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Gametophytic selection for early maturity in tomato (Lycopersicon esculentum Mill)

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GAMETOPHYTIC SELECTION FOR EARLY MATURITY IN TOMATO (LYCOPERSION ESCULENTUM MILL.)

BY

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DISSERTATION

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DEDICATION

"Every gun that is made, every warship launched, every rocket fired signifies, in the final sense, a theft from those who hunger and are not fed, those who are cold and not clothed. This world in arms is not spending money alone. It is spending the sweat of its laborers, the genius of its scientists, the hopes of its children...This is not a way of life at all in any true sense. Under the cloud of threatening war, it is humarity hanging from a cross of iron.

Dwight D. Eisenhower, 1890-1969.
From a speech before the American Society of Newspaper Editors, April 16, 1953.

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ABSTRACT

GAMETOPHYTIC SELECTION FOR EARLY MATURITY IN TOMATO
(LYCOPERSICON ESCULENTUM MILL.)

by

Mark Crispi
University of New Hampshire, May, 1991

The feasibility of applying low temperature as a screen for selecting early gametes during pollination of tomato flowers was investigated. Pollen germination and tube growth on solidified agar were evaluated at 8, 15, or 25 C after 6, 12, 18, or 24 hours of incubation for two early-, two intermediate-, and two late-maturing tomato cultivars. Pollen from early cultivars showed superior germination and tube growth at 25 C, whereas pollen germination and tube growth of late cultivars were greatest at 15 C. Pollen germination and tube growth of the two early and two late cultivars were evaluated at three (three hour incubation) or five (six hour incubation) temperatures between 10 and 16 C. The relationships between temperature and in vitro pollen germination or tube growth fit a second degree polynomial for all cultivars. The relationship between optimal temperatures for pollen germination and tube growth and the maturity of the cultivar was further investigated by placing pollen from early and late cultivars on intermediate-maturing females and exposing plants to 25 C day/ 20 C night

and 15 C day/ 10 C night temperatures. Low temperature reduced pollen germination and tube growth rate for all cultivars, regardless of maturity class.

To simulate a population of segregating gametes, pollen mixtures from early and late cultivars were applied to intermediate females under normal and low temperatures. After 48 or 72 hours, styles of pollinated pistils were cut. Low temperatures severely reduced percent fruit set and number of seeds per fruit. However, pollen of early-maturing cultivars had no competitive advantage at either temperature.

Hybrids from crosses of similar and different maturity classes were artifically self-pollinated under low and normal temperatures, and after 72 or 96 hours, styles were cut. Low temperature self-pollinations reduced percent fruit set and number of seeds per fruit. Field comparisons of F_2 populations derived from low and normal temperature self-pollinations revealed no significant shifts in earliness.

Under low or normal pollination temperatures, in vitro pollen germination and tube growth did not correspond to in vivo germination and tube growth. Furthermore, the gametophytic selection scheme used did not cause significant shifts in maturity.

INTRODUCTION

The life cycle of a plant alternates between sporophytic (diploid) and gametophytic (haploid) phases. Because the sporophytic phase is more prominent than the gametophytic phase, both in duration and in economic importance, plant breeders traditionally have favored sporophytic selection schemes. However, when the number of loci controlling a desirable character is high (e.g. a quantitatively inherited character), large plant populations are needed to increase the opportunity to obtain superior zygotes. Selection progress during the sporophytic phase, therefore, often is limited by the number of plants that can be screened at one time. If selection for a quantitative trait could be effected in the male gametophytic phase, the number of plants required for selection would be less than that required for a sporophytic selection scheme (Zamir, 1983). For example, the smallest F_2 population showing all genotypes from an F_1 heterozygous for ten loci would be 4^{10} = 1,048,576. Growing this many tomato plants in the field would be virtually impossible. However, it would be relatively simple to screen and select among the 2^{10} (1024) different haploid gametes and then evaluate a smaller, preselected F_2 population of sporophytes. A major question in the development of a gametophytic selection scheme is whether the desired trait is expressed in the haploid stage.

Another concern is the appropriateness of the selection pressure employed in terms of selecting the desired genotype(s).

The development of early-maturing tomato (Lycopersicon esculentum Mill.) cultivars has been and continues to be a main objective of both processing and fresh market tomato breeding programs. Tomato earliness has been measured several ways, including length of time from seeding to fruit ripening (expressed in days and in growing degree-days), and number and/or weight of ripe fruit by a particular harvest date (Bernier and Ferguson, 1962; George and Peirce, 1969; Gibrel et al., 1982). To facilitate genetic studies of the inheritance of earliness, investigators have divided the time from seeding to date of first ripe fruit into components (Fogle and Currence, 1962), including number of days to first flower (anthesis), first flower to first fruit set, and first fruit set to first ripe fruit (Bernier and Ferguson, 1962; Fogle and Currence, 1950; Gibrel et al., 1982; Honma et al., 1963; Kerr, 1955; Powers and Lyon, 1941). Inheritance studies of earliness and its components in field-grown tomatoes have indicated that earliness (and its components) are quantitatively inherited and greatly influenced by environmental conditions (Bernier and Ferguson, 1962; Cuarto and Cubero, 1982; Fogle and Currence, 1950; Gibrel et al., 1982; Kerr, 1955; Powers and Lyon, 1941). If a gametophytic selection scheme were to be

effective, shifts in the resulting population for one or more of the components of earliness must occur. The number of days from seeding to first flower is associated with gene expression in the sporophyte, and any shift in that component after gametophytic selection would imply an overlap between gene expression in the gametophyte and the sporophyte (Figure 1). The time between first flower to first set is controlled by an interaction between gametophytic and sporophytic gene expression (Knox et al., 1986; Stephenson and Bertin, 1983; etc.) and a gametophytic selection scheme that shifts this component would imply overlap in gene expression between the gametophyte and the gametophyte-sporophyte interaction (Figure 1). A change in number of days from first fruit set to first ripe fruit after gametophytic selection implies that there is an overlap in gene expression between the gametophyte and the sporophyte-zygote interaction that governs this component (Figure 1). For the plant breeder, a gametophytic selection scheme that can reduce the number of any of these components would be of great value, because it would improve earliness as defined by days from seeding to first ripe fruit.

One factor that contributes to the earliness of a tomato cultivar is its capacity to set fruit early in the season when ambient temperatures are low (Daubeny, 1961; Kerr, 1955; Stevens and Rick, 1986; Wittwer et al., 1948). Robinson and Echim (1988) found that in vitro pollen

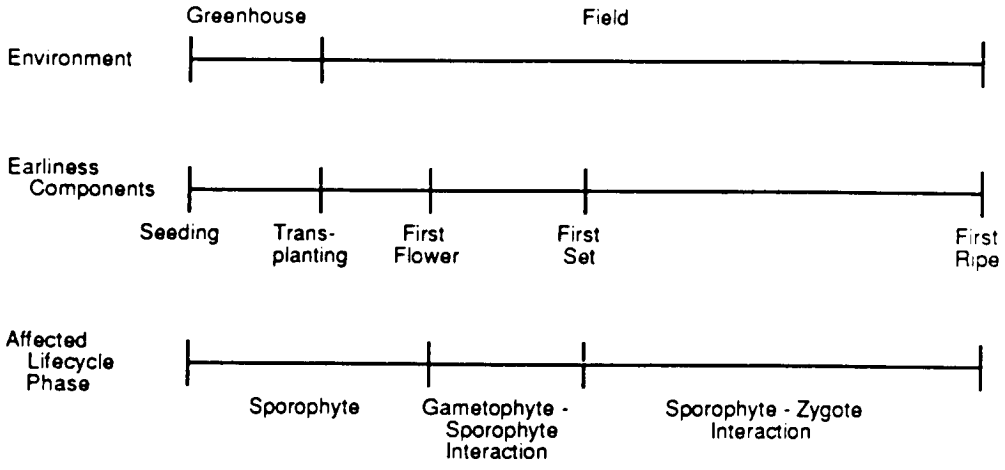


Figure 1. Tomato earliness components, lifecycle phases associated with the components, and the environment encountered by field-grown tomato plants in this study.

function at 10 C is correlated with a cultivar's capacity to set fruit on the first inflorescence when field temperatures are low. They theorized that application of low temperature stress might be useful in selecting gametes that carry genes contributing to early maturity. In this dissertation, pollen function (germination, tube growth, and fertilization) of early and late maturing tomato cultivars under conditions of low temperature was examined, and field tests of progeny derived from selective fertilizations under normal and low temperatures were evaluated for days from seeding to first flower and days from seeding to first ripe fruit. The predictive association of number of days from seeding to first flower and days from seeding to first ripe fruit was enhanced by transplants and black polyethylene mulch presumably because environmental extremes had been eliminated (Peirce and Crispi, 1989). It is hoped that the results of this study will provide the foundation for the development of a gametophytic selection scheme specific for early maturity in tomato. If a successful gametophytic selection scheme can be developed, future studies would be designed to determine the precise nature of any possible gene expression overlap between lifecycle phases.

The objectives of this study were:

1) Determine if pollen function of early-maturing cultivars differs from that of late-maturing cultivars under low temperature conditions.

2) Determine if in vitro pollen behavior (germination, tube growth) corresponds with in vivo pollen behavior (fertilization) under low-temperature conditions.

3) Determine if gametophytic selection under low-temperature stress can be used to select early-maturing tomato plants.

LITERATURE REVIEW

Earliness in tomato

Early production of tomato (Lycopersicon esculentum Mill.) may be improved through cultural systems and genetic enhancement. Genetic improvement of earliness has been a major objective of both fresh market and processing tomato breeders (Gould, 1974; Kerr, 1955). Earliness in tomatoes is important because "early fruit brings the highest price on the fresh market" (Daubeny, 1961), and because earliness "advances, extends and makes more manageable the tomato harvest" for the processing tomato grower (Gould, 1974). Hence, an understanding of the components and inheritance of earliness is of great importance to the plant breeder.

Tomato earliness has been measured several ways, including length of time from seeding to fruit ripening (expressed in days and in growing degree-days), and number and/or weight of ripe fruit by a particular harvest date (Bernier and Ferguson, 1962; George and Peirce, 1969; Gibrel et al., 1982). To facilitate genetic studies of the inheritance of earliness, investigators have divided the time from seeding to date of first fruit ripe into components (Fogle and Currence, 1962), including number of days to first flower (anthesis), first flower to first fruit set, and first fruit set to first ripe fruit (Bernier and Ferguson, 1962; Fogle and Currence, 1950; Gibrel et al.,

1982; Honma et al., 1963; Kerr, 1955; Powers and Lyon, 1941). Inheritance studies of earliness and its components in field-grown tomatoes have indicated that earliness (and its components) are quantitatively inherited and greatly influenced by environmental conditions (Bernier and Ferguson, 1962; Cuarto and Cubero, 1982; Fogle and Currence, 1950; Gibrel et al., 1982; Kerr, 1955; Powers and Lyon, 1941). Under controlled greenhouse conditions, Honma et al. (1963) studied days from seeding to first flower and concluded that this component of earliness was controlled by a single gene that "interacted so greatly with the environment that field studies would indicate a quantitative pattern of inheritance." Regardless of the number of genes, it is clear that earliness and its components are highly variable in response to environment (Cuarto and Cubero, 1982).

Peirce and Crispi (1989) were able to enhance the predictive association between days from seeding to first flower and days from seeding to first ripe fruit in the field by using transplants, black polyethylene mulch, and clear polyethylene row covers. These cultural treatments tended to remove extremes of environment, enabling a breeder to improve earliness by selecting genotypes that flower early.

Low temperature fruit set

One factor that may contribute to earliness of a tomato cultivar is its capacity to set fruit early in the season when ambient temperatures are low (Daubeny, 1961; Kerr, 1955; Stevens and Rich, 1986; Wittwer et al., 1948). Optimal temperatures for fruit set generally are considered to be 15 to 20 C night and above 25 C day (Went, 1944; Went, 1945). Varietal differences for capacity to set fruit at low temperatures (night temperatures below 12.8 C) have been observed by several authors (e.g. Bohn, 1955; Daubeny, 1961; Frazier, 1951; Hornby and Daubeny, 1956; Kemp, 1965; Kemp, 1966; Kemp, 1968; Learner and Wittwer, 1953; Went, 1945; Wittwer et al., 1948). Successful fruit set at a night temperature of 4.5 C was thought to be controlled by a single, environmentally-variable, dominant gene in the cultivar 'Earlinorth' (Kemp, 1965). At 10 C and above, the rate of fruit set increased rapidly (Kemp, 1965) for lines possessing the ft (fruit temperature) gene (Kemp, 1966).

Picken (1984), in a review of pollination and fruit set in tomato, concluded that "temperatures below 10 C or above 30 C are detrimental to one or more of the processes leading to fruit set. Pollen development, especially after meiosis, is most affected at low temperature, but poor pollen germination and slow pollen tube growth due to low temperature may also contribute to poor fruit set in some cultivars."

Low temperature in vivo pollen function studies in tomato

Dempsey and Boynton (1962) investigated low-temperature effects on field pollinations of tomato. Pollen was collected from field-grown plants in the morning and used to pollinate 16 flowers each from three crosses ('Pearson' x 'Pearson', 'San Marzano' x 'San Marzano', 'Pearson' x 'San Marzano'). Flowers were pollinated every three hours for 24 hours. Pollen germination and rate of pollen tube growth were assessed by observing pollen tube fluorescence at 6, 9, and 12 hours after pollinations. Pollen germination and pollen tube growth were "greatly reduced" in blossoms pollinated during the midnight to 6AM period (10 to 15 C air temperatures) when compared to those pollinated during the rest of the 24-hour period (20 to 35 C air temperatures). Reduced fruit set and seed set also were observed in the midnight to 6AM pollinations.

Dempsey (1970), in a similar study with excised flowers incubated at 5, 10, or 15 C, found that pollen germination and pollen tube growth were slower when compared to pollinated, excised flowers incubated at 20 and 25 C. Pollen germination time was inversely related to temperature, and rate of pollen tube growth was directly related to temperature. Charles and Harris (1972) obtained similar in vivo results for pollen tube growth of two cold-setting and two heat-tolerant tomato selections. Pollen,

produced at 18.3 or 26.7 C, was applied to the stigmas of excised male-sterile flowers and incubated at 10, 18.3, or 26.7 C. Pollen tubes penetrated the ovary in 24 hours in the 18.3 C and 26.7 C incubated flowers, but tubes required 84 hours to reach the ovary in flowers incubated at 10 C.

Low temperature in vitro pollen function studies in tomato

In vitro evidence for low temperature inhibition of pollen function has been obtained by several investigators. Charles and Harris (1972) collected pollen from two cold-setting and two heat-tolerant selections. Collected pollen was incubated separately on artificial medium at 10, 18.3 or 26.7 C. Pollen germination was greatest for the 18.3 C incubation and lowest for the 10 C incubation. Zamir et al. (1981), obtained pollen from cultivated tomato and L. hirsutum Humb. and Bonpl. (a high-altitude Peruvian ecotype) and incubated the pollen separately on an artificial medium at 5 C (100 hours duration) or 15 C (8 hours duration). At 5 C, pollen germination was inhibited to a greater extent in the cultivated tomato than in the cold-tolerant Peruvian ecotype.

Maisonneuve and Den Nijs (1984) reviewed pertinent literature and found that tomato cultivar differences for in vitro pollen germination and tube growth had been observed at both low and normal temperatures. In their own study, Maisonneuve and Den Nijs compared in vitro pollen

germination and tube growth (at 10, 14, 22C) with sporophyte growth (dry weight, number of leaves) at 19 C day/ 10 C night for six cultivars of tomato. Their results indicated no clear relationship between pollen germination or tube growth and eventual sporophyte growth.

Recently, Robinson and Echim (1988) studied in vitro pollen function of different cultivars, breeding lines, mutants and species of Lycopersicon. Field-collected pollen was incubated on artifical medium at 10, 22 or 35 C. The "magnitude of pollen germination and the average length of pollen tubes were subjectively evaluated by microscopic examination." Their results suggested that in vitro pollen germination and average pollen tube length at 10 C are correlated with the capacity to set fruit at low temperature.

Gametophytic selection

Traditionally, plant breeders have favored sporophytic (diploid) selection schemes. When the number of genes controlling a desirable character is high (e.g. a quantitatively inherited character), selection progress during the sporophytic phase is often limited by the large number of plants that must be screened to obtain a desired genotype.

Gametophytic selection implies the ability to recognize desirable genotypes in the haploid or gametophytic phase of

a plant's life cycle. The theory and evidence favoring gametophytic selection over sporophytic selection has been eloquently reviewed by Hutton (1988) and can be summarized as follows:

a) male gametophytes (which develop into pollen) greatly outnumber female gametophytes during a typical pollination event;

b) pollen grains successfully compete prior to fertilization of female gametophytes by germinating rapidly and/or having rapid pollen tube growth rates;

c) competitive ability due to transcription of loci in both the haploid and diploid phases of the life cycle would result in the overlap of gametophyte and sporophyte gene expression;

d) a high degree of overlap in gene expression between the gametophytic and sporophytic phases indicates "that selection in one phase should have a high probability of influence in the remaining phase;" and

e) if the correct selective agent is found and the pollen-selected genes are expressed in the sporophyte, then the breeder could drastically reduce the number of field plants required to screen for a desired genotype in progenies produced by selected pollen.

Sporophytic and gametophytic gene expression overlap in tomato

Evidence for overlap of sporophytic and gametophytic gene expression in tomato (L. esculentum) was obtained by Tanksley et al (1981). In that study, isozyme profiles of sporophytic tissue (roots, leaves, and seeds) were compared with those of the male gametophyte (pollen) for nine enzyme systems. Sixty percent of the 30 isozymes present in the sporophyte were found in the gametophyte, and 18 of the 19 gametophytic isozymes also were found in the sporophytic tissue. According to the authors, these results "support the concept that selection for genes expressed in the gametophytic stage could have a marked effect on the sporophytic generation, since many of these genes are expressed in both generations."

