

Extensive conclusions concerning this correlation cannot be made without controlled genetic experiments.

*A priori*, it was thought that the coefficient of variability for attributes of the inbreds B6274 and B3615 would be less than those for the open-pollinated 'Imperator 58' and 'Nantes'. Perhaps this was not observed because selection for flavor had not been made in the development of these lines or it may have been masked by variation among panelists. The more often lower simple correlations between parameters of 'Imperator 58' and 'Nantes' (Table 3) may be in part due to their greater variation. Two panelists noted substantial piece-to-piece variation for Florida-grown 'Nantes' and 'Imperator 58'.

The variation of flavor within a root suggested 2 ways to improve carrot flavor: reduce the contribution of the crown and tip to the total carrot root and reduce the xylem size. Except for wasteful trimming, the former solution is not very feasible. Carrot breeders have in large part produced the latter solution by selecting for "coreless" (small xylem) types. The sugar coating experiment demonstrates that the expression of harshness and sweetness are independent so that the improvement of flavor and preference can be fully realized only by improving both attributes. The reduction in harshness upon storage suggests a need for postharvest experiments in this area.

Carrot flavor attributes were influenced by genetic and environmental variation. Harsh flavor and sweetness accounted for most variation in overall preference and intensity of carrot flavor difference but only a part of overall carrot flavor. Varia-

tion within the root also affected flavor. The objective components accounting for these attributes are discussed elsewhere (5).

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## Influence of Environment and Flower Maturity on Hybrid Seed Production of Exserted Stigma Tomatoes Crossed without Emasculation<sup>1</sup>

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**Abstract.** Two inbreds of tomato (*Lycopersicon esculentum* Mill.) with exserted stigmas, one without and the other with positional sterility (*ps*), were crossed without emasculation, at 3 stages of maturity under 3 environmental conditions. Seed production was maximal when flowers at anthesis were pollinated during cloudy weather with relative humidities (RH) of about 70% and temperatures about 24°C. Seed production was poor when flowers were pollinated 3 days before anthesis during hot (32°C), clear, dry weather (RH - 48%). The *ps* inbred had less than 1% selfing at all stages of flower development and environments. Selfing contamination for the *ps*<sup>+</sup> inbred was less than 4% per line except in some cases when flowers were crossed before anthesis. Selfing of 35% occurred when flowers were crossed 3 days before anthesis during favorable pollinating weather.

The self-pollinating nature of cultivated tomato has made production of hybrid seed difficult and expensive since manual operations are required for both emasculation and pollen

transfer. Several reports indicated considerable time savings for hybrid seed production by using seed parents with stamenless (*sl*) (9), positional sterile (*ps*) (2, 12, 15) or *ps* plus exserted stigmas (15) to eliminate emasculation. Seed parents with either exserted stigmas (4, 19) or male sterility (*ms*) (17) would likely result in similar time savings. The use of non-emasculating schemes have other drawbacks such as maintenance of parental seed (*sl*, *ps*, *ms*) or stigma exertion of F<sub>1</sub> plants (19). Consequently commercial development of non-emasculating schemes or natural crossing schemes (16, 22), which eliminate manual pollen transfer as well as emasculation, has been limited. However, some of these labor-saving methods may become more commercially feasible in the future if the benefits can be shown to outweigh the disadvantages.

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Genetic emasculation schemes must permit adequate cross-pollination and virtually eliminate self-pollination. The objective of our study was to evaluate both hybrid seed production and selfing contamination of tomato inbreds with 1) stigma exertion and 2) stigma exertion plus *ps*, pollinated at 3 stages of flower development under several environmental conditions.

### Materials and Methods

Two previously described (19) inbreds designated EX-1 and EX-2 were used. Both have exerted stigmas and the recessive potato leaf (*c*) character. In addition, EX-2 also has green stem and *ps*. These and pollinator plants were transplanted to a silty clay loam soil previously broadcast with 183 kg/ha 12N-12P-12K fertilizer at The Ohio State University Horticulture Farm during the summer of 1977. Both inbreds were pollinated at 3 stages of flower development during 3 environments with the same cut leaf (*c*<sup>+</sup>) inbred which was grown in an adjacent plot. A split-plot design was used with inbreds and stage as main plots and environments as subplots. There were 6 plants per experimental unit and 4 blocks.

The stages of flower development at pollination are depicted in Fig. 1 and described as follows:

1. Early-sepals beginning to reflex, petals white and not reflexed, anthers green, 3 days before anthesis.
2. Mid-sepals (and petals for EX-1) reflexed 45-90°, petals and anthers greenish yellow, about 1-2 days before anthesis.
3. Late-sepals (and petals for EX-1) reflexed 90°, petals and anthers bright yellow, at anthesis.

