SOME FACTORS AFFECTING POLLEN VIABILITY IN A TOMATO BREEDING PROGRAM.

by

WINSTON BENSON CHARLES

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN AGRICULTURE
in the Department
of
Horticulture

We accept this thesis as conforming to the
standard required from candidates for the
degree of MASTER OF SCIENCE IN AGRICULTURE

THE UNIVERSITY OF BRITISH COLUMBIA

November, 1962.

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Horticulture.

The University of British Columbia,
Vancouver 8, Canada.

Date 30/11/62.

ABSTRACT

In the tomato-growing areas in southern Canada, it is desirable to have commercial varieties having the character of being able to set fruit at relatively cool temperatures. Puck a non-commercial variety sets fruit at temperatures between 55° and 65°F. Experiments attempting to identify the mechanism of the desirable character of Puck in terms of pollen viability were done.

The tomato varieties, Puck, Bonny Best, Earliana 498, and their reciprocal crosses, were grown both in the field and in greenhouses. Various factors affecting variability in pollen samples and pollen viability determinations, were studied.

Experimental results indicated that a sample of pollen taken at anthesis consists of mature, immature, and empty grains. The relative proportions of the three classes of grains depended upon the method of collection used, the time of collection and the location of the pollen source on the plant. The greater the variability in the sample, the larger is the representative sample size required for microscopic examination at a chosen degree of tolerance. The staining technique gave the highest percentages of viable pollen. Low viability percentages were obtained *in vitro*, and these are attributed to bursting of some of the normal mature grains during pollen tube initiation. A quantitative *in vivo* procedure of counting pollen tubes by callose fluorescence under ultra violet light, was developed. Variations in the *in vivo* results can be ascribed to variation in

the concentration of pollen growth factor (PGF) which appeared to vary with the number and density of grains used in pollinations. Results showed increased germination percentages when the number of grains used was increased. The most suitable number and density for maximum group effect has not been investigated. The variety Puck gave higher viability percentages than Bonny Best in most cases. Further investigations of the problem would require more precise control of environmental factors during pollen development, and exposure of pollen following anthesis.

ACKNOWLEDGEMENTS

The writer wishes to express his thanks to Dr. C.A. Hornby, Associate Professor of Horticulture and Chairman of the thesis Committee, for supervision of the experimental work and for his valuable assistance in the preparation of the thesis.

Acknowledgement is given to the other members of the thesis Committee; Dean B.A. Eagles, Dr. V.C. Brink, Professor of Agronomy and Chairman of the Division of Plant Science; Dr. G.H. Harris, Professor of Horticulture; Dr. N.A. Mac Lean, Assistant Professor of Plant Pathology; Dr. A.J.Renney, Associate Professor of Agronomy, and Dr. G.E. Rouse, Instructor, Department of Biology and Botany, for their interest and concern with the project and preparation of the thesis.

It is wished to offer special thanks to Dr. D.P. Ormrod, Assistant Professor of Agronomy, and member of the thesis Committee for his invaluable help in the statistical analyses used in evaluating data during the preparation of the manuscript.

The writer is indebted for financial assistance during the course of the work, to the Division of Plant Science.

TABLE OF CONTENTS.

INTRODUCTION

It is desirable to have commercial varieties of tomato that will set fruit under the relatively cool temperatures which prevail in the early spring and occasionally in the early summer months in Canada. Existing commercial varieties, when grown under these conditions frequently fail to set fruit on the first and sometimes second trusses, resulting in a reduction in potential yield of early fruit. Early fruit-set in tomatoes grown in Canada is important for at least three reasons:

1. The fruit which sets in early May and throughout June brings the highest price on the early market.
2. In most of the northern tomato growing regions, the first killing frost often occurs early enough that a significant portion of the tomato crop is not harvested (Kerr, 1955, and Seelig, 1956). Early fruit-set would allow time for the ripening of a greater bulk of fruits before the first killing frost.
3. Early fruit-set would enable the extension of commercial tomato production into areas where the short season is the limiting factor at present.

The non-commercial variety Puck was found to have the character of setting fruit in England, Oregon and British Columbia, at temperatures 55° - 65°F. which are too low for commercial tomato production, (Crane, 1948, Heydecker, 1948, Frazier, 1951, and Daubeny, 1955).

Fluctuating temperature conditions in the field make it impossible to rely on identification of the low temperature

character for any given plant. Thus the breeding program requires a controlled environment to ensure identification of the desired germ plasm. In previous work, some evidence has indicated a correlation between the percentage of good pollen and the early fruit-setting character of Puck (Daubeny, 1955). If such a correlation could be established in terms of pollen viability, it would permit the rapid evaluation in greenhouses or growth chambers of crosses and breeding lines possessing the desired characteristic. This would reduce the time required to bring plants to the fruiting stage. Also, this procedure would permit the handling of larger populations for making selections, and this would hasten the breeding program for originating a new, true-breeding variety.

Daubeny (1955) and Guccione (1959) working with Bonny Best and Puck tomato pollen, reported results which showed tremendous variability in pollen viability among samples taken from each of the two varieties. Hence, further investigation of the causes which may have been responsible for the variability in pollen, has remained desirable.

In the present investigation, experiments were designed to study methods of sampling pollen along with a comparative study of the techniques used in determining pollen viability. The objective was to evaluate the suitability of the methods for use in the identification of the desired low-temperature character of Puck variety and in segregating populations.

LITERATURE REVIEW

The review of literature is reported under the following three parts: (1) sampling (2) methods used in pollen viability determination (3) use of pollen as a means of plant selection.

1. Sampling.

It is surprising to find that so little attention has been paid to sampling procedures and techniques used in making pollen counts in the literature dealing with pollen studies. Several reports dealing with pollen viability and abortion, have expressed percentages without stating the number of grains examined. Even where mentioned, the precise method of extracting samples, along with the method of selection of a microscopic field for obtaining a random sample, are not reported in detail.

(a) Microscopic field.

Few reports have mentioned how the spread of pollen grains on the slide affect the microscopic field from which counts are made.

Oberle and Goertzen (1952) used a haemacytometer for making counts in fruit varieties. Marks (1954) stated that empty grains being lighter tend to move towards the edges of the coverslip when it is lowered so that a random distribution of viable and non-viable grains is not obtained.

(b) Sample size.

It is necessary to know the minimum sample size that is required to give a true estimate of the pollen viability of a sample.

Among the several investigators who have expressed percentages of viable pollen without stating the sample size used are Sandsten (1908), Nebel (1939), Hough (1939) working with pollen from different fruit varieties, and Poole (1932) working with Crepis pollen.

The choice of sample size appears to be arbitrarily chosen in the following reports, except in the case of Flory and Tomes (1943).

Becker (1932) working with Prunus used 3,000 to 8,000 pollen grains for most plum hybrids but only 400 and 500 in certain cases. Jaranowski (1961) used several thousand pollen grains in a Melilotus interspecific hybrid to determine the percentage of normal pollen. Flory and Tomes (1943) reported from a statistical test for homogeneity that 1,000 grain samples from well-mixed pollen from many flowers on the same plant of fruit trees were sufficiently large to give a reliable varietal index. Oberle and Goertzen (1952) evaluated the number of grains per anther by suspending the contents of 100 anthers in 2.5 ml. solution containing 5 grams of calgomite to 100 ml. of water. Hence it was assumed that the contents of each anther were suspended in 0.025 ml. of solution, and one of the 4 corner squares of the counting chamber occupied 16 areas each 0.25 millimeter square.

Smith (1942) used 370-379 grains in pollen of Antirrhinum and over 1,000 grains in pollen of Bryophyllum to base his germination percentages. He emphasized that size of pollen grains determines the number of grains that would be present

in the microscopic field. Daubeny (1955) working with tomato pollen used three samples of 100 grains each, to determine percentages. Guccione (1959) working also with tomato pollen based percentages on samples of 200 grains. Dorsey (1914) used 200 grains to determine percentages in each grape variety, and five years later, without stating any reason for his choice, based counts on less than 100 grains of several plum varieties. Martin (1913) used 107-209 grains in Trifolium pratense and calculated viability percentages on the number of pollen grains that germinated. Vasil (1960 a) used for each germination test on Cucurbitaceae, random counts of 100-200 grains (in groups of 25 or more from different microscopic fields on the same slide) in order to determine the percentage germination. King (1960) working with tomato and potato pollen stated that "viability percentages were taken from an average of at least 100 grains counted in each of two areas in a pollen culture".

(c) Method of collecting pollen.

(i) Method of extraction:- Rarely does the literature state how pollen samples are extracted from flowers.

Among the several workers who have failed to state precisely how pollen samples were actually removed from flowers, are Sandsten (1908), Becker (1932), Judkins (1939), Nebel (1939), Hough (1939), Smith (1942), Oberle and Watson (1953), Kurtz and Liverman (1958), and Vasil (1960 a).

Martin (1913) working with Trifolium pratense reported that the keel of the flower was sprung with a scalpel, and as the pollen was thrown from the anthers, it was collected on the ins-

trument and spread on the medium. Anthony and Harlan (1920) dusted barley pollen from already dehisced anthers, on a slide. Poole (1932) working with Crepis species removed 6-8 new florets daily with a pair of forceps and placed them in a drop of aceto-carmine into which the grains immediately settled of their own accord. The florets were then removed and the drop sealed. Daubeny (1955) and Guccione (1959) extracted tomato pollen from freshly dehisced anthers by means of a dissecting needle. Brewbaker and Majumber (1959) transferred small samples of Petunia pollen with a dissecting needle directly from dehiscing anthers to hanging drops of 0.01 ml. stock solution (10% sucrose + 100 ppm. H_3BO_3 in deionized distilled water).

(ii) Time of collection.

Several workers used anthesis as a guide for collecting pollen samples. Sisa (1932) observed that pollen grains germinated more readily the day preceding anthesis and less readily after noon on the day of anthesis in Cucurbita species. Judkins (1939), without stating the reason for his choice, carried out pollination for in vivo studies in the tomato in the early afternoon. Flory and Tomes (1943) gathered buds just prior to anthesis when working with plum pollen. Vasil (1960 a) working with Cucurbita pollen collected pollen samples soon after dehiscence of the anthers early in the morning, and observed that collections made on a warm day, even a few hours after dehiscence, became desiccated and lost viability. Jaranowski (1961) working with the interspecific hybrid Melilotus palonica X M. alba collected samples from recently dehisced anthers.

(d) Variability of pollen at different sources.

As early as 1849 and 1869, Schleiden and Van Tieghem respectively reported that in vitro germination results varied from species to species and also from variety to variety. Valleau (1918) observed flower-to-flower variations between five strawberry flowers from a single plant which had 9.9 to 25.5 percent of aborted pollen. Further investigation showed that the percentage of pollen abortion varied from 17.09 to 50 in nine different anthers of a single flower. Brink (1924 b) reported that pollen from different flowers or even from different anthers of a flower gave variable results and suggested that differences in exposure to light may be responsible. Smith (1942) also noted anther-to-anther pollen variation in the same flower of Antirrhinum and Bryophyllum. Oberle and Goertzen (1952) reported that the number of grains per anther in fruit varieties appears to vary from year to year for the same variety of fruit, and attributed this variation to fluctuating environmental or nutritional conditions which exist during blossom development. Jaranowski (1961) also reported flower-to-flower variation in the percentage of normal grains taken from the same Melilotus hybrid. Kubo (1954), and Kurtz and Liverman (1958) also reported flower-to-flower variation and anther-to-anther variation in tomato and cocklebur.

Wanscher (1941) reported on the effect of flower position on pollen abortion in plums and showed that the percentages of stainable grains were highest in flowers at the base of the twigs, the values being gradually lower and usually reaching zero at the twig tips.

2. Methods used in pollen viability determinations.

It is apparent from the literature that great variability exists among the three methods used in pollen viability determinations.

The standard methods which have been used for determining pollen viability are:

(a) staining - examination of pollen using stain to show differences within sample,

(b) *in vitro* - the germination of pollen on artificial media,

(c) *in vivo* - the germination of pollen in natural state on flower pistil.

(a) Staining.

The staining procedures have been questioned as a valid measure of pollen viability. Johri and Vasil (1961) stated that the commonest and most reliable methods are the *in vitro* and *in vivo*. The major objection to the staining method is that the staining capacity of the contents of the pollen grains may not be indicative of viability. Leitner (1938) reported that pollen of *Lolium perenne* taken from herbarium material stained normally although known to lose viability one day after anthesis.

Several workers consider both the iodine and acetocarmine methods which are indicative of the presence of starch to be correlated with pollen viability. Daubeny (1955), and Guccione (1959) used iodine-potassium iodide and acetocarmine for determining pollen viability in tomato. Marks (1954) used acetocarmine jelly for estimating pollen viability of *Solanum* sp. Bajpai and Lal (1958), also considered pollen of fruit trees and vegetables

to be viable as long as they are stainable with acetocarmine. King (1960) used the peroxidase method and reported reliable results from the test. This method is dependent on the oxidation of benzidine by peroxidase (present in the pollen grain) in the presence of hydrogen peroxide.

Ostapenko (1956) used the acetocarmine and peroxidase methods for determining viability of pollen in apple, peach, apricot, plum and pear and regarded the methods as unreliable in determining pollen viability.

Mattson et al (1947) reported on the use of triphenyl tetrazolium chloride (T.Z.) as an indicator of viability. In the presence of viable tissue the colourless solution of 2,3,5 - triphenyl tetrazolium chloride forms the insoluble red triphenyl formazan.

Vieitzev (1952) using triphenyl tetrazolium chloride for testing pollen viability reported that the method is only applicable where the pollen possesses a thin and colourless exine. Oberle and Watson (1953) working on pollen viability of fruit trees compared this method of determination with the in vitro method and found that all varieties showed a higher percentage of viable pollen with the tetrazolium salt. It was further observed that pollen samples heated at 80°C. for 15 minutes gave negative in vitro test, but positive T.Z. reaction, but when heated at 110°C. both tests gave negative results. The results suggested that temperatures over 100°C. were necessary to inactivate the enzyme. It was then concluded that the 2,3,5 - triphenyl tetrazolium chloride is of little value as an indicator of pollen germinability for certain fruit trees.

There seems to be some controversy over the nature of the food reserves in pollen grains at functional maturity.

Maheshwari (1950) working on angiosperm pollen reported that, as functional pollen grains matured they synthesized starch. Daubeny (1955), and Guccione (1959) working on Maheshwari's assumption regarded Puck and Bonny Best tomato pollen to be viable only when they were stained a dark colour by iodine-potassium iodide solution. Lesley and Lesley (1939) used dilute iodine-potassium iodide solution to stain tomato pollen and concluded from the intensity of staining that starch is hydrolysed to sugars as functional grains matured. Iwanami (1959) reported on the disappearance of starch in pollen grains just prior to dehiscence of the anthers and considered the disappearance of starch to be due to its conversion into sugars.

(b) In vitro germination.

The literature on the in vitro technique is so extensive that no attempt is made to give a complete review, since that work has been done by Johri and Vasil (1961).

Conflicts of opinion exist on the reliability of the in vitro determination of pollen viability. Howlett (1936) reported that tomato pollen grains that appeared normal in shape and external appearance did not always germinate in vitro. King (1955) reported cases when stored potato pollen did not germinate in vitro but produced satisfactory fruit set in vivo. He stated further that failure to get in vitro germination is not necessarily an indication of dead pollen, thus the method is not a reliable prepollination estimate of the potency of pollen in the field.