Gametophytic selection schemes for tomato

Richardson and Currence (1953) developed an in vivo gametophytic selection scheme to alter the segregation of several traits in the F_2 of a cross between an early- and a late-maturing cultivar. Selection was accomplished by cutting the F_1 style at different intervals after natural pollination. Stylar cutting 24 hours after pollination altered the segregation of several characters (fruit set, seed set, weight per seed, fruit shape) but had no effect on two components of earliness (days from seeding to first

flower and days from seeding to first ripe fruit). Seeds also were selected, based on location in the F_1 fruit. Early yield (amount of fruit harvested prior to September 8th) was slightly but significantly greater (14.3 to 12.3 pounds) on plants grown from stem-half seeds of naturally set fruit than it was on those grown from comparable blossom-end halves.

Zamir et al. (1981) devised a gametophytic selection scheme using low temperature as the selective agent. Mixtures were prepared from pollen harvested from L. esculentum and L. hirsutum. Greenhouse grown plants of L. esculentum were transferred to growth chambers and pollinated with the mixtures. Fertilizations occurring during a 12 C light/ 6 C dark regime resulted in the doubling of the frequency of L. hirsutum gametes contributing to hybrid formation as compared to fertilizations with the same mixtures at 24 C light/ 19 C dark. These results suggested that selection at the pollen level occurs in response to low day/night temperature treatments.

In a continuation of their previous study, Zamir et al. (1982) harvested pollen from a greenhouse-grown inter-specific F_1 hybrid between L. esculentum and L. hirsutum and applied this pollen to greenhouse-grown plants of L. esculentum. The flowers were pollinated within two temperature regimes (24 C light/19 C dark and 12 C light/ 6

C dark). BC_1 plant tissue then was analyzed electrophoretically for loci that mark six of the 12 tomato chromosomes. Isozyme analysis indicated that two chromosomal regions of L. hirsutum, linked to the enzymatic genes Pgi-1 and Adh-2, are highly favored in crosses at low temperature. The authors concluded "that the low temperature tolerance of L. hirsutum pollen grains is determined by genes expressed in the pollen genome."

Zamir and Vallejos (1983) expanded on the work of Zamir et al. (1982) by electrophoretically comparing BC_1 plants ((L. esculentum x L. hirsutum) x L. esculentum) obtained from pollinations performed under two different temperature regimes (24 C light/19 C dark and 12 C light/ 6 C dark) with those pollinated using pollen developed under the same two temperature regimes. They found that "haploid selection for low temperature tolerance is stronger during pollen function than during pollen formation."

In order to develop salt-tolerant tomato plants, Sacher et al. (1983) developed a gametophytic selection scheme that employed NaCl as the selective agent. F_1 plants from the cross L. esculentum cv. 'New Yorker' (salt sensitive) x L. pennellii (Corr.) D'Arcy (salt tolerant) were grown under control and salt stress conditions. It was found that F_2 plants derived from salt-stressed F_1 plants were more vigorous when grown under both saline and control conditions than the F_2 plants from control F_1 plants.

Maisonneuve et al. (1986) developed a method to select for growth capacity at low temperature. F_2 and BC_1 progenies were produced by crossing tomato cultivars differing in low temperature adaptation. Flowers were pollinated under two temperature regimes (22 C light/15 C dark and 15 C light/8 C dark) with pollen formed under both regimes. Progenies were compared for vegetative growth (leaf and stem dry weights) at a low temperature regime (19 C light/ 10 C dark). From the results obtained, the authors concluded that "low temperature treatment of a heterozygous plant during pollen formation or pollen germination did not appear to aid sporophytic selection."

Using low temperature as a selective agent, Zamir and Gadish (1987) developed a system to select for tomatoes adapted to low temperature. Pollen harvested from an F_1 hybrid between L. esculentum and L. hirsutum, was applied to stigmas of male-sterile L. esculentum plants in growth chambers set at 12 C light/ 7 C dark, and 24 C light/ 18 C dark. BC_1 seeds from both temperature treatments were germinated, and root elongation rates were measured at 9 C or 24 C. The results indicated that root elongation of seedlings from the low-temperature crosses was less inhibited by the cold than root elongation for progenies of the normal-temperature crosses.

METHODS AND MATERIALS

Five experiments were developed based upon the previously stated objectives. Several procedures and materials used repeatedly within these experiments are described under "General Procedures". All experiments were conducted in facilities located at either the University of New Hampshire or the US Forest Service Northeast Forest Experiment Station (NFES), Durham, NH.

General Procedures

Plant Material

To reveal evidence of gametophytic selection, six tomato (L. esculentum) cultivars were required that differed in maturity type (early, intermediate, or late), specific single-gene varietal characteristics (i.e. mature fruit color and uniform unripe color genes), and pollen germinability and pollen tube growth rates at low temperature (15 to 8 C). The six cultivars (Table 1) were chosen from among 21 cultivars evaluated for these differences at Woodman Farm, Durham, NH, during the summer of 1987. Pollen germination and tube growth were measured for each of the 21 cultivars at three temperatures (8, 15, or 28 C) after 24 hours of incubation on an artifical medium. The results of this preliminary study suggested that within the chosen cultivars, low temperature (15 to 8

Table 1. Maturity class, uniformity of unripe fruit color, flesh color, and seed source of six tomato cultivars used for gametophytic selection and pollen analysis.[1]

Cultivar	Maturity class[2]	1987 Days from seeding to First flower	First ripe	Genotype[3]	Seed source[4]
Gemstate	E	46	87	UU, $Og^c Og^c$	J
Rocket	E	46	85	uu, $Og^c Og^c$	S
Earlirouge	I	53	95	uu, $og^c og^c$	"
Bellstar	I	52	96	uu, $og^c og^c$	"
Crimsonvee	L	50	109	ugug, $og^c og^c$	"
Harvestvee	L	57	108	uu, $og^c og^c$	"

[1] Pedigree of each cultivar is in Appendix 1.

[2] Maturity class based on catalog description: E = early; I = intermediate; L = late.

[3] Gene symbols: U = green shoulder; u = uniform ripening; ug = uniform grey-green; R = red flesh; Og^c = red flesh; og^c = crimson flesh.

[4] J = Johnny's Selected Seeds, Albion, Maine; S = Stokes Seeds Inc., Buffalo, New York.

C) could be used to inhibit pollen germination and pollen tube growth rates selectively (data not shown).

For all subsequent winter growth chamber and greenhouse tests, seeds were sown in September or October in seedling flats filled with a peat-vermiculite mix (Redi-earth, W.R. Grace and Co., Cambridge, MA) and placed in the UNH greenhouse. Greenhouse temperatures were maintained as indicated for each specific experiment. When seedlings reached the two-leaf stage, they were transplanted to 7.5 cm Jiffy strip pots (Jiffy Products, Shippegan, Canada). Redi-earth, amended with 14N-6.1P-11.6K controlled-release fertilizer (Osmocote, Grace Sierra, Milpitas, CA) at 0.7 kg N/ha, was used as the growing medium. When seedlings were approximately 12 cm in height, each was transplanted into a larger pot containing a growing medium of Redi-earth (amended with Osmocote at the previous rate) to which 1.6 kg/m^3 of dolimitic lime was added. Plants were staked, tied and pruned when necessary.

Pollen Collection and Pollinations

Pollen collected from flowers of each cultivar one day after anthesis by means of a vibratory collector (Figure 2, design adapted from Rick, 1980) was placed in gelatin capsules (No. 0, Eli Lilly and Co., Indianapolis, IN). Pollen was collected daily (9:30AM to 10:30AM) to provide a fresh supply for each day of pollinations.

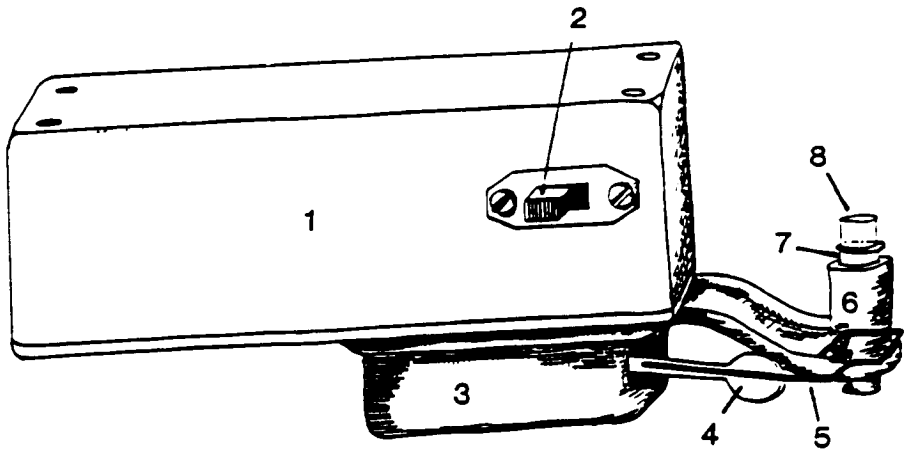


Figure 2. Vibratory collector for tomato pollen, modified from Rick (1980). Four "C" batteries within the battery box (1) are wired in series to an on/off switch (2) and a doorbell ringer (3). A bent paper clip (5) is attached to the bell ringer arm (4) with silicone caulk. The bell is replaced by surgical tubing (6) that has paper balling in the end that is screwed to the bell ringer. In the upper end of the tubing is a micropipet tip (7) that is the diameter of a #0 gelatin capsule (8). Pollen is collected by placing the anther cone of a flower into a capsule held within the micropipet tip and completing the circuit. The capsule and flower are vibrated, releasing pollen into the capsule.

Female plants were emasculated and then pollinated as described by Rick (1980), and each pollinated flower was labeled to show pollination parentage and date.

Seeds and pulp were extracted from mature fruit, and fermented (Rick, 1980). Seeds were then cleaned, dried and bulked by pedigree. All seeds were stored at room temperature in labeled paper envelopes.

Plants for Field Studies

Each spring, seeds were sown in Speedling trays (72 cells per tray) filled Redi-earth amended with Osmocote), and placed in the UNH greenhouse (24 C day and 19 C night). When seedlings reached the two- to three-leaf stage, they were hardened in the greenhouse by periodic wilting. Four weeks after seeding, the plants were soaked in starter solution (16N - 13.9P - 13.3K), then hand transplanted into a prepared field (896 kg/ha of 15N-6.5P-12.5K fertilizer disked in) at Woodman Farm, Durham, NH. Fungicides were applied when necessary, primarily for control of early blight [*Alternaria solani* (Ellis and Martin) Jones and Grout].

Statistical Analyses and Graphs

Data were analyzed using the computer program MSTAT, MS-DOS version 4.0 (Freed *et al.*, 1985). Graphs were drawn with Cricketgraph, Macintosh version 1.2.

In Vitro Pollen Germination and Pollen Tube Growth of Early and Late Maturing Tomato Cultivars

The procedures used for Experiments 1 and 2 were adapted from Maisonneuve and Den Nijs (1984), and Zamir et al. (1981).

Experiment 1. A 24-Hour In Vitro Study of Pollen Germination and Pollen Tube Growth at Three Temperatures

In September, 1987, nine seedlings each of 'Gemstate', 'Rocket', 'Crimsonvee', and 'Harvestvee', and three seedlings each of 'Earlirouge' and 'Bellstar' were grown in the greenhouse (24 C day and 19 C night) as described under "General Procedures". Each was then transplanted into a 25 cm diameter plastic pot and grown to flowering.

Pollen was collected from each cultivar as previously described, and applied with a 5 µl Drummond microcap apparatus (Drummond Scientific Co.) to a medium composed of 18% sucrose, 1% agar, and 0.015% boric acid, adjusted to pH 6.00. The autoclaved medium was poured into six-well tissue culture dishes (Nunc, Inc., Naperville, IL), and pollen was plated, one cultivar to a well, 0.15 µl of pollen per well, at room temperature (approximately 22 C). The dishes then were incubated in dark growth chambers, simultaneously, at 8, 15, or 25 C for 6, 12, 18, or 24

hours. Pollen germination and pollen tube growth were terminated by spraying 5% acetocarmine on the medium when the tissue culture dishes were removed from the chambers. Dishes were sealed with Parafilm M (American Can Co., Greenwich, CT) and stored at 5 C.

This experiment was designed as a split-split plot, with temperatures as whole plots, incubation times as subplots, and cultivars as sub-subplots. There were four replications (one replication every other day). To minimize growth chamber effects, the following were randomized: temperature of each growth chamber after each replicate series; position of each multiwell dish within a chamber; and position of each cultivar within the six-well tissue culture dish.

Dishes were scored for percent pollen germination (based on 200 pollen grains per well) using a microscope at 100x magnification. A pollen grain was considered germinated if the pollen tube length was at least equal to the diameter of the pollen grain. Individual pollen tube lengths were measured and mean pollen tube length was calculated as the average of 50 (or 25 for the 8 C treatment) randomly chosen pollen tubes per well. Measurements were taken at 100x magnification, using an ocular micrometer.

Percentage pollen germination data, subjected to angular transformation (Steel and Torrie, 1980), and pollen

tube lengths were analyzed by analysis of variance.

Experiment 2. In Vitro Temperature Gradient Study of Pollen Germination and Pollen Tube Growth

In August, 1990, four seedlings each of 'Gemstate', 'Rocket', 'Crimsonvee', and 'Harvestvee' were grown in the greenhouse (29 C day and 24 C night), and each was then transplanted into a 25 cm diameter plastic pot.

Pollen was collected from each cultivar as previously described and was applied with a 5 µl Drummond microcap apparatus (Drummond Scientific Co.), on the medium used for Experiment 1, in LabTek four-chamber tissue culture slides (Nunc, Inc., Naperville, IL). Before pollen application, slides containing medium were preincubated at their respective treatment temperatures for 42 hours. Pollen from each cultivar then was plated, one cultivar to a chamber, 0.15 µl of pollen per chamber, on eight duplicate four-chamber slides. The slides were placed within a temperature gradient device (Figure 3) in a dark growth chamber. The temperature gradient device was calibrated to provide treatment temperatures ranging from 10 to 16 C. Pollen-treated slides were removed from the gradient device after three (three slides) or six (five slides) hours of incubation. Pollen germination and pollen tube growth were terminated by spraying 5% acetocarmine on the medium when the slides were removed from the gradient device. Slides

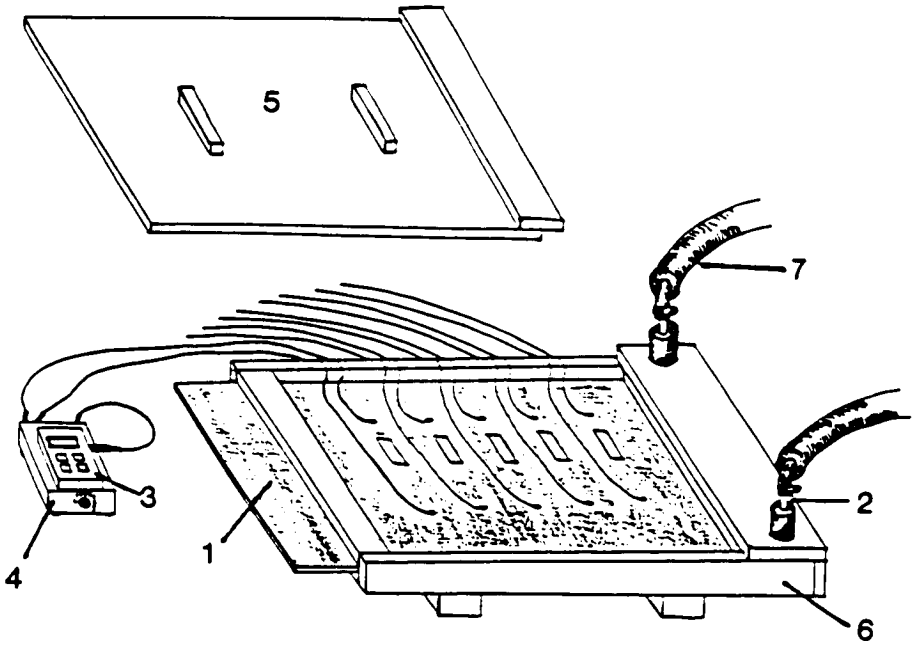


Figure 3. Temperature gradient device, modified from Chatterton and Kadish (1969) and Hensley et al. (1982). The device was placed inside a growth chamber. Chamber temperature controlled temperature of the exposed end of the 122 cm x 51 cm x .635 cm aluminium plate (1). At the opposite end of the plate, an enclosed water bath provided energy transfer between the aluminium plate and the temperature-controlled 1.27 cm diameter copper tubing (2). The copper tubing was connected, via 13 mm ID Buna N tubing, to a refrigerated circulating water bath located outside of the growth chamber. The temperature gradient was established by adjusting the minimum (temperature of the chamber) and the maximum (temperature of the circulating bath) temperatures of the desired gradient. The temperature gradient across the plate was monitored by ten thermistor surface probes (model YSI409A, YSI, Inc., Yellow Springs, OH) connected to an electronic digital thermometer (3) via a ten probe benchtop thermistor switchbox (4)(Cole-Parmer Instrument Co., Chicago, IL). The gradient cover (5) and the plate insulation (6) was made of 2.54 cm thick rigid foam, assembled with silicone caulk. The copper and Buna N tubing were insulated with 1.27 cm thick foam rubber tubing insulation (7).

were placed in a slide tray within a plastic bag and stored at 5 C. There were four replications (one replication every other day) of this procedure.