Weather data for the pollination environments are given in Table 1. In general, environment I was hot, clear, and dry, environment II was moderate, clear, and dry and environment III was moderate, cloudy, and humid. All pollination procedures were done between 11:30 AM to 2:00 PM, an optimal time according to both Kretchman (11) and Dempsey and Boynton (6). A battery-powered vibrator was used to collect pollen into a gelatin capsule (size 00) on each pollination day. Stigmas to be pollinated (tagged earlier) were then dipped into the capsule. A maximum of 3 fruit per cluster were pollinated and allowed to set to minimize possible competition between fruits (1). Later, data were taken as to the percentage of successful pollinations and seeds per fruit. Seeds from successful

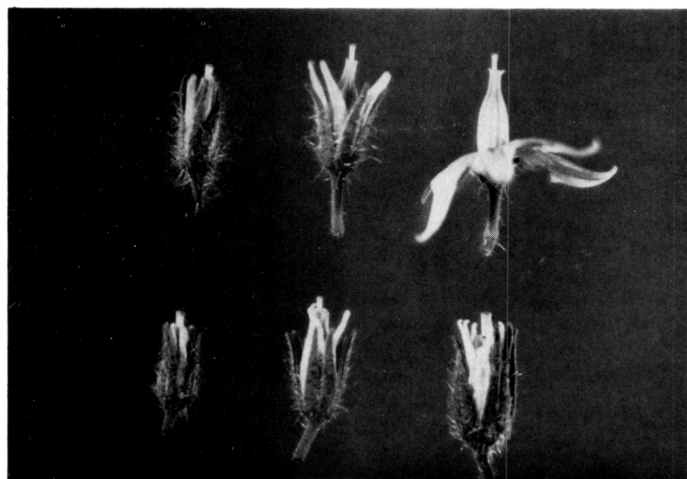


Fig. 1. Stages of flower development used for crossing exerted tomato inbreds. The upper row is EX-1, the lower row is EX-2. The left column is Stage 1, about 3 days before anthesis, the middle column is Stage 2, about 1-2 days before anthesis, and the right column is Stage 3 at anthesis.

pollinations were planted in the greenhouse to calculate the percentage of self-pollination using the *c* locus.

### Results

EX-1 set a higher percentage of fruit but had fewer seeds per fruit than EX-2, resulting in a similar number of seeds per pollination for both inbreds (Table 2). The percentage of fruit set was lower for flowers pollinated 3 days before anthesis than those pollinated at later stages (Table 3). Percentage fruit set was unaffected by environment. However, there was a significant interaction between flower stage at pollination and environment for seeds per fruit and seeds per pollination (Table 3). Thus, ranking of seed set per environment is not the same for all flower stages. Seed set during environment III was greatest and seed set during environment I was the least for the mid- and late stages, but not for the early stage where seed set was low for all environments. Seed set from pollinations made at the late (anthesis) stage was greatest and seed set from the early stage was the least within environments. Optimal seed set was attained when flowers at anthesis were pollinated during environment III (moderate, humid, cloudy).

Selfing contamination was less than 1% for EX-2 under all environments and flower stages (Table 4). The only statistically significant amount of selfing occurred when EX-1 was pollinated 3 days before anthesis during environment III. Non-significant trends suggest selfing occurred more often during favorable pollination weather (environment III) and/or with flowers pollinated before anthesis (Table 4).

### Discussion

**Seed production.** Seed production is best discussed with seeds per pollination data since this parameter takes differences in percent fruit set and seeds per fruit into account (Table 2). Furthermore, environment interacted with the seed count variables but not with percent fruit set which tends to negate the value of percent fruit set data alone in seed production studies.

It appears that exerted inbreds either alone or with *ps* are similar for hybrid seed production (Table 2). Since there was no interaction of genotype with stage and/or environment, it can be assumed that either inbred would result in optimal seed production if pollinated at anthesis during weather similar to environment III.

The poor fruit and seed set for flowers pollinated 3 days before anthesis could be due to poor stigma receptivity (3) or non-receptive ovules or both. Iwahori (10) reported polar nuclei do not fuse until 1 to 2 days before anthesis, hence fertilization at an earlier stage may be impossible. Our results confirm earlier reports (12, 15, 20) that anthesis is the optimal stage for fertilization and seed set (Table 3).