Several suggestions have been made with respect to the failure of stored pollen to germinate *in vitro*. Johri and Vasil (1961) stated that the loss of certain vital systems during storage are later compensated by the stigmatic tissue at the time of germination. Nebel and Ruttle (1937) believed that desiccation, and utilization of reserve food and inactivation of the enzymes may cause failure of the metabolic processes responsible for germination.

On the contrary there are cases where stored pollen gave a moderate *in vitro* germination but a correspondingly poor fruit set *in vivo* (Sandsten, 1908; and Knowlton, 1922).

Medium

Guccione (1959) reported on choice of medium for germination of tomato pollen, and confirmed the common procedure of using 10% sucrose solution containing 30 ppm. of boron.

Temperature

Temperature effects on stored pollen have been observed in the *in vitro* procedure. The pollen of several species remains viable for longer periods at lower temperatures than at higher (Sandsten, 1908). Knowlton (1922) stored the pollen of *Antirrhinum* at five temperatures and concluded that the lower the temperature the longer the viability.

Sexsmith and Fryer (1943) found that a linear relationship existed between pollen tube growth and temperature in *Medicago sativa*.

The correlation between temperature and pollen germination in apple was found to be represented by an optimum curve (Roberts and Struckmeyer, 1948; Visser, 1955), and maximum tube length

is obtained between 20° and 30°C. in pollen of Vitis vinifera (Winkler, 1926) and Irish potato, (King and Johnston, 1958).

At sub-optimal temperatures satisfactory tube lengths can be obtained only if the period of growth is not a limiting factor. Adams (1916) reported 90-100 per cent germination in apple at 14°C. after 24 hours. Ostlind (1945) observed only 10-36 per cent germination in apple after 69 hours at 2°C., but there was a marked increase to 90-100 per cent after 119 hours.

Smith (1942) reported that the diameter of the pollen tubes in Antirrhinum majus and Bryophyllum daegremontianum increases with an increase in temperature.

Sutton and Wilcox (1912) found that the optimum temperature required for tomato pollen germination was 93°F.

Guccione (1959) found that the optimum temperature required for the in vitro germination of tomato varieties of Puck and Bonny Best pollen was 20°C.

Irrespective of temperature the growth curve of the tube remains sigmoid (Vasil, 1958, a; Vasil and Bose, 1959).

Bursting

A drawback to the in vitro procedure is the bursting of pollen grains and tubes after placement in the media.

Brink (1924 a) commented on the role of sugar as an osmotic agent and stated that "A source of no little annoyance and perplexity to those who have attempted to cultivate pollen artificially has been the bursting of pollen and pollen tubes".

There are several conflicting reports on the cause of bursting. Van Tieghem (1869), Molisch (1893), and Lidfors (1896) have stated that bursting cannot be due to an osmotic phenomenon. Lloyd (1918), Anthony and Harlan (1920), Brink

(1924 c) and Vasil (1958 a,b; 1960 a) attributed bursting to be due to an osmotic phenomenon, and showed experimentally that bursting of the pollen of Cucumis melo var. utilissimus and Mormordica charantia invariably decreased with an increase in the osmotic concentration of the medium.

(c) In vivo germination of pollen.

In vivo procedures have been very difficult to use and have depended on killing, sectioning, and staining material (Smith and Cochran 1935). Procedures, like those used by Buchholtz (1931), Chandler (1931), Nebel (1931) and, Pandey and Henry (1959), are successful in plants having large pistils and relatively large pollen tubes. Linskens and Esser (1957) and Martin (1959) have reported tracing pollen tube extension down the styles of tomato and petunia respectively by fluorescent stain. The method enabled them to examine small pollen grains, and the tiny slender tubes in the small pistils by means of the aniline blue dye resulting in fluorescent staining of callose tissue under ultra violet light. They used the procedure for descriptive and qualitative work only.

Collose plugs.

It has been observed that as the pollen tube elongates in vitro certain plug-like structures are formed at regular intervals along its length and divide into small segments. These plugs are made up of callose, a substance very similar to cellulose (Currier, 1957).

Callose is a polyglucoside derived from glucose but its exact chemical nature is still uncertain. Its formation depends

on the limited capacity of pollen tubes to utilize the glucopyranose component of the metabolised sucrose during respiration (Tupý, 1960). This explains the increased accumulation of callose in incompatible and slow growing tubes (Tupý, 1959; Vasil, 1960 a).

Brink (1924 d) and Iwanami (1959) have demonstrated experimentally that the tips of pollen tubes cut off from the older portions by callose plugs, are capable of independent growth, and that the plugs give mechanical strength to the tube and restricts protoplasmic streaming to the actively growing region. Group effect or Pollen Growth Factor (PGF).

Several reports have shown that the aggregation of pollen grains in culture media has a marked stimulatory effect on number of grains germinating and on the ultimate length of tubes. Brink (1924 d), Kuhn (1938), Visser (1955), Savelli and Caruso (1940) have demonstrated convincingly "the grouping effect" on the increase of germination percentage and pollen tube growth.

Visser (1955) suggested that the substance with the "germination promotion" properties which diffuses from the grains is neither boric acid nor an enzyme but perhaps a glucoside.

Brewbaker and Majumber (1959) have shown that the pollen growth factor (PGF), was dialyzable, insoluble in ether, relatively heat stable, and was not replaceable by kinetin or auxin. It was suggested that lack of pollen tube growth in incompatible styles is due to insufficient (PGF).

3. Use of pollen as a means of plant selection.

The use of a pollen character as a basis for plant selection

to separate plants of different genotypes has not been used with much success.

Oberle and Goertzen (1952) stressed that unfavourable environmental or nutritional conditions may account for unfavourable blossom development and some of the variation in number of normal grains per anther which may vary from year to year for the same variety of fruit.

Bennett (1959) used artificial pollen germination of Dallis grass to evaluate differences between high and low fertility lines, but found the method to be unreliable as a basis of selection for improved seed production.

Attempts to use pollen size as an indicator of polyploidy or as a taxonomic characteristic, have failed due to the variations in pollen size brought about by the environmental factors. Schoch - Bodmer (1940) reported that a positive correlation existed between available moisture and pollen size in Lythrum salicaria. Bell (1959) showed that mineral nutrition affects the amount of variability of pollen size. He observed that the largest pollen size obtained in tomato, came from plants treated with nitrogen.

Blanco (1951) has reported that in maize a correlation has been found between pollen size and combining ability.

No doubt the limited usefulness of pollen as a selection character has been limited by the variation produced by environmental conditions.

MATERIALS AND METHODS.

A. Materials.

The plant materials used in the experiments consisted of the tomato varieties, Puck, Bonny Best, Earliana 498 and their reciprocal crosses.

Puck.

Puck was introduced in England in 1948 by M.B. Crane (1948). He described it as a dwarf bush variety that produced an early and a heavy crop. Heydecker (1948) described the fruit of Puck as medium in size and possessing the uniform colour gene. The fruit wall is differentiated from the placenta and at maturity lacks the fleshy character of commercial varieties. As reported by Daubeny (1955), the variety requires a greater number of days between blossoming and ripening than some of the commercial varieties grown in British Columbia. The leaves are dark green, thick, with characteristic roughness of surface. The plant has a determinate growth habit which is synonymous with self-pruning and is controlled by a single recessive gene sp. located in the sixth chromosome and belongs to the fourth linkage group (Yeager, 1927; Rick and Butler, 1956). The stem is woody, and is strong enough to bear the weight of the fruits in the early period of growth. The main stem terminates in a leaf and carries a double inflorescence in the last internode. Axillary side-shoots develop, and are determinate in growth habit. These axillary shoots carry single clusters but in the last internode double clusters occur.

The variety was selected from the F_2 of a cross between the

American bush type variety, Victor, and an unidentified dwarf type variety released from Russia, at the end of World War II.

The variety has the outstanding feature of setting fruit at temperatures between 55° and 65°F., (Frazier, 1951, Smith and Cochran, 1935), which makes it a valuable source of germ plasm for improving commercial varieties so as to increase present day commercial tomato production.

Bonny Best.

Bonny Best was developed from a single plant selection of Chalk's Early Jewel, (Boswell, 1933). It is an old and well-known commercial variety. It has been extensively cultivated as a second early variety on the North American continent since the early part of this century subsequent to its introduction to Philadelphia by the firm of Johnson and Stokes.

Boswell (1933) observed that the fruit set of Bonny Best was considerably delayed under low temperature conditions. The variety has fleshy, coloured fruits, medium in size, and slightly oblong in shape. The plant has indeterminate growth habit and medium vine growth. There are four or five flowers per cluster, with two or three fruits being set per cluster.

Although there are earlier commercial varieties, Bonny Best was chosen in preference to others since more is known about the effect of temperature on its pollen germination.

Earliana.

Earliana was one of the earliest important tomato varieties grown in British Columbia. The number of flowers per cluster is seven to nine with normally three or four fruits being set per

cluster (Boswell, 1933). This variety is regarded as a popular early commercial tomato variety and thus was chosen for comparison with Puck. The strain of Earliana used in this work was Morse's Special 498 and will subsequently be referred to as 498.

Hybrids.

The hybrids used in this work are listed as follows, showing the maternal parent first. The list of crosses shows corresponding symbols which will be used for future reference.

1.	(Bonny Best x Puck) F_1	(BxP) F_1
2.	(Puck x Bonny Best) F_1	(PxB) F_1
3.	(Bonny Best x Puck) F_2	(BxP) F_2
4.	(Puck x Bonny Best) F_2	(PxB) F_2
5.	(Bonny Best x Puck) F_3	(BxP) F_3
6.	(Puck x Bonny Best) F_3	(PxB) F_3
7.	(Puck x Earliana 498) F_3	(Px498) F_3

B. Methods.

1. Culture of plants.

The experiments were conducted both in the field and greenhouses of the Division of Plant Science at the University of British Columbia, 1960 - 1962.

Six lots of plants were grown.

Lot 1. Seeds of Puck and Bonny Best were sown October 20, 1960, and plants were grown in two benches in both warm (60°-70°F.) and cool (50°-60°F.) greenhouses.

Lot 2. Seeds of Puck, Bonny Best, Earliana 498 along with their reciprocal crosses were sown April 21, 1961, and plants were grown in the field during the spring and summer. This was an excellent season for the tomato crop and

plants were in a vigorous state all the time.

Lot 3. Seeds of Puck, Bonny Best, Earliana 498 along with their reciprocal crosses were sown August 20, 1961 and plants were grown in the south benches of the warm and cool greenhouses.

Lot 4. A set of plants similar to those in lot 3 was started October 10th, 1961 and were grown in the benches on the northern sides in both warm and cool greenhouses.

Lot 5. Plants of Bonny Best and Puck were started March 10, 1962 and were grown in the southern benches in both houses.

Lot 6. A set of plants similar to those in lot 5 was started April 16, 1962, and was planted in the northern benches in both houses.

The plants in lots 5 and 6 were essentially subjected to similar temperatures since the differential between cool and warm temperatures became less pronounced as the spring advanced.

In general, seedlings were pricked out two weeks after seed sowing and set in 2" x 2" veneer bands in flats. The plants were transplanted two weeks later to benches of sterilized soil in the greenhouses or to the field. In the greenhouses the experiments were planted in a simple randomized block design with 15 plants of each variety, Puck and Bonny Best, randomized within a single block. When the 10 varieties were grown the experimental design used was 3 randomized blocks with 10 treatments or single plant plots per block. All plants in benches were spaced at 20 inches apart between rows and approximately 15 inches apart within rows. In the field the experimental design consisted of 8 randomized blocks with 10 treatments per block, with single plant plots

spaced 3 feet apart within and between rows.

Prior to planting, fertilizer was applied at the rate of 800 lbs per acre of 4:10:10. The plants were sprayed periodically with 5 percent malathion to protect them from aphids and white flies in the greenhouses. In the field 5 percent D.D.T. dust was put in a circular band around each plant soon after planting to prevent cutworm damage.

The benches used for the greenhouse experiments were parallel to the north-south walls in both houses.

In both cool and warm houses, temperatures were recorded by thermographs. The temperature in the cool house was kept as closely as possible between the range of 50°F.- 60°F. and in the warm house between 60°F.- 70°F.

All seedlings were kept in the warm house until transplanting time.

During the winter 1960-61 the temperature in the cool house went above 65°F. on eleven days and lasted for a total period of 44 hours. For the greater part of the time, temperatures were between 55°F. and 60°F. In the warm house, temperatures went a little below 65°F. at night, and only five times below 60°F. for very short periods lasting between two to three hours.

During the winter 1961-62 the temperature in the cool house went above 65°F. on eighteen days and for a cumulative total period of 64 hours. For the greater part of the time, temperatures were between 55° and 60°F. In the warm house, temperatures went below 65°F. at night, and sixteen times below 60°F., for very short periods.

Supplementary lighting was used during the course of the experiments to provide extra-illumination in the morning and

evening under the seasonal conditions of low light intensity and short photoperiod. An eighteen hour photoperiod was then ensured.

All plants except those grown in the field, were pruned to a single stem and staked.

Pollen samples were taken from flowers, which were marked prior to opening, when stamens had reached full anthesis, except in experiment 4, on methods of pollen collection. Sampling was done on the second day after the flower opened.

Pollen samples were examined and grains counted under the microscope using a magnification of 100X. Counts were made on the total number of grains (which usually range from between 300-400 grains), occupying the 24 squares of the ocular micrometer. It was not possible to compare pollen viability of the two varieties, Puck and Bonny Best under cool house conditions because of lack of Bonny Best pollen. When pollen was produced by Bonny Best in the cool house, there was no guarantee that the pollen was formed within the experimental temperature range of 55^{o}-65^{o}F. since short exposures to high temperatures during sunny days in the winter months were adequate to cause pollen development in the variety.

2. Laboratory methods.

(1) Staining.

After evaluating the four staining methods, namely the aceto-carmine, the peroxidase test, tetrazolium salt, and standard iodine-potassium iodide (I-KI), reported in the literature for determining pollen viability, only the iodine-potassium iodide procedure was satisfactory for the work after it had been modified. Daubeny (1955)

and Guccione (1959) reported on the customary use of I-KI solution in which darkly stained grains were considered viable.

Experiments were carried out dealing with the concentration of I-KI solution and stages of maturity of pollen. Pollen samples stained with dilute I-KI (one drop of a 1% solution of I-KI in 10cc of water) showed different staining intensities for the 3 flower stages. In vitro and in vivo experiments were done using mature and immature pollen samples to confirm the results of the staining. As the staining experiments were conducted, pollen was classified as (1) viable - large and lightly stained grains (2) immature - darkly stained grains and (3) non viable - empty grains which were unstained.

(2) In vitro germination.

Considering Howlett's (1936) report that tomato pollen grains which appear normal in shape and external appearance are often non-viable, it was considered desirable to compare the germination percentages of pollen in vitro with those obtained from staining. As a result in vitro experiments were run concurrently with the staining experiments in several cases throughout the work by using subsamples from samples for both pollen viability determinations.

The medium used for germinating pollen grains contained 10% sugar, 0.5% agar, and 30 ppm. boron, (Guccione, 1959).