Pollen germination and pollen tube lengths were measured as described in Experiment 1. Curves were drawn from the data to express the relationships between temperature and percentage pollen germination and temperature and mean pollen tube length.

In Vivo Pollen Germination and Pollen Tube Growth of Early and Late Maturing Tomato Cultivars.

Experiment 3. Four Day Study

During September, 1987, nine seedlings each of 'Gemstate', 'Rocket', 'Crimsonvee', and 'Harvestvee', and 12 seedlings each of 'Earlirouge' and 'Bellstar' were grown in the greenhouse (24 C day and 19 C night) and transplanted into 25 cm diameter plastic pots. The 24 plants of 'Earlirouge' and 'Bellstar' served as female parents and were maintained at 24 C day and 19 C night at the NFES greenhouse.

When female parent plants, 'Earlirouge' and 'Bellstar', were 45 to 60 cm in height, six of each were moved to two growth chambers located in the main building of the NFES, three plants per cultivar in each chamber. After the first pollination series, the six plants of each cultivar were

returned to the NFES greenhouse and the remaining six were placed in the chambers for the second pollination series. Conditions in one growth chamber were set at 12 hour cycles of 25 C day (light period) and 20 C night (dark period). The photosynthetically active radiation was approximately 200 µmol s^{-1} m^{-2}, and relative humidity was approximately 50%. The second chamber had an identical time cycle, light intensity, and relative humidity, but was maintained at 15 C (light) and 10 C (dark). Both chambers had a temperature variation of ± 1 C.

The nine plants of each pollen donor were maintained in the UNH greenhouse. For each series of crosses, pollen was collected from these donor plants, as described under "General Procedures", and applied to females in the growth chambers. The types of crosses in each of the two pollination series are listed in Table 2.

One, two, three and four days after initiating a pollination series, two pistils per cultivar cross, per chamber, were harvested, fixed, stored and stained as described in Figure 4. Pistils were squashed between coverslip and slide as described by Martin (1959). Evaluations of pollen germination and measurements of pollen tube length were made by observing slides under UV illumination (hv = 360 nm) with a Leitz Orthomat microscope. Pollen germination per pistil was classified in the following manner: no germination; low germination (less than

Table 2. Crosses made in each growth chamber for Experiments 3.1 and 3.2.

Pollination series	Experiment[1]	
	3.1	3.2
1	E x G	E x G
	B x G	B x G
	E x R	E x C
	B x R	B x C
2	E x C	E x R
	B x C	B x R
	E x H	E x H
	B x H	B x H

[1] Parental cultivars designated as follows: Gemstate (G); Rocket (R); Earlirouge (E); Bellstar (B); Crimsonvee (C); and, Harvestvee (H).

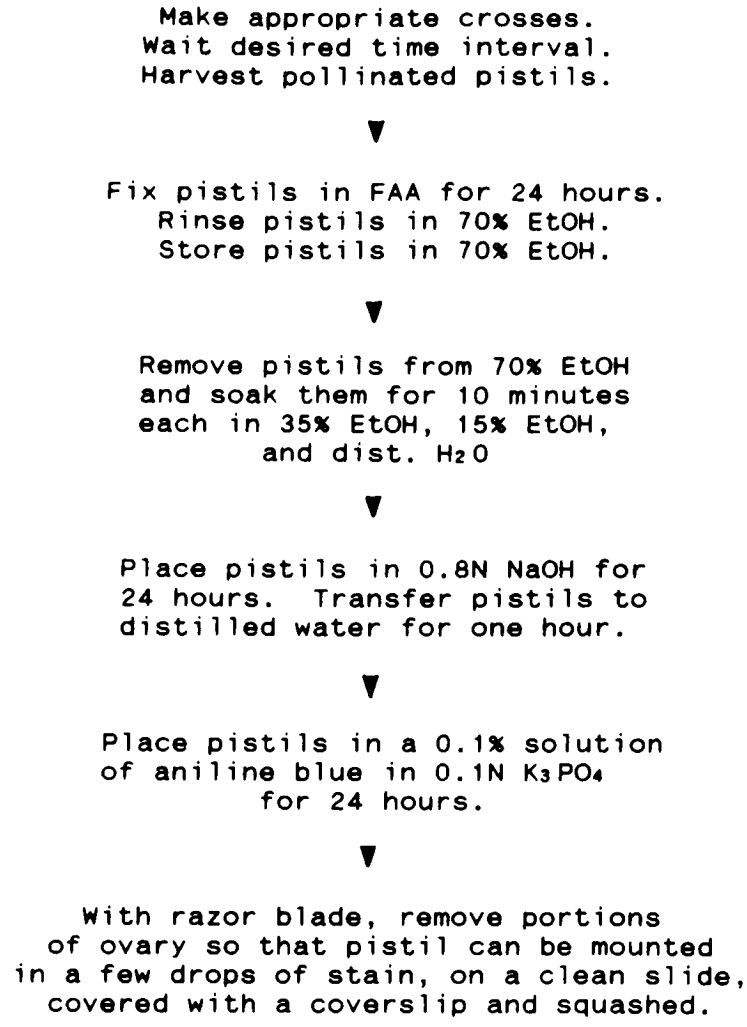


Figure 4. Staining procedure for Experiment 3, modified from Martin (1959). See Martin (1959) for solution recipes.

Table 3. Procedural differences between Experiments 3.1 and 3.2.

Procedure	Experiment	
	3.1	3.2
Date of greenhouse seeding	9/87	8/90
Greenhouse temperature (day / night)	24 C / 19 C	29 C / 24 C
Plants of each pollen donor cultivar	9	4
Plants of each female parent cultivar	12	16
Pot diameter for female parent plants (cm)	25	20
Date of pollination events	2/88	11/90
No. plants per female parent cultivar per chamber	3	4
Chamber light intensity (PAR: umol s^{-1} m^{-2})	200	200 - 400
Temperature variation within low-temperature chamber	± 1 C	± 2 C

10 tubes per pistil); intermediate germination (10 to 30 tubes per pistil); and high germination (more than 30 tubes per pistil). Pollen tube lengths were measured for the 10 longest tubes by determining the average length of the tubes relative to the length of the style. Pollen tube lengths were designated as 1/4, 1/2, 3/4, or full length styles. All observations were made at 100x magnification.

Modification of Experiment 3

In the Fall of 1990, Experiment 3 was repeated in facilities located at the UNH greenhouse, with modifications as listed in Table 3. The two versions are referred to as Experiments 3.1 and 3.2.

Selective Fertilizations by Pollen Mixtures of Early and Late Maturing Tomato Cultivars.

Experiment 4

Selective Fertilizations. The selective fertilization procedure was adapted from Zamir et al. (1981). 'Earlirouge' and 'Bellstar' were chosen as female parents based on maturity type (intermediate), and homozygousity for recessive genes, old gold crimson fresh color (og^c og^c) and uniform unripe fruit color (u u). The four remaining cultivars served as pollen donors and differed in maturity type, flesh color, and uniform unripe fruit color.

In September, 1987, nine seedlings of each pollen-donor cultivar and 30 seedlings of each female-parent cultivar were grown in the UNH greenhouse (24 C day and 19 C night) as described under "General Procedures". Each seedling was transplanted into a 25 cm diameter plastic pot. The 60 female parent plants then were transferred to the NFES greenhouse (24 C day and 19 C night).

When female parent plants were 45 to 60 cm in height, six of each female parent cultivar were moved to two growth chambers located in the main building of the NFES, three plants per cultivar per growth chamber. When pollinations were completed, the six plants were moved back into the greenhouse, and another six were brought to the chambers to be pollinated. This six-plant cycling continued until each female parent plant was pollinated. Conditions in one growth chamber were set at 12 hour cycles of 25 C day (light period) and 19 C night (dark period). Photosynthetic photon flux (PPF) was 200 $\mu mol\ s^{-1}\ m^{-2}$. The second chamber was set at an identical time cycle and PPF but was maintained at 15 C (light) and 10 C (dark). Both chambers were set at approximately 50 % relative humidity and had a temperature variation of ± 1 C.

The nine plants of each pollen donor cultivar were maintained in the UNH greenhouse. Pollen was collected from these plants, and six different pollen mixtures were prepared representing equal volumes of pollen harvested from

any two of the four cultivars, and thoroughly mixed with the vibratory pollen collector. Pollen mixtures were used immediately after preparation.

Female parents were emasculated and pollinated as previously described. The types of crosses made with pollen mixtures, in each growth chamber, are listed in Table 4. In addition, crosses were made with unmixed pollen in the UNH greenhouse as a control: these crosses are listed in Table 4. Enough flowers were pollinated to provide an adequate amount of F_1 seed per cross. Natural selfing of two flowers per cultivar was allowed to take place to obtain parental seed.

Forty-eight hours after pollination, plants were moved from the growth chambers to the NFES greenhouse. Immediately after removing plants from the growth chambers, styles of flowers receiving a pollen mixture were cut at midlength with a sterile razor blade to prevent late fertilizations under subsequent greenhouse conditions (24 C day and 19 C night).

Only three fruits were set on all plants pollinated at low temperature resulting in an insufficient amount of seed for the planned field trials. To salvage some aspect of this experiment, the harvested fruits of normal-temperature pollinations were sliced into two portions, with 2/3 of the fruit at the receptacle end, 1/3 of the fruit at the blossom end. Seeds were harvested from each portion and stored.

Table 4. Crosses made for Experiments 4.1 and 4.2.

Crosses[1]	
Pollen mixture	Non-mixture
E x (G + R)	E x G
E x (G + C)	E x R
E x (G + H)	E x C
E x (R + C)	E x H
E x (R + H)	
E x (C + H)	
B x (G + R)	B x G
B x (G + C)	B x R
B x (G + H)	B x C
B x (R + C)	B x H
B x (R + H)	
B x (C + H)	

[1] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Earlirouge (E); Bellstar (B); Crimsonvee (C); and, Harvestvee (H).

Field Experiment. During April, 1988, seeds were sown and seedlings transplanted to Speedling trays for eventual transplanting. After four weeks of growth, the plants were transferred to a field location arranged in five consecutive blocks of 30 plots each (30 plots wide by 1 plot long), surrounded by guard rows of tomato and eggplant. Each experimental plot consisted of a bed of double rows of five plants (ten plants per plot), spaced 0.45 m apart on black plastic mulch (1 m wide and 38 µm thick). Plots were 1.8 m apart on center. The entries are listed in Table 5.

Data were recorded on unripe fruit color (uniform or non-uniform) and ripe fruit color (og^c og^c or Og^c og^c), the latter using light reflectance color determination on 2 fruits per plant (Watada et al., 1976). Data from progeny tests using seeds from blossom and receptacle ends of the fruit were compared for segregation of genetic markers, using the contingency Chi-square test (Strickberger, 1976).

Modification of Experiment 4

When Experiment 4 was attempted again in Winter, 1989/90, enough seed was obtained from the low temperature pollination treatment to plant a field experiment in Summer, 1990, with the modifications as listed in Table 6. This version is referred to as Experiment 4.2 and the version referred to as Experiments 4.1 compared segregation of genetic markers in the blossom and receptacle ends of

Table 5. Entries planted in Field Experiments 4.1 and 4.2.

Entries [1,2,3]	
Experiment 4.1	Experiment 4.2
E x (G + R)	E x (G + R)
E x (G + C)	E x (G + H)
E x (G + H)	E x (R + C)
E x (R + C)	E x (R + H)
E x (R + H)	B x (G + C)
E x (C + H)	B x (R + C)
B x (G + R)	E x G
B x (G + C)	E x R
B x (G + H)	E x C
B x (R + C)	E x H
B x (R + H)	B x G
B x (C + H)	B x R
E x G	B x C
E x R	
E x C	
E x H	
B x G	
B x R	
B x C	
B x H	
Parents (controls)	

[1] There were two populations for every pollen mixture entry for Experiment 4.1. One population was derived from seed located in the blossom end of the fruit and the other population was derived from seed located in the receptacle end of the fruit.

[2] There were two populations for every pollen mixture entry in Experiment 4.2. One population was derived from the low-temperature pollination treatment (15 C light/ 10 C dark) and the other population was derived from the normal-temperature pollination treatment (25 C light/ 20 C dark).

[3] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Earlirouge (E); Bellstar (B); Crimsonvee (C); and, Harvestvee (H).

Table 6. Procedural differences between Experiments 4.1 and 4.2

Procedure	Experiment	
	4.1	4.2
Date of greenhouse seeding	9/87	9/89
Plants of each pollen-donor cultivar	9	3
Plants of each female-parent cultivar	30	48
Cuttings made and date of transplanting	No	Yes: 2/90
Pot diameter for female-parent plants (cm)	25	15
Greenhouse temperature for female-parent plants (day/night)	24 C / 19 C	29 C / 24 C
Date of pollination events	1/88	3/90
No. plants per female parent cultivar per chamber	3	4
Chamber light intensity (PAR: umol s-1 m-2)	200	200-400
No. hours after pollination when styles were cut	48	72
Location of cut in style	midlength	just below stigma
Seed bulked by location in fruit	Yes	No
Field experiment arranged in blocks	Yes	No: continuous beds
Field experiment plant spacing (m)	0.45	0.61

harvested fruit.

Selective Self-Fertilizations of F_1 Hybrids of Early and Late Maturing Tomato Cultivars.

Experiment 5

Selective Self-Fertilizations. During October, 1988, eight seedlings of each F_1 hybrid (Table 7) were grown in the UNH greenhouse at 24 C day and 19 C night). Each seedling was transplanted into a 20 cm diameter plastic pot. When plants were 45 to 60 cm in height, eight plants each of two F_1 hybrids were placed in two growth chambers (environmental settings as described for Experiment 4.2), four plants per hybrid per growth chamber. When the pollination treatment was completed, these 16 plants were moved back to the greenhouse and another 16 plants (eight plants each of two other F_1 hybrids) were brought to the chambers to be pollinated. This two-hybrid cycling continued until all F_1 hybrid plants had been pollinated.

Pollen was collected from each plant to be pollinated, and these plants also were emasculated. Fresh self-pollen then was applied to obtain F_2 seeds (see "General Procedures"). These hand-emasculations were made to insure against any self-pollination that may have occurred before the temperature treatment was applied. Enough flowers were

Table 7. F_1 hybrids self-pollinated under selective conditions in Experiments 5.1, 5.2, and 5.3.

Experiment no.[1]	
5.1 and 5.2	5.3
G x R	G x R
G x C	G x C
G x H	G x H
R x G	R x G
R x C	R x C
R x H	R x H
C x G	C x G
C x R	C x R
C x H	C x H
H x G	H x R
H x R	H x C
H x C	

[1] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Crimsonvee (C); and, Harvestvee (H).

pollinated to provide an adequate amount of F_2 seeds per cross.

Seventy-two hours after pollination, plants were moved from the growth chambers into the greenhouse. Immediately after removing plants from the growth chambers, pollinated styles were cut at midlength with a sterile razor blade to prevent late fertilizations under greenhouse conditions (24 C day and 19 C night). Seeds were harvested from ripe fruit and stored.

Field Experiment. In May, 1989, F_2 seeds were sown and eventually transplanted as described under "General Procedures". The plants were transferred to an experimental area that was divided into four consecutive blocks, each of which contained 24 randomized plots (24 plots wide by 1 plot long). Eggplant and tomato guard rows surrounded the experiment. Each experimental plot consisted of staggered, double rows of 10 plants (20 plants per plot), spaced 0.61 m apart on beds mulched with black plastic (1 m wide and 38 μm thick). Plots were 1.8 m apart on center. The experimental plot entries are listed in Table 8.

The dates of first flower (anthesis) and first ripe fruit (breaker stage) were recorded for each plant in each plot. Dates of first flower and first ripe fruit were converted to days from seeding. Summary statistics were calculated for each plot, and frequency distributions were constructed for these data. Contingency Chi-squares

Table 8. Entries planted in Field Experiments 5.1, 5.2, and 5.3.

Entries [1,2]		
Experiment 5.1	Experiment 5.2	Experiment 5.3
G x R F_2, F_1	G x C F_2	G x R F_2
G x C " , "	G x H "	G x C "
G x H " , "	R x G "	G x H "
R x G " , "	R x C "	R x G "
R x C " , "	R x H "	R x C "
R x H " , "	C x G "	C x G "
C x G " , "	C x R "	C x R "
C x R " , "	H x G "	H x R "
H x G " , "	H x R "	H x C "
H x R " , "	Parents	Parents
H x C " , "		
Parents		

[1] There were two populations for every F_2 entry for Experiments 5.1, 5.2, and, 5.3. One population was derived from the low temperature self-pollination treatment (15 C light/ 10 C dark) and the other population was derived from the normal temperature pollination treatment (25 C light/ 20 C dark).

[2] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Crimsonvee (C); and, Harvestvee (H).

(Strickberger, 1976) were used to compare frequency distributions of populations derived from the two pollination temperature treatments.

Modifications of Experiment 5

Experiment 5 was performed three times with procedural modifications as listed in Table 9. The three versions are referred to as Experiments 5.1, 5.2, and 5.3.

Table 9. Procedural differences among Experiments 5.1, 5.2, and 5.3.

Procedure	Experiment		
	5.1	5.2	5.3
Date of greenhouse seeding	10/88	10/88	12/89
Cuttings made and date transplanted	No	Yes: 3/89	No
Date of pollination events	2/89	4/89	3/90
No. plants per hybrid per chamber	4	2	3
No. hybrids per chamber	2	4	3
No. hours after pollination when styles were cut	72	96	72
Location of cut in style	midlength	midlength	just below stigma
Date of seeding for field	5/5/89	5/18/90	5/18/90
Date of transplanting into field	6/5/89	6/18/90	6/18/90
Fertilizers applied in field prior to planting	896 kg/ha 15N-6.5P-12.5K	448 kg/ha Ammonium nitrate	896 kg/ha 15N-6.5P-12.5K
Fertilizers applied after planting	none	224 kg/ha Ca nitrate	224 kg/ha Ca nitrate

RESULTS AND DISCUSSION

In Vitro Pollen Germination and Pollen Tube Growth of Early and Late Maturing Tomato Cultivars.