Since seed set was low for early stage pollinations under all environments, environmental effects on seed set will be discussed for only mid- and late stage pollinations. Within these 2 stages seed set increased in order from environments I to III (Table 3). The relatively low seed set of environment I could have been due to high temperatures (9, 23) or high temperature and dry winds (1, 14, 21). These 2 parameters were greater for environment I than environment II while cloud cover and humidity were similar (Table 1). Although the effects of temperature and wind were not tested separately, when pollinating it appeared that temperature was the primary factor. The superior seed set of environment III over environment II within stages was probably due to greater humidity and cloud cover of the former since temperature and wind speed were similar (Tables 1, 3). The humidity at pollination (68%) for environment III was close to the 70% deemed optimal by Kretchman (11) who also tested higher humidities not tested in this study. Use of shade cloths improved seed set in previous work (1, 14) and this could relate to a reduction in temperature and wind,

Table 1. Weather data during the 3 pollination environments used for hybrid seed production on exerted stigma tomato inbreds, 1977.<sup>z</sup>

Variable	Environment I <sup>y</sup>			Environment II			Environment III		
	General <sup>x</sup>	Daily <sup>w</sup>	At pollination <sup>v</sup>	General	Daily	At pollination	General	Daily	At pollination
<i>Temperature (°C)</i>									
Mean high	31.0	33.7	---	26.1	24.4	---	27.7	27.2	---
Mean low	19.9	21.1	---	13.8	11.5	---	19.0	18.1	---
Average	25.5	27.4	32.6	19.9	18.3	24.6	23.2	22.6	24.2
<i>Relative humidity (%)</i>									
Mean high	83.1	83.8	---	84.7	82.3	---	88.7	90.0	---
Mean low	51.0	43.8	---	50.3	43.7	---	64.2	65.0	---
Average	67.0	63.3	48.4	67.5	62.1	48.6	79.2	79.6	68.0
<i>Sky cover (%)</i>	59.5	46.0	38.0	63.0	40.0	37.0	87.0	95.0	90.0
<i>Wind (km/hr)</i>	11.6	25.2	18.5	12.1	10.2	13.5	10.0	5.8	9.4

<sup>z</sup>Data taken from National Weather Service at Port Columbus International Airport (approx. 6.5 km due east of the O.S.U. Horticulture Farm) except for temperatures which were measured at the O.S.U. Farm.

<sup>y</sup>Pollinations made for Environment I: July 6-8, 14, 20; II: July 26-28; III: Aug. 12, 14.

<sup>x</sup>General = means of all days from 3 days before to 3 days after the first and last pollination dates per environment respectively.

<sup>w</sup>Daily = means from pollination days per environment.

<sup>v</sup>At pollination = means at 1 PM of each pollination day per environment (approx. pollination time).

and increased humidity near exposed stigmas. In our experiment the cloudy weather would not affect wind but could have reduced temperatures and increased humidity near the flowers.

*Selfing contamination.* The inbred with *ps* (EX-2) had less than 1% selfing under all stages and environments in agreement with previous reports (7, 8, Table 5). Selfing of the *ps*<sup>+</sup> inbred

(EX-1) varied with flower stage and environment. Interestingly, the most selfing (35.6%) occurred when pollinations were made 3 days before anthesis during favorable (environment III) pollination weather. Apparently during this early stage the cross pollinations were largely unsuccessful which permitted selfing when anthesis took place later. Selfing of early stage flowers was most prevalent when the humid environment

Table 2. Efficiency of hybrid seed production for 2 tomato inbreds with exerted stigmas and with and without *ps*.

Inbred	Fruit set (%)	Seeds/fruit	Seeds/pollination
EX-1 ( <i>ps</i> <sup>+</sup> )	73.2	47.0	34.4
EX-2 ( <i>ps</i> )	62.5	59.1	37.0
LSD 5%	10.1	6.2	6.2

Table 3. Interaction of the stage of flower development at pollination with environment on pollination efficiency.

Developmental stage <sup>z</sup>	Pollination environment <sup>y</sup>	Fruit set (%)	Seeds per fruit	Seeds per pollination
Early	I	44.8	18.1	6.4
	II	29.1	23.6	6.9
	III	37.8	13.4	4.6
Mid	I	82.4	26.7	21.6
	II	82.2	50.9	42.7
	III	77.6	80.6	62.9
Late	I	85.6	59.9	50.6
	II	79.2	80.0	64.0
	III	89.1	124.3	111.5
LSD 5%		14.7	16.4	15.7

<sup>z</sup>Early: pollinated 3 days pre-anthesis, Mid: pollinated 1-2 days pre-anthesis, and Late: pollinated at anthesis.

<sup>y</sup>Pollination dates for environment I: July 6-8, 14, 20; II: July 26-28; III: Aug. 12, 14. See Table 1 for weather detail.

Table 4. Relationship of genotype, stage of flower development at pollination, and environment on selfing contamination from crosses of two tomato inbreds with exerted stigmas.

