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Influence of High Temperature on Pollen Grain Viability and Pollen Tube Growth in the Styles of *Phaseolus vulgaris* L.[1]

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Abstract. **Four cultivars of *Phaseolus vulgaris* L. were studied in a growth chamber experiment to determine the effect of high temperature stress on pollen viability and pollen tube growth in styles. Compared with an optimal growing temperature (25/20°C, day/night), stress temperatures (35/20°, day/night or 35° constant) reduced the percentage of viable pollen for all cultivars, but cultivar differences were apparent. Beans generally produced only small quantities of pollen under normal growing temperatures but much more under high temperatures, again with cultivar differences. Finally, stress temperatures did not reduce the ability of pollen tubes to grow to the base of the style. Our results suggest that injury to pollen at high temperatures up to 35° is not likely to hinder the ability of beans to set pods.**

High temperature adversely affects fruit set in *P. vulgaris* (4, 15, 20). Maximum growth rate occurs between 20 and 25°C; higher temperatures are harmful (7, 13, 24). Davis (8) found that a temperature of 24.5° reduced pod set, and Stobbe et al. (23) observed little or no set when the temperature approached 35°. A large percentage of fruit that does set at high temperatures has been reported to be parthenocarpic (3, 23).

Information relating high-temperature stress to bean yields is limited, and few investigations have been made of the mechanisms involved in stress. Likewise, there is little information on how cultivars differ in their tolerance of high temperatures. A primary objective of this study was to determine if there is a differential response of the pollen of bean cultivars to heat stress. Consequently, we investigated the effects of high temperatures on the fertility of beans, especially pollen viability and pollen tube growth.

Materials and Methods

Four cultivars were used: 'Oregon 1604', received from Dr. J. R. Baggett, Department of Horticulture, Oregon State University, Corvallis; 'UI 111', from Mr. M. LeBaron, Research-Extension Center, University of Idaho, Kimberly; 'Bontoc' and PI 271997, from Dr. H. M. Munger, Department of Plant Breeding and Biometry, Cornell University, Ithaca, N.Y. The cultivars were selected because they differed in response to temperature stress. 'Bontoc' and PI 271997, were reported to be tolerant to high temperature (25; and Dr. D. P. Coyne, Department of Horticulture, University of Nebraska, personal communication). 'Oregon 1604', a snap bean cultivar with prolific flowering capacity, was developed for processing at Oregon State University. In the field at Manhattan, Kansas, under high summer temperatures this cultivar flowers freely but sets poorly and thus is intolerant to heat. 'UI 111' is a semi-determinate pinto bean grown commercially in Kansas. Since it sets fairly well under Kansas conditions, it obviously has some tolerance to high temperatures.

The cultivars were studied in a growth chamber under 3 temperature regimes: 25° (day)/20°C (night), 35°/20°, and 35° constant, all ± 1°. Relative humidity was controlled at 35% ± 2%. The photoperiod, 14 hr, corresponded to the thermoperiod. Light quanta flux density between 400 and 700 nm at the top of the plants was 40 nE cm^{-2} sec^{-1}. Full sunlight ≈ 200 nE cm^{-2} sec^{-1}.

Seeds were planted in 15.2 cm clay pots in a medium composed of 3 silt loam soil:2 coarse builders' vermiculite:1 sphagnum peat:1 perlite. Two plants of each cultivar were grown in each replication, and there were 2 replications at each treatment temperature. From day of planting until day 19 the plants were in a growth chamber at 25°/20°C temperature under a 14-hour photoperiod. On day 20 they were transferred to the appropriate treatment.

Beginning the third day of flowering, which for the earliest cultivar was day 31, and on alternate days for 5 sample dates, 2 fully open flowers were randomly selected from each plant for counts of pollen viability, which was estimated by staining the pollen with propionic carmine and counting 500 grains for each sample under 10x magnification. Stained grains regular in shape were considered "viable." From each flower a single slide preparation was made containing all the pollen of that flower. Randomly selected fields were observed until a total of 500 grains had been counted. Estimates of total grains per flower were made by extrapolating from the field areas for the 500 grain count to that of the entire cover slip. Several estimates verified by total counts were accurate within ± 8%.