Experiment 1. A 24-Hour In Vitro Study of Pollen Germination and Pollen Tube Growth at Three Temperatures

Temperature and incubation time affected pollen germination and pollen tube lengths similarly in all cultivars (Figures 5, 6, 7). Analysis of variance of percent pollen germination (angularly transformed) and mean pollen tube length data indicated no differences among the four replications over time; however, there were significant temperature by cultivar interactions for both data sets (Table 10). When these interactions were examined graphically, the cultivar differences were expressed primarily as differences in slope (Figure 5). From 15 to 25 C, 'Gemstate' and 'Rocket', the early cultivars, showed stable or increased germination whereas the late cultivars, 'Crimsonvee' and 'Harvestvee', showed declining germination. Mean pollen tube length, from 15 to 25 C, increased at a greater rate for the early cultivars than for the late cultivars. Pollen germination percentages and mean pollen tube lengths were lower at 8 C than at 15 or 25 C for all cultivars.

These results suggest that of 8, 15, or 25 C, the

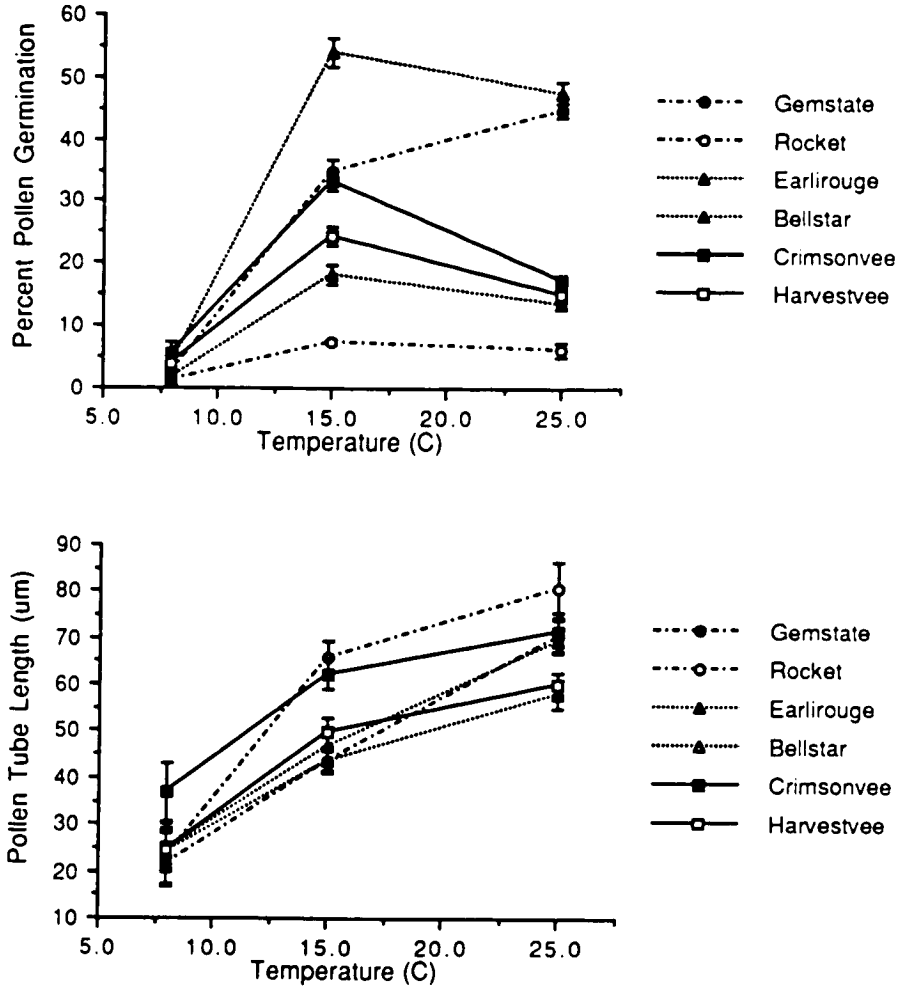


Figure 5. Temperature by cultivar interactions for (top) percent pollen germination and (bottom) mean pollen tube length. Each data point ± SE represents a mean of four replications and four incubation times.

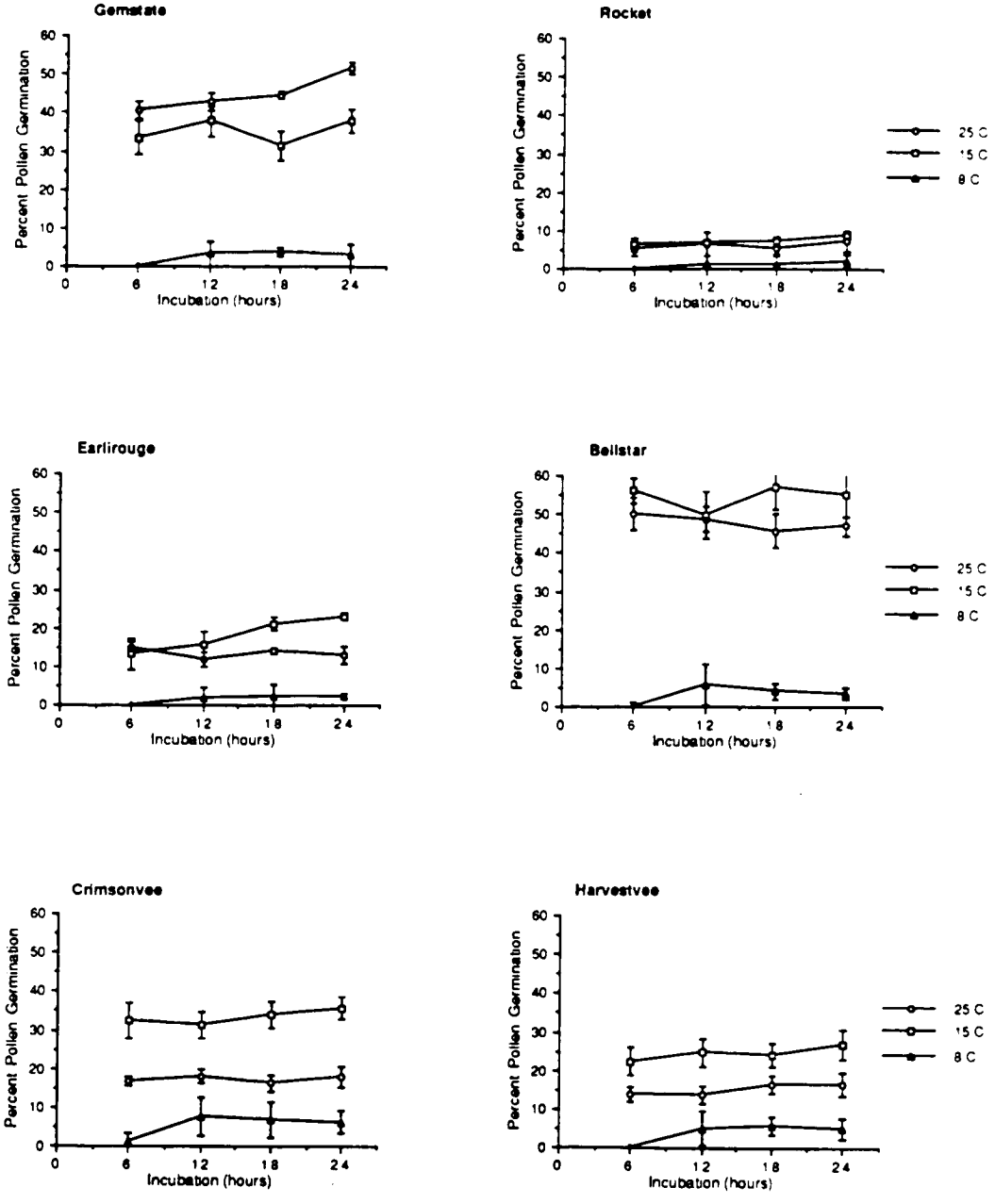


Figure 6. Percent pollen germination at three temperatures and four incubation times for six tomato cultivars. Each data point ± SE represents a mean of four replications.

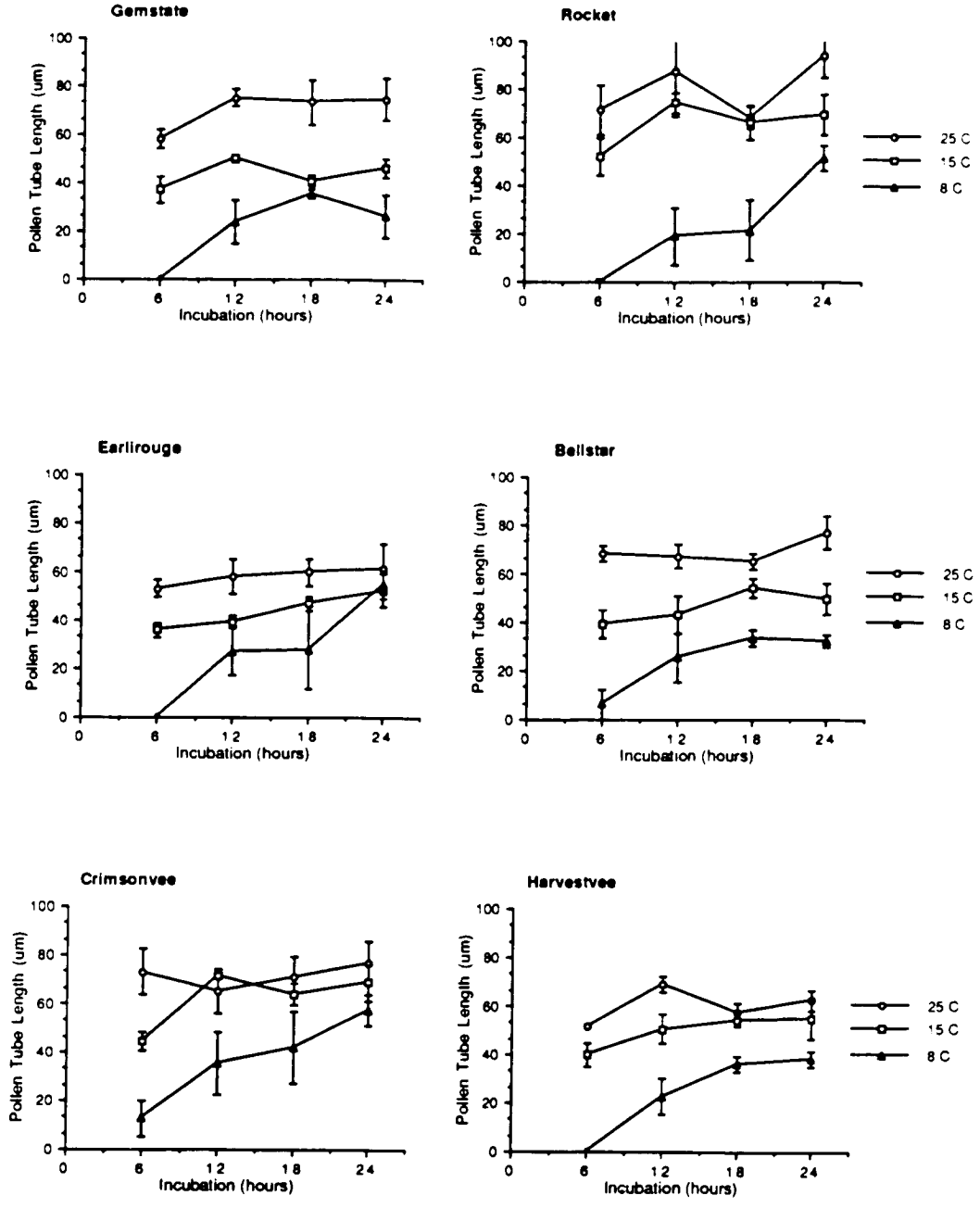


Figure 7. Mean pollen tube lengths at three temperatures and four incubation times for six tomato cultivars. Each data point ± SE represents a mean of four replications.

Table 10. Variances from analyses of percentages of pollen germination, and mean pollen tube lengths for six tomato cultivars.

		Mean squares[1]	
Source of variation	df	Germination[2]	Tube length
Replicates	3	326.3	240.7
Temperatures	2	15,951.0**	43,325.2**
Rep x Temp (error a)	6	401.0	1,558.7
Incubation times	3	278.2**	6,403.4**
Temp x Incub	6	128.3**	1,190.8*
Error b	27	30.9	416.5
Cultivars	5	2,999.3**	1,828.6**
Temp x Cult	10	682.6**	618.0**
Incub x Cult	15	7.1	171.9
Temp x Incub x Cult	30	11.3	137.2
Error c	180	18.2	125.3
Total	287		
CV (%)		19.1	22.9

[1] Variances significant at 5% (*) or 1% (**) probability level.

[2] Percentage pollen germination data subjected to angular transformation.

optimal temperature for in vitro pollen germination of 'Gemstate', an early cultivar, is 25 C and optimal temperature for pollen germination of the late cultivars is 15 C. Dempsey (1970) reported that the optimal temperature for pollen germination was 25 C for the 16 tomato cultivars and lines that he tested. Other workers (Charles and Harris, 1972; Maisonneuve and Den Nijs, 1984) have found that the optimal temperature is closer to 15 C for tomato pollen germination (18.3 C and 14.0 C , respectively). In any case, there appears to be variation among tomato cultivars with respect to optimal temperatures for in vitro pollen germination.

Growth of pollen tubes in this study appeared to be directly related to temperature as reported by Dempsey (1970), Charles and Harris (1972), and Maisonneuve and Den Nijs (1984), but there was variation among cultivars for rates of pollen tube growth at different temperatures as noted by Robinson and Echim (1988). This variation may be associated with early maturity, since the late cultivars had the lowest rates of tube growth from 15 to 25 C. However, this association between maturity type and rate of tube growth was not evident from 8 to 15 C (Figure 5).

A possible source of confounding in this test may have been applying pollen to dishes held at room temperature rather than preincubating the dishes at their respective treatment temperatures. A small-scale comparison where

pollen was applied to dishes either held at room temperature or preincubated at designated temperatures, indicated that germination percentages for the 8 and 15 C treatments were slightly inflated in the room temperature dishes (approximately 1.5 and 4.0 percentage points, respectively) and that there was no difference for the 25 C treatment.

Experiment 2. An In Vitro Temperature Gradient Study of Pollen Germination and Pollen Tube Growth

The relationships between temperature and percent pollen germination, and between temperature and mean pollen tube growth were best described by a second degree polynomial for all cultivars, from data taken after three or six hours of incubation (Figures 8, 9, 10, 11).

After three hours of incubation, the relationship between temperature and percent pollen germination most closely fit a positive second degree partial regression coefficient (increasing slope) for 'Gemstate' (1.0924), 'Rocket' (1.0017), 'Crimsonvee' (1.3815), and 'Harvestvee' (0.70554)(Figure 8). After six hours of incubation, the relationship between temperature and pollen germination was best described by a positive second degree partial regression coefficient for 'Gemstate' (0.77697) and a negative second degree partial regression coefficient for 'Rocket' (-0.39024), 'Crimsonvee' (-1.4477), and 'Harvest-vee' (-0.9255)(Figure 9).

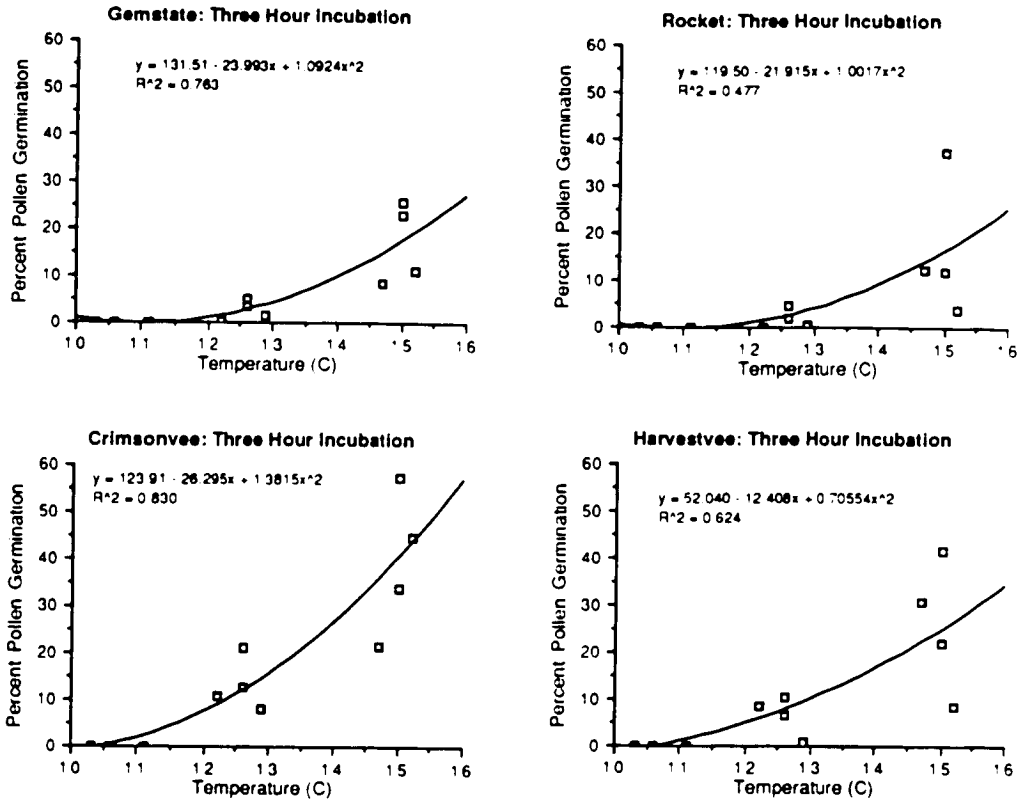


Figure 8. Relationship between temperature and percent pollen germination, after three hours of incubation, for four tomato cultivars. Data from four replicates are included.

