469	2104de
MD03-5872	2004e

† means followed by the same letter are not significantly different from each other

Table 3.16 Average seed yield across ten soybean entries (Group A) at four Kansas environments during 2006 – 2007.

Environment	Yield
	kg ha^{-1}
Dryland 2006	1950d†
Irrigated 2006	2622ab
Dryland 2007	2555bc
Irrigated 2007	2757a

† means followed by the same letter are not significantly different from each other

Table 3.17 Average seed yield for ten soybean entries (Group A) grown in four Kansas environments during 2006 – 2007.

	Yield			
	Irrigated		Dryland	
Entry	2006	2007	2006	2007
	kg ha^{-1}			
HS4-3143	2777ab†	2596bc	2354a	2623bc
K03-2897	3107a	3030ab	2293a	2885ab
LD00-2817W	2118c	2838b	1708bcd	2556bcd
LD00-3309	2650ab	3510a	2192ab	2589bc
LG03-3853	26033b	2650bc	1580cd	3194a
LS03-4993	2629ab	3026b	1896a-d	2569bc
LS93-0375	2771ab	2670bc	1957ab	1775e
MACON	2717ab	2616bc	2111ab	2656bc
MD03-5469	2744ab	1984d	1439d	2266cd
MD03-5872	2085c	2293cd	1574cd	2071de

† means, within a column, followed by the same letter are not significantly different from each other

Table 3.18 Analysis of variance of seed yield (Group B) for soybeans grown in four Kansas environments during 2006 - 2007.

Effect	df	F	Pr > |t|
Env	1	4.22	0.0447
Entry	18	4.51	<0.0001
Env*Entry	18	2.54	0.004

Table 3.19 Average seed yield for 19 entries (Group B) grown in four Kansas environments during 2006 - 2007.

Entries	Yield
	kg ha^{-1}
KS4607	2878a†
KS4702sp	2851ab
KS4402sp	2804abc
K05-4624	2798abc
KS4602	2697abc
K06-6325	2629abc
K06-6643	2616abc
K06-6597	2589abc
K06-6536	2482a-d
KS4303sp	2468a-d
IA3023	2441a-d
K05-4602	2441a-d
KS4302sp	2441a-d
KS4202	2401a-d
KS4103sp	2293bcd
K06-6017	2246cde
K06-6081	1991de
KS4694	1675e
K06-6219	1015f

† means followed by the same letter are not significantly different from each other

Table 3.20 Average seed yield across 19 soybean entries (Group B) for two Kansas environments during 2007.

Environment	Yield
	kg ha^{-1}
Irrigated 2007	2482a†
Dryland 2007	2334b

† means followed by the same letter are not significantly different from each other

Table 3.21 Average seed yield for 19 soybean entries (Group B) grown in two Kansas environments during 2007.

Entry	Yield	
	Irrigated	Dryland
	kg ha^{-1}	
IA3023	2224bc†	2666a-f
K05-4602	2480abc	2408b-f
K05-4624	2606abc	3020ab
K06-6017	2550abc	1952fgh
K06-6081	2374bc	1619gh
K06-6219	1237d	799i
K06-6325	2569abc	2689a-f
K06-6536	2745abc	2227c-g
K06-6597	3143a	2048fgh
K06-6643	2591abc	2644a-f
KS4103sp	2128bc	2469a-f
KS4202	2627abc	2175d-g
KS4302sp	2477abc	2406b-f
KS4303sp	2856ab	2088efg
KS4402sp	2682abc	2932abc
KS4602	2512abc	2893a-d
KS4607	2539abc	3174a
KS4694	2031c	1321hi
KS4702sp	2864ab	2843a-e

† means, within a column, followed by the same letter are not significantly different from each other

Table 3.22 Analysis of variance of pods at the fifth node for soybean entries (Group A) grown in four Kansas environments during 2007.

Effect	df	F	Pr > F
Env†	3	61.71	<0.0001
Entry	9	4.74	<0.0001
Env*Entry	27	3.91	<0.0001

†Env = environment

Table 3.23 Number of pods at the fifth node, averaged across ten soybean entries (Group A), for four Kansas environments during 2006 and 2007.

Environment	Pods at the 5th node
	no.
2006 Irrigated	5a†
2006 Dryland	4a
2007 Irrigated	2b
2007 Dryland	2b

† means followed by the same letter are not significantly different from each other.

Table 3.24 Number of pods per fifth node on ten soybean entries (Group A) averaged across four Kansas environments during 2006 and 2007.