Pollen stainability has been used widely as a rapid, conservative estimate of pollen sterility due to cytogenetic abnormalities or environmental treatments (2, 6, 12, 17, 18, 19). Although some reports indicate that pollen stainability may not be so accurate as *in vitro* germination of pollen for estimating male fertility (11), *in vitro* response is not always correlated with pollen tube behavior in the styles under increasing temperature (5). Seed set would have been the most reliable means of evaluation, as has been done with tomato (22), but the greater difficulty of pollinating beans precluded this approach. In any case our interest was in comparative, long-term effects of different temperature regimes on pollen viability; and for this purpose pollen stainability can provide a reliable, conservative estimate of fertility reduction.

Styles were fixed in formalin:80% ethanol:acetic acid, (1:8:1) and pollen tube growth as measured by the number of tubes reaching the base of the style was studied by the technique of Martin (16). Sampling dates were the same as for the pollen stainability study. In this experiment we were interested only in determining whether or not high temperature prevented

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pollen tubes from growing through to the base of the style. Therefore, we did not do a time sequence study of this event under the influence of high temperature. From a practical point of view all that matters with respect to the event of pod set is whether high temperature does or does not prevent tubes from growing to the base of the style.

Statistical analysis of each sampling date showed that the duration of time spent under a treatment regime had no significant effect on either pollen stainability or the number of tubes reaching the base of the style. Therefore, the data as presented are pooled over all sampling dates.

Results

None of the 4 cultivars differed significantly from the others in % stainable pollen at the lowest, control temperature regime (25°/20°C) (Table 1). At the higher temperature treatment (35°/20°) each cultivar had a significantly lower percentage of stainable pollen, but PI 271997 was more severely affected than the others. Pollen stainability at 35° was not significantly lower than at 35°/20°, but pollen stainability of 'Oregon 1604' and 'UI 111' was severely reduced. In contrast, 'Bontoc' was least affected by the constant 35°, as evidenced by its significantly higher proportion of stainable pollen at that temperature.

Table 2 shows temperature effects on the number of pollen grains produced by the 4 cultivars. Under the optimal (25°/20°C) regime PI 271997 produced significantly more pollen grains than the other cultivars. Pollen grain production by each cultivar increased significantly when temperature was increased to 35°/20°, but production at 35° constant did not differ significantly from that at 35°/20° except for PI 271997. Its production at 35° constant did not differ significantly from that at 25°/20° but was significantly lower than that at 35°/20°.

Estimates of the number of viable pollen grains per flower for each cultivar under each heat treatment are presented in Table 3. For each cultivar, except PI 271997, the estimated number of viable grains is higher under the 2 stress temperatures than under the control temperature. The number of viable grains for PI 271997 is less under both high temperature treatments.

Effects of temperature on the ability of pollen tubes to grow to the base of the style are shown in Table 4. Temperature had no effect on pollen tube growth of any cultivar, except 'UI 111', which had significantly more tubes reaching the base of the style at 35°C constant than at other temperatures tested. Accurate determination of the number of pollen tubes reaching the base of the style was somewhat difficult. Although the tubes were easy to observe under UV fluorescence, they generally coalesced at the base of the style so that it was difficult to count individual tubes. Tube counts, therefore, are conservative and represent the minimum number reaching the base of the style.

Discussion

The effect of high temperatures in reducing pollen viability has been demonstrated for several crops. including tomato (21), cucumber (9), and petunia (19). Failure of tomato fruit to set at high temperature has been attributed to lack of viable pollen (1), even though development of only a few seeds is sufficient to ensure ovary retention and expansion.

Few studies have been made of the effect of temperature on pollen fertility in *Phaseolus.* Inoue and Shibuya (10), using 'Kentucky Wonder', examined the effects of temperature, time, and humidity on *in vitro* germination of pollen collected from field-grown plants. Maximal germination (90.7%) occurred for pollen collected at anthesis and dropped after 1 hr storage at 20-25°C to 59.6% or at 30-35° to 12.4%. However, this pollen was produced under uncontrolled, undescribed environmental conditions.

Our concern was to see whether the extreme temperature conditions common in Kansas could reduce the amount of

Table 1. Effect of day/night temperatures under a 14 hr day on percentages of stainable pollen grains of 4 bean cultivars.

Cultivar	Stainable pollen (%) 25°/20°C	35°/20°C	35°C constant
Bontoc	76.4A[z]A[y]	53.5A B	50.0A B
PI 271997	85.9A A	24.6B B	16.0B B
Oregon 1604	81.7A A	53.8A B	23.0B B
UI 111	75.6A A	43.1A B	16.5B B

[z]Mean separation within temperature regimes by LSD, 1% level.
[y]Mean separation within cultivars by LSD, 1% level.