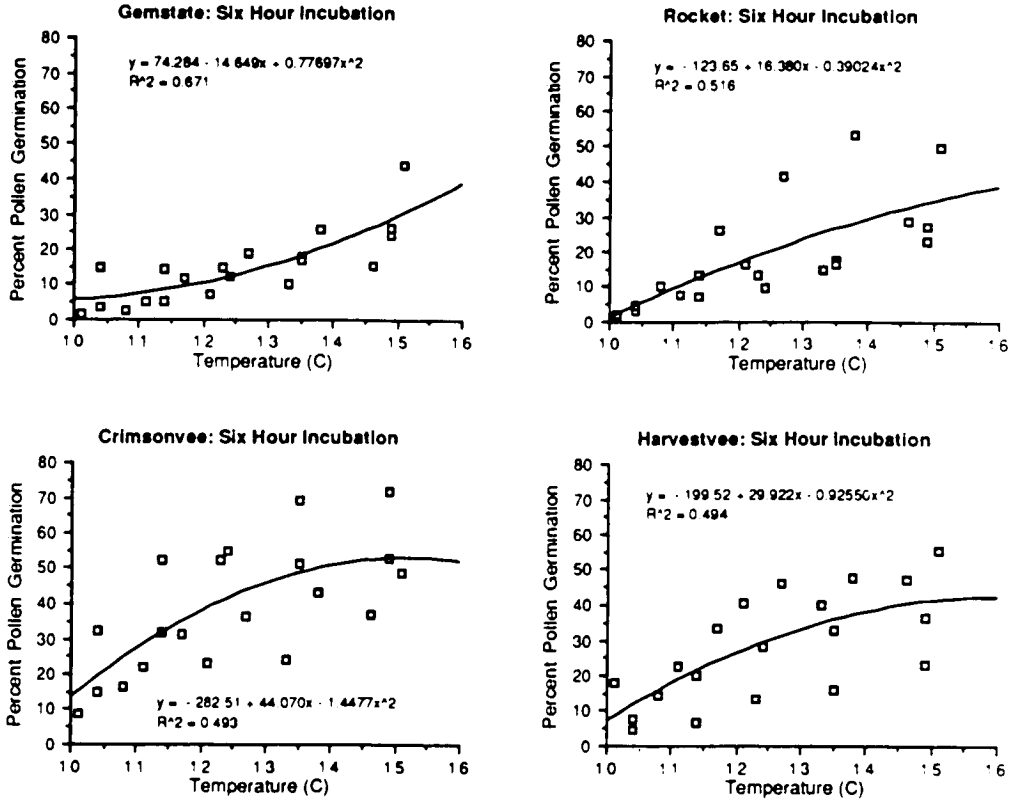


Figure 9. Relationship between temperature and percent pollen germination, after six hours of incubation, for four tomato cultivars. Data from four replicates are included.

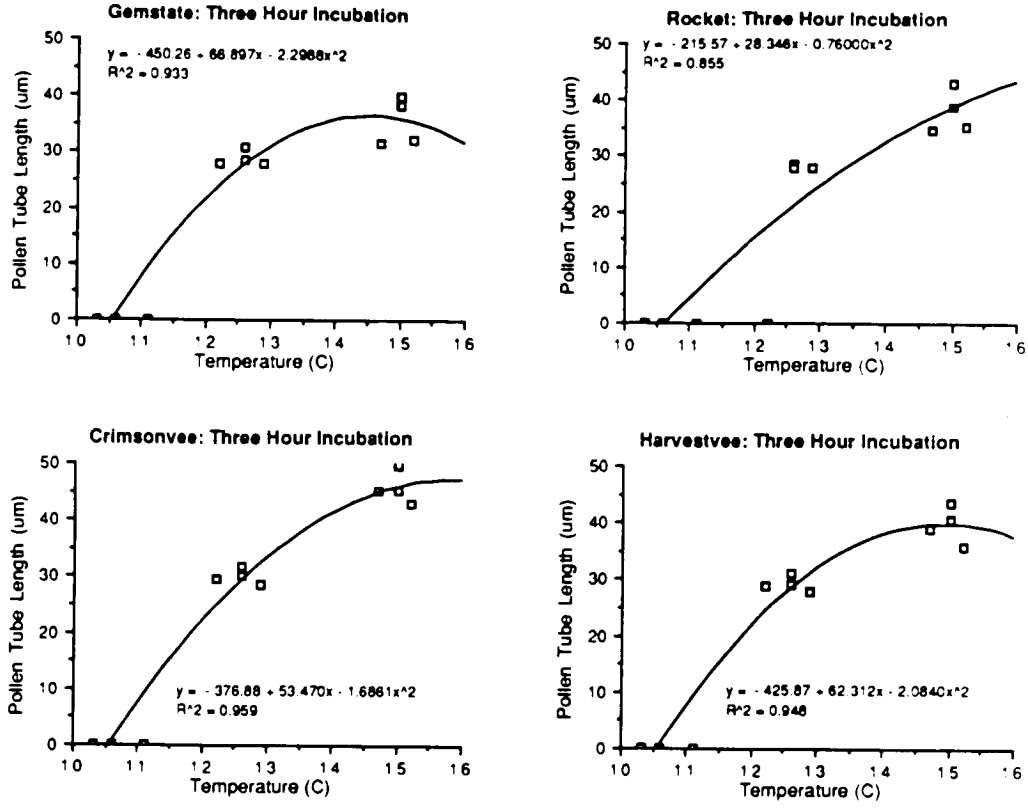


Figure 10. Relationship between temperature and mean pollen tube length, after three hours of incubation, for four tomato cultivars. Data from four replicates are included.

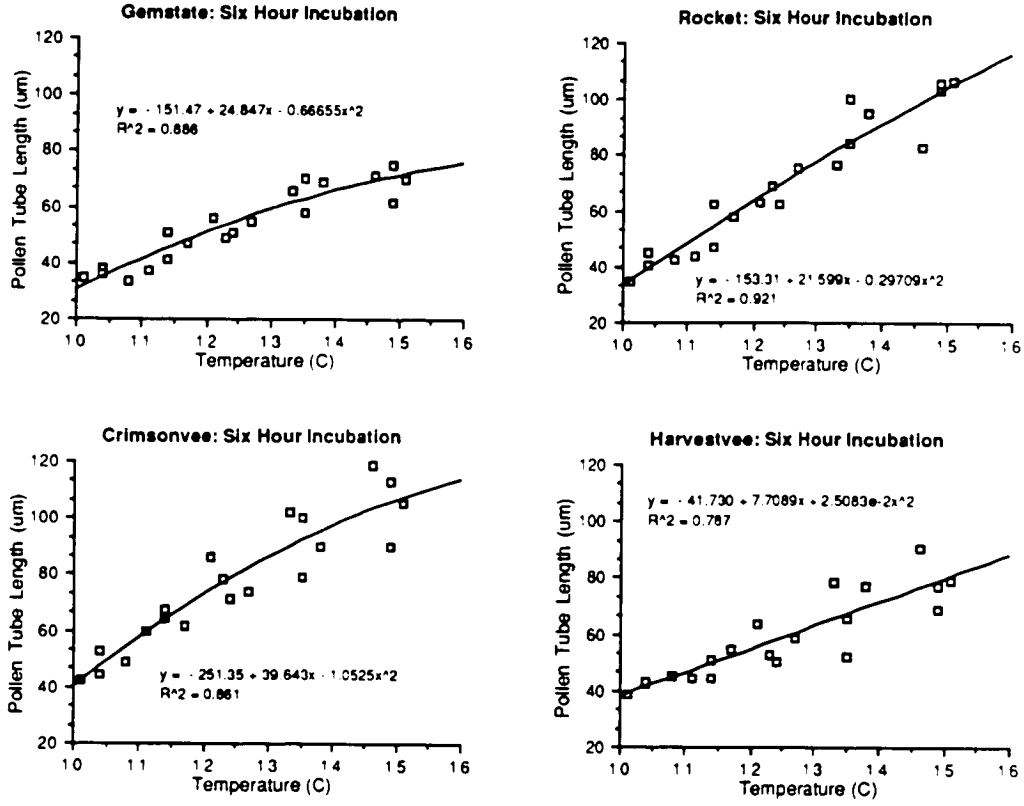


Figure 11. Relationship between temperature and mean pollen tube length, after six hours of incubation, for four tomato cultivars. Data from four replicates are included.

The relationship between temperature and mean pollen tube length after three or six hours of incubation was described by a negative second degree partial regression coefficient for all cultivars, except for 'Harvestvee' data from the six hour incubation period (0.33946)(Figures 10, 11). These results are similar to those obtained in Experiment 1 in that mean tube length was directly related to temperature and in that there was no association between cultivar maturity type and pollen tube growth rate in the low-temperature range tested (8 and 15 C for Experiment 1, 10 to 16 C for Experiment 2).

The most obvious effect of length of incubation period was a difference in percentage germination. After three hours of incubation at the lowest temperature, there was no germination for any of the cultivars. However, all cultivars germinated when incubation time was extended to six hours incubation (Figures 8, 9). Thus, given sufficient time, a certain level of in vitro germination can be observed even at suboptimal temperatures (Vasil, 1987).

An experiment designed to investigate pollen germination and pollen tube growth over a wide range of temperature (e.g. 8 to 40 C) would provide information pertaining to optimum temperatures for in vitro pollen germination and pollen tube growth. In such an experiment, it is likely that the relationship between temperature and percent pollen germination and between temperature and mean

pollen tube length would be described by a sigmoidal curve (Dempsey, 1970; Luza et al., 1987; and, Vasil, 1987).

In Vivo Pollen Germination and Pollen Tube Growth of Early and Late Maturing Tomato Cultivars.

Experiment 3.1.

In all instances, the percentage of germinated pollen was higher and pollen tubes grew faster under the normal-temperature regime compared to the low-temperature regime (Figures 12, 13). These results agree with the findings of other workers (Dempsey and Boynton, 1962; Dempsey, 1970; Charles and Harris, 1972). However, percentage germination and rates of tube growth under low temperature conditions were lower and slower, respectively, than those reported by Dempsey (1970), and Charles and Harris (1972).

When 'Bellstar' plants were used as the female, 'Crimsonvee' and 'Harvestvee' pollen germinated better and exhibited more rapid pollen tube growth under the low-temperature regime than did pollen from 'Gemstate' and 'Rocket' (Figure 12). Under normal temperatures, 'Harvest-vee' had the highest germination percentage and the most rapidly-growing tubes when compared to the other pollen donor cultivars, all of which were similar in terms of germination and speed of tube growth (Figure 12).

When 'Earlirouge' plants served as females, 'Gemstate'

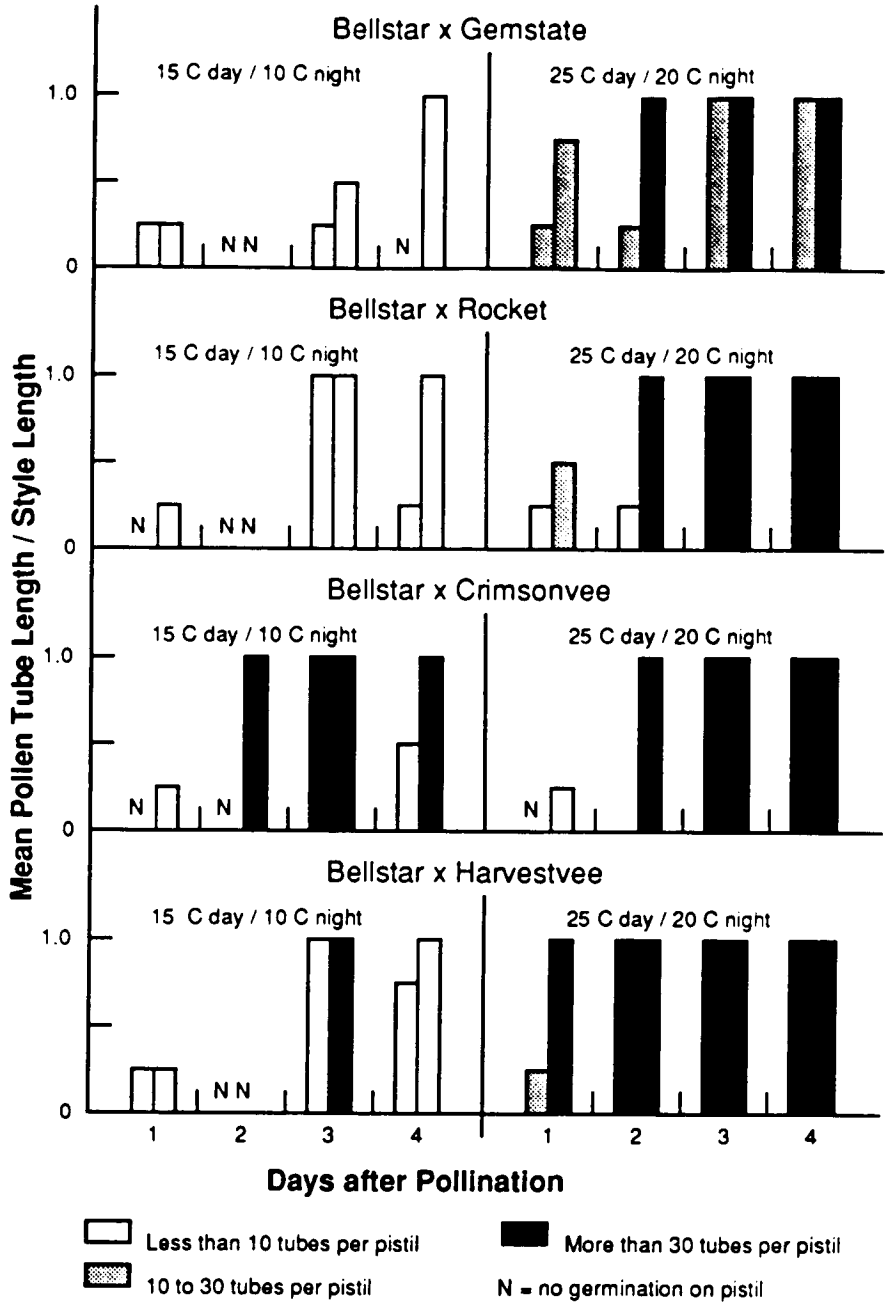


Figure 12. Effect of temperature regime and duration on mean pollen tube length (relative to style length) and pollen tube number for Bellstar pistils pollinated in 1988 by four tomato cultivars. Each bar represents a pistil.

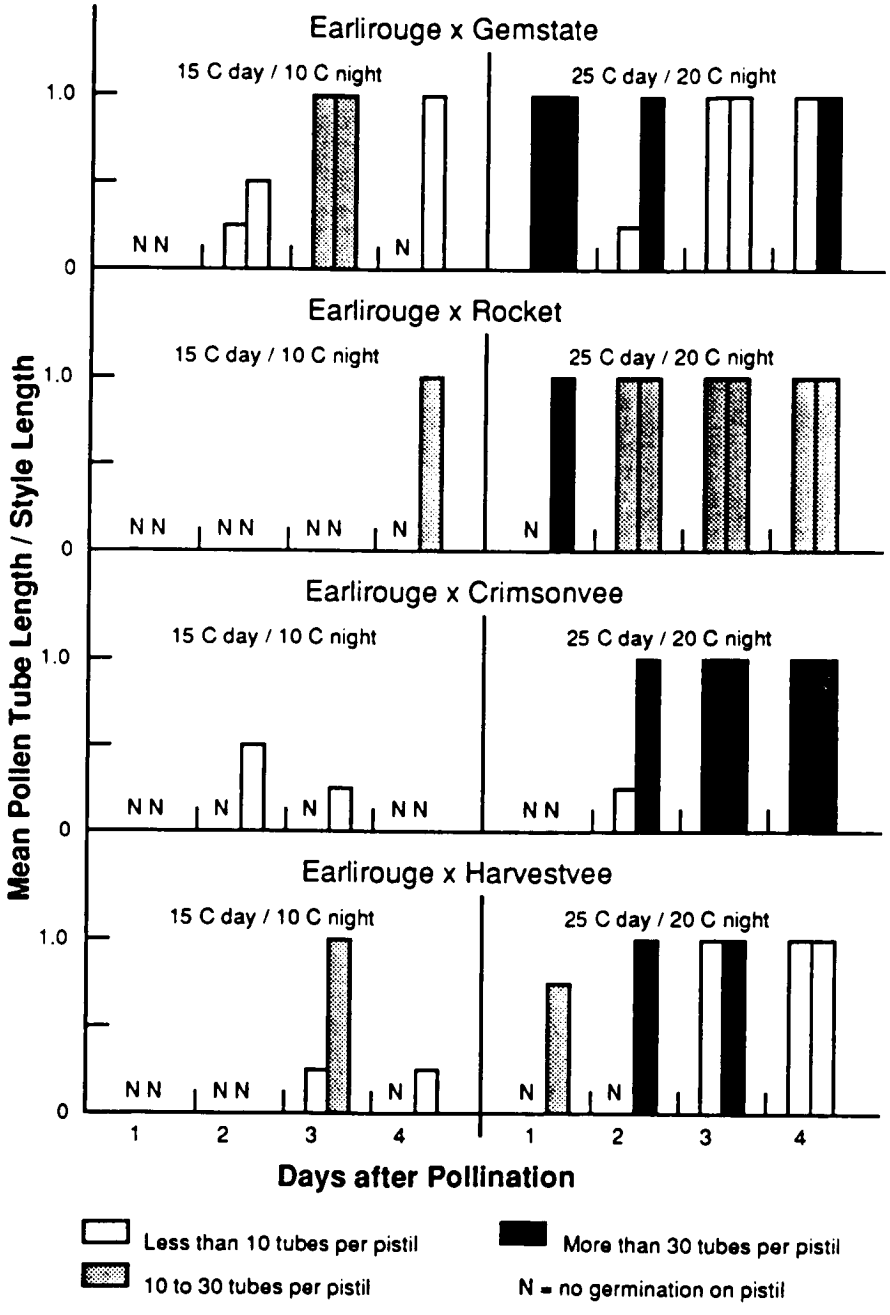


Figure 13. Effect of temperature regime and duration on mean pollen tube length (relative to style length) and pollen tube number for Earlirouge pistils pollinated in 1988 by one of four tomato cultivars. Each bar represents a pistil.

pollen was superior in germination and showed more rapid pollen tube growth under the low-temperature regime than did the other cultivars, whereas 'Crimsonvee' pollen had the lowest percentage germination and slowest pollen tube growth (Figure 13). Under the normal temperature regime, 'Gemstate' and 'Rocket' pollen tubes grew faster than those of 'Harvestvee' and 'Crimsonvee'. 'Gemstate' and 'Crimsonvee' showed superior pollen germination in comparison to 'Rocket' and 'Harvestvee' under normal temperatures (Figure 13).