A small drop of medium was placed on a slide at room temperature, and the sample of pollen was stirred into the drop. The slide was then inverted over well-slides containing two drops of water. The slide was then incubated in an oven at a temperature of 20°C. On one occasion in the summer of 1962 germination was poor due to high temperatures of 24°C. in the laboratory. Sub-

sequently by placing a beaker of water in the oven, germination percentages were back to normal. It is likely that the increase in humidity at the higher temperature, or that a lowering of temperature created the favourable conditions for germination. After eleven hours, counts were made of the three classes: (1) viable (2) burst and (3) empty grains.

(3) In vivo germination.

The determination of pollen viability in vivo is a problem in plants with a relatively small stigma, e.g. the tomato. Several attempts were made to observe pollen germinating and traversing the style by staining. None of the methods used by Buchholtz (1931), Chandler (1931) etc., proved successfull. Martin's (1959) qualitative procedure of observing pollen tubes by fluorescence was adapted, and yielded results.

Several preliminary experiments were carried out to test the suitability of Martin's method for giving a quantitative expression of pollen viability in vivo. The difficulties encountered during the course of the early investigation were as follows.

1. Finding a procedure to have fluorescent streaks of clustered bands of germinated pollen grains distinct enough to count accurately under the dissecting microscope.

2. The plants on which preliminary in vivo germination studies were carried out were in a declining state of vigour and the pollen samples had extreme variability in the amount of functional pollen. More uniform germination results were obtained when flowers from vigorous plants of Puck and Bonny Best had come into flower.

3. It was difficult to arrive at an appropriate number of grains

necessary to give a germination percentage on the stigma because of the pollen group effect.

The procedure developed was as follows. Flowers were emasculated and tagged before the petals were reflexed, and this operation was done with a pair of tweezers, so that the anthers were removed as a cone.

Pollen grains were counted under a dissecting microscope, picked up by means of a single camel hair, and transferred to the stigmatic surfaces of the emasculated flowers at the time when the petals became reflexed and fluid was noticeable on the stigmatic surface. Care was taken to place the grains in the areas where there was an accumulation of stigmatic fluid. These areas occurred in small pockets on the stigma. After pollen was transferred to the stigmatic surface, the camel hair was inspected under the microscope. If pollen grains persisted the camel hair was passed along the stigmatic surface repeatedly until free of grains. Pollen grains were allowed to germinate for 48 hours, after which the stigmas were removed. The styles were fixed in formalin-acetic-acid- 80% alcohol (1:1:8) for 48 hours, the time being double that used by Martin (1959) so as to attain satisfactory penetration. The styles were washed for 1.5 hours then softened and cleared in a strong 8N sodium hydroxide solution for 24 hours. They were then washed again for 2 hours and stained with a 0.1% solution of water soluble aniline blue dye dissolved in 0.1N K_3PO_4. Stain was allowed to penetrate over a 4 hour period. Then styles were smeared by pressing them between a coverslip and a microscope slide, and examined under a dissecting microscope of 40X, using direct illumination with ultraviolet light

of a wavelength of about 356 mμ. Counts were made in a dark room of the number of grains which had germinated. Under these conditions the callose in germinated pollen tubes appeared fluorescent with a bright-yellow-green colour and contrasted strongly with the bluish or greyish colour of the stylar tissue. The germinated pollen tubes were outlined by a callose lining and irregularly spaced callose plugs.

3. Experiments on sampling.

Experiments on sampling pollen were conducted to ascertain (1) the number of pollen grains which must be examined to give a reliable estimate of pollen viability, (2) the variability between methods of extracting or collecting pollen for examination and (3) the variability between pollen from different sources. Both the staining and in vitro germination of pollen were employed for viability determinations.

(a) Sample size.

Experiment 1.

The experiment was conducted to determine the number of grains necessary to give a reliable estimate of the pollen viability of a sample. Pollen samples were collected in small glass vials by means of a pollen vibrator, using each of three single flowers from two plants of Bonny Best variety. Each sample was taken on a different date, namely March 3, 1962, May 5, and May 7, 1962. From these three samples the pollen viability of ten subsamples per sample was examined using the staining procedure. Results were obtained by counting (1) the number of grains occupying the 24 squares on a graded occular micrometer (Method A); and (2) in the same microscopic field for the same subsample, 100 grains were counted in squares taken at random in the microscopic

field (Method B). The data were converted to percentages. Only data on mature pollen grains were analyzed statistically.

Sample 1 used in the experiment was taken from a flower on a truss of a Bonny Best plant of lot 4, which was approximately five months old, and already laden with fruit. The vigour of the plant for the production of new flowers was low.

Samples 2 and 3 were taken from single flowers from the first and second clusters respectively on the same three-month-old Bonny Best plant of lot 5. The plant was in a relatively high state of vigour. Results are presented in table 1.

(b) Methods of pollen collection.

It was realized that a pollen sample taken from a fully reflexed tomato flower would not contain grains of equal and uniform maturity, but would consist of pollen at different stages of maturity which could be distinguished by the intensity of staining. It was decided to study further the maturity of pollen as it affected viability measurements, especially since it was found experimentally that immature pollen does not germinate in vitro. Hence age of pollen or stage of maturity was considered a major source of variation in pollen viability determinations.

The methods of pollen collection were: (1), the effect of different methods of pollen extraction on the percentage of mature and immature pollen in samples; and (2), the effect of time of sampling on the degree of maturity and viability of pollen grains in a sample.

(i) Methods of pollen extraction.

Experiment 1.

The experiment was conducted to observe the effect of two methods of collection of pollen on maturity and viability in two varieties, Puck and Bonny Best. Pollen viability determinations were started December 1, 1961, on plants in lot 1, using the staining and *in vitro* procedures concurrently.

In the first method, a dissecting needle was used for collecting pollen samples. The point of the needle was inserted into the anther and the needle was then moved along the anther groove on one side of the stamen. Each of four stamens from the same flower of truss 1 was examined, and one plant of each variety was sampled. Thus 2 flowers were used to provide 8 stamens for pollen samples.

In the second method, a vibrator was used to collect pollen samples in glass vials. Each sample was taken from a single flower. Two flowers on truss 1, and two on truss 2 were used from each variety. Thus 8 flowers were used to provide 8 samples.

Three subsamples per sample were examined on the second day of anthesis. The plants were young and vigorous in appearance. Results are presented in table 3.

Experiment 2.

The objectives of the experiment were identical with those of experiment 1, except that the effects of the two methods of extraction were studied on the ten varieties of plants in lot 1. The needle method of extraction was modified by taking pollen samples from the surface of a single stamen of a single flower of each variety since it was thought that collection from the surface would give a more uniform sample of mature grains than

extraction from the interior. The experiment was started on January 5, 1961.

The plants at the time of sampling were advanced in age and laden with fruits. Results are presented in table 4.

Experiment 3.

A preliminary experiment was conducted to test further the differences in pollen grain maturity. Samples were obtained from 3 stamens per flower of each of the two varieties, Puck and Bonny Best. Sampling was performed by: (1), passing the needle in the interior of the anther along one side of the anther groove; and (2), by passing the needle over the surface of the anther; and (3), by passing the anther through water to suspend the pollen. Five subsamples per anther for each method of extraction were examined for one flower of each of Puck and Bonny Best plants of lot 3. Pollen viability was determined by staining. The experiment was begun on December 20, 1961. Results are presented in table 5.

Experiment 4.

A further experiment was conducted to obtain information on the percentage of immature grains associated with ten subsamples from each pollen sample derived from each of four methods of collection. The four methods used for obtaining pollen samples were:

1. from a single flower by means of the pollen vibrator;
2. from a single stamen of a flower by passing the needle over the surface;
3. from a single flower by tapping it over a drop of stain on the slide;

4. from each of 5 stamens of a single flower, by passing each stamen twice through water.

Viability determinations were made by the staining technique. The flowers used were obtained by taking one flower from the first truss on each of four healthy Puck plants of lot 5, which were young and vigorous. The experiment was begun on May 10, 1962. Results are presented in table 6.

(ii) Time of pollen collection in relation to age of flower.

(1) Pollen maturity at different bud and flower stages. Experiment 5.

It was considered desirable to study the effect of stage of maturity of buds and flowers on the maturity of pollen grains. Clusters were chosen on plants in lot 2, to provide four flowers or buds at different stages of maturity on each cluster, namely
(1) a flower with petals fully reflexed and at full anthesis,
(2) a bud beginning to open, with sepals starting to spread,
(3) a bud at an earlier stage than number 2,
(4) a very young bud at an earlier stage than number 3.

Such clusters were chosen on a single plant of each of ten varieties, and there were two replications. Thus eighty buds or flowers were used in the experiment. All pollen samples were taken by inserting a dissecting needle into anthers on July 7, 1961. Maturity and viability were determined by the staining procedure.

All flowers were on second trusses of selected plants in the two blocks.

Results are presented in table 7.

(2) Pollen viability as affected by longevity of the flower.

Experiment 6.

After considering the effect of immature pollen on the viability results in samples taken from flowers at different ages on the same cluster, it was considered desirable to obtain information on pollen maturity and viability in flowers over the life period beginning at full anthesis, which occurred on the second day after opening, to the time petals wilted. This span was five days under the conditions for the experiment.

Previous workers (Daubeny, 1955, and Guccione, 1959), used the fully reflexed flower as a guide to time of pollen sampling. However, a fully reflexed flower could cover a period of probably 5 days and variability in pollen viability could be a function of this time. It was considered desirable to check this assumption.

A single flower from each of ten varieties in plants of lot 2, was selected, and one stamen per flower was examined on each of five successive days. Thus five of the six stamens in each flower were sampled. Each stamen had to be removed before extracting a pollen sample, resulting in a daily removal from each flower. Sampling was started on August 8, 1961, and the field-grown plants were very vigorous. Removal of anthers was a delicate operation, but there was no apparent damage to remaining anthers and other floral parts. Results are presented in table 8.

(c) Variation due to differences in location of pollen sources on plants.

The inconsistent results obtained by Daubeny (1955), and Guccione (1959), on pollen viability determinations in tomato pollen suggested variability in pollen samples. No mention was made in these reports of the location of the flowers on the plant from which pollen samples were obtained. It was desirable to check more carefully the variability in pollen viability which may have resulted from:

(i) different zones of the same stamen;
(ii) different stamens of the same flower;
(iii) different flowers on the same truss and;
(iv) different trusses on the same plant.

(i) Pollen viability at different zones of the same stamen. Experiment 1.

The experiment was designed to determine the viability of pollen of the two varieties, Puck and Bonny Best, at three zones, namely, tip, middle, and base, of each of three relatively long stamens. Pollen samples were taken by means of an inserted needle, and pollen viability determinations were made by staining and <u>in vitro</u> techniques. One flower from a plant of each of the two varieties in lot 4, was used. Three stamens from each flower were divided into zones and three subsamples were examined per zone. The experiments were started January 17, 1962, on flowers on the third trusses of the varieties. Results are presented in table 9.

Experiment 2.

The experiment was started July 15, 1961. The objectives

were the same as experiment 1, except that a single stamen from a single flower of the 10 varieties of plants in lot 2, was used. Results are presented in table 10.

(ii) Pollen viability of different stamens of the same flower.

Experiment 3.

The experiment was intended to determine the pollen viability of six stamens of each of four flowers of Puck. Four pollen samples were obtained by the needle method of extraction from the interior of the stamen. Pollen viability was determined by staining and in vitro methods. Samples were taken on July 13, 1961, from flowers on the first truss of four Puck plants of lot 2. The plants were young and vigorous. Results are presented in table 11.

(iii) Pollen viability of different flowers on the same truss.

Experiment 4.

The experiment was designed to determine the pollen viability of different flowers on the same truss. The samples were three flowers from one truss per plant, on three plants from each of two varieties in two blocks. Thus a single pollen sample was taken from each of 36 flowers by means of the vibrator, and each sample was subdivided to give 3 subsamples and each of the three subsamples was stained and cultured in vitro.

Pollen samples were taken on January 23, 1961, from flowers on each of the two varieties, Puck and Bonny Best in lot 1. Results are presented in table 12.

(iv) Pollen viability of different trusses on the same plant.

Experiment 5.

The experiment was designed to determine differences in pollen viability among each of 3 trusses of Puck. Samples were collected on June 28, 1961, from truss 1, on June 30, 1961 from truss 2, and July 4, 1961 from truss 3, on the same plant of lot 2. Four flowers per truss were used and one sample was collected from each of the 4 flowers by the vibrator. Pollen viability was determined on 3 subsamples per sample by the staining technique.

The plant was young and vigorous at the time sampling was done. Results are presented in table 13.

4. Comparison of methods of pollen viability determinations.

It was considered imperative to study more closely the variations in pollen viability which may be attributed to the different methods used in measuring this viability namely, the staining, the in vitro and in vivo techniques.

Throughout the course of the research both the staining and in vitro methods of pollen viability determinations were used and their values compared.

The experiments on the comparison of methods were conducted in three parts.

(a) Comparison of the staining and in vitro techniques.

(b) Experiments on in vivo germination.

(c) Comparison of staining, in vitro and in vivo techniques.

(a) Comparison of staining and *in vitro* techniques.

Experiment 1.

The experiment was designed to compare the techniques of staining and *in vitro* germination for revealing the pollen viability of ten varieties. A single flower at full anthesis from each of the 10 varieties of plants of lot 3, was used. Pollen samples were collected on November 24, 1961, with the vibrator. Six subsamples per sample were used for pollen viability determinations. The plants were young and vigorous. Results are presented in table 14.

(b) Experiments on *in vivo* germination.

In the previous experiments the staining and *in vitro* determinations of pollen viability gave widely differing results. These differences raise the question as to which method most closely reveals the potency of pollen under natural conditions. Consequently, it was desirable to investigate the relationship between pollen viability determinations made *in vivo* (under natural conditions) with those determined artificially, namely the staining and *in vitro* techniques, using the same pollen sample for the three methods.

Experiment 1.

The extension of pollen tubes in tomato styles was clearly visible *in vivo* when Martin's (1959) fluorescent technique was employed. A mass of pollen was used for pollination. However, the resulting thick fluorescent bands of aggregated tubes, made it impracticable to count the number of grains which had germinated in the first samples examined.

The first modification towards the development of a quantitative procedure was to decrease the sample size used in previous pollinations. In a preliminary experiment, an arbitrary number of 10 grains was used to pollinate each of six stigmas of each of the varieties, Puck and Bonny Best. The experiment was done on plants of lot 4, and was started on March 7, 1962. These plants were in a relatively low state of vigour.

Experiment 2.

The fact that no germination was observed when 10 grains were used for the in vivo germination in experiment 1, necessitated repeating the experiment using an increased number of grains. An arbitrary number of 100 grains was used to pollinate each of five stigmas of each of the varieties, Puck and Bonny Best, plants of lot 4, and the plants were in a low state of vigour. The experiment was started on March 12, 1962. Results are presented in table 15.

Experiment 3.

Having obtained successful germination in experiment 2, within limit of counting pollen tubes in the styles, an experiment was designed to study the effect of number of grains on germination on stigmas of the varieties, Puck and Bonny Best. Thus 15, 50, 100 and 200 pollen grains were applied respectively to four stigmas of each of the two varieties. Three replications were used. A total of 24 separate flowers were pollinated. The first flower on a truss was used in each case in order to employ large

vigorous buds. The experiment was begun on April 15, 1962, using plants in lot 4. Results are presented in table 16.