Inbred	Developmental stage <sup>z</sup>	Pollination environment <sup>y</sup>	Potato leaf progeny (%) <sup>x</sup>
EX-1 ( <i>ps</i> <sup>+</sup> )	Early	III	35.6
EX-1 ( <i>ps</i> <sup>+</sup> )	Early	I	9.6
EX-1 ( <i>ps</i> <sup>+</sup> )	Mid	III	7.5
EX-1 ( <i>ps</i> <sup>+</sup> )	Late	III	3.1
EX-1 ( <i>ps</i> <sup>+</sup> )	Early	II	2.7
EX-1 ( <i>ps</i> <sup>+</sup> )	Late	I	0.4
EX-1 ( <i>ps</i> <sup>+</sup> )	Late	II	0.4
EX-2 ( <i>ps</i> )	Late	II	0.2
EX-1 ( <i>ps</i> <sup>+</sup> )	Mid	I	0.2
EX-2 ( <i>ps</i> )	Mid	I	0.1
EX-2 ( <i>ps</i> )	Late	III	0.1
EX-2 ( <i>ps</i> )	Late	I	0.1
EX-1 ( <i>ps</i> <sup>+</sup> )	Mid	II	0.0
EX-2 ( <i>ps</i> )	Early	I	0.0
EX-2 ( <i>ps</i> )	Early	II	0.0
EX-2 ( <i>ps</i> )	Early	III	0.0
EX-2 ( <i>ps</i> )	Mid	II	0.0
EX-2 ( <i>ps</i> )	Mid	III	0.0
LSD 5%			10.9

<sup>z</sup>Early: pollinated 3 days pre-anthesis, Mid: pollinated 1-2 days pre-anthesis, and Late: pollinated at anthesis.

<sup>y</sup>Pollination dates for environment I: July 6-8, 14, 20; II: July 26-28; III: Aug. 12, 14. See Table 1 for weather detail.

<sup>x</sup>EX-2 seedlings also had green stems indicating EX-2 was the pollen source.

sustained receptive stigmas (Tables 1, 4). Previous reports on exerted seed parents have been variable. In a greenhouse experiment Smith (20) found no selfing when cross-pollinations were made at the "bud" stage and only 0.26% selfing when cross-pollinations were made at the "open" stage. In field experiments, Currence (4) found 10 to 33% selfing when an exerted line was crossed with several pollinators, and Roevers (18) obtained 69% selfing when a slightly exerted seed parent was cross-pollinated. Different environments, genotypes, and/or pollination procedures could have caused the variable results. Although not studied, we do not suggest pollinating flowers which have been dehiscing pollen for a long time, which might have been done in other experiments. Also, using gelatin capsules with abundant pollen for cross-pollinating provides a competitive advantage for pollinator over seed parent pollen.

*Applied aspects.* Our results indicate seed set is greater when pollinations are made at anthesis than at earlier stages, but this may not be true when emasculation is required. Under some environmental conditions parts of exposed pistils could dessicate between emasculation and subsequent pollination at anthesis. This may explain why Barrons and Lucas (1) obtained 76% fruit set when pollinations were made at emasculation and 57% fruit set when pollinations were made the next day at anthesis. Yet Daskaloff (5) reported 55% fruit set at emasculation 1 day before anthesis with fruit set increasing on succeeding days to 95% 3 days later. Advantages of the production schemes studied include time saved without emasculation and greater, more stable seed set due to pollination at anthesis without exposure of entire pistils to dessication.

The major problem with using exerted seed parents for hybrid seed production is that the resulting hybrids are somewhat exerted and yield problems could result (19). If a recessive exerted mutant could be found, this objection would be eliminated. The use of *ps* insures almost no selfing but parental seed maintenance requires manual extraction of pollen from the indehiscent anthers and *ps* is an additional gene to work with. With exertion alone selfing could be a problem under some conditions, but our results suggest pollination at anthesis limits selfing to less than 4%. This would be too much selfing for greenhouse hybrids but might be acceptable for field grown tomatoes. Use of seed parent seedling markers such as *c* could be used to eliminate inbreds from being transplanted but would also be a source of parental seed to other seed companies. Perhaps use of seed markers such as brown seed (*bs*) would be more useful so self pollinations could be eliminated before planting (22).

Our results indicate seed production would be more efficient on mild, cloudy, relatively humid days and inefficient on hot, dry, and possibly windy days (Table 1, 3). Much commercial seed production takes place in hot, dry environments to avoid problems such as disease or early frost which also could limit seed production. Yet, minimal pollination during the extremely hot, dry, periods is advisable. McGuire (13) and Bullard (2) reported on the successful storage of tomato pollen over  $\text{CaCl}_2$  and Larson and Paur (12) and Hafen (9) found stored pollen resulted in greater pollination success at high temperatures. Therefore, seed producers might also consider collecting and storing pollen during favorable weather for use during hot weather if pollinating is necessary during these periods.

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