The results of this experiment suggest no clear relationship between pollen donor maturity type and pollen germination and mean pollen tube growth under different temperature regimes. Although it appeared that pollen from late cultivars had a clear advantage under low temperature conditions when 'Bellstar' was the female, 'Gemstate' pollen was superior to all other cultivars under low temperatures when 'Earlirouge' was the female.

The results of this experiment may have been compromised somewhat by the health of the female plants. These plants were noticeably brittle and were heavily infested with white fly (Trialeurodes vaporariorum Westwood). An interaction between plant condition and low temperature conditions may have resulted in low callose production in pollen tubes and thereby decreased the likelihood of their detection (Martin, 1959) or may have

impeded pollen germination (Heslop-Harrison, 1987). It is also possible that certain combinations of style and pollen are more important in determining germination percentage and subsequent pollen tube growth than the genotype of the pollen alone.

Experiment 3.2.

In all instances, pollen germination percentages and pollen tube growth rates at normal temperatures exceeded those at low temperatures (Figures 14, 15). Under low temperatures, levels of pollen germination in this study were higher and rates of pollen tube growth were more rapid than those observed in Experiment 3.1 and were similar to those reported by Charles and Harris (1972).

On 'Bellstar' females, percent pollen germination of 'Harvestvee' and 'Rocket', exposed to low temperatures, was higher and resulting pollen tubes grew faster as compared to pollination with 'Gemstate' and 'Crimsonvee' (Figure 14). Under normal temperatures, all cultivars were similar in both pollen germination percentage and speed of pollen tube growth (Figure 14).

When 'Earlirouge' plants served as female, all cultivars showed similar percentages of pollen germination under the low-temperature regime, whereas 'Crimsonvee' and 'Harvestvee' pollen tubes grew faster than those of 'Gemstate' and 'Rocket' (Figure 15). Under normal-

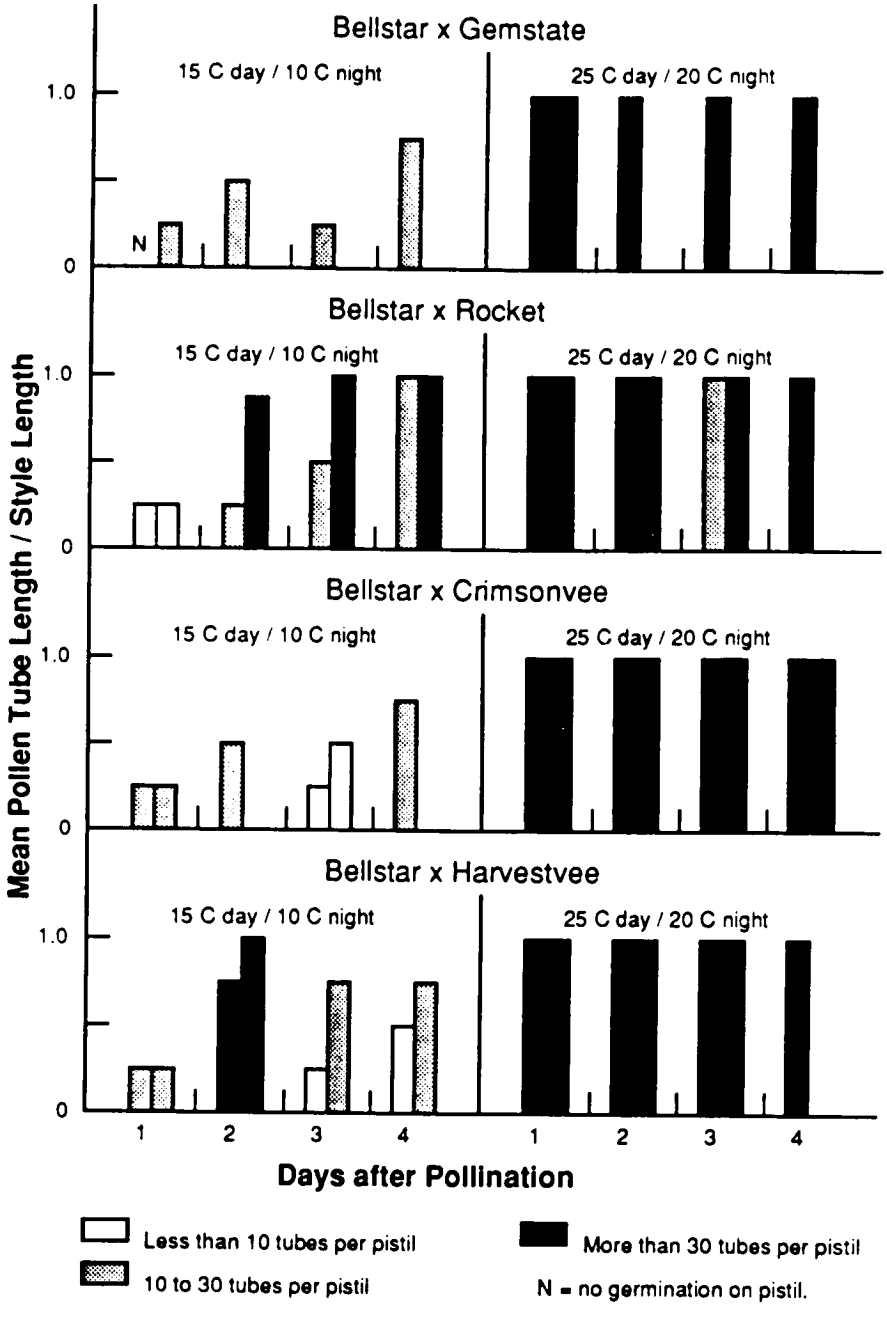


Figure 14. Effect of temperature regime and duration on mean pollen tube length (relative to style length) and pollen tube number for Bellstar pistils pollinated in 1990 by one of four tomato cultivars. Each bar represents one pistil.

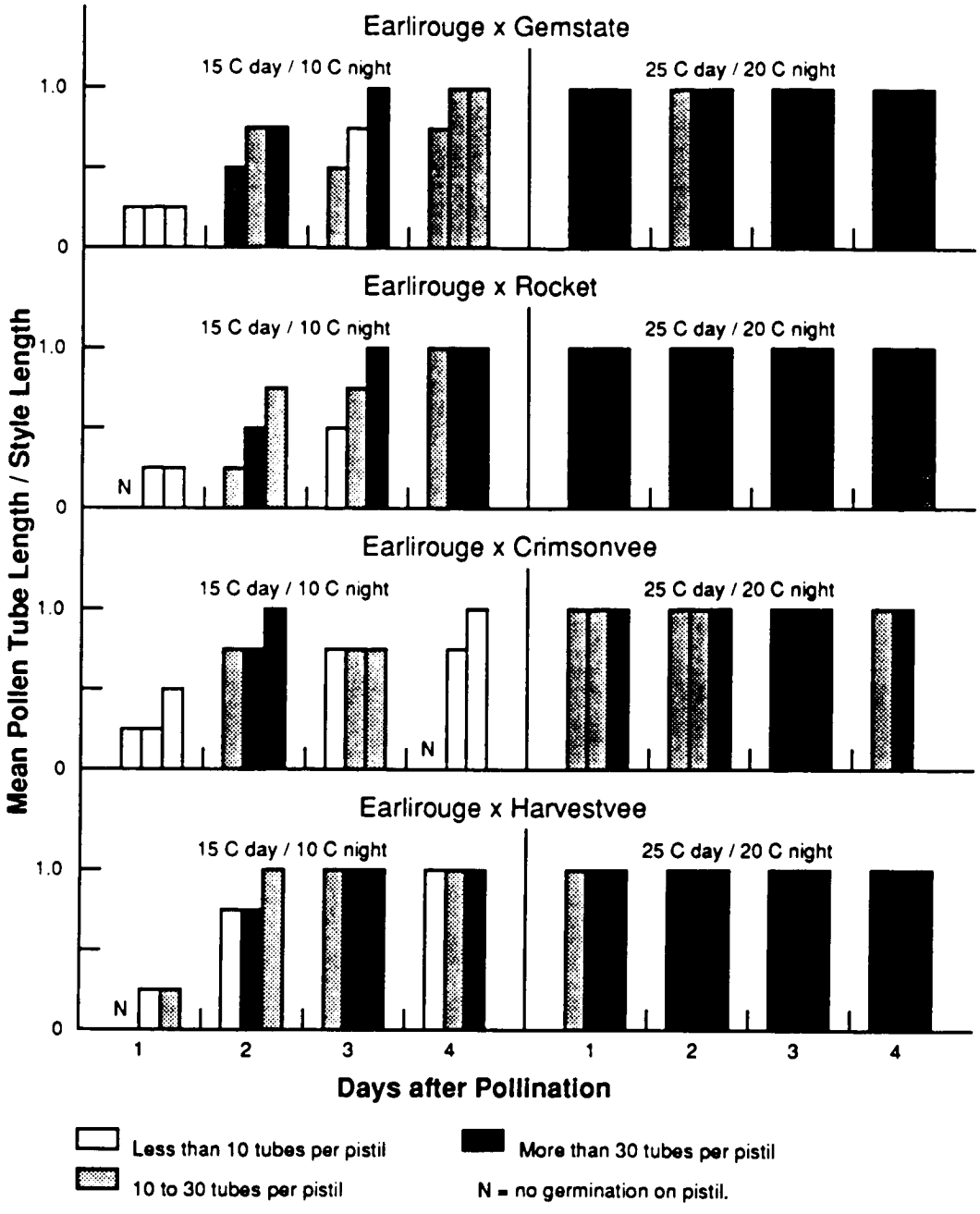


Figure 15. Effect of temperature regime and duration on mean pollen tube length (relative to style length) and pollen tube number for Earlirouge pistils pollinated in 1990 by one of four tomato cultivars. Each bar represents one pistil.

temperature conditions, 'Gemstate', 'Rocket', and 'Harvestvee' pollen germination was superior to that of 'Crimsonvee', whereas the rate of pollen tube growth was similar for all cultivars (Figure 15).

The results of this experiment support the conclusions of Experiment 3.1. There appeared to be no clear relationship between pollen donor maturity type and in vivo pollen germination and pollen tube growth. Furthermore, there was no consistent pattern of pollen donor superiority, either in percentage of pollen germination or rate of tube growth between the two experiments. This lack of consistency may have been due to differences in condition of the female plants in the two experiments, and/or to the different greenhouse temperatures at which pollen developed in the two experiments (Table 3).

Selective Fertilizations by Pollen Mixtures of Early and Late Maturing Tomato Cultivars.

Experiment 4.1.

Only three fruits were obtained from pollinations at low temperatures. The most likely reason for the relatively small number of set fruit was that pollen tubes of the early and late cultivars did not grow more than one half the

length of the style within the 48 hours before midlength stylar cutting and removal from the low temperature chamber. Contrary to these results, Dempsey (1970) found that tomato pollen tubes growing at 15 or 10 C required 16 or 34 hours, respectively, to reach the ovules of the pollinated flowers. Results from Experiment 3.1 and 3.2 suggested that 48 to 72 hours was required for pollen tubes to grow more than half the style length. The differences between this study and results obtained by Dempsey may have been due to cultivar choice and/or to the vigor of the female plants as previously discussed for Experiment 3.1

Progeny tests of seeds from blossom and receptacle ends of fruit from flowers pollinated at normal temperatures were compared for segregation of genetic markers. Results of contingency Chi-square analyses of the segregation of genetic markers are summarized in Table 11. Of eleven comparisons of receptacle and blossom end segregation, only one (Gemstate x Crimsonvee) was significant (P = 5%). In that comparison, the 'Crimsonvee'-associated marker (og^c) was predominant in the receptacle end of the fruit, whereas the 'Gemstate'-associated marker (Og^c) was predominant in the blossom end of the fruit (Table 11). Though population sizes were small, these results suggest that seed position within harvested fruit is not related to any competitive difference among pollen grains of the early and late maturing cultivars, and they are in agreement with the

Table 11. Contingency Chi-square analyses of segregation of genetic markers in Experiment 4.1, planted in Summer, 1988.

Entry[1]	Fruit portion[2]	Unripe fruit color		Ripe fruit color		Contingency Chi-square[3]
		Uu	uu	Og^c og^c	og^c og^c	
E x (G+R)	RE	41	28			
	BE	39	45			2.07
E x (G+C)	RE			16	26	
	BE			52	31	5.82*
E x (R+C)	RE	1	1			
	BE	4	5			0.41
E x (R+H)	RE			36	24	
	BE			100	48	0.77
E x (C+H)	RE	29	29			
	BE	53	43			0.21
B x (G+R)	RE	0	1			
	BE	0	2			NC
B x (G+C)	RE			31	40	
	BE			21	31	0.03
B x (G+H)	RE	1	4			
	BE	5	5			0.31
B x (R+C)	RE	7	6			
	BE	4	12			1.46
B x (R+H)	RE			2	4	
	BE			2	5	0.17
B x (C+H)	RE	0	1			
	BE	0	2			NC

[1] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Earlirouge (E); Bellstar (B); Crimsonvee (C); and, Harvestvee (H). E x (G+H) did not germinate.

[2] Fruit portions are designated as follows: receptacle end (RE); blossom end (BE).

[3] Significant at the 5% (*) probability level. NC designates that Chi-square could not be calculated.

findings of Richardson and Currence (1953) regarding seed location and two components of earliness.

Experiment 4.2.

Only six of the twelve planned low-temperature crosses produced enough seeds to be planted in a field experiment (Tables 4, 5). The lack of fruit and seed set was in part caused by severe blossom end rot but also may have been affected by poor pollen quality. Percent fruit set data and mean seeds per fruit after pollination treatment are summarized in Table 12. Results are presented for the three crosses not affected by blossom end rot, either in fruit set or number of seeds. The percent fruit set and number of seeds per fruit were reduced following pollination under low temperatures (Table 12), a response consistent with those of Charles and Harris (1972) and Zamir et al. (1982).

When segregation classes of genetic markers were compared using contingency Chi-squares in populations developed from low- and normal-temperature pollinations, none of the comparisons was significant (Table 13). Though populations were small, these results suggest that differential selection at the gametophytic level did not occur under low temperatures when using pollen mixtures of early and late cultivars, whereas Zamir et al. (1981) were able to effect gametophytic selection under low temperatures when using pollen mixtures of L. esculentum and L. hirsutum.

Table 12. Percent fruit set and mean seed per fruit after pollination treatment in Experiment 4.2.

Cross[1]	Temperature (C)	No. flowers pollinated	No. fruit set	Percent fruit set[2]	No. seeds	Seeds per fruit
E x (R+H)	25/20	11	9	81.8	870	96.7
"	15/10	14	4	28.6	41	10.3
E x (R+C)	25/20	15	8	53.3	386	48.3
"	15/10	24	9	37.5	175	19.4
B x (R+C)	25/20	12	7	58.3	133	19.0
"	15/10	17	2	11.8	9	4.5

[1] Parental cultivars are designated as follows: Rocket (R); Earlirouge (E); Bellstar (B); Crimsonvee (C); and Harvestvee (H). Fruit set and seed per fruit data were not recorded for the other crosses made in Experiment 4.2 due to blossom end rot.

[2] Percent fruit set equals the number of fruit set divided by the number of flowers pollinated multiplied by 100.

Table 13. Contingency Chi-square analyses of segregation of genetic markers in Experiment 4.2, planted in Summer, 1990.

Entry[1]	Temperature (C)	Unripe fruit color		Ripe fruit color				Contingency Chi-square[2]
		Uu	uu	Og^c	og^c	og^c	og^c	
E x (G+R)	25/20	11	25					
	15/10	6	9					0.11
E x (G+H)	25/20	10	11					
	15/10	8	4					0.48
E x (R+C)	25/20	15	19					
	15/10	10	28					1.78
E x (R+H)	25/20			31		4		
	15/10			12		0		0.39
B x (G+C)	25/20			12		21		
	15/10			4		1		1.84
B x (R+C)	25/20	11	27					
	15/10	3	5					0.003

[1] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Earlirouge (E); Bellstar (B); Crimsonvee (C); and, Harvestvee (H).