Entry	Pods at the 5th node
	no.
HS4-3143	4.3a†
MACON	3.9abc
LG03-3853	3.7abc
LS03-4993	3.7abc
LD00-3309	3.5a-d
LD00-2817W	3.4bcd
LS93-0375	3.2b-e
MD03-5469	3.0cde
K03-2897	2.7de
MD03-5872	2.0e

† means followed by the same letter are not significantly different from each other.

Table 3.25 Average number of pods per fifth node on soybean entries (Group A) grown in four Kansas environments during 2006 and 2007.

Entry	Pods			
	Irrigated		Dryland	
	2006	2007	2006	2007
	no.			
HS4-3143	5.1abc†	2.8a	6.3a	3.1ab
K03-2897	2.8d	2.7a	4.0bc	1.5bc
LD00-2817W	4.8a-d	0.5b	5.2ab	3.0ab
LD00-3309	4.0bcd	1.7ab	4.7b	3.5a
LG03-3853	5.8ab	2.1a	5.5ab	1.5bc
LS03-4993	4.6a-d	2.7a	5.2ab	2.2abc
LS93-0375	3.0cd	3.0a	5.2ab	1.5bc
MACON	6.5a	2.3a	5.5ab	1.5bc
MD03-5469	5.3ab	2.3a	2.5c	1.9bc
MD03-5872	6.0ab	1.0b	0.3d	0.9c

† means, within a column, followed by the same letter are not significantly different from each other

3.26 Analysis of variance of pod-set at the fifth node for soybean entries (Group A) grown in four Kansas environments during 2006 and 2007.

Effect	df	F	Pr > F
Entry	9	1.65	0.1173
Env.†	3	3.57	0.0507
Entry*Env.	27	1.02	0.4579

†Env. = environment

3.27 Average rate of pod-set at the fifth node for soybean entries grown in four Kansas environments during 2006 and 2007.

Env.	Pod Set
	%
2006 irrigated	24a
2006 dryland	21ab
2007 irrigated	19b
2007 dryland	17b

Table 3.28 Analysis of variance of the number of pods at the fifth node for soybean entries (Group B) grown in two Kansas environments during 2007.

Effect	df	F	Pr > F
Env†	1	0.26	0.6135
Entry	17	1.37	0.1470
Env*Entry	17	5.12	<0.0001

†Env = environment

Table 3.29 Average number of pods per fifth node on soybean plants for entries (Group B) grown in four Kansas environments during 2007.

Entry	Pods	
	Irrigated	Dryland
	no.	
IA3023	2.9a†	3.6a
K05-4602	2.1ab	2.6a-d
K05-4624	1.7a-d	1.0de
K06-6017	5.8a	0.5e
K06-6081	2.2ab	1.5de
K06-6219	1.5bcd	2.5bcd
K06-6325	1.7a-d	1.6de
K06-6536	2.5ab	1.5de
K06-6597	3.6a	1.6cde
K06-6643	2.5a	1.7cde
KS4103sp	1.2bcd	3.3ab
KS4202	1.8a-d	2.9abc
KS4302sp	1.6bcd	2.5bcd
KS4303sp	3.6a	0.9e
KS4402sp	0.8d	3.0abc
KS4602	0.9cd	2.7abc
KS4607	1.2bcd	2.5bcd
KS4702sp	1.8abc	2.3bcd

† means, within a column, followed by the same letter are not significantly different from each other

Table 3.30 Correlation coefficients between pollen germination rate, yield component factors, and yield for entries (Group A) grown in four Kansas environments (n=40) or two Kansas environments (n=20).

	ta	t1	t2	t3
		n=40		
t1	0.70			
	(0.01)			
t2	0.28	0.01		
	(0.08)	(0.95)		
t3	0.27	-0.19	-0.53	
	(0.002)	(0.000)	(0.01)	
y	0.08	0.24	-0.11	-0.06
	(0.74)	(0.14)	(0.50)	(0.71)
		n=20		
p1	0.02	-0.15	-0.02	0.32
	(0.93)	(0.53)	(0.95)	(0.17)
p2	0.22	0.12	0.17	0.28
	(0.35)	(0.61)	(0.46)	(0.24)
p3	0.02	-0.09	0.24	-0.10
	(0.93)	(0.70)	(0.30)	(0.67)
s	0.13	0.11	0.00	0.20
	(0.58)	(0.63)	(0.98)	(0.39)
f	-0.02	-0.05	0.09	0.28
	(0.93)	(0.83)	(0.69)	(0.744)

ta	=	*average of the three germ temps*
t1	=	*mean pollen germ @ 28 C*
t2	=	*mean pollen germ @34 C*
t3	=	*mean pollen germ @ 38 C*
f	=	*average pod set at 5th node*
p1	=	*mean number pods/plants*
p2	=	*mean number pods/plot*
p3	=	*mean number pods/5th node*
y	=	*mean seed yield*

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