Experiment 4.

Lack of germination of pollen on three Bonny Best stigmas when 50 grains were used for pollinations in experiment 2, suggested the existence of a varietal difference in germination between the two varieties, Puck and Bonny Best. The indication of this varietal response was not considered conclusive because there were only 3 flowers used. Consequently an experiment was designed to investigate further the effect on in vivo germination when 50 and 100 grains were applied respectively to two stigmas of each of the two varieties. Six replications giving a total of 24 separate flowers were used. The experiment was started on April 22, 1962, using plants of lot 4. Results are presented in table 17.

Experiment 5.

The experiment was designed to investigate the effect of self- and cross-pollinations on pollen viability determinations, since it was considered desirable to see whether varietal differences in pollen and stigma could affect the percentage germination of pollen in vivo. Consequently self- and cross-pollinations were carried out on each of two stigmas respectively of each of the two varieties, Puck and Bonny Best. Two densities of pollen, namely 50 and 100 grains, were used in both self- and cross-pollinations. Two replications giving a total of 16 separate

flowers were used. The experiment was begun on April 23, 1962, using plants of lot 4. Results are presented in table 18.

The plants of lot 4, from which flowers were obtained for the previous *in vivo* experiments, were in a low state of vigour at the time the experiments were conducted. As a consequence two further experiments, 6 and 7, were carried out using flowers from young vigorous plants of lot 6, to study (1) the effect of number of grains on *in vivo* germination of pollen from each of the two varieties, Puck and Bonny Best, and (2) the effect of varietal differences of pollen and stigma on germination of pollen *in vivo*.

Experiment 6.

In this experiment 50 and 100 grains were used to pollinate each of two stigmas respectively of each of the two varieties. Twelve replications were used, giving a total of 48 separate flowers. Two stigmas of the first two flowers on truss three on each of the 24 plants of lot 6 were used. The remaining flower buds on the truss along with the first two clusters were removed from the plants. Results are presented in table 19.

Experiment 7.

Since the effects of 50 and 100 grains on germination were already investigated in experiment 5, it was desirable to find out the effect of larger numbers of grains on germination. Reciprocal cross-pollination was compared with self-pollination, using 200 grains on each of 24 stigmas of each of the two varieties,

Puck and Bonny Best. The experiment was started on July 24, 1962, using vigorous plants of lot 6. Results are presented in table 20.

(c) Comparison of the staining, in vitro and in vivo techniques used in pollen viability determinations.

Experiment 1.

The previous in vivo experiment made possible the comparison of artificial pollen viability determinations (staining and artificial germination), with natural pollen viability determination made by in vivo germination. The in vivo results from the Puck and Bonny Best self-pollinations in experiment 7, were also used for this experiment. Twelve subsamples of Puck and Bonny Best pollen used for the pollinations in the in vivo experiment 7, were used to obtain data on the staining and in vitro pollen viability determinations. The experiment was started July 24, 1962. Results are presented in table 21.

The statistical methods of analysis of data varied with the experiment. The data were analyzed as simple randomized block experiments, factorial experiments, and a combination of factorial with nested classification as outlined in Steel and Torrie (1960).

RESULTS

I. Modification of the staining procedure.

The results of staining pollen, from flowers at different stages, with dilute iodine-potassium iodide solution showed that different stages of pollen development exhibited a variation in the intensity of staining which was related to stage of maturity of the flowers.

Pollen from flowers at the very early bud stage gave little staining indicating little or no starch.

In the intermediate bud stage prior to anthesis, when the anther showed a greenish to very light yellow colour, pollen samples gave a very high intensity of staining. The staining was so intense that the typical deep blue colour was observed under the microscope. This stage obviously marks the point of greatest accumulation of starch in the pollen cell.

In pollen samples taken from flowers at anthesis the majority of pollen grains showed little to no staining which is indicative of little or no starch. Darkly stained grains similar to those found in the intermediate bud stage, were also present among the lightly-stained mature, functional pollen grains. The darkly stained grains were regarded as immature grains. Non-viable or empty grains were also present in the samples.

In vitro germination tests of these dark immature grains showed that they absorbed less water and were less dilated than the viable mature grains when the latter were placed in the media. None of these immature grains gave any sign of pollen tube initiation.

In vivo germination tests using immature grains showed no germination.

On this basis it was considered justified to use the three classes (1) mature (2) immature and (3) empty or non-viable grains in all subsequent staining of pollen samples.

II. Experiments on sampling.

(a) Sample size.

Experiment 1.

The difficulties in chosing the number of pollen grains to be examined and counted for a reliable estimate of viability is illustrated in the data of table 1.

Table 1. Percentages of mature pollen in samples examined by two procedures; A= 24 squares of the microscopic field, and B= 100 grains taken at random in squares.

Samples	Methods	Subsamples 1	2	3	4	5	6	7	8	9	10	$\bar{x}$	$\bar{x}$
1	A	69	69	60	68	69	63	67	69	61	68	663	586
	B	55	64	28	21	71	65	47	68	34	56	509	
2	A	82	85	87	89	90	95	92	91	95	92	898	874
	B	65	83	75	90	84	94	89	90	93	87	850	
3	A	74	89	82	74	94	89	91	90	83	89	855	838
	B	80	93	82	66	74	91	76	94	79	86	821	

Analysis of variance.

Source	D.F.	M.S.	F
Total	59	-	-
Treatments	5	2241.8	-
Methods (M)	1	928.0	10.82**
Samples (S)	2	4975.0	53.84**
M x S	2	215.5	2.33
Error	54	92.4	

Duncan's test.

Samples	1	3	2
$\bar{x}$	58.6	83.8	87.4

The analysis shows that the differences between the two factors, choice of counting procedure and source of samples, are each highly significant. The interaction of method x sample was non-significant which suggested that these factors acted independently. The results indicated that the method A, (counting the total number of grains in 24 squares of the ocular micrometer), gave significantly higher percentages of mature pollen than method B, (counting a total of 100 grains in squares at random in the microscopic field).

When the three sample means of 58.6, 83.8, and 87.4 were compared by Duncan's test, it was noted that the differences between the percentages of mature pollen in samples 2 and 3 were significantly greater than in sample 1. The inference is drawn that samples 2 and 3 were taken from the same population, the young vigorous plants (lot 6), whereas sample 1 was taken from another population, namely, the older plants bearing many fruits (lot 4).

Each sample was considered separately in the calculations of required sample size. The ten subsamples of sample 1 showed standard deviations of $\pm$ 3.56 and $\pm$ 17.70 for methods A and B respectively. The data from both methods of microscopic determination are comparable because their standard deviations are derived from the same number of subsamples. It should be noted that the standard deviation for method A is almost five times less than that for B. It is evident that less variability existed between subsamples in the former case than in the latter.

The number of subsamples required to give a reliable estimate

would vary with the degree of tolerance chosen. Consequently table 2, was calculated from the data of table 1 to give a range from 1-50 percent degree of tolerance at the 95% level.

Table 2. Values of n= number of subsamples, when degree of tolerance (D) ranges from 1% to 50% at 95% level, in application to data in table 1.

	Samples					
	1		2		3	
Methods	A	B	A	B	A	B
D	n	n	n	n	n	n
1	48.58	1203.39	67.40	200.78	189.06	311.52
2	12.14	300.84	16.85	50.19	47.26	77.88
3	5.39	133.35	7.48	22.28	20.98	34.57
4	3.04	75.22	4.21	12.54	11.81	19.47
5	1.94	48.14	2.69	8.03	7.56	12.46
6	1.34	33.33	1.86	5.56	5.23	8.62
7	0.99	24.55	1.37	4.09	3.85	6.35
8	0.75	18.77	1.05	3.13	2.94	4.85
9	0.59	14.80	0.83	2.47	2.33	3.84
10	0.48	12.00	0.67	2.40	1.89	3.11
11	0.44	10.09	0.61	1.82	1.71	2.83
12	0.40	10.02	0.61	0.67	1.57	2.59
24	0.20	5.01	0.28	0.83	0.78	0.12
50	0.09	0.60	0.13	0.42	0.37	0.62

The calculations were performed as follows.

If $\bar{x}$ = sample mean

μ = true mean

s = standard deviation

$s_{\bar{x}}$ = standard error of the mean

n = required number of subsamples

$t_{\alpha df} = 1.96$

$\bar{x} - \mu = p$ the chosen percent degree of tolerance.

Then $t_{\alpha} = \dfrac{\bar{x} - \mu}{s_{\bar{x}}} = \dfrac{\bar{x} - \mu}{\frac{s}{\sqrt{n}}}$

Solving for n

$$n = \left(\frac{t \times s}{\bar{x} - \mu}\right)^2$$

When $n = 1$ $\quad \bar{x} - \mu = t_{\alpha} \times s.$

Example.

Suppose the degree of tolerance was arbitrarily chosen to give a deviation about the true mean of not more than $\pm$ 5 at the 95% level. Then the calculation for sample 1 would be.

$$n \text{ for } A = \left(\frac{1.96 \times 3.56}{5}\right)^2 = 1.94$$

$$n \text{ for } B = \left(\frac{1.96 \times 17.70}{5}\right)^2 = 48.14$$

Consequently with a 5% degree of tolerance the number of subsamples to be examined for method A would be approximately 2, and for method B approximately 49.

On the basis of this formula, the degree of tolerance for the two methods of the 3 samples examined when n = 1, would be as follows.

Sample 1	A = 3.56 x 1.96	=	6.97 %	degree of tolerance
	B = 17.70 x 1.96	=	34.69 %	" " "
Sample 2	A = 4.19 x 1.96	=	8.21 %	" " "
	B = 7.23 x 1.96	=	14.17 %	" " "
Sample 3	A = 7.02 x 1.96	=	13.75 %	" " "
	B = 9.01 x 1.96	=	17.65 %	" " "

The standard deviations of the subsamples of A and B for sample 2 were $\pm$ 4.19 and $\pm$ 7.23 respectively. Once again method A showed less variation between subsamples than method B.

In sample 3, the standard deviations for A and B were $\pm$ 7.02 and $\pm$ 9.01 respectively. Again greater variability existed in the latter.

It is of interest to note that the number of grains to be counted would depend upon the degree of tolerance chosen and would be indicative of the more efficient method in saving time.

The average number of grains counted in the 24 squares per subsample in method A for samples 1, 2 and 3 was 364.6, 344.5 and 359.8 respectively. The average number of grains counted per subsample for B for the three samples was 100.

An example of the use of table 2 for computing actual pollen sample size needed for obtaining a reliable estimate of viability, is the comparison of two arbitrary choices of level of tolerance, 2% and 10% in the variability.

The number of grains required for counting by the two methods allowing a 2% tolerance is as follows.

Sample 1	A = 364.6	x	12.14	=	4,426.24	grains	to	be counted
	B = 100	x	300.84	=	30,084.00	"	"	"
Sample 2	A = 344.5	x	16.85	=	5,804.82	"	"	"
	B = 100	x	50.19	=	5,019.00	"	"	"
Sample 3	A = 359.8	x	47.26	=	17,004.14	"	"	"
	B = 100	x	77.88	=	7,788.00	"	"	"

The number of pollen grains required when a 10% tolerance was permitted would be.

Sample 1	A = 364.6	x	0.48	=	175.00	grains	to	be counted
	B = 100	x	12.00	=	1,200.00	"	"	"
Sample 2	A = 344.5	x	0.67	=	230.81	"	"	"
	B = 100	x	2.40	=	240.00	"	"	"
Sample 3	A = 359.8	x	1.89	=	680.02	"	"	"
	B = 100	x	3.11	=	311.00	"	"	"

From the calculations above it is seen that the degree of tolerance desired would determine the labour involved in counting. For example in sample 1 where 2% tolerance was allowed, using table 2, the number of subsamples required to give a reliable estimate for B was 300.84, and where the degree of tolerance was 10% the number of subsamples would be 12. This difference is

magnified when transposed to 30,084 and 1,200 pollen grains. Obviously over 28,000 more grains would need to be counted to reduce the tolerance from 10% to 2%.

By further inspection of the calculations in both cases it can be seen that for sample 1, method A is more efficient than B, since fewer grains need to be counted.

In sample 2, the values for n in both instances are higher for B than A, method B appears to be more efficient at the 2% tolerance because approximately 800 grains less need to be counted than for A. At the 10% tolerance level, both methods require almost identical numbers of grains, and thus could be considered equally efficient.

Similarly in sample 3, method B was more efficient at the 2% and 10% degree of tolerance.

The foregoing considerations of the limited data emphasize that tomato plants may be very variable in producing viable pollen. A larger number of grains was required to give a reliable estimate for the older plants of sample 1 under the growing conditions for plants of lot 4. In contrast, the young vigorous plants of lot 6 provided samples 2 and 3, which had similar high percentages of mature pollen. The former plants could be assessed by method A, counting grains in the microscopic field per subsample, whereas the latter plants could be confidently assessed using method B of choosing 2 or 3 groups of 100 grains giving a range of 200 - 300 grains.

Obviously counts of 100 grains per sample would be inadequate for these studies. Thus percentages given here are based on counts ranging between 300-400 grains per subsample.

(b) Methods of pollen collection.

Among the several sources contributing to the inaccurate estimate of pollen viability, differences in stages of maturity of pollen grains in samples could account for great variation in pollen viability determinations.

(i) Methods of extraction.

The methods employed in extracting pollen samples influence the relative amounts of immature and mature grains present in samples, as shown in the data of tables 3 to 6 inclusively.

Experiment 1.

Analysis of data in table 3, shows all interactions to be significant. The differences in pollen viability between the needle and vibrator methods of pollen collection were affected by the staining and <u>in vitro</u> techniques. Also the differences in pollen viability between the staining and <u>in vitro</u> methods varied with each of the two varieties, Puck and Bonny Best.

Inspection of the data shows consistently lower percent ages of mature pollen for the vibrator than the needle methods when the staining technique was used.

Experiment 2.

The two methods of pollen extraction were then tested on all ten varieties as shown in the data of table 4.

Analysis of data shows all interactions to be significant. The differences in pollen viability between the needle and vibrator methods of pollen collection were affected by (1) the staining and <u>in vitro</u> techniques and (2) the variation in viability results of all 10 varieties, due to genetic constitution of the tomato lines.

Table 3. Viability percentages of pollen collected by (1) needle in the interior of the anther (NI) and (2) the pollen vibrator, of Puck (P) and Bonny Best (B).

Varieties	Tests	Sub-samples	Needle Stamens 1	Needle Stamens 2	Needle Stamens 3	Needle Stamens 4	Vibrator Flowers 1	Vibrator Flowers 2	Vibrator Flowers 3	Vibrator Flowers 4
P	Staining	1	95	80	53	81	47	47	26	31
		2	75	89	67	83	47	47	33	32
		3	16	93	76	58	46	40	33	29
		$\bar{x}$	61.9	87.2	65.2	73.9	46.6	44.6	30.6	30.6
		$\bar{x}$		72.1				38.1		
	In vitro	1	59	63	78	25	32	45	28	58
		2	63	61	67	32	27	48	30	66
		3	64	61	72	56	29	41	19	56
		$\bar{x}$	61.9	61.6	72.2	37.6	29.3	44.6	25.6	59.9
		$\bar{x}$		58.3				39.9		
B	Staining	1	87	83	73	77	12	53	50	36
		2	95	79	81	77	15	52	51	31
		3	73	81	72	67	15	49	53	35
		$\bar{x}$	74.9	80.9	75.2	73.5	13.9	51.2	51.2	33.9
		$\bar{x}$		78.7				37.6		
	In vitro	1	59	66	51	57	67	64	71	63
		2	75	55	60	52	66	61	69	59
		3	73	61	54	27	55	62	75	65
		$\bar{x}$	68.9	60.6	54.9	45.2	62.6	62.2	71.5	62.2
		$\bar{x}$		57.4				64.7		

Analysis of variance.