[2] Values exceeding a Chi-square of 3.84 are significant at the 5% (*) probability level.

The probable reason for this difference is that the genetic and adaptive dissimilarities between L. esculentum and L. hirsutum provided more variation for gametophytic selection than did the dissimilarities among early and late cultivars of L. esculentum (Maisonneuve et al., 1986).

Selective Self-Fertilizations of F_1 Hybrids of Early and Late Maturing Tomato Cultivars.

Experiment 5.1.

Percent fruit set and mean number of seeds per fruit following artificial self-pollination are presented in Table 14. In seven of eleven hybrids, percent fruit set was reduced following self-pollination at low temperature in comparison to self-pollination at normal temperature (Table 14). Fewer seeds per fruit were obtained under low-temperature pollinations in comparison to normal-temperature pollinations in nine of eleven hybrids following self-pollination (Table 14). These results agree with those of Maisonneuve et al. (1986) and appear to be biologically significant. However, pollination temperature did not affect percent fruit set and mean seeds per fruit according to Wilcoxon's Signed Rank Test (P = 5%).

Eleven F_2 populations derived from F_1 plants self-pollinated at normal temperatures were compared with similar populations derived from low temperature-pollination for

Table 14. Percent fruit set and mean seeds per fruit after self-pollination at normal (25 C day / 20 C night) or low (15 C day / 10 C night) temperature in Experiment 5.1.

Hybrid[1]	Temperature (C)	No. flowers pollinated	No. fruit set	Percent fruit set[2]	No. seeds	Seeds per fruit
G x R	25/20	11	8	72.7	440	55.0
"	15/10	11	9	81.8	393	43.6
G x C	25/20	10	8	80.0	359	44.9
"	15/10	14	1	7.1	20	20.0
G x H	25/20	11	9	81.8	420	46.7
"	15/10	10	7	70.0	88	12.6
R x G	25/20	12	12	100.0	938	78.2
"	15/10	13	8	61.5	243	30.4
R x C	25/20	27	18	66.7	1,104	61.3
"	15/10	24	10	41.7	439	43.9
R x H	25/20	12	3	25.0	190	63.3
"	15/10	7	2	28.6	86	43.0
C x G	25/20	24	18	75.0	1,129	62.7
"	15/10	22	13	59.1	692	53.2
C x R	25/20	11	8	72.7	423	52.9
"	15/10	12	1	8.3	20	20.0
C x H	25/20	8	0	0.0	0	0.0
"	15/10	17	0	0.0	0	0.0
H x G	25/20	16	9	56.3	359	39.9
"	15/10	19	3	15.8	26	8.7
H x R	25/20	7	4	57.1	74	18.5
"	15/10	14	9	64.3	200	22.2
H x C	25/20	13	1	7.7	10	10.0
"	15/10	10	3	30.0	68	22.7

[1] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Crimsonvee (C); and Harvestvee (H).

[2] Percent fruit set equals the number of fruit set divided by the number of flowers pollinated multiplied by 100.

days from seeding to first flower and days from seeding to first ripe (Tables 15, 16, 17, 18). Mean number of days from seeding to first flower differed by one or more days for three comparisons (G x H, R x H, H x G) (Table 15), but in only one of these three comparisons (R x H) was the difference statistically significant (t-test, P = 5%). In this comparison, the mean number of days to first flower of the population derived from low-temperature self-pollination was 1.5 days less (earlier) than that of the population derived from the normal-temperature treatment (Table 15). When the frequency distributions of days from seeding to first flower of F_2 populations derived from normal-temperature self-pollination were compared to those from similar genetic populations derived from low-temperature self-pollinations, the differences in two of eleven comparisons (R x H, H x G) were significant at the 1% level (Table 16). Significance in one of these two comparisons (H x G) was most likely due to the widely different sizes of the F_2 populations compared (Table 15) and to the limitations of the contingency Chi-square in handling such wide differences (Steel and Torrie, 1980). The mean number of days from seeding to first ripe differed by one or more for five comparisons (G x C, G x H, R x H, H x G, H x C) (Table 17). In four of these comparisons (G x H, R x H, H x G, H x C), the differences were statistically significant (t-test, P = 5%). In one of these four comparisons (R x H),

Table 15. Summary of days from seeding to first flower from Experiments 5.1, 5.2, and 5.3.

Entry[1]	Temperature (C)	Days from seeding to first flower								
		Exp. 5.1			Exp. 5.2			Exp. 5.3		
		Mean	SE	n	Mean	SE	n	Mean	SE	n
G x R	25/20	46.3	0.4	79	-----			42.7	0.4	67
"	15/10	46.3	0.3	72	-----			41.7	0.4	73
G x C	25/20	48.7	0.3	80	43.3	0.5	29	43.3	0.4	71
"	15/10	48.8	0.5	16	43.9	0.5	52	43.0	0.4	65
G x H	25/20	49.1	0.4	49	45.3	0.3	78	45.1	0.4	74
"	15/10	50.4	0.5	41	46.0	0.6	31	44.0	0.3	80
R x G	25/20	47.9	0.3	80	42.3	1.3	7	43.7	0.5	68
"	15/10	48.1	0.6	48	43.1	0.4	74	42.0	0.4	65
R x C	25/20	47.3	0.3	78	45.5	0.3	73	45.0	0.4	74
"	15/10	47.8	0.3	78	44.0	0.3	65	45.7	0.4	77
R x H	25/20	48.3	0.3	80	46.4	0.3	78	44.5	0.4	79
"	15/10	46.8	0.2	79	45.9	0.3	80	45.6	0.4	72
C x G	25/20	47.4	0.3	77	-----			43.4	0.4	73
"	15/10	47.4	0.3	78	42.8	0.8	13	45.8	0.4	63
C x R	25/20	47.5	0.3	80	43.6	0.3	79	43.7	0.3	76
"	15/10	47.9	0.4	16	44.5	0.4	76	43.7	0.4	68
H x G	25/20	48.2	0.3	77	43.9	0.4	80	-------		
"	15/10	50.3	0.9	13	44.5	0.3	80	-------		
H x R	25/20	48.6	0.3	53	46.2	0.3	80	44.9	0.3	69
"	15/10	48.3	0.3	74	46.9	0.3	70	45.8	0.6	26
H x C	25/20	50.5	0.8	11	-----			49.1	0.4	68
"	15/10	49.8	0.3	58	-----			50.3	1.3	7
G	---	47.6	0.5	36	42.7	1.1	15	43.1	0.9	17
R	---	44.8	0.3	40	41.7	0.4	17	42.8	0.7	16
C	---	50.3	0.2	40	46.9	0.3	18	46.8	0.4	18
H	---	52.5	0.3	40	49.9	0.4	17	48.5	1.0	20

[1] Parental cultivars designated as follows: Gemstate (G); Rocket (R); Crimsonvee (C); Harvestvee (H).

Table 16. Contingency Chi-square analyses of Experiments 5.1, 5.2, and 5.3. The tests compare frequency distributions of days from seeding to first flower of progeny derived from normal and low temperature self-pollinations of F_1 hybrids.[1]

	Experiment[2]					
	5.1		5.2		5.3	
Progeny group	Chi-square	df	Chi-square	df	Chi-square	df
G x R	13.01 NS	13	-------		17.81 NS	13
G x C	9.13 NS	13	9.91 NS	12	7.97 NS	12
G x H	10.90 NS	14	21.14 NS	13	20.59 NS	14
R x G	22.63 NS	14	12.47 NS	11	26.35 *	15
R x C	11.78 NS	11	27.71 **	11	19.66 NS	15
R x H	27.19 **	11	17.54 NS	12	30.98 **	15
C x G	10.06 NS	13	-------		27.39 *	15
C x R	8.06 NS	13	23.72 *	13	10.35 NS	13
H x G	28.51 **	13	13.85 NS	13	-------	
H x R	6.26 NS	11	11.12 NS	13	16.93 NS	11
H x C	7.77 NS	10	-------		24.63 *	14

[1] Normal and low temperature regimes were 25 C day and 20 C night, and, 15 C day and 10 C night, respectively.

[2] Chi-square value significant at the 5% (*), or 1% (**) probability level, or not significant (NS).

Table 17. Summary of days from seeding to first ripe fruit from Experiments 5.1, 5.2, and 5.3.

Entry[1]	Temperature (C)	Days from seeding to first ripe								
		Exp. 5.1			Exp. 5.2			Exp. 5.3		
		Mean	SE	n	Mean	SE	n	Mean	SE	n
G x R	25/20	82.9	0.4	79	-----			80.4	0.4	66
"	15/10	82.8	0.4	72	-----			80.8	0.4	73
G x C	25/20	90.6	0.4	80	86.9	0.6	29	87.5	0.4	71
"	15/10	91.6	0.7	16	87.2	0.4	51	87.8	0.5	64
G x H	25/20	88.7	0.7	49	87.2	0.3	78	87.5	0.4	74
"	15/10	91.8	0.9	41	86.9	0.6	31	86.6	0.3	80
R x G	25/20	83.6	0.4	80	78.1	1.2	7	80.7	0.5	67
"	15/10	84.5	0.6	48	80.8	0.4	74	80.4	0.5	65
R x C	25/20	90.0	0.4	77	88.3	0.4	69	87.7	0.4	74
"	15/10	90.7	0.4	78	86.5	0.3	64	88.3	0.4	77
R x H	25/20	88.9	0.6	80	87.1	0.4	78	87.2	0.3	79
"	15/10	86.6	0.4	79	87.0	0.4	80	86.6	0.4	71
C x G	25/20	89.9	0.5	77	-----			87.6	0.4	73
"	15/10	89.6	0.5	78	86.7	0.8	13	88.2	0.4	63
C x R	25/20	90.3	0.4	80	87.2	0.3	79	87.3	0.4	75
"	15/10	90.1	0.8	16	87.8	0.5	76	88.1	0.5	67
H x G	25/20	88.3	0.5	76	86.8	0.3	79	-------		
"	15/10	91.4	1.3	13	87.7	0.3	79	-------		
H x R	25/20	88.1	0.5	53	87.0	0.3	80	86.8	0.4	69
"	15/10	87.5	0.5	74	87.7	0.4	70	88.0	0.7	26
H x C	25/20	98.4	1.9	11	-----			97.6	0.4	68
"	15/10	101.3	0.5	58	-----			99.6	0.8	7
G	---	85.3	0.6	36	82.6	1.0	10	81.4	0.5	17
R	---	81.8	0.3	40	81.2	0.4	17	82.0	0.6	16
C	---	104.0	0.4	40	100.7	0.7	15	96.1	0.7	18
H	---	104.8	0.5	40	97.7	0.9	18	97.8	1.5	17

[1] Parental cultivars designated as follows: Gemstate (G); Rocket (R); Crimsonvee (C); Harvestvee (H).

Table 18. Contingency Chi-square analyses of Experiments 5.1, 5.2, and 5.3. The tests compare frequency distributions of days from seeding to first ripe fruit of progeny derived from normal and low temperature self-pollinations of F_1 hybrids.[1]

Progeny group	Experiment[2] 5.1 Chi-square	df	5.2 Chi-square	df	5.3 Chi-square	df
G x R	17.82 NS	15	-------		17.05 NS	16
G x C	27.80 *	16	10.55 NS	13	18.39 NS	18
G x H	18.56 NS	21	12.78 NS	14	16.76 NS	16
R x G	18.22 NS	15	15.05 NS	14	17.31 NS	17
R x C	14.43 NS	16	19.04 NS	13	20.23 NS	16
R x H	31.99 *	19	15.83 NS	15	22.73 NS	15
C x G	17.01 NS	19	-------		13.37 NS	17
C x R	24.15 NS	16	17.03 NS	18	9.86 NS	17
H x G	32.19 *	17	18.31 NS	13	-------	
H x R	15.92 NS	16	25.58 NS	16	23.99 NS	16
H x C	24.20 *	14	-------		14.76 *	15

[1] Normal and low temperature regimes were 25 C day and 20 C night, and, 15 C day and 10 C night, respectively.

[2] Chi-square value significant at the 5% (*) probability level, or not significant (NS).

the mean of the population derived from the low-temperature self-pollinations was lower than the mean of the population derived from the normal self- pollinations (Table 17). Of the eleven comparisons of frequency distributions of days from seeding to first ripe of F_2 populations derived from the two self-pollination temperatures, four (G x C, R x H, H x G, H x C) showed significant differences at the 1% level (Table 18). In only one of these four comparisons (R x H) were F_2 populations close enough in size to support a valid use of the contingency Chi-square (Table 17).

The results of this experiment suggest that superior gametes could not be selected for days from seeding to first flower and days from seeding to first ripe fruit, under either temperature regime, even though pollination at low temperature, in most instances, reduced the number of seeds per fruit.

Experiment 5.2.

The data for percent fruit set and mean seeds per fruit following self-pollination are presented in Table 19. In ten of the eleven hybrids, percent fruit set was less following low-temperature self-pollination than following normal-temperature self-pollination (Table 19). Fewer seeds per fruit were obtained in all hybrids following self-pollination under low temperature in comparison to self-

Table 19. Percent fruit set and mean seeds per fruit after self-pollination at normal (25 C day / 20 C night) or low (15 C day / 10 C night) temperature in Experiment 5.2.

Hybrid[1]	Temperature (C)	No. flowers pollinated	No. fruit set	Percent fruit set[2]	No. seeds	Seeds per fruit[3]
G x R	25/20	8	8	100.0	309	NC
"	15/10	5	2	40.0	15	NC
G x C	25/20	14	7	50.0	65	NC
"	15/10	13	5	38.5	88	NC
G x H	25/20	9	7	77.8	387	NC
"	15/10	13	4	30.8	51	NC
R x G	25/20	6	2	33.3	12	NC
"	15/10	12	4	33.3	243	NC
R x C	25/20	14	12	85.7	1,467	122.3
"	15/10	13	9	69.2	229	25.4
R x H	25/20	6	6	100.0	638	106.3
"	15/10	11	6	54.5	135	22.5
C x G	25/20	8	3	37.5	17	NC
"	15/10	13	3	23.1	25	NC
C x R	25/20	12	12	100.0	1,085	90.4
"	15/10	15	11	73.3	258	23.5
C x H	25/20	6	3	50.0	31	10.3
"	15/10	10	0	0.0	0	0.0
H x G	25/20	13	10	76.9	681	68.1
"	15/10	13	8	61.5	314	39.3
H x R	25/20	12	8	66.7	437	54.6
"	15/10	11	6	54.5	78	13.0
H x C	25/20	10	9	90.0	631	70.1
"	15/10	9	0	0.0	0	0.0

[1] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Crimsonvee (C); and Harvestvee (H).

[2] Percent fruit set equals the number of fruit set divided by the number of flowers pollinated multiplied by 100.

[3] Not calculatable (NC) due to blossom end rot.

pollination under normal temperature (Table 19) (Wilcoxon's Signed Rank test, P = 5%). These results agree with those obtained by Maisonneuve et al. (1986).

Eight F_2 populations derived from normal-temperature self-pollination treatments were compared with genetically similar populations derived from low-temperature pollination (Tables 15, 16, 17, 18). The mean number of days from seeding to first flower differed by one or more days for only one (R x C) of eight comparisons (Table 15). For this comparison, the mean number of days for the population derived from low-temperature self-pollination was significantly less (t-test, P = 5%) than that for the population derived from the normal temperature pollination (Table 15). When the frequency distributions of days from seeding to first flower of F_2 populations derived from normal temperature self-pollinations were compared to similar populations derived from low-temperature self-pollination, two of eight comparisons (R x C, C x R) showed significant differences (Table 16). The mean number of days from seeding to first ripe fruit differed by one or more days for two (R x G, R x C) of eight comparisons (Table 17). In one of these comparisons (R x C) the difference was significant (t-test, P = 5%). In this comparison, the mean number of days of the population derived from low temperature self-pollination was less than that of the population derived from normal temperature self-pollination (Table 17). None

of the comparisons of frequency distributions of days from seeding to first ripe of F_2 populations derived from the two self-pollination treatments were significant (Table 18).

As was the case in Experiment 5.1, the results obtained in this experiment indicate that gametophytic selection did not occur during either low- or normal-temperature self-pollination for days from seeding to first flower and days from seeding to first ripe fruit, even though the numbers of seeds per fruit were reduced after self-pollination at low temperature.

Experiment 5.3.

Percent fruit set and mean number of seeds per fruit following self-pollination are summarized in Table 20. In nine of the eleven hybrids, percent fruit set was lower following low-temperature self-pollination in comparison to the normal-temperature self-pollination treatment (Table 20). Fewer seeds per fruit were obtained following self-pollination under low temperature than following self-pollination under normal temperature in all eleven hybrids. These results are in general agreement with those of Experiments 5.1 and 5.2, even though low-temperature self-pollination, while reducing the number of seeds per fruit, did not significantly affect percent fruit set (Wilcoxon's Signed Rank Test, P = 5%). The differences in statistical results among Experiments 5.1, 5.2, and 5.3, with respect to

Table 20. Percent fruit set and mean seeds per fruit after self-pollination at normal (25 C day / 20 C night) or low (15 C day / 10 C night) temperature in Experiment 5.3.