Table 2. Effect of day/night temperature treatments under a 14 hr day on the total number of pollen grains/flower produced by 4 bean cultivars.

Cultivar	Total pollen grains/flower 25°/20°C	35°/20°C	35°C constant
Bontoc	881B[z]B[y]	1834B A	1948B A
PI 271997	2140A B	2844A A	1687B B
Oregon 1604	416B B	1592B A	2031B A
UI 111	429B B	2023B A	3066A A

[z]Mean separation within temperature regimes by LSD, 1% level.
[y]Mean separation within cultivars by LSD, 1% level.

Table 3. Estimated number of viable pollen grains produced per flower by each cultivar at each day/night treatment regime.[z]

Cultivar	Number of viable grains 25°/20°C	35°/20°C	35°C constant
Bontoc	673	981	974
PI 271997	1838	700	270
Oregon 1604	340	856	467
UI 111	324	870	406

[z]Computed by multiplying the estimated number of grains (Table 2) by the percentage of grains staining (Table 1) for each cultivar under each treatment.

Table 4. Effect of day/night temperatures under a 14 hr day on total number of pollen tubes growing to the base of the style in 4 bean cultivars.

Cultivar	Number of pollen tubes growing to style base 25°/20°C	35°/20°C	35°C constant
Bontoc	5.1A[z]	4.5A	5.4A
PI 271997	4.1A	4.3A	3.4A
Oregon 1604	5.1A	5.4A	5.0A
UI 111	5.5B	5.1B	8.5A

[z]Mean separation within cultivars by LSD, 1% level.

viable pollen sufficiently to account for the extensive blossom drop observed under field conditions. Wide diurnal temperature fluctuations typically occur during the growing season in Kansas. In the eastern part of the state, at Manhattan, the average daily maximum and minimum in July are 32.7°C and 20.4°, respectively, representing a 12.3° diurnal fluctuation; the diurnal fluctuation for the same month in western Kansas at Goodland is 16.4°.

Dale has suggested (7) that a diurnally fluctuating thermoperiod is more antagonistic to optimum growth than a constant temperature. We used the 35°C constant temperature to examine the influence of thermoperiod on pollen viability. It also served as an even greater high-temperature stress, because it is 6.24° above the daily average at 35°/20°. Under this extreme it would not be surprising to observe a significant reduction in viability for all cultivars, but this did not happen. Pollen viability for 'Bontoc' was maintained at 50.0%, barely different from the 53.5% viability at the diurnally fluctuating high temperature. This suggests that 'Bontoc' may prefer a constant temperature regime. But our results do not permit us to draw the same conclusion for the other cultivars.

We did not find that exposure of pollen throughout its developmental stages to high temperatures significantly reduced pollen stainability or the number of pollen tubes growing to the base of the style. In fact, the high temperatures caused all cultivars to produce significantly more *total* pollen grains per flower (Table 2). This is exactly the opposite of the effect of high temperature on pollen production reported for tomato (1, 22) and carnation (12).

Because of the observed increase in total pollen grains per flower, estimated numbers of viable grains per flower increased at high temperatures for all but one cultivar (Table 3). The net increase at high temperatures resulted from a much larger increase in pollen grain production than decrease in viability percentage. This was not true of PI 271997, which produced significantly more grains at the low temperature than did the other cultivars. While its total grain production at the higher temperatures did increase, the increase was small compared with increases for the others, and its decline in pollen viability was larger.

The results of this experiment clearly demonstrate that cultivar differences exist for pollen grain production in *P. vulgaris*. Although all cultivars were equal in percentage of viable pollen assayed at the optimum temperature, PI 271997 produced significantly more grains. But high temperature apparently stressed it more than the others. 'Bontoc' showed more stress tolerance than the others in its ability to maintain a significantly higher percentage of viable grains at 35°C constant, which substantially stressed the other cultivars.

However, the observed adverse effects of high temperature stress do not lead to the conclusion that high temperature effects on pollen significantly contribute to failure to set pods. Even with viability reduced to as low as 16%, enough pollen grains surely remain for fertilization. As Lambeth (14) pointed out, only 1 ovule need be fertilized to prevent pod drop. Results of this experiment show that even under high stress temperatures, enough tubes reach the base of the style so that high temperature stress should not impede fertilization. The effect of high temperatures in reducing pod set most likely occurs after fertilization, at least for temperatures up to 35°C.

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