USE OF SAMPLING TIME AND TYPE OF ACCLIMATION IN THE ELECTRICAL CONDUCTIVITY ASSAY FOR HEAT TOLERANCE IN BEAN CULTIVARS

by

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LITERATURE REVIEW

Dry beans, Phaseolus vulgaris L., are a major horticultural crop in Kansas. An important problem with this crop, however, is the reduced yield that comes with high summer temperatures, those over 30 C. Ormrod (13) found that the lack of pod set at 35/26.5 C day/night temperatures could be attributed to degeneration of the embryo sac contents. Although at two lower temperature regimes, 29.5/21 C and 24/15.5 C day/night, the embryo sacs appeared functional, a significant percent failed to develop and the exact cause of this was unknown. Halterlein et al. (6) showed that reduced yield at high temperatures was not due to lack of viable pollen and suggested that "injury to pollen at high temperatures up to 35 C is not likely to hinder the ability of beans to set pods." Likewise, Weaver et al. (16) found that pollen of heat tolerant bean plants was at least 50% viable at 41 C, as determined by a phyloxin-methyl green dye. However, they did not determine whether or not the pollen grains could produce pollen tubes and fertilize the egg cell.

Heyne and Laude (7), while looking at combined heat and drought tolerance in corn, calculated the percent injured tissue and the percent dead plants. They found that young plants were most heat tolerant. At a certain point, which seemed to correlate with the stage when the endosperm no longer contributed to the plant, heat tolerance declined. After that point, no real differences were found. They also examined heat and drought tolerance during dark and light periods and found that light increased the heat tolerance, possibly due to a buildup of photosynthates.

Yarwood (17) introduced the idea of acquired heat tolerance by reporting that ten-day-old seedlings could be acclimated by dipping leaves into hot water for a brief period of time. Leaves so treated were less injured than untreated leaves by subsequent heat stress temperature treatment.

Ng and Bouwkamp (12) attempted to rank different cultivars in the field on the basis of visible signs of damage, such as the stage at which the flower or immature pod abscised. Benepal and Rangappa (1) counted the number of pods set under high temperature as compared to that set under a control temperature. Cultivars with the smallest differences were judged to be heat tolerant.

While differences in heat tolerance are known to exist among cultivars, determining these differences by field trials is not practical, due to the difficulty of separating the stresses due to heat, drought, and insects. Also, field trials take several months to complete. Laboratory tests that reduce the environmental variables of the field and decrease the time involved would be preferable.

Several such laboratory procedures have been developed. One test utilizes the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC). If the tissue turns a charateristic red color, then the sample is viable (15).

A second laboratory test was performed by Weaver et al. (16). When phyloxin-methyl green dye was applied to pollen from fully-opened flowers or from buds in the late white-petal stage of heat-stressed bean plants, aborted pollen grains could be distinguished from potentially functional pollen grains. Sibling pairs of bean lines in which one of the pair was heat tolerant and the other heat intolerant always showed greater pollen abortion by the heat intolerant sib. While this test may serve to identify differences in heat tolerance, beans will set pods containing only one seed, and this requires only one pollen grain.

Consequently, heat intolerant selections identified by such a screen may still be as capable of setting pods under field conditions as their heat tolerant sibs.

A third laboratory procedure that has been developed is the electrical conductivity test. Dexter et al. (5) studied the winter hardiness of alfalfa and other small grains. Electrical conductivity was used in two ways. In one experiment the leakage of electrolytes into distilled water by stressed pieces of alfalfa roots was measured by testing the electrical conductivity of the solution. The other method involved measuring changes in the resistance of the tissue itself, before and after freezing injury. The first method provided early researchers with a fairly reliable means of determining hardiness, while the second one gave a wide range of values, even within individual plants.

Kinbacher et al. (9) refined Dexter's procedure by cutting discs from the leaves, washing the discs, placing them in test tubes subjected to a range of temperatures in a water bath for appropriate times, and then testing the leachate for conductivity. A similar technique was used by Bouslama and Schapaugh (3) to evaluate soybeans for stress tolerance and Blum and Ebercon (2) to evaluate wheat for drought and heat tolerance. Other workers (4, 11) have heat-treated washed, whole leaves and then cut discs for testing by electrical conductivity. This technique can result in leachate from the cutting process itself being combined with that from heat damaged cells.