Source	D.F.	M.S.	F
Total	95	-	-
Exp. Units	31	861.96	6.22
Treatments	7	3045.85	13.53
Methods (M)	1	11180.00	49.68**
Varieties (V)	1	1350.00	5.99*
Tests (T)	1	57.00	n.s +
M x V	1	533.00	2.36
M x T	1	5012.00	22.27**
V x T	1	1477.00	6.56*
M x V x T	1	1712.00	7.60*
Exp. Error	24	225.00	1.30
Sampling Error	64	172.22	

+ n.s. is used to denote F values of less than 1 and thus non-significant.

Table 4. Viability percentages of pollen collected by (1) the needle on the surface of the stamen (N) and (2) the vibrator (V), using a single flower of each of the two varieties, Puck (P) and Bonny Best (B), and their reciprocal crosses.

Tests		Varieties																			
		P		B		(BxP)F		(PxB)F		(BxP)F		(PxB)F		(BxP)F		(PxB)F		E 498		(Px498)F	
	Methods	N	V	N	V	N	V	N	V	N	V	N	V	N	V	N	V	N	V	N	V
Staining	Sub-samples																				
	1	63	33	70	35	79	35	78	49	79	48	65	21	71	9	31	14	79	12	31	39
	2	57	39	69	42	79	29	73	48	57	50	58	15	74	6	34	14	77	15	70	35
	3	73	66	65	30	70	27	77	47	80	51	62	22	70	6	26	16	47	14	52	41
	$\bar{x}$	643	459	679	356	759	303	759	479	719	496	616	193	716	69	303	146	676	136	509	383
	$\bar{x}$	551		518		531		619		608		404		393		224		406		446	
In vitro	1	53	55	17	33	9	21	16	20	17	42	35	34	14	8	13	26	36	19	30	17
	2	61	57	18	39	18	23	16	21	15	46	23	33	37	6	18	30	36	17	29	16
	3	61	53	19	33	21	32	16	21	22	47	33	28	46	7	15	29	43	19	23	18
	$\bar{x}$	583	549	179	349	159	253	179	206	179	449	303	316	323	69	153	283	383	183	273	169
	$\bar{x}$	566		264		206		193		314		309		196		218		283		221	

Analysis of variance.

Source	D.F.	M.S.	F
Total	119	-	-
Treatments	39	-	-
Varieties (V)	9	609.7	12.7**
Methods (M)	1	9048.0	189.2**
Tests (T)	1	11175.0	233.7**
V x M	9	932.0	19.4**
V x T	9	772.3	16.1**
M x T	1	7906.0	165.3**
V x M x T	9	154.4	3.2**
Error	80	47.8	

Inspection of the data shows consistently lower percentages of viable pollen in the staining technique for the vibrator than for the needle methods of collection. The same trend exists in the in vitro technique except in the Bonny Best variety and the $(BxP)F_1$, $(BxP)F_2$, $(PxB)F_1$, and $(PxB)F_3$ crosses.

The inconsistency in results caused by the interactions of factors in experiments 1 and 2 led to further work, and experiments 3 and 4 were done to obtain further information on methods of extraction.

Experiment 3.

Table 5. Viability percentages of pollen collected by (1) water (W), (2) the needle in the interior of the stamen (NI) and (3) the needle on the surface of the stamen (NS); viability being determined by staining.

	Puck			Bonny Best		
Methods	W	NI	NS	W	NI	NS
1	86	81	96	81	78	89
2	94	95	97	80	79	93
3	91	89	91	75	79	82
4	94	91	91	77	80	79
5	92	89	81	84	85	90
$\bar{x}$	91.4	89.0	91.2	79.4	80.2	86.6
$\bar{x}$		90.5			82.0	

Analysis of variance.

Source	D.F.	M.S.	F
Total	29	-	-
Treatments	5	-	-
Varieties (V)	1	538.0	24.9**
Methods (M)	2	52.5	2.4
M x V	2	34.0	1.5
Error	24	21.6	

Analysis of data in table 5 shows the interaction, variety x method, is non-significant. No significant differences in viability

existed between the three methods of extraction, but there was a significant difference attributed to variety. Puck had a greater percentage viable pollen than Bonny Best.

Experiment 4.

Results of experiment 3 failed to show any differences between the 3 methods of extracting pollen samples by the needle. Consequently four methods of pollen extractions were studied in experiment 4. To emphasize the effect of the methods of extraction, data are reported only on the percentages of immature pollen grains obtained in the samples.

Table 6. Percentages of immature grains of Puck using 4 methods of extraction, viability being determined by staining.

Methods	Subsamples 1	2	3	4	5	6	7	8	9	10	$\bar{x}$
Vibrator	10.0	7.0	7.0	9.0	8.0	7.0	12.0	9.0	7.0	8.0	8.4
Needle (NS)	0.5	1.2	0.5	0.4	0.5	1.5	0.3	1.2	0.3	0.3	0.6
Tapping	6.0	2.0	4.0	5.0	4.0	5.0	4.0	3.0	4.0	4.0	4.1
Water	3.0	4.0	4.0	2.0	3.0	2.0	5.0	5.0	3.0	3.0	3.4
$\bar{x}$	4.8	3.5	3.8	4.1	3.8	3.8	5.3	4.5	3.5	3.8	

Analysis of variance.

Source	D.F.	M.S.	F
Total	39	-	-
Subsamples	9	1.37	n.s
Methods	3	102.45	78.80**
Error	27	1.30	

Duncan's test.

Methods	Needle	Water	Tapping	Vibrator
$\bar{x}$	0.67	3.40	4.10	8.40

In table 6, it can be seen that highly significant differences existed between the methods of sampling. Duncan's test revealed further that the vibrator gave significantly higher percentages of immature pollen than any of the remaining three methods. No significant differences were found between the water and tapping methods,

both of which gave significantly greater percentages of immature pollen than the needle method.

Thus passing a dissecting needle on the surface of the stamen appears to be the most desirable procedure.

(ii) Time of pollen collection in relation to age of flower.

The age at which the flower is sampled can affect the percentages of immature grains in pollen samples, as shown in tables 7 and 8.

(1) Pollen maturity at different bud and flower stages.

Experiment 5.

Four flowers were sampled simultaneously, thus providing flowers in a sequence of maturity from the anthesis stage to increasingly immature buds.

The effects of this gradient in maturation are shown in table 7.

There were highly significant differences between the percentages of immature pollen in the four flowers on the same truss.

The Duncan's test showed that the first two flowers had less immature pollen than the last two which were essentially buds, and this difference was highly significant.

(2) Pollen viability as affected by longevity or life-span of the flower.

Experiment 6.

The time at which pollen samples are taken during the period the flower remains open appears to have great influence on pollen viability as shown in table 8.

Table 7. Percentages of immature grains from four flowers at different stages on the same truss of each of 10 varieties, viability determined by staining..

Block	Flowers	Varieties										
		P	B	(BxP)F	(PxB)F	(BxP)F	(PxB)F	(BxP)F	(PxB)F	498	(Px498)F	$\bar{x}$
I	1	14	12	8	9	15	7	15	9	24	9	12.2
	2	58	10	9	89	22	15	97	16	32	6	35.4
	3	99	93	64	95	99	22	99	83	77	97	82.8
	4	99	99	99	96	98	97	99	98	90	99	97.7
	$\bar{x}$	67.0	53.5	45.00	72.2	58.5	35.2	77.5	51.5	55.7	52.7	
II	1	20	21	21	16	39	32	9	13	39	25	23.5
	2	16	19	10	7	10	31	21	16	68	36	23.4
	3	25	86	97	7	95	92	86	93	98	47	72.6
	4	58	95	97	95	99	94	98	95	98	55	88.4
	$\bar{x}$	29.7	55.2	56.2	31.2	60.7	62.2	53.5	54.2	75.7	40.7	

Analysis of variance.

Source	D.F.	M.S.	F
Total	79	-	-
Blocks	1	495.0	n.s
Treatments	39	-	-
Flowers (F)	3	27331.0	48.2**
Varieties (V)	9	379.2	n.s
F x V	27	244.1	n.s
Error	39	567.0	

Duncan's test.

Flowers	1	2	3	4
$\bar{x}$	17.8	29.4	77.7	92.9

Table 8. Percentages of viable pollen from five stamens of each of 10 varieties with one stamen from each flower being examined successively over a period of 5 days.

Tests	Days	Varieties										
		P	B	(BxP)F	(PxB)F	(BxP)F	(PxB)F	(BxP)F	(PxB)F	498	(Px498)F	$\bar{x}$
Staining	1	95	85	75	85	91	81	49	65	71	86	78.3
	2	91	71	56	23	96	76	23	45	12	79	57.2
	3	81	65	45	89	83	71	65	6	29	66	60.0
	4	93	54	67	33	69	56	71	18	26	61	54.8
	5	75	61	52	67	74	33	56	7	7	64	49.6
	$\bar{x}$	87.0	66.0	59.0	59.4	82.6	63.4	52.8	28.2	29.0	71.2	
In vitro	1	69	74	49	37	66	56	65	45	41	71	57.3
	2	75	65	38	21	86	73	51	65	19	53	54.6
	3	49	31	51	5	70	23	46	15	23	61	37.4
	4	38	6	2	9	21	9	10	25	5	12	13.7
	5	51	3	11	15	50	11	12	16	6	19	19.4
	$\bar{x}$	56.4	35.8	30.2	17.4	58.6	34.4	36.8	33.2	18.8	43.2	

Analysis of variance.

Source	D.F.	M.S.	F
Treatments	99	-	-
Tests (T)	1	13806.0	91.36**
Days (D)	4	4129.5	27.32**
Varieties (V)	9	2404.1	15.91**
V x D	36	239.2	1.58
T x D	4	999.8	6.66**
T x V	9	538.6	3.56**
Error	36	151.1	

Analysis of data in table 8 shows main effects and interactions involving T (method of estimating viability) to be highly significant. The differences between staining and in vitro had effects similar to those reported in previous experiments. The differences between stamens on days of sampling a given flower were highly significant, with decreases in the amount of viable pollen associated with increasing age. Thus, it appears that variability in results of pollen counts could be due to differences in the time of collection when the flower had been opened, even if the flowers appeared to be fresh or in good condition. Maximum viability would be assured if pollen was sampled when the flower reached full anthesis, i.e second day after first reflexing of petals.

Although, all 10 varieties showed a decline in viability, there were significant differences among varieties with increasing time.

(c) Variation due to differences in location of pollen sources on plants.

Differences in location of pollen sources on the plant may account for some of the variability in pollen viability determinations.

(i) Pollen viability at different zones of the same stamen. Experiments 1 and 2.

The effect of zonal differences of pollen samples, taken from the same stamen, on pollen viability determinations, is shown in the data of tables 9 and 10.

Table 9. Pollen viability at 3 zones in the same stamen of each of 3 stamens in a single flower of each of the two varieties, Puck (P) and Bonny Best (B). Viability determined by staining and *in vitro*.

Varieties	Tests	Samples	Stamen 1			Stamen 2			Stamen 3		
			Tip	Mid	Base	Tip	Mid	Base	Tip	Mid	Base
P	Staining	1	99	97	97	99	97	96	95	97	97
		2	98	99	94	95	93	98	99	94	94
		3	89	95	97	98	96	93	98	93	96
		$\bar{x}$	95.2	96.9	95.9	97.2	95.2	95.7	97.2	94.5	95.5
		$\bar{x}$		95.1			95.1			94.9	
	In vitro	1	51	50	52	60	55	50	31	27	41
		2	56	49	31	51	56	52	58	43	45
		3	16	49	52	49	55	53	40	31	27
		$\bar{x}$	40.9	49.2	44.9	53.2	55.2	51.6	42.9	33.6	37.6
		$\bar{x}$		44.6			52.9			37.7	
B	Staining	1	86	90	77	84	78	77	91	82	85
		2	94	85	89	95	96	72	78	83	86
		3	90	79	85	89	74	81	87	94	79
		$\bar{x}$	89.9	84.5	83.5	89.2	82.5	76.5	85.2	86.2	83.2
		$\bar{x}$		85.2			82.0			84.1	
	In vitro	1	58	53	31	47	52	53	19	44	53
		2	49	52	18	36	47	43	51	26	25
		3	51	27	41	24	19	23	17	25	19
		$\bar{x}$	52.6	43.9	29.9	35.6	39.2	39.6	28.9	31.6	32.3
		$\bar{x}$		41.8			37.8			30.6	

Analysis of variance.

Source	D.F.	M.S.	F
Total	107	-	-
Rep. (Stamens)	5	718.6	11.31**
Treatments	11	-	-
Varieties (V)	1	2640.0	41.57**
Tests (T)	1	64827.0	1020.89**
Zones (Z)	2	113.0	1.77
V x T	1	59.0	n.s
V x Z	2	60.0	n.s
T x Z	2	10.0	n.s
V x T x Z	2	3.0	n.s
Error	91	63.5	

Duncan's test.

Stamens	3 B	2 B	1 B	3 P	1 P	2 P
$\bar{x}$	57.9	60.5	64.1	66.9	70.6	74.7

Analysis of data in tables 9 and 10, shows no significant differences between the viability of pollen from the three zones (tip, middle, and base) of the same stamen.

Duncan's test in table 9, revealed that significant differences existed between stamens 3 and 2, and stamens 3 and 1, in the same flower from the varieties of Puck and Bonny Best respectively. Puck gave significantly higher percentages of viable pollen than Bonny Best. The staining technique gave significantly higher and more uniform percentages of viable pollen than the *in vitro* germination.

It was not possible from the data in table 10 to differentiate between the effects of pollen viability which are due to variety and those which are due to method of viability determinations since both factors interacted.

Table 10. Pollen viability at 3 zones in one stamen of a single flower of each of ten varieties; viability determined by staining and *in vitro*.

Block	Tests	Zones	Varieties										
			P	B	(BxP)F	(PxB)F	(BxP)F	(PxB)F	(BxP)F	(PxB)F	498	(Px498)F	$\bar{x}$
I	Staining	Tip	89	78	75	96	89	47	75	87	69	77	78.2
		Mid	88	66	73	86	94	87	73	89	65	84	80.5
		Base	94	88	72	86	82	71	71	82	76	84	80.6
		$\bar{x}$	90.3	77.3	73.3	89.3	88.3	68.3	72.9	85.9	69.9	81.6	
	In vitro	Tip	49	36	69	49	67	56	77	86	39	64	59.2
		Mid	49	45	66	79	67	69	77	87	67	67	67.3
		Base	63	41	54	51	83	75	59	82	37	66	61.1
		$\bar{x}$	53.6	40.6	62.9	59.6	72.2	66.6	70.9	84.9	47.6	65.6	
II	Staining	Tip	94	69	81	89	87	38	78	86	72	87	78.1
		Mid	83	66	79	87	81	58	79	91	79	85	78.8
		Base	88	67	84	82	89	71	95	85	79	78	81.8
		$\bar{x}$	88.3	67.3	81.3	85.9	85.6	55.6	83.9	87.3	76.6	83.3	
	In vitro	Tip	59	62	42	48	78	69	78	59	67	73	63.5
		Mid	65	61	67	52	89	57	76	83	61	81	69.2
		Base	63	61	70	53	40	75	70	78	69	75	65.4
		$\bar{x}$	62.2	61.2	59.6	50.9	68.9	66.9	74.5	73.2	65.6	76.2	

Analysis of variance.