Hybrid[1]	Temperature (C)	No. flowers pollinated	No. fruit set	Percent fruit set[2]	No. seeds	Seeds per fruit
G x R	25/20	19	18	94.7	1,955	108.6
"	15/10	23	22	95.7	1,087	49.4
G x C	25/20	16	15	93.8	1,142	76.1
"	15/10	18	15	83.3	564	37.6
G x H	25/20	14	14	100.0	1,120	80.0
"	15/10	20	13	65.0	156	12.0
R x G	25/20	24	22	91.7	1,841	83.7
"	15/10	23	14	60.9	190	13.6
R x C	25/20	11	9	81.8	510	56.6
"	15/10	12	10	83.3	333	33.3
R x H	25/20	15	7	46.7	483	69.0
"	15/10	19	8	42.1	170	21.3
C x G	25/20	20	18	90.0	1,316	73.1
"	15/10	27	13	48.1	407	31.3
C x R	25/20	13	12	92.3	1,313	109.4
"	15/10	15	7	46.7	119	17.0
C x H	25/20	9	2	22.2	109	54.5
"	15/10	8	0	0.0	0	0.0
H x R	25/20	13	10	76.9	818	81.8
"	15/10	16	4	25.0	49	12.3
H x C	25/20	11	7	63.6	221	31.6
"	15/10	13	1	7.7	17	17.0

[1] Parental cultivars are designated as follows: Gemstate (G); Rocket (R); Crimsonvee (C); and Harvestvee (H).

[2] Percent fruit set equals the number of fruit set divided by the number of flowers pollinated multiplied by 100.

the affect of pollination temperature of percent fruit set and number of seeds per fruit, are probably due to inherent limitations of Wilcoxon's Signed Rank Test. As the number of comparisons increases above ten, the efficiency of the test increases (Snedecor and Cochran, 1967). The number of comparisons for each experiment, for percent fruit set or number of seeds per fruit, were eleven and in one instance only seven. When data from the three experiments were combined, low temperature self-pollination significantly reduced both percentage fruit set (number of comparisons = 33) and the number of seeds per fruit (number of comparisons = 29) (Wilcoxon's Signed Rank Test, P = 5%).

Days from seeding to first flower and days from seeding to first ripe fruit of F_2 populations derived from normal-temperature, self-pollination treatments were compared with similar genetic populations derived from the low-temperature treatment (Tables 15, 16, 17, 18). The mean numbers of days from seeding to first flower differed by one or more days for six comparisons (G x R, G x H, R x G, R x H, C x G, H x C) (Table 15). In four of these six comparisons (G x H, R x G, R x H, C x G) the differences were significant (t-test, P = 5%). In two comparisons (G x H, R x G) the means of the populations derived from low-temperature pollination were less than those of populations derived from normal-temperature self-pollinations (Table 15). When the frequency distributions of days from seeding to first flower

of F_2 populations derived from normal- and low-temperature self-pollinations were compared, four of ten comparisons (R x G, R x H, C x G, H x C) showed significant differences (Table 16). In one comparison (H x C), significance may have been caused by a large difference in the size of the populations compared (Table 15) (Steel and Torrie, 1980). The mean number of days from seeding to first ripe differed by one or more days for two (H x R, H x C) of ten comparisons (Table 17). For both comparisons, this difference was not significant (t-test, p = 5%). Only one of ten comparisons (H x C) of frequency distributions of days from seeding to first ripe of F_2 populations derived from the two self-pollination treatments was significant (Table 18). Significance of this comparison was most likely due to the difference in size of the populations compared.

The results of this experiment agree with those of Experiment 5.1 and 5.2 in that they suggest that gametophytic selection was not successful for either days from seeding to first flower or days from seeding to first ripe fruit, under low or normal pollination temperature. The results, furthermore, were not changed by the timing of the style cut after pollination or by the location of the style cut (Table 9). Although the results of these experiments seem to cast doubt on using low temperature to select early maturity components, other workers have used temperature stress during pollen development and pollen

storage successfully in gametophytic selection schemes (Mulinix and Iezzoni, 1988; Rodriquez-Garay and Barrow, 1988). Several aspects of these experiments could be modified to increase the likelihood of significant shifts in the mean of progeny generations, as for example, decreasing the time after pollination before style cutting from 72 to 48 hours to increase selection pressure; increasing the size of the F_2 populations planted in the field to improve sampling of progeny derived from the self-pollination treatments; and, fine-tuning the stress temperatures to enhance selection pressure.

Choice of parents also plays an important role. Parents should have large enough differences to achieve significant and useful population shifts (Maisonneuve et al., 1986). In this study, the differences between early and late cultivars for number of days from seeding to first flower and number of days from seeding to first ripe were 3 to 8 days (Table 15) and 14 to 23 days (Table 17), respectively. To date, the only successful gametophytic selection schemes that have been developed for tomato utilize interspecific variation (Sacher et al., 1983; Zamir et al., 1981; Zamir et al., 1982; Zamir and Vallejos, 1983; Zamir and Gadish, 1987).

Planting early in the season to increase exposure to suboptimal temperatures might have improved the separation of genetic differences in capacity to set fruit at low

ambient temperatures. The maximum and minimum daily temperatures in Durham, NH, during the duration of field Experiments 5.1 (1989), 5.2 and 5.3 (1990) are presented in Appendix 2.

The cornerstone of any gametophytic selection scheme is that the genes of the desired sporophytic character also must be expressed in the gametophytic phase (Tanksley et al., 1981) and that an appropriate selection pressure must be employed (Zamir, 1983). In this study, the gametophytic selection system was utilized to to try to select for characters that were not exclusively controlled by the sporophyte (Figure 1). A possible reason for the lack of response to gametophytic selection in Experiment 5 and the other in vivo experiments (3 and 4) is that the genes controlling days from seeding to first flower and days from seeding to first ripe fruit are not expressed in the pollen phase. Unlike the results of Maisonneuve et al. (1986), the temperature by cultivar interactions for in vitro pollen germination and tube growth observed in Experiment 1 suggested that temperature could be used to favor and select pollen with early maturity genes. But, as has been the case in muskmelon (Cucumis melo L.), effects of temperature on in vitro pollen germination and pollen tube behavior cannot always be used to predict pollen germination or pollen tube behavior in vivo (Maestro and Alvarez, 1988).

CONCLUSIONS

It is theorized that gametophytic selection can increase selection efficiency by decreasing the number of F_2 plants required to evaluate a quantitatively inherited trait, compared to the number of F_2 plants required in a sporophytic selection scheme. Furthermore, successful application of selection pressure at the gametophytic stage should shift the F_2 population mean toward the desired extreme for the trait selected. Because early maturity frequently implies flowering and pollination early in the season when temperatures are low, it seemed plausible that temperature might be used as an effective agent for applying selection pressure at the gametophytic stage. In theory, the most vigorous gametes would compete successfully within the stress imposed by low temperature. This competitive advantage might be realized as greater pollen grain germination or as more rapid pollen tube growth of "early" gametes as compared with "late" gametes.

In this study, differences for in vitro pollen germination and pollen tube growth under low and normal temperatures were related to the maturity class of the cultivar. Optimal temperatures for in vitro germination and tube growth appeared to be greater than 15 C for early cultivars and approximately 15 C for late cultivars. In vivo pollen germination percentage and rate of tube growth

under low and normal temperatures was not related to cultivar maturity class. Low temperature reduced pollen germination percentage and tube growth rate for all cultivars, regardless of maturity class. Pollen of early-maturing cultivars had no competitive advantage at either temperature. Hence, under the low- and normal-temperature regimes used in this study, in vitro pollen germination and pollen tube growth did not correspond to in vivo germination and tube growth.

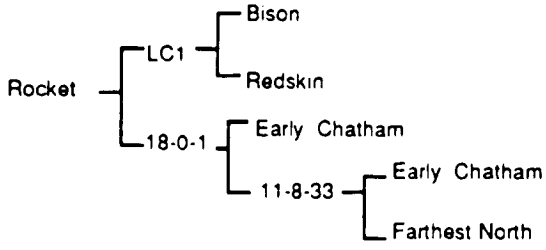
Artificial self-pollinations under low temperatures of early by early, early by late, late by early, and late by late hybrids resulted in reduced percent fruit set and number of seeds per fruit compared to similar self-pollinations under normal temperatures. However, no significant changes in population means for either number of days from seeding to first flower or number of days from seeding to first ripe were observed when F_2 progenies derived from low- and normal-temperature self-pollinations were compared in the field. Although this particular gametophytic selection scheme did not improve average maturity date, the results of this study are not conclusive evidence that the components of earliness evaluated are not gametophytically selectable under low temperature. Only after testing such modifications as (1) decreasing the time after pollination before style cutting to increase selection pressure; (2) increasing the size of plantings to improve

sampling of progeny derived from the self-pollination treatments; (3) fine tuning the stress temperatures to enhance selection pressure; (4) varying the stage of pollen development at which the stress was applied; and (5) planting earlier in the season to facilitate detection of differences in capacity to set fruit at low temperatures could low temperature be accepted or ruled out as an effective selection pressure.

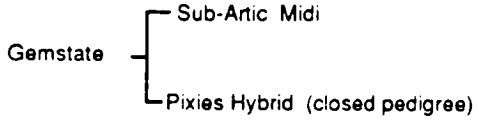
APPENDIX 1

Pedigrees of six tomato cultivars selected for study.

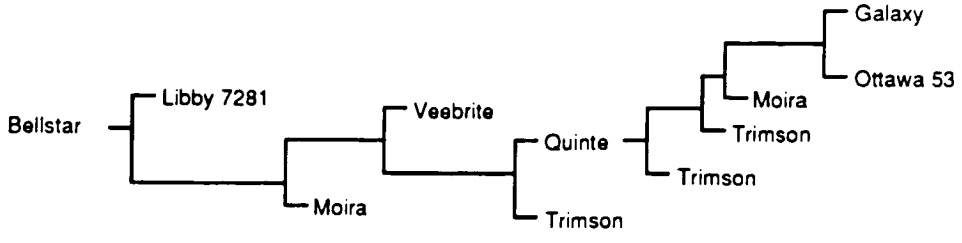
A. Rocket (Allen and Walkof, 1967).



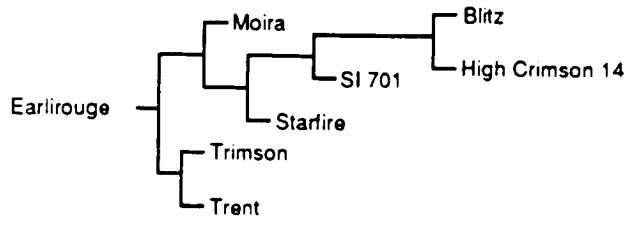
B. Gemstate (Boe, Pelofske, and Bakken, 1980).



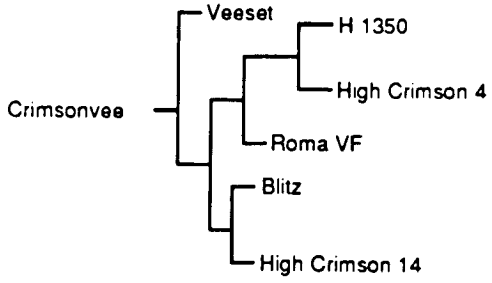
C. Bellstar (Metcalf, 1983).



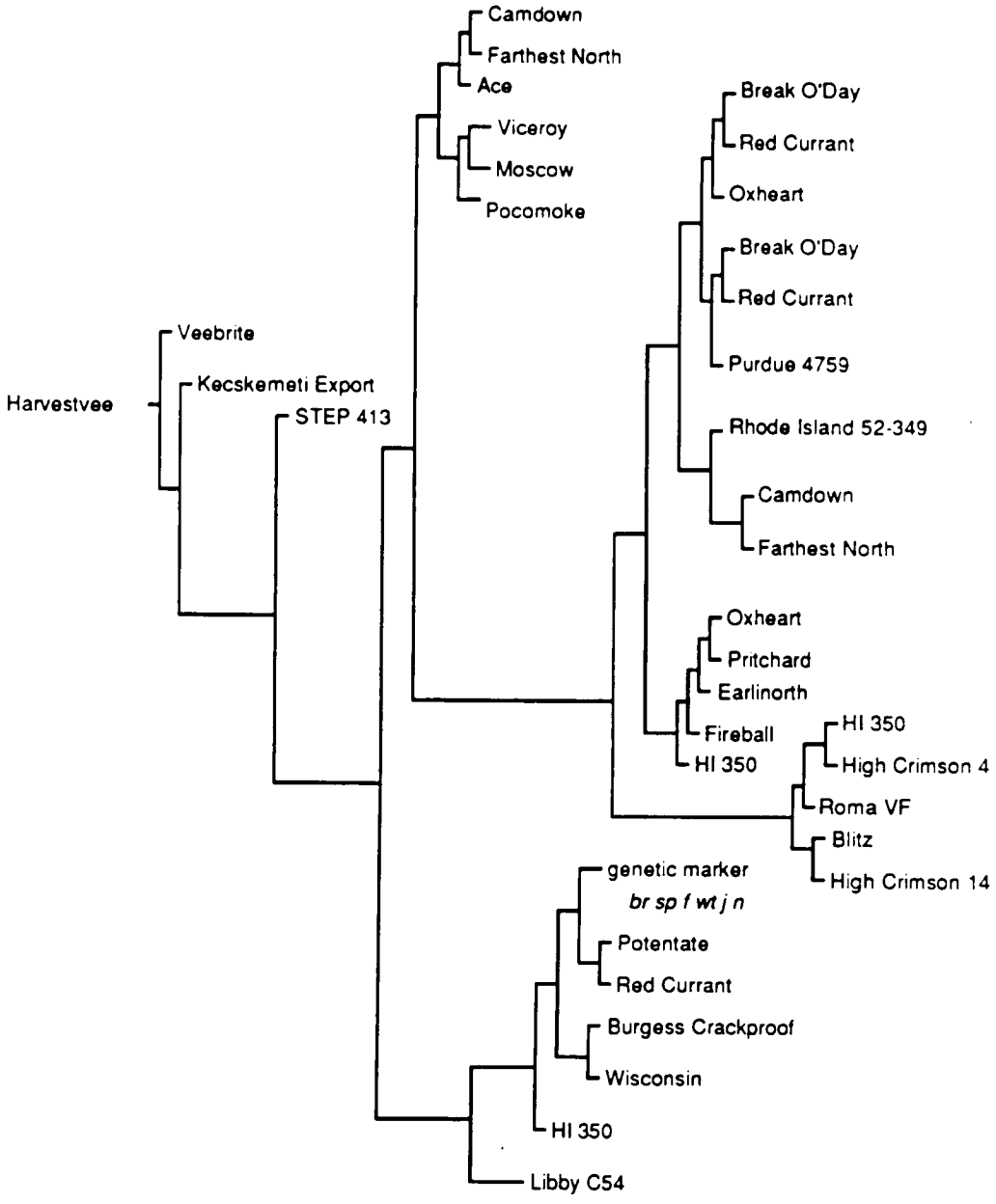
D. Earlirouge (Metcalf, 1977).



E. Crimsonvee (Kerr, 1981).

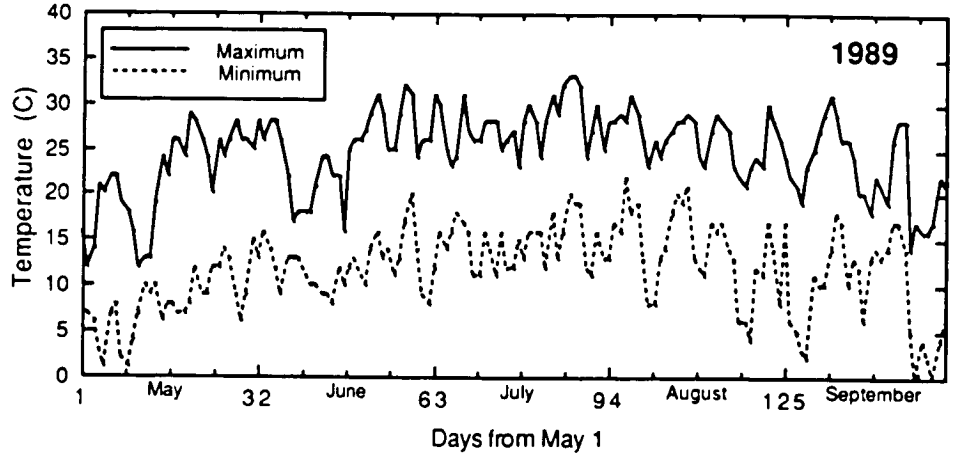


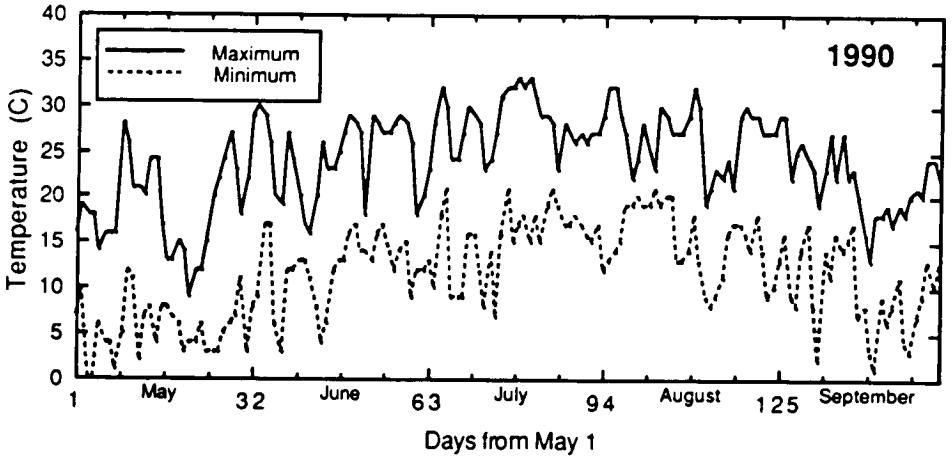
F. Harvestvee (Kerr, 1981).



APPENDIX 2

Maximum and minimum daily temperatures recorded in Durham, NH, from May to September, 1989 and from May to September, 1990. Measurements were made six feet above the ground, at a weather station 0.3 miles away from Woodman Farm.





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