Ingram (8) modified the method described by Kinbacher et al. (9) to compare the relative heat tolerance of rootstalks. After root tissue segments were subjected to a range of temperatures for a range of times, they compared temperatures corresponding to the midpoint (50% electrolyte loss), as determined by a fitted sigmoidal curve. The model, as developed by Ingram (8), is similar to one used by Schaff (14) to fit data from heat-stressed bean leaf discs.

Li and Davis (10) compared non-stressed (20/15 C day/night) bean cultivars of heat-tolerant and heat-susceptible cultivars and found no differences. The same comparison done under stress levels (35 C) showed differences between heat-tolerant and heat-susceptible cultivars. They concluded that "the tolerant beans were able to more rapidly adapt to the high temperature condition than the sensitive one."

Chen et al. (4) compared TTC and electrical conductivity techniques in tests of one heat tolerant and one heat susceptible cultivar each of bean, tomato, soybean, and potato. They compared the heat-killing times with the heat-killing temperatures and found that heat-killing times were more precise indicators of relative heat tolerance.

Schaff (14) also compared the TTC and electrical conductivity techniques and found that they were not significantly different, but the electrical conductivity method correlated more closely with the results of field trials conducted under heat stress. In his study Schaff (14) attempted to separate 26 cultivars of common bean based on the heat-killing temperature. The range of estimates of killing temperatures was only 4.5 C.

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MANUSCRIPT

Use of sampling time and type of acclimation in the electrical conductivity assay for heat tolerance in bean cultivars.[1]

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Abstract: Eight cultivars of beans, *Phaseolus vulgaris* L., previously identified as differing in heat tolerance, were evaluated in the laboratory by the electrical conductivity test using time as the variable. Two different acclimation treatments were also compared: 24 hours at a constant 37.5 C and a two-hour stress at 45 C for each of four consecutive days. The lethal time, the time causing 50% electrolyte leakage, was estimated both by fitting the data to a sigmoidal model and by linear interpolation. After the killing times were estimated, weighted and unweighted analyses of variance and a corresponding LSD procedure were used to compare cultivars and tests. The cultivars differed greatly in their responses. While significant differences among them were not observed with the four-day acclimation, this was achieved with the 24-hour acclimation treatment.

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[1] Received for publication . . . . .

[2] Graduate student and Professor.

Introduction

Dry beans, Phaseolus vulgaris L., are a major horticultural crop in Kansas. An important problem with this crop, however, is the reduced yield that comes with high summer temperatures, those over 30 C. While differences in heat tolerance are known to exist among cultivars (7), determining these differences by field trials is not practical, since field trials take several months to complete. Laboratory tests that reduce the environmental variables of the field and decrease the time involved would be preferable.

Greenhouse tests (1) and laboratory tests (4, 6) have been used to determine heat tolerance in bean cultivars. Schaff (7), using temperature as the variable compared the 2,3,5-triphenyl tetrazolium chloride reduction (TTC) and electrical conductivity tests and found that cultivars performed similarly in both tests, although results for the electrical conductivity method correlated more closely with field trial performance under severe heat stress. Chen et al. (2) found that heat killing times were more precise indicators of relative heat tolerance than heat killing temperatures and also observed that the TTC and electrical conductivity tests gave similar results.

Two different acclimation treatments have been used to harden bean plants prior to evaluation for heat tolerance by electrical conductivity. Kinbacher et al. (3) used a brief heat stress period on each of four consecutive days to approximate field conditions, while Marsh (5) used a continuous 24-hour stress.

Since shorter acclimation times facilitate more rapid testing, the present study was designed to compare the relative effectiveness of the 24-hour and four-day acclimation treatments and to use killing times to distinguish among previously evaluated, putative heat-tolerant cultivars, since prior testing of them was only by killing temperature (7).

Materials and Methods

Plant material. Eight cultivars, PI 271998, PI 324607, Oregon 1604, UI 114, ND 364, Wyoming 166, Valley, and 5BP7 were used. Five cultivars have previously been identified as being heat tolerant (PI 271998, PI 324607, UI 114, and 5BP7) or heat susceptible (Oregon 1604). The remainder were cultivars that were tested in the 1980 dry bean yield trials at Manhattan, KS. Other than 5BP7, all of the cultivars were obtained from D. Schaff, Kansas State University, Manhattan. 5BP7 was obtained from L. Marsh and D. Davis, University of Minnesota, St. Paul.