Source	D.F.	M.S.	F
Total	119		
Blocks	1	81.0	n.s
Treatments	59		
Varieties (V)	9	567.0	5.5**
Zones (Z)	2	178.0	1.7
Tests (T)	1	7099.0	69.1**
V x Z	18	72.5	n.s
V x T	9	388.0	3.7**
Z x T	2	122.5	1.2
V x Z x T	18	69.6	n.s
Error	59	102.7	

(ii) Pollen viability of different stamens of the same flower.

Experiment 3.

No variation in pollen viability was found to be due to zonal sources of pollen, hence a more detailed study of the variation in pollen viability between stamens of the same flower was made, and data are shown in table 11.

Analysis of data in table 11 shows all interactions to be significant. Hence it was not possible to evaluate the difference in pollen viability between the stamens within a flower, because the pollen viability results were affected by the variability due (1) to the staining and *in vitro* methods and (2) the four flowers from which the samples were taken. Also by inspection it is clear that there is great variation from stamen to stamen within a flower.

(iii) Pollen viability of flowers on the same truss.

Experiment 4.

The effect on viability of pollen samples taken from different flowers on the same truss, is shown in the data of table 12.

Table 11. Pollen viability percentages of six stamens from the same flower of Puck; pollen viability determined by staining and *in vitro*.

Flowers	Samples	Staining						In vitro					
		Stamens						Stamens					
		1	2	3	4	5	6	1	2	3	4	5	6
1	1	64	81	93	88	59	77	55	50	75	66	61	59
	2	57	77	95	86	54	73	43	58	65	59	80	63
	3	69	78	98	88	61	78	49	65	42	34	71	75
	4	71	82	97	83	62	71	53	72	66	71	86	61
	$\bar{x}$	65.2	79.5	95.7	86.2	59.0	74.5	50.0	61.2	62.0	57.5	74.5	64.5
	$\bar{\bar{x}}$	76.7						61.6					
2	1	48	59	81	89	95	68	33	63	19	71	34	21
	2	49	75	94	77	87	56	65	29	44	65	81	19
	3	56	75	97	78	91	51	41	45	53	49	63	35
	4	54	64	95	67	93	41	52	61	31	52	43	18
	$\bar{x}$	51.7	68.2	91.7	77.7	91.5	54.0	47.5	49.5	36.7	59.2	55.2	23.2
	$\bar{\bar{x}}$	72.4						45.2					
3	1	98	96	93	78	64	71	30	43	16	42	49	54
	2	79	95	81	69	72	68	44	61	21	37	78	46
	3	83	95	96	81	69	65	54	21	51	65	43	34
	4	95	81	94	75	71	61	46	39	33	36	55	61
	$\bar{x}$	88.7	91.7	91.0	75.7	69.0	66.2	43.5	41.0	30.2	45.0	56.2	48.7
	$\bar{\bar{x}}$	84.0						44.1					
4	1	89	69	78	91	47	85	65	45	32	68	61	45
	2	94	68	95	82	71	78	34	45	23	65	42	18
	3	87	69	82	89	86	87	36	29	61	57	19	56
	4	89	71	52	91	56	77	21	33	28	50	25	51
	$\bar{x}$	89.7	69.2	76.7	88.2	65.0	81.7	39.0	38.0	36.0	60.0	36.7	42.5
	$\bar{\bar{x}}$	78.4						42.0					

Analysis of variance.

Source	D.F.	M.S.	F
Total	191	-	-
Treatments	47	-	-
Tests (T)	1	39704.0	320.1**
Flowers (F)	3	1037.0	8.36**
Stamens (S)	5	547.8	4.41**
T x F	3	1214.3	9.79**
T x S	5	488.6	3.94**
F x S	15	610.4	4.92**
T x F x S	15	539.8	4.35**
Error	144	124.0	

Data in table 12 were not analysed statistically due to the complexity involving factorials, split-plot, subsampling and non-interpretable interactions.

Inspection of the data, however, revealed no appreciable differences in pollen viability among the three flowers examined from the same truss. The in vitro viability determinations are comparatively lower than the staining. Puck pollen showed consistently higher viability percentages than Bonny Best.

(iv) Pollen viability of different trusses of the same plant. Experiment 5.

The variation in pollen viability of different trusses on the same plant could influence the results of viability determinations as shown in table 13.

Analysis of data in table 13 showed that some highly significant differences existed between the subsamples taken from a single flower. Although the F-test did not show differences among the pollen viability of the three trusses, the Duncan's test revealed significant differences among them. This test showed the differences in pollen viability of truss 1, and truss 3, to be significant, but differences between truss 1 and 2, and also between 2 and 3 were non-significant.

Table 12. Viability percentages of pollen of Puck (P) and Bonny Best (B) of different flowers on the same truss.

Varieties	Plant No.	Flowers	Staining						In vitro						
			Block I			Block II			Block I			Block II			
			Subsamples			Subsamples			Subsamples			Subsamples			
			1	2	3	1	2	3	1	2	3	1	2	3	$\bar{x}$
P	1	1	93	94	91	95	91	98	71	74	56	31	78	53	77.0
		2	95	92	84	97	93	95	62	49	31	44	78	64	73.6
		3	86	90	93	89	93	97	57	53	53	53	21	62	70.5
	2	1	80	98	97	87	95	93	61	64	54	45	76	53	75.2
		2	92	97	98	97	91	96	36	78	44	44	42	29	70.3
		3	93	95	86	94	99	98	57	76	78	19	27	27	70.7
	3	1	96	94	97	91	95	97	44	60	45	31	35	60	70.4
		2	96	92	95	87	93	99	49	51	75	58	24	15	69.4
		3	91	91	93	92	92	98	29	48	61	27	71	62	71.2
B	1	1	89	85	92	99	98	94	31	34	41	44	51	21	64.9
		2	83	89	86	93	93	95	21	62	59	24	19	61	65.4
		3	93	90	96	97	91	87	65	58	45	18	23	51	67.8
	2	1	95	95	92	84	93	94	41	56	47	16	19	41	64.4
		2	85	89	87	88	81	85	57	45	62	44	35	23	65.0
		3	93	93	93	86	82	79	35	50	41	61	21	62	66.3
	3	1	94	86	89	93	93	92	53	47	53	36	37	29	66.8
		2	87	89	89	89	92	93	28	61	46	35	46	60	67.9
		3	94	87	91	91	87	91	31	33	33	47	60	43	65.6

Table 13. Viability percentages of pollen from three trusses of the same Puck plant, determined by staining.

		Truss 1				Truss 2				Truss 3			
Flowers		1	2	3	4	1	2	3	4	1	2	3	4
Sub-samples	1	91	85	78	87	88	81	73	91	65	90	64	75
	2	89	82	87	89	73	86	69	86	66	87	51	67
	3	93	94	82	84	89	83	71	84	73	88	57	65
	$\bar{x}$	90.9	86.9	82.5	86.5	83.2	83.2	70.9	86.9	67.9	88.2	57.2	68.9
	$\bar{x}$		86.7				81.1				70.6		

Analysis of variance.

Source	D.F.	M.S.	F
Total	35	-	-
Exp. Units	11	332.09	-
Samp. Error	24	22.08	-
Trusses	2	800.00	3.50
Exp. Error	9	228.11	10.33**

Duncan's test.

Trusses	3	2	1
$\bar{x}$	70.6	81.1	86.7

A trend can be observed that the percentage viability fell off from truss 1 to truss 3. This situation may not always be the case since the vigour of the plant may determine the extent of decreases in pollen viability.

III. Comparison of methods of pollen viability determinations.

One of the greatest difficulties encountered in the estimation of pollen viability of flowers taken from a single plant is the extreme variability which exists in pollen samples. The variable results are due not only to various locations from which the sample is taken, but also to the variability resulting from the techniques used in determining the viable count.

(a) Comparison of the staining and *in vitro* techniques.

Experiment 1.

Differences between the staining and *in vitro* procedures as seen in data in table 14 are highly significant because the percentages of viable pollen determined by staining are consistently very much higher than the results from the *in vitro* tests on the same samples. The analysis of data shows a significant interaction between variety x viability, indicating that the differences between the two techniques were not the same for all varieties.

These results confirm the observations of previous experiments, where the two methods were employed simultaneously, and these experiments raise the question as to which procedure is the more reliable for estimating the pollen which would be functional under natural conditions for reproduction on the tomato plant.

(b) Experiments on *in vivo* germination of pollen.

The validity of the staining and *in vitro* techniques could only be ascertained by comparison with the natural conditions for pollination and growth of pollen i.e examination *in vivo*. To count the number of tiny tubes from germinated pollen on the pistil of a tomato plant, in order to establish an *in vivo* quantitative measure, is very difficult as shown by the following experiments.

Experiment 1.

Preliminary trials using Martin's (1959) *in vivo* procedure indicated the feasibility of the test, but when large amounts of pollen were used the resulting thick fluorescent bands of aggregated pollen tubes prevented reasonable counts of the germinated pollen within the style.

Table 14. Pollen viability percentages determined by staining and *in vitro* germination on a single flower from each of 10 varieties.

Tests	Sub-samples	Varieties									
		P	B	(BxP)F	(PxB)F	(BxP)F	(PxB)F	(BxP)F	(PxB)F	498	(Px498)F
Staining	1	86	73	80	85	88	82	88	84	73	79
	2	89	78	76	86	75	76	70	82	71	65
	3	87	74	79	83	86	79	81	82	70	67
	$\bar{x}$	87.2	74.9	78.2	84.5	82.9	78.9	79.5	82.5	71.2	70.2
In vitro	1	53	46	40	57	77	69	33	37	42	45
	2	33	25	44	62	49	49	42	54	48	51
	3	56	49	43	60	63	59	38	46	41	49
	$\bar{x}$	47.2	39.9	42.2	59.6	62.9	58.9	37.6	45.6	43.6	48.2

Analysis of variance.

Source	D.F.	M.S.	F
Total	59	-	-
Treatments	19	-	-
Tests (T)	1	13923.0	287.6**
Varieties (V)	9	221.0	4.5**
V x T	9	107.6	2.2*
Error	40	48.4	

The *in vivo* qualitative test would have been easiest when a small number of grains were used; and in the first experiment, 10 pollen grains were placed on each of the stigmatic surfaces.

No germination occurred on any of the 12 stigmas used in experiment 1.

Experiment 2.

The surprisingly negative results in experiment 1, and the successful germination of masses of pollen in preliminary work, required a study between these extremes. An arbitrary choice of 100 grains resulted in the germination data in table 15.

Table 15. Percentage pollen germination *in vivo* on 5 stigmas of each of two varieties, Puck (P) and Bonny Best (B), using 100 grains per stigma.

Varieties	Stigmas					$\bar{x}$
	1	2	3	4	5	
P	98	57	35	30	12	46.4
B	21	9	41	23	17	22.2

Analysis of variance.

Source	D.F.	M.S.	F
Total	9	-	-
Varieties	1	1465.00	2.38
Error	8	614.25	

Data in table 15, show that successful germination was obtained, when 100 grains were used as compared with 10 grains used in experiment 2. The density of fluorescent pollen tubes was well within limits of counting.

Inspection of the data suggests that Puck gave higher germination percentages than Bonny Best, but the difference is not significant.

Experiment 3.

The fact that number of grains appeared to have an effect on germination *in vivo* suggested that further investigation was warranted.

Table 16. Pollen viability percentages of Puck (P) and Bonny Best (B) varieties using 15, 50, 100,and 200 grains in pollinations.

Grain No.	Varieties						$\bar{x}$
	P	B	P	B	P	B	
15	0	0	0	0	0	0	0.0
50	6	0	13	0	14	0	5.5
100	32	18	17	18	20	6	18.5
200	73	7	18	5	20	19	23.6

Analysis of variance on data transformed by the angular method (A).

Source	D.F.	M.S.	F
Total	23	-	-
Treatments	7	-	-
Varieties (V)	1	743.26	12.08**
Grain No. (N)	3	1014.96	16.50**
V x N	3	129.08	2.09
Error	16	61.48	

Duncan's test.

Grain No.	15	50	100	200
$\bar{x}$	0.0	5.5	18.5	23.6

The more comprehensive experiment 3 confirmed the results of experiments 1 and 2. Data in table 16, indicate that no germination occurred when the relatively small number of 15 grains was used per stigma, but that some germination occurred on all stigmas when 100 grains were applied. The intermediate number of 50 shows a distinct varietal response in that Puck pollen germinated but none of the Bonny Best pollen germinated. The varietal response was not consistent when the number of pollen grains was increased to 200. Thus the analysis did not show a significant interaction of pollen grain number and variety.

Puck gave a highly significantly greater percentage of pollen germination *in vivo* than Bonny Best. Duncan's test revealed no significant differences between the germination of 15 and 100 grains, or between 100 and 200 grains, but the latter pair gave significantly higher germination percentages than the former pair.

Experiment 4.

The indication of a distinct varietal response in experiment 3, when 50 grains were used, was very interesting, but due to the limited sample of three Bonny Best flowers used, the experiment was repeated using a larger number of flowers.

Table 17. Percentage germination *in vivo* of Puck and Bonny Best using 50 and 100 grains per stigma.

Grain No.	Puck flowers						Bonny Best flowers						$\bar{x}$
	1	2	3	4	5	6	1	2	3	4	5	6	
50 grains	26	24	30	20	28	30	6	14	12	0	16	0	17.1
100 grains	52	41	51	46	54	46	46	63	58	25	48	51	48.4

Analysis of variance.

Source	D.F.	M.S.	F
Total	23	-	-
Treatments	3	-	-
Varieties (V)	1	495.0	7.63*
Grain No. (N)	1	5859.0	90.41**
V x N	1	523.0	8.07*
Error	20	64.8	

Analysis of data in table 17, showed the interaction (V x N) to be significant. This indicated that the differences between the viability percentages of 50, and 100 grains, varied with the two varieties, Puck and Bonny Best.

Although the percentages of viable pollen in Bonny Best were lower than Puck, Bonny Best showed a higher germination percentage

increase *in vivo* when the number of grains used in pollination was increased from 50 to 100 grains.

Experiment 5.

The experiment was designed to investigate the effects of varietal differences of pollen and stigma, on pollen viability determinations *in vivo*, resulting from controlled self- and cross-pollinations of the two varieties, Puck and Bonny Best.

Table 18. Pollen germination percentages *in vivo* from reciprocal pollinations and self-pollinations of Puck (P) and Bonny Best (B).

Pollen	P	P	B	B	P	P	B	B	$\bar{x}$
Stigmas	P	B	B	P	P	B	B	P	
50 grains	32	36	40	14	38	44	12	6	27.75
100 grains	71	76	68	64	69	63	58	13	60.25

Analysis of variance.

Source	D.F.	M.S.	F
Total	15	-	-
Treatments	7	-	-
Grain No. (N)	1	4225.0	16.98**
Pollen (P)	1	1482.0	5.95*
Stigmas (S)	1	506.0	2.03
N x P	1	0.0	n.s
N x S	1	102.0	n.s
P x S	1	224.0	n.s
N x P x S	1	50.0	n.s
Error	8	248.8	

Analysis of data in table 18, showed no significance for the interactions. There were no significant differences in varietal effects on germination, between stigmas of the varieties, Puck and Bonny Best. One hundred grains applied to stigmas gave significantly higher germination percentages than fifty grains.

Puck pollen gave significantly higher percentages germination *in vivo* than Bonny Best pollen regardless of stigma, thus suggesting the differences are inherent in the pollen only.

Experiment 6.

The plants of lot 4, from which flowers were obtained for the previous *in vivo* experiments, were in a rather low state of vigour at the time the investigations were made. Consequently two additional experiments (6 and 7,) were performed on flowers from young vigorous Puck and Bonny Best plants of lot 6. This was done in order to compare the effects of vigour of plants on pollen germination which had previously been found in experiments 4 and 5. The precision of experiments 6 and 7 was increased by using twice the number of replications that had been used in experiments 4 and 5.

Table 19. Percentage germination *in vivo* of Puck (P), and Bonny Best (B), using 50 and 100 grains.

Varieties	Grain No.	Subsamples												$\bar{x}$
		1	2	3	4	5	6	7	8	9	10	11	12	
P	50	76	92	56	50	70	88	94	42	46	72	56	84	68.80
	100	78	65	80	56	97	73	73	55	82	91	80	71	75.05
B	50	42	46	66	70	50	66	52	81	46	68	70	38	57.89
	100	98	83	51	65	82	67	71	85	92	81	91	56	76.80

Analysis of variance.

Source	D.F.	M.S.	F
Totals	47	-	-
Treatments	3	-	-
Varieties (V)	1	252.0	1.19
Grain No. (N)	1	1900.0	8.99**
V x N	1	481.0	2.27
Error	44	211.3	

Analysis of data in table 19, showed no significance for the interaction. There were no varietal differences in pollen germination between Puck and Bonny Best pollen. Germination percentages were significantly greater from 100 grains than 50 grains.

Germination percentages were considerably improved when flowers from vigorous plants were used.

Experiment 7.

The choice of 200 grains for carrying out self- and cross-pollinations in the experiment, was used since the effects of number of grains using 50, and 100 grains, were already evident from the results in experiment 5.

Table 20. Pollen percentage germination *in vivo* from reciprocal pollinations of Puck (P) and Bonny Best (B), 200 grains being applied to each stigma.

Pollen	Stigma	Stigmas 1	2	3	4	5	6	7	8	9	10	11	12	$\bar{x}$
B	B	86	37	61	58	95	93	65	85	25	82	81	36	73.0
P	B	76	80	96	68	80	78	90	92	58	95	73	62	
B	P	80	60	56	82	78	60	76	58	61	75	61	52	71.8
P	P	91	64	74	86	44	73	82	78	81	97	85	70	

Analysis of variance.

Source	D.F.	M.S.	F
Total	47	-	-
Treatments	3	-	-
Pollen (P)	1	1519.0	5.88*
Stigmas (V)	1	16.0	n.s
P x V	1	7.0	n.s
Error	44	258.3	

Analysis of data in table 20, showed no significance for the interaction. There were no significant differences in varietal effects on germination between stigmas of the varieties, Puck, and Bonny Best. When 200 grains were used in pollinating flowers on vigorous plants, the variety Puck gave significantly higher viability percentages than Bonny Best.

(c) Comparison of the staining and *in vivo* techniques used in pollen viability determinations.

Experiment 1.

The development of a quantitative procedure for counting pollen tubes in styles made it possible to compare the artificial methods of pollen viability determinations, namely the staining and *in vitro* techniques, with the *in vivo* method. The comparisons are shown in table 21.

Table 21. Pollen viability percentages determined by the staining, *in vitro*, and *in vivo* techniques.

Varieties	Tests	Stigmas 1	2	3	4	5	6	7	8	9	10	11	12	$\bar{x}$	$\bar{x}$
P	Staining	92	96	86	93	90	99	89	95	87	82	92	95	91.2	
	In vitro	75	67	32	45	58	64	72	78	63	31	64	64	59.3	75.9
	In vivo	91	64	74	86	44	73	82	78	81	97	85	70	77.0	
B	Staining	85	83	94	87	93	83	91	93	92	89	86	79	87.8	
	In vitro	60	55	27	31	54	63	57	46	28	51	41	30	45.2	66.7
	In vivo	86	37	62	58	95	93	65	85	25	82	81	36	67.0	

Analysis of variance.

Source	D.F.	M.S.	F
Total	71	-	-
Treatments	5	-	-
Varieties (V)	1	1552.0	7.57**
Tests (T)	2	8354.0	40.75**
V x T	2	161.0	
Error	66	205.0	

Duncan's test.

Tests	*In vitro*	*In vivo*	Staining
$\bar{x}$	52.3	72.0	89.6

Analysis of data in table 21, showed no significance for the interaction. Puck variety gave highly significantly greater differences in pollen viability than Bonny Best variety.

There were highly significant differences between the three methods of pollen viability determinations. Duncan's test revealed that the three methods were significantly different from each other with the staining technique giving the highest percentage viable pollen, followed by the *in vivo* and *in vitro* techniques.

DISCUSSION

The literature contains many references to variability in pollen viability determinations. This variability has made it difficult to obtain consistent pollen viability measurements,and to have confidence in the results of evaluating pollen samples. In order to provide more information, three important sources of variability were studied in the foregoing experiments. These were (1), methods of sampling (2), the plant material itself and, (3) methods used in pollen viability determinations.

(1) Methods of sampling.

The choice of a number of grains necessary to give a representative sample size, varies with the following: (1), the degree of tolerance chosen at a fixed confidence limit; and (2), the amount of variability existing in the sample. In general, it was found that the larger the degree of tolerance chosen, the smaller was the sample required. A sample of pollen taken from a flower on an old plant already laden with fruit, showed tremendous variability among measurements of viability of subsamples. Such extreme variability necessitated the use of a large sample to represent the population. In contrast, pollen taken from flowers

on young vigorous plants showed more uniformity among subsamples, and consequently a small sample size was adequate to give reliable results. It is reasonable to expect that a young vigorous plant would have enough food resources to develop large quantities of viable pollen at anthesis, and that plants in an advanced fruiting stage would be likely to have more undeveloped or abnormal pollen. This condition is apparently normal, considering the life cycle and morphology of the tomato plant, in which fruits already set and allowed to develop have priority on the food resources (Murneek, 1926, Mc Collum, 1934, and Dearborn, 1936). Apart from age of plant, several factors in the environment appear to affect the variability in pollen samples, among which are: soil conditions, temperature, light, relative humidity and maturity of flower at the time of sampling. It is probable that vigour of plant may have greater effects on variability in pollen samples than age of plant.

The choice of sample size of 300-400 pollen grains used in the present experiments can be considered within limits of desired reliability in cases where pollen was obtained from vigorous plants. This number is of less value where pollen was obtained from plants in a non-vigorous state, since variability would be measured at a large degree of tolerance.

Experimental results on sample size show how much confidence should be placed in pollen viability measurements made by previous workers, referred to in the literature, where viability percentages are expressed (1) without stating the number of grains used and (2) by using an arbitrary number of grains as a representative sample size regardless of source and growing conditions.

Experimental results have also indicated that the extent of variability in a single pollen sample is in part dependent on the method used in its collection. It was observed that whenever the vibrator was used for pollen collection, large quantities of immature grains were obtained. This mixture was not expected, but apparently vibration dislodges a considerable quantity of immature grains in samples, and the quantity probably varies with the duration of vibration of the flower. In striking contrast to this situation, the procedure of passing the needle along the surface of the stamen gave the least amount of immature grains. It was generally observed that the method of collection which employed the least mechanical force resulted in the highest percentages of functional pollen. It has also been shown that the time at which a tomato flower is sampled may account for lack of consistency of pollen viability measurements. This situation results from the different stages of pollen maturity associated with different bud and flower stages. Hence pollen sampled at early bud stages would give lower viability percentages just before or at anthesis, due to the high immature grain content. Results on sampling the same flower over successive days indicated that more viable pollen is present on the first 2 days after full anthesis occurs than sampling on subsequent periods during the time the flower remains open.

It is apparent that lack of the consideration of sample size, methods of pollen collection, and time of collecting samples, could explain some of the discrepancies encountered in previous pollen viability determinations.

(2) The plant material.

When dealing with a plant like the tomato, which bears a succession of flowers, it is quite possible that differences in environmental conditions under which separate flowers are initiated and developed, could have subsequent effects on pollen development and viability at different locations on the same plant.

Considering the stamen as the smallest unit of different location of pollen on a plant, it was found that no differences in pollen viability existed within three zones of the relatively long stamens studied. These results suggested that the rates of pollen development were the same in the three zones. In contrast, pollen viability differed greatly from stamen to stamen in the same flower. It is possible that the various factors in the environment acting on a developing flower, have greater influence on pollen development among stamens rather than within a single stamen. This variability from stamen to stamen is quite possible when it is realised that there may be differences in quantities of nutrients translocated, and other effects such as light intensity, temperature and relative humidity. It is also possible that the fluctuating environmental factors resulted in a high variation in pollen viability among stamens of the same flower, and may account for the lack of significant differences among flowers.

Differences in pollen viability among trusses on the same plant may be again attributed to the varying environmental conditions under which each of the three trusses was initiated and developed. The fact that pollen viability declined from truss 1 to

truss 3 would suggest that plant food was being utilized by developing fruit on older trusses and that, under the prevailing environmental conditions, plant growth limited the food supplied to developing pollen in the upper trusses.

The results show the variability that may be obtained when pollen samples are taken from (1) single stamens by needle, (2) flower to flower on the same truss and also (3) different trusses of the sample plant. This variability could account for the apparent lack of consistency experienced by other workers.

(3) Methods used in pollen viability determinations.

Experimental results have indicated that pollen viability determined by different procedures, shows variation within the same pollen sample. This situation was noticeable when the staining technique showed comparatively higher viability percentages than the in vitro technique.

The modification of the iodine-potassium iodide procedure for pollen viability determinations was based on the nature of the food reserve revealed by staining. Maheshwari (1950) reported that synthesis of starch occurs as functional grains mature in angiosperm pollen. Daubeny (1955) and Guccione (1959) regarded tomato pollen to be viable only when they stained a dark colour by iodine-potassium iodide solution. Current investigation (using dilute iodine-potassium iodide solution to stain pollen from different bud and flower stages) suggested that (1) tomato pollen grains are non-viable at their highest peak of starch content which occurs at the intermediate bud stage and (2) functional mature grains give little to no stain reaction with dilute iodine-

potassium iodide solution, indicating the presence of little or no starch. This difference is no doubt due to the conversion of starch to sugars as grains mature, and confirms reports of Lesley and Lesley (1939) and Iwanami (1959).

Consequently a sample of pollen taken from a fully reflexed tomato flower at anthesis, is found to consist of a mixture of pollen grains which vary in stages of maturity. These stages are apparently correlated with the degree of conversion of starch to sugars within the grains.

Not all of the normal mature grains germinate when placed in artificial medium. The failure to germinate is attributed to (1), frequent bursting of mature grains during pollen tube initiation and (2), a decrease in the group stimulation effect on germination, caused by small localized areas of uneven density. This results from uneven distribution when pollen samples are being stirred into the medium.

Bursting of mature grains occurs after the first fifteen minutes of germination. Bursting of tubes can occur at any time during pollen tube elongation. The reason for bursting is unknown. Vasil (1960 a) reported that bursting of pollen grains decreases with an increase in the osmotic concentration of the medium.

Experimental results have indicated that the greater the stage of pollen maturity, the higher is the sugar concentration within the grain. When grains are placed in a germinating medium of fixed concentration, the amount of water absorbed by each grain is affected by its osmotic concentration. Consequently grains having higher osmotic concentrations than the surrounding medium will absorb greater volumes of water than those of lower osmotic concentrations. Hence bursting will result when the volume of

water absorbed exerts a pressure beyond the mechanical strength of the intine during its initial stage of protrusion through the germ pore. It is quite possible that the mechanical strength of the intine may be genotypically controlled.

The inconsistent behaviour of samples of Bonny Best pollen and a few reciprocal crosses, is questionable since the general trend resulting from experiments on the comparison of staining with in vitro determinations, showed higher viability percentages for the former technique. It is possible that the pollen samples of these varieties are very easily affected by slight differences in density of grains during germination.

The relative value of pollen viability determined under artificial and natural conditions, was only assessed after a quantitative in vivo procedure was developed.

The phenomenon of the mutual stimulation caused by increasing density of pollen grains was obvious throughout the course of the in vivo work. Both Puck and Bonny Best showed an increased germination percentage when the number of grains used for pollinations was increased.

Although the in vivo technique shows great promise as a valid test of pollen viability, further investigation is required to ascertain the sample size that would give the most appropriate density for the group stimulation effect on germination. This pollen growth factor (PGF) has been reported by various workers for in vitro cultures, but its occurrence in nature has not been previously recorded.

The present investigation has revealed varietal differences

in pollen between Puck and Bonny Best tomato varieties, with Puck yielding higher viability percentages than Bonny Best in most cases. It is possible that differences between the two types of pollen might be genetic. It is also possible that Puck may be more efficient in carrying out photosynthesis at a greater rate than Bonny Best. Subsequently, greater amounts of carbohydrates are made available to maturing pollen in Puck plants.

Fluctuating environmental conditions, both in the field and under greenhouse conditions when pollen was produced, probably obscured genetic differences among the varieties, Puck, Bonny Best, Earliana 498, and their reciprocal crosses. It is possible that if plants are developed in growth chambers, the environmental effects on pollen development would be more uniform. As a result, it would be possible to assess genetic differences in the laboratory, and use such differences as the basis for selection of plants carrying the desired genetic constitution.

SUMMARY AND CONCLUSIONS

Experiments on pollen viability in the tomato varieties Puck, Bonny Best, Earliana 498, and their reciprocal hybrids were done to ascertain suitability of using pollen tests as a basis of selection in a breeding program aiming to originate a new, true-breeding commercial variety possessing the characteristic of setting fruit at cool temperatures (below 65°F.) in southern Canada.

Plants were grown in the field and under two levels of temperature in greenhouses. The temperatures in the warm house were regulated at 65°-75°F., and in the cool house 55°-65°F. All pollen

studies utilized pollen from plants in the field and warm greenhouses.

The experiments included a study of the sources of variability which affect pollen viability determinations. The sources studied were (1) methods of sampling (2) the plant material itself and (3) the methods used in pollen viability determinations.

The experimental results showed that a sample of pollen taken from a tomato flower at full anthesis possesses variability, which is due to the presence of mature, immature and empty grains. Different pollen samples showed different proportions of these classes.

The variability of pollen sampled, is affected by method of collection, the time of collection and the location of the pollen source on the plant. Pollen samples collected from the surfaces of stamens with a dissecting needle, gave highest percentages of viable pollen. Pollen samples taken on the first day when the flower attained maximum anthesis gave higher percentages of mature pollen than samples collected subsequently during the period the flower petals were open. Pollen samples taken from different locations on the same plant, showed no differences in pollen viability between pollen taken from zones of the same stamen. The great variation in pollen viability between single stamens within a flower could account for the variability between flowers on the same cluster and also between clusters on the same plant. The variability of pollen which results from methods of sampling, has subsequent effects on the representative sample size. The greater the variability, the larger is the number of grains required to give a reliable sample size at a chosen degree of tolerance.

The staining technique showed higher percentages of viable pollen than the *in vitro* and *in vivo* techniques, for the same sample of pollen. Reduction in the viable percentages *in vitro* is believed to be due to bursting of some mature pollen grains.

Variations in germination percentages *in vivo* are related to the density and number of grains used in pollination. Both varieties, Puck and Bonny Best showed response to group stimulation since increased germination percentages were obtained by increasing the number and density of grains.

Although the pollen viability results of the two varieties, Puck and Bonny Best, lacked consistency, there was some indication that Puck gave higher viable percentages than Bonny Best.

It is recommended that further work dealing with pollen viability as a means of tomato plant character identification should be done in relation to a consideration of sources accounting for variability in pollen samples.

1. Method of pollen collection - pollen samples should be collected by passing a needle over the surface of an anther using as little mechanical force as possible.
2. Time of pollen collection - pollen samples should be collected on the first day when the flower attains maximum anthesis.
3. Location of pollen source - sampling of pollen should be restricted only to flowers developed under similar environmental conditions.
4. Sample size - samples possessing a high percentage of mature grains are desirable, since sample size is easily assessed when it is possible to use a small number of grains at a small degree of tolerance.

5. *In vivo* - viability determinations *in vivo* appear to be the most valid measure of pollen viability, but further investigation of number and density of grains on group stimulation effect on germination is desirable.

6. Further investigation of the subject would call for more precise control in the environmental conditions during pollen development, and exposure of pollen following anthesis.

LITERATURE CITED

1. Adams, J.
   1916. On the germination of the pollen grains of apple and other fruit trees. Bot. Gaz. 61: 131-47.

2. Anthony, S., and H.V. Harlan.
   1920. Germination of barley pollen. Jour. Agr. Res. 18: 525-36.

3. Bajpai, P.N., and A.B. Lal.
   1958. Storage experiments with pollens of cultivated fruit trees and vegetables. Sci. and Cult. 24: 616-7.

4. Becker, C.L.
   1932. Studies of pollen germination in certain species and interspecific hybrids of Prunus. Proc. Amer. Soc. Hort. Sci. 29: 122-6.

5. Bell, C.R.
   1959. Mineral nutrition and flower to flower pollen size variation. Amer. Jour. Bot. 46: (9) 621-4.

6. Bennett, H.W.
   1959. Artificial pollen germination for selection of improved seed production in Paspalum dilatatum. Poir. Agron. Jour. 51: 109-11.

7. Blanco, J.L.
   1951. Report of the FAO hybrid maize meeting held in Clermont-Ferrand, France, 23-6.

8. Boswell, V.R.
   1933. Description of types of principal American varieties of tomatoes. U.S. Dep. Agr. Misc. Pub. 160.

9. Brewbaker, J.L., and S.K. Majumber.
   1959. Cultural studies of the population effect and the self-incompatibility inhibition. Amer. Jour. Bot. 48: 457-64.

10. Brink, R.A.
    1924 a. The physiology of pollen. I. The requirements for growth. Amer. Jour. Bot. 11: 218-28.

11. _____
    1924 b. The physiology of pollen. II. Further considerations regarding the requirements for growth. Amer. Jour. Bot. 11: 283-94.

12. _____ 1924 c. The physiology of pollen. III. Growth in vitro and in vivo. Amer. Jour. Bot. 11: 351-64.

13. _____ 1924 d. The physiology of pollen. IV. Chemotropism; effects on growth of grouping grains; formation and function of callose plugs; summary and conclusions. Amer. Jour. Bot. 11: 417-36.

14. Buchholtz, J.T. 1931. The dissection staining and mounting of styles in the study of pollen-tube distribution. Stain Techn. 6: (2) 13-25.

15. Chandler, C. 1931. A method for staining pollen-tubes within the pistil. Stain Techn. 6: (2) 25-6.

16. Correns, C. 1889. Kulturversuche mit dem pollen von Primula acaulis L. Ber. Deut. Bot. Ges. 7: 265-72.

17. Crane, M.B. 1948. Thirty-ninth annual report of the John Innes Hort. Inst.

18. Currier, H.B. 1957. Callose substances in plant cells. Amer. Jour. Bot. 44: 478-88.

19. Daubeny, H.A. 1955. Some effects of cool temperatures on flower production and pollen germination in certain lines of tomato. M.S.A. Thesis, University of British Columbia.

20. Dearborn, R.B. 1936. Nitrogen nutrition and chemical composition in relation to growth and fruiting of the cucumber plant. Cornell Univ. Agr. Exp. Sta. Mem. 192: 1-25.

21. Dorsey, M.J. 1914. Pollen development in the grape with special reference to sterility. Minn. Agr. Exp. Sta. Bul. 144.

22. _____ 1919. A study of sterility in the plum. Genetics 4: 417-88.

23. East, E.M., and J.B. Park.
1918. Studies on self-sterility. II. Pollen tube growth. Genetics 3:353-66.

24. Flory, W.S., and M.L. Tomes.
1943. Studies of plum pollen, its appearance and germination. Jour. Agr. Res. 67: 337-67

25. Frazier, W.A.
1951. A variety with the unusual ability to set fruit at low temperatures. Report of the Tomato Genetics Cooperative 1:6.

26. Gessner, F.
1948. Stoffwanderungen in bestäubten Orchideenblüten. Biol. Zentralbl. 67: 457-77.

27. Guccione, G.M.
1959. Some effects of temperature on flower production, compatibility relations and pollen development in certain lines of the tomato. (Lycopersicon esculentum Mill.) M.S.A. Thesis, University of British Columbia.

28. Heydecker, W.
1948. A labour-saving tomato. Grower 29:469.

29. Hornby, C.A., and H.A. Daubeny.
1956. Genetic differences in pollen production, germination and growth. Report of the Tomato Genetics Cooperative 6: 16.

30. Hough, L.F.
1939. The pollen value of 134 apple varieties as determined by germination tests and field trials. Proc. Amer. Soc. Hort. Sci. 37: 133-40.

31. Howlett, F.S.
1936. The effect of carbohydrate and nitrogen deficiency upon microsporogenesis and development of the male gametophyte in the tomato. (Lycopersicon esculentum. Mill.). Amer. Jour. Bot. 50: 767-803.

32. Iwanami, Y.
1959. Physiological studies of pollen. Jour. Yokohama. Munich. University. 116: 1-137.

33. Jaranowski, J.K.
1961. Semisterility in the interspecific hybrid Melilotus polonica x M. alba. Amer. Jour. Bot. 48: 28-35.

34. Johri, B.M., and I.K. Vasil.
1961. Physiology of pollen. Bot. Rev. 27: (3) 325-81.

35. Judkins, W.P.
1939. Time involved in pollen tube extension through style and rate of fruit growth in tomato. (Lycopersicon esculentum. Mill.).Proc. Amer. Soc. Hort. Sci. 37: 891-4.

36, Kerr, E.A.
1955. Some factors affecting earliness in the tomato. Can. Jour. Agr. Sci. 35: 302-9.

37. King, J.R.
1955. The rapid collection of pollen. Proc. Amer. Soc. Hort. Sci. 66: 155-6.

38. _____
1960. The peroxidase reaction as an indicator of pollen viability. Stain Techn. 35: 225-7.

39. _____ , and T.M. Johnston.
1958. Factors affecting Irish potato pollen germination in an artificial environment. Amer. Potato Jour. 35: 689-700.

40. Knowlton, H.E.
1922. Studies in pollen with special reference to longevity. Cornell Univ. Agr. Exp. Sta. Mem. 52: 751-93.

41. Kubo, A.
1954. The unstable germination ability of pollen grains of Cosmos bipinnatus Cavanilles A.J. Jour. Sci. Hiroshima Univ. 6: 237-50.

42. Kuhn, E.
1938. Zur physiologie der Pollenkeimung bei Matthiola. Planta. 27: 304-33.

43. Kuhn, R.
1943. Uber die biologische Bedentung der Borsaure. Wien. Chem. Zeit. 46:1-9.

44. Kurtz, E.B., and J.L. Liverman.
1958. Some effects of temperature on pollen characters. Bul. Torrey. Bot.Cl. 85: 136-8.

45. Leitner, J.
1938. Karminessigsaure als Hilfsmittel zur Untersuchung des Inhaltes reifer, vollkommen ausgetrockneter Pollenkorner. Zeits. Wiss. Mikros. Techn. 55: 48-50.

46. Lesley, J.W., and M.M. Lesley.
1939. Unfruitfulness in the tomato caused by male sterility. Jour. Agr. Res. 58: 621-30.

47. Lidfors, B.
1896. Zur Biologie des Pollens. Jour. Wiss. Bot. 29: 1-38.

48. Linskens, H.F.
1954. Biochemical studies on the incompatibility reaction in the style of Petunia. VIII. Int. Bot. Congr. (Paris) Sec. 10: 146-7.

49. _____ , and K. Esser.
1957. On a specific colouration of the pollen tubes in the style and the number of callose plugs after selfing and cross-pollination. Naturwissenschaften. 44: 16.

50. Lloyd, F.E.
1918. The effect of acids and alkalies on the growth of protoplasm in pollen-tubes. Mem. Torrey Bot. Cl. 17: 84-9.

51. Maheshwari, P.
1950. An introduction to the embryology of angiosperms. Mc Graw-Hill. New York.

52. Marks, G.E.
1954. An acetocarmine glycerol jelly for use in pollen fertility counts. Stain Techn. 29: 277-8.

53. Martin, J.A.
1913. The physiology of the pollen of Trifolium pratense. Bot. Gaz. 56: 112-26.

54. _____
1915. Relations of moisture to seed production in alfalfa. Iow. Agr. Exp. Sta. Res. Bul. 23: 303-24.

55. Martin, F.W.
1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Techn. 34: 125-8.

56. Mattson, A.M., C.O. Jensen and R.A. Dutcher.
1947. Triphenyl tetrazolium chloride as a dye for vital tissue. Science 106: 294-5.

57. Mc Collum, J.P.
1934. Vegetative and reproductive responses associated with fruit development in cucumber. Cornell Univ. Agr. Exp. Sta. Mem. 163: 1-26.

58. Molisch, H.
1893. Zur physiologie des pollens mit besonderer Rucksicht auf die chemotropische Bewegungen der Pollenchlauche. Sitzungsber. Wien. Akad. Wiss. Mathnaturw. Kl. 102: 423-48.

59. Murneek, A.E.
1926. Effects of correlation between vegetative and reproductive functions in the tomato. Plant Physiol. 1: 3-56.

60. Nebel, B.R.
1939. Longevity of pollen in apple, pear, plum, peach, apricot, and sour cherry. Proc. Amer. Soc. Hort. Sci. 37: 130-2.

61. _____, and M.L. Ruttle.
1937. Storage experiments with pollen of cultivated fruit trees. Jour. Pomol. 14: 347-59.

62. Oberle, G.D., and K.L. Goertzen.
1952. A method for evaluating pollen production of fruit varieties. Proc. Amer. Soc. Hort. Sci. 59: 263-5.

63. _____ , and R. Watson.
1953. The use of 2,3,5 - triphenyl tetrazolium chloride in viability tests of fruit pollens. Proc. Amer. Soc. Hort. Sci. 61: 299-303.

64. Ostapenko, V.I.
1956. The question of estimating different methods of determining pollen viability. Bjul. Central. Genet. Labor. I.V. Micurina, No. 2.

65. Ostlind, N.
1945. Investigation concerning pollen germination in artificial substances. Beratt. Alnarps Zandtbr.—O. Mej Inst. Tradg. : 143-72.

66. Pandey, K.K., and R.D. Henry.
1959. Use of pectinase in pollen tube and pollen mother cell smears. Stain Techn. 34: 19-22.

67. Poole, C.F.
1932. Pollen grains as an indication of fertility in hybrids. Genetics. 17: 125-36.

68. Rick, C.M., and L. Butler.
1956. Cytogenetics of the tomato. Advances in Genetics. 8: 268-382. Academic Press, New York.

69. Roberts, R.H., and B.E. Struckmeyer.
1948. Notes on pollination with special reference to Delicious and Winesap. Proc. Amer. Soc. Hort. Sci. 51: 54-61

70. Sandsten, E.P.
1908. Some conditions which influence the germination and fertility of pollen. Wisc. Agr. Exp. Sta. Bul. 4: 149-72.

71. Savelli, R., and C. Caruso.
1940. Stimulation mutuelle dans la germination des grains de pollen de Nicotiana. Compt. Rend. Acad. Sci. (Paris) 210: 184-6.

72. Schleiden, M.J.
1849. Principles of scientific botany. (Trans. by E.R. Lankester) London.

73. Schoch - Bodmer, H.
1940. The influence of nutrition upon pollen grain size in Lathrum salicaria. Jour. Genetics 40: 393-402.

74. Seelig, R.A.
1956. Facts and pointers on marketing fresh tomatoes. U.S. Dep. Agr. Market Report.

75. Sexsmith, J.J., and J.R. Fryer.
1943. Studies relating to fertility in alfalfa (Medicago sativa. L.). I. Pollen viability, as affected by seasonal age of plant. Sci. Agr. 24: 95-100.

76. Sisa, M.
1932. Relationship between the age and viability of pollen in different cucurbits. Japanese Jour. Genetics. 8: 19-25.

77. Smith, P.F.
1942. Studies on the growth of pollen with respect to temperature,auxins, colchicine, and vitamin B1. Amer. Jour. Bot. 29: 56-66.

78. Smith, O., and H.L. Cochran.
1935. The effect of temperature on pollen germination and tube growth in the tomato. Cornell Univ. Agr. Exp. Sta. Mem. 175: 1-11.

79. Steel, R.G.D., and J.H. Torrie.
1960. Principles and Procedures of statistics. Mc Graw-Hill. New York.

80. Straub, J.
1947. Zur Entwicklungs physiologie der Selbststerilitat von Petunia. II. Das Prinzip des Hemmungsmechanismus. Zeits. Naturf. 26: 433-44.

81. Sutton, L.F., and R.B. Wilcox.
1912. Factors influencing pollen germination. Thesis Univ. Wisconsin.

82. Terao, H. , Y. Otani, Y. Doi., and Z. Tyo.
1940. Physiological studies of the rice plant with special reference to the crop failure caused by the occurrence of unseasonable low temperature. III. On the impotency of pollen and pistils under low temperatures. Proc. Crop. Sci. Soc. Japan. 12: 196-202.

83. Tupý, J.
1959. Callose formation in pollen tubes and incompatibility. Biol. Plantarum 1: 192-8.

84. _____
1960. Sugar absorption, callose formation and the growth rate of pollen tubes. Biol. Plantarum. 2: 169-80.

85. Valleau, W.D.
1918. Sterility in the strawberry. Jour. Agr. Res. 12: 613-70.

86. Van Tieghem, P.
1869. Recherches physiologiques sur la