Seeds were planted into 7.6-liter pots containing a soil mix consisting of soil : peat : perlite : vermiculite (3:3:2:1 v/v). Plants were maintained in a greenhouse for six to eight weeks, free of water stress, at temperatures of 21 to 26 C. Plants were acclimated at half-bloom stage.

Acclimation treatments. Two different acclimation treatments, in addition to an unacclimated control, were tested:

1) Four-day treatment: Plants were transferred to a growth chamber set for a 16 hour photoperiod, 900 $\mu E\ sec^{-1}\ m^{-2}$, and 25/20 C

day/night temperature, with a two-hour period of 45 C for four days (7).

2) Twenty-four-hour treatment: Plants were transferred to a growth chamber set for a 14-hour photoperiod, 900 $\mu E \ sec^{-1} \ m^{-2}$, and 37.5 C temperature, for 24 hours (5).

Viability tests. Control and heat acclimated leaf samples were evaluated for heat tolerance using the electrical conductivity method of Kinbacher et al. (3) as modified by Schaff (7). Discs 1 cm in diameter were punched from fully expanded young leaves with a cork borer, washed in double-distilled deionized water for one hour with three water changes, and then placed in test tubes, each receiving five discs and 1 ml of double-distilled deionized water. Test tubes containing leaf discs from plants hardened for four days were placed in a water bath maintained at 48 C, while those containing discs from plants hardened for 24 hours were treated at 47 C. Preliminary experiments with the 24-hour acclimation treatment indicated that 48 C was slightly too high for discrimination among cultivars. Tests consisted of five leaf discs per test tube, three test tubes per time period, including an untreated control of three test tubes per cultivar, eight cultivars per test, and three or four replications of each test/acclimation treatment.

The first three replications with the 24-hour acclimation treatment were done in split runs of four plants each, selected at random, and sampled at 15 minute intervals from 0 to 180 minutes. The analysis of these results and a separate experiment led us to realize that testing all eight cultivars at the same time was more important than sampling so often, and subsequent replications were conducted testing all eight cultivars together at 30-minute intervals over the same time span.

After removal from the water bath, 20 ml of double-distilled deionized water were added to each test tube, and the tubes were stored for 24 hours at room temperature. The conductance reading (C1) was then measured at 25 C with a YSI model 32 conductivity meter (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio). The leaf discs were killed by placing the test tubes in boiling water for 15 minutes. After holding the tubes for 24 hours at room temperature to equilibrate, a second conductance reading (C2) was taken at 25 C. Relative leakage was estimated by the equation:

$$\text{Relative Leakage} = 1 - [1 - (C1 / C2) / 1 - (C1c / C2c)],$$

where C1 is the first conductivity reading and C2 is the conductivity reading of the same test tube after boiling. C1c and C2c are the first and second readings for the control group (7).

Data analysis. The lethal time, Time50, is calculated by finding the time at which half of the cell solutes have leaked out of the cells. Nonlinear estimations of average killing time for each cultivar were made, according to the procedure of Schaff (7), by fitting the data to a sigmoidal curve with the following equation:

$$\text{Viability} = 1 / (1 + e^{-B(\text{Time} - \text{Time50})}) + \epsilon ,$$

where B is a rate parameter, not the coefficient of variance, and ϵ represents the deviation of the observations about the nonlinear regression model. The lethal time, Time50, is calculated by finding the time at which half of the cell solutes have leaked out of the cells. Time50 was also calculated by linear interpolation, using the two data points to either side of the 50% relative leakage level for comparative purposes with the nonlinear estimation.

After the killing times were estimated, a weighted and unweighted analyses of variance and corresponding LSD procedure were used to compare cultivars and treatments.

Results and Discussion

In the four-day acclimation treatment some cultivars showed great variability among runs in heat killing times: 5BP7 and UI 114 ranged from most to least heat tolerant (Table 1). Other cultivars, like Valley and ND 364, were relatively consistent in their performance. Similar variability among runs was observed for the 24-hour acclimation treatment (Table 2), for which Valley and ND 364 were more variable. The split runs of Table 2 are explained in the previous section.

Althought variability within runs was fairly low, as indicated by the standard errors, the great variation in cultivar values among runs necessitated use of mean killing times (Table 3). While significant differences among them were not observed with the four-day acclimation, this was achieved with the 24-hour acclimation period. Differences in cultivar ranking between the two acclimation treatments are not meaningful due to the lack of signficance in the four-day acclimation. Schaff (7) was able to obtain significant cultivar differences in killing temperatures with the same four-day acclimation treatment, but it should be noted that, due to the large number of cultivars tested

(26), he was unable to test all of them at one time.

Appreciable changes in relative cultivar rankings in successive tests were responsible for the lack of signficance, when tests were averaged, for our four-day acclimation treatment and also caused the relative lack of significant differences among cultivars in the 24-hour acclimation treatment, despite the substantial differences in average killing times (Table 3). Because of this problem, every effort was made to minimize variation between tests by performing them the same way each time. But certain variables were unavoidable. Some of the cultivars tested were bush types and others of vining habit, which caused differences in floral initiation, as did differences in photoperiodic response.

Killing times were calculated by the sigmoidal curve fitting procedure of Schaff (7) as well as by linear interpolation, in order to compare how effectively the two methods permitted distinctions to be made among cultivars. Schaff's procedure utilizes all of the data points obtained and weights them equally, while the latter method uses only the two data values closest to the estimated killing time. Only slight, nonsignificant differences were observed between the two methods for the 24-hour acclimation treatment, so that linear interpolation was as effective in separating cultivars as the nonlinear model. Consequently, it would appear that at least for evaluation of cultivars by killing time, either method could be used. Figure 1 illustrates data points and the calculated curves for estimation of Time50 for one cultivar.

Seven of the eight cultivars we tested were also evaluated by Schaff (7). If we compare their performance in his tests with ours, only one cultivar performed appreciably differently in the two experiments: Wyoming 166. Wyoming 166 was the most tolerant cultivar in our tests (Table 3), while it was one of the most intolerant in Schaff's results. However, when he subsequently retested six of his cultivars in a diallel crossing series (7), Wyoming 166 was second in heat tolerance, only being surpassed by PI 271998, suggesting, together with our results, that Wyoming 166 is more heat tolerant than his first test indicated.

Using a 24-hour acclimation treatment of detached leaflets at 37 C, Marsh (5) tested 17 bean cultivars for heat tolerance by the electrical conductivity method. Fifteen of these were putatively heat tolerant and two intolerant. Her two cultivars with the longest killing times were also tested by us: PI 271998 (108.6 min.) and 5BP7 (98.6 min.). In our tests these two cultivars were not significantly different, as she also observed, although 5BP7 was significantly lower than Wyoming 166.

It is clear from the results of our tests, as well as the others reported here, that the electrical conductivity test needs further modification it if is to give repeatable results for use in large-scale testing to identify heat tolerant cultivars or selections consistently in a breeding program. Our results have verified the tolerance of previously identified heat tolerant cultivars, but the degree to which these cultivars really differ in heat tolerance, if at all, as measured by electrical conductivity must await greater refinement of this test.

Figure 1. Estimation of killing time (Time50) for PI 324067 by linear interpolation and by fitting to sigmoidal equation.

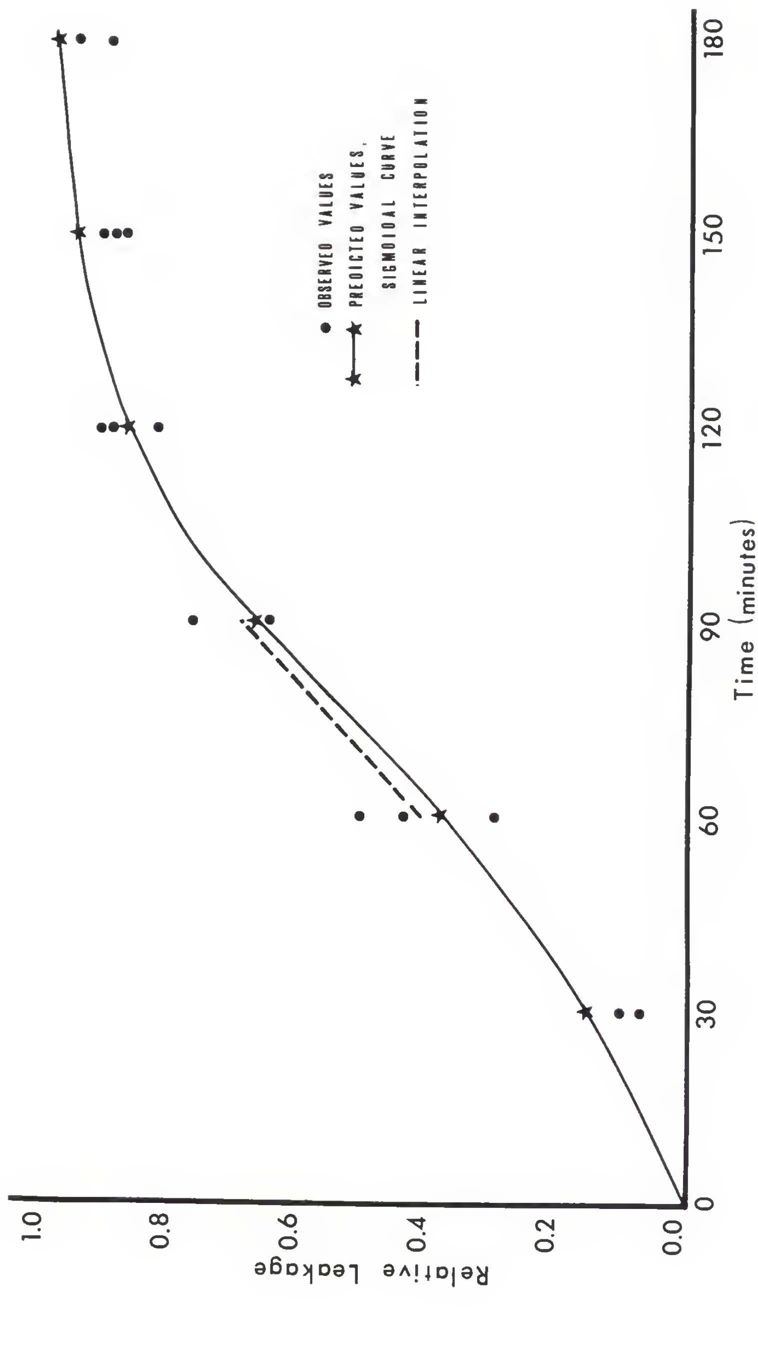
Relative Leakage
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
120
150
180
Time (minutes)
OBSERVED VALUES
PREDICTED VALUES, SIGMOIDAL CURVE
LINEAR INTERPOLATION

Table 1. Four-day acclimation treatment: killing times and standard errors (in minutes) for each run.

Cultivar	Run 1	Run 2	Run 3
Nonlinear model			
Wyoming 166	85.8 ± 5.6	66.2 ± 4.3	87.4 ± 25.5
ND 364	111.2 ± 10.9	106.5 ± 4.0	120.0 ± 3.7
UI 114	39.0 ± 7.1	94.8 ± 8.7	167.9 ± 18.0
Valley	92.7 ± 5.4	81.8 ± 4.0	95.3 ± 9.5
PI 271998	69.4 ± 3.8	76.6 ± 6.0	110.2 ± 6.8
PI 324067	117.8 ± 11.3	87.1 ± 6.2	74.2 ± 5.7
5BP7	157.3 ± 6.7	59.5 ± 7.6	122.0 ± 6.0
Oregon 1604	80.0 ± 9.4	132.6 ± 6.7	123.1 ± 5.3
Linear interpolation			
Wyoming 166	81.9	66.8	122.8
ND 364	85.7	99.5	120.0
UI 114	38.0	90.0	168.8
Valley	93.0	75.0	98.0
PI 271998	66.0	68.3	102.0
PI 324067	91.7	78.2	82.0
5BP7	162.0	58.7	122.4
Oregon 1604	70.4	126.6	113.5

Table 2. Twenty-four-hour acclimation treatment: killing times and standard errors (in minutes) for each run.

Cultivar	Run 1	Run 2	Run 3	Run 4
Nonlinear model				
Wyoming 166	110.7 ± 3.7(2)[z]	153.7 ± 7.6(2)	127.5 ± 5.1(2)	101.5 ± 5.8(1)
ND 364	108.9 ± 4.3(1)	159.5 ± 9.4(2)	40.0 ± 2.4(1)	107.3 ± 12.6(1)
UI 114	116.5 ± 6.3(1)	91.5 ± 8.1(1)	53.9 ± 3.8(1)	131.3 ± 10.3(1)
Valley	53.7 ± 3.7(2)	144.1 ± 7.2(1)	61.8 ± 1.9(1)	68.1 ± 8.1(1)
PI 271998	53.8 ± 3.9(2)	90.3 ± 6.9(1)	123.2 ± 6.4(2)	30.6 ± 6.8(1)
PI 324067	105.4 ± 9.4(2)	71.0 ± 2.6(1)	54.3 ± 2.5(2)	46.5 ± 4.6(1)
5BP7	85.4 ± 4.0(1)	60.6 ± 4.2(2)	47.4 ± 2.6(1)	52.9 ± 7.3(1)
Oregon 1604	93.1 ± 4.1(1)	75.5 ± 3.3(2)	94.2 ± 7.3(2)	0.0 ± 14.8(1)
Linear interpolation				
Wyoming 166	114.2(2)	156.0(2)	120.0(2)	104.3(1)
ND 364	110.4(1)	168.8(2)	39.9(1)	112.5(1)
UI 114	122.0(1)	100.9(1)	52.5(1)	133.1(1)
Valley	41.2(2)	151.7(1)	58.6(1)	77.3(1)
PI 271998	58.3(2)	82.5(1)	113.8(2)	31.0(1)
PI 324067	87.9(2)	70.0(1)	43.5(2)	45.3(1)
5BP7	95.4(1)	53.1(2)	42.9(1)	45.5(1)
Oregon 1604	73.0(1)	75.5(2)	55.0(2)	21.7(1)

[z] Numbers in parentheses refer to the first or second group of the split runs.

Table 3. Unweighted estimates of mean killing times (in minutes) for high temperature tolerance in common beans receiving different acclimation treatments.

Cultivar	4-day acclimation	24-hour acclimation
Nonlinear model		
Wyoming 166	79.80 a[z]	123.35 a
ND 364	112.57 a	103.92 ab
UI 114	100.57 a	98.30 ab
Valley	89.93 a	81.92 ab
PI 271998	85.40 a	74.47 ab
PI 324067	93.03 a	69.22 b
5BP7	112.93 a	65.70 b
Oregon 1604	111.90 a	61.57 b
Linear interpolation		
Wyoming 166	90.50 a	123.62 a
ND 364	101.76 a	107.90 ab
UI 114	98.93 a	102.12 abc
Valley	88.67 a	82.20 abc
PI 271998	78.77 a	71.40 bc
PI 324067	83.97 a	61.67 bc
5BP7	114.37 a	59.22 c
Oregon 1604	103.50 a	56.30 c

[z]separation in columns by LSD, 5% level

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APPENDICES

LIST OF APPENDICES

Appendix A. Computer Model in SAS

```
DATA ALL;
INPUT DATE 3-8 RUN 13 ACC 18 TEMP 21-13 1 CULT 27-28 PLANT
31-33 TIME 36-38 A1 40-13 1 A2 45-48 1 A3 50-53 1 A4 55-58 1 A5
60-63 1 A6 65-68 1;
REP=1; R = 1 - (A1/A4); OUTPUT;
REP=2; R = 1 - (A2/A5); OUTPUT;
REP=3; R = 1 - (A3/A6); OUTPUT;
CARDS;
DATA ONE; SET ALL;
PROC SORT; BY CULT ACC PLANT;
DATA TWO; SET ONE;
IF TIME > 0 THEN DELETE;
PROC MEANS NOPRINT; BY CULT ACC PLANT; VAR R;
OUTPUT OUT = NEW MEAN = RC;
DATA THREE; SET NEW;
PROC SORT; BY CULT ACC PLANT;
DATA TEST1; MERGE ONE THREE; BY CULT ACC PLANT;
IF TIME = 0 THEN DELETE;
READ = 1 - (R/RC);
PROC SORT; BY CULT ACC PLANT;
PROC PRINT;
PROC NLIN; BY CULT ACC PLANT;
PARMS B=.01 TO .1 BY .02 U = 20 TO 160 BY 20;
BOUNDS U >0;
L = EXP(-B*(TIME-U));
MODEL READ = 1/(1+L);
DER. B=(TIME-U)*L/(1+L)**2;
DER. U=-L*B/(1+L)**2;
OUTPUT OUT=NEWA P=PREAD PARMS=BP UP ESS=SSRESP;
PROC PLOT; BY CULT ACC;
PLOT PREAD*TIME='#' READ*TIME='@'/0;
PROC PRINT
```

Appendix B. An Example of Linear Interpolation

The computer print out would read:

15 minutes	.46
?	.50
30 minutes	.61

30 minutes - 15 minutes = 15 minutes

.61 - .46 = .15 difference between 15 and 30 minutes
.61 - .50 = .11 difference between 30 minutes and the killing point

.11/.15 = 73.3%; 73.3% x 15 minutes = 10.99 minutes

30 minutes - 10.99 minutes = 19.01 minutes

19.01 is the estimated killing time by linear interpolation.

Appendix C. Weighted estimates of mean killing times (in minutes) for high temperature tolerance in common beans receiving different acclimation treatments.

Cultivar	4-day acclimation	24-hour acclimation
<u>Nonlinear model</u>		
Wyoming 166	80.28 a[z]	119.34 a
ND 364	112.66 a	107.01 ab
UI 114	87.75 a	102.79 ab
Valley	89.47 a	97.17 ab
PI 271998	85.96 a	72.11 b
PI 324067	94.23 a	66.17 b
5BP7	107.25 a	71.41 b
Oregon 1604	110.51 a	64.45 b

[z]separation in columns by LSD, 5% level

Appendix D. Four-day acclimation treatment: the following data, derived from the non-linear model, was used to calculate the weighted estimates (Appendix C).

Cultivar	Run	Time50	STDER	MSRES
1	1	69.4	3.8397	0.01108
1	2	76.6	5.9703	0.01058
1	3	110.2	6.8499	0.01554
2	1	117.8	11.3082	0.02307
2	2	87.1	6.1660	0.01492
2	3	74.2	5.7333	0.00941
3	1	80.0	9.3755	0.01880
3	2	132.6	6.7106	0.00986
3	3	123.1	5.3332	0.00697
4	1	39.0	7.1481	0.02342
4	2	94.8	8.6798	0.01219
4	3	167.9	18.0061	0.01481
5	1	111.2	10.8955	0.02146
5	2	106.5	4.0444	0.00615
5	3	120.0	3.7010	0.00611
6	1	85.8	5.5802	0.01351
6	2	66.2	4.3160	0.00432
6	3	87.4	25.5463	0.06761
7	1	92.7	5.3527	0.01361
7	2	81.8	3.9889	0.00521
7	3	95.3	9.4997	0.00521
8	1	157.3	6.7486	0.00639
8	2	59.5	7.5672	0.01488
8	3	122.0	5.9732	0.00993

Appendix E. Twenty-four-hour acclimation treatment: the following data, derived from the non-linear model, was used to calculate the weighted estimates (Appendix C).

Cultivar	Run	Time50	STDER	MSRES
1	1	53.8	3.9141	0.00571
1	2	90.3	6.8871	0.01217
1	3	123.2	6.3551	0.01568
1	4	30.6	6.7782	0.01801
2	1	105.4	9.4192	0.02789
2	2	71.0	2.5965	0.00444
2	3	54.3	2.5025	0.00916
2	4	46.5	4.6055	0.01614
3	1	93.1	4.0699	0.00634
3	2	75.5	3.3167	0.00655
3	3	94.2	7.2533	0.03382
3	4	0.0	14.8385	0.00650
4	1	116.5	6.3027	0.01162
4	2	91.5	8.1057	0.01788
4	3	53.9	3.8400	0.01784
4	4	131.3	10.2945	0.01206
5	1	108.9	4.3109	0.00885
5	2	159.5	9.4071	0.01161
5	3	40.0	2.3850	0.01343
5	4	107.3	12.6417	0.04116
6	1	110.7	3.7452	0.00945
6	2	153.7	7.6149	0.01395
6	3	127.5	5.0748	0.01165
6	4	101.5	5.8412	0.01141
7	1	53.7	3.6527	0.00780
7	2	144.1	7.2100	0.02188
7	3	61.8	1.9018	0.00589
7	4	68.1	8.0584	0.01357
8	1	85.4	4.0472	0.00792
8	2	60.6	4.1592	0.00978
8	3	47.4	2.5633	0.01302
8	4	52.9	7.2643	0.01986

Appendix F. Killing time (in minutes) of two cultivars sampled 24 hours hours apart (24-hour acclimation treatment).

Cultivar	Day 1	Day 2	Cultivar Mean
PI 320467	166.8	61.3	121.8 a[z]
	153.8	108.8	
	135.9	91.6	
	161.9	95.0	
5BP7	114.5	53.9	90.6 b
	123.0	65.8	
	145.4	71.1	
	81.5	64.7	
Mean by days	135.3 c[z]	76.5 d	

[z]LSD (0.05) = 19.39 in colums and rows

Appendix G. A comparison of 1980 yield trials at Manhattan, KS, 4-day acclimation treatment (in minutes), and 24-hour acclimation treatment (in minutes).

Cultivar	yield[z] kg/ha	4-day acclimation	24-hour acclimation
ND 364	2303 a[y]	122.57 a	104.37 ab
Wyoming 166	1531 b	79.80 a	122.85 a
UI 114	1311 bc	100.57 a	99.65 ab
Valley	344 de	111.90 a	54.30 b

[z]Schaff, D. 1984. Screening and inheritance of heat tolerance in common bean (*Phaseolus vulgaris* L.). Ph.D. Thesis, Kansas State University, Manhattan, Kansas.

[y]separation in columns by LSD test at 5% level

Appendix H. Comparison of variability between runs within treatment.

Acclimation Treatment	Run	killing time (in minutes)
4-day[z]	1	94.15 a[y]
	2	88.14 a
	3	112.51 a
24-hour[x]	2	107.15 a
	1	89.21 ab
	3	75.22 ab
	4	67.27 b

[z]4-day treatment had no significant differences among runs, which was desirable, but also had no significant differences in cultivars (from Table 3).

[y]separation in columns by LSD, 5% level

[x]24-hour treatment had significant differences between runs and also had significant differences between cultivars (from Table 3).

Appendix I. No acclimation treatment: killing times (in minutes) for each run, and unweighted estimates of mean killing times (in minutes).

Cultivar	Run 1	Run 2	Run 3	Mean
Nonlinear model				
5BP7	133.60	26.20	43.20	67.67 a[z]
ND 364	96.32	51.60	35.60	61.17 ab
Oregon 1604	103.60	31.90	25.10	53.53 ab
Wyoming 166	75.00	58.40	34.10	55.83 ab
Valley	39.70	57.00	50.70	49.13 ab
UI 114	73.00	70.80	24.80	56.20 ab
PI 324067	106.20	39.00	00.00	48.40 ab
PI 271998	20.30	23.60	17.80	20.57 ab
Linear Interpolation				
5BP7	133.60	26.20	43.20	14.30 a
ND 364	71.60	65.80	35.20	44.40 a
Oregon 1604	98.20	28.00	18.20	43.63 ab
Wyoming 166	44.20	50.70	38.30	44.37 ab
Valley	35.00	54.60	43.50	67.67 ab
UI 114	58.90	49.50	22.50	57.53 ab
PI 324067	83.90	26.30	15.70	41.97 ab
PI 271998	14.20	18.90	9.81	14.30 b

[z] separation in columns by LSD, 5% level

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USE OF SAMPLING TIME AND TYPE OF ACCLIMATION IN THE ELECTRICAL CONDUCTIVITY ASSAY FOR HEAT TOLERANCE IN BEAN CULTIVARS

by

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ABSTRACT

Eight cultivars of beans, Phaseolus vulgaris L., previously identified as differing in heat tolerance, were evaluated in the laboratory by the electrical conductivity test using time as the variable. Two different acclimation treatments were also compared: 24 hours at a constant 37.5 C and a two-hour stress at 45 C for each of four consecutive days. The lethal time, the time causing 50% electrolyte leakage, was estimated both by fitting the data to a sigmoidal model and by linear interpolation. After the killing times were estimated, weighted and unweighted analyses of variance and a corresponding LSD procedure were used to compare cultivars and tests. The cultivars differed greatly in their responses. While significant differences among them were not observed with the four-day acclimation, this was achieved with the 24-hour acclimation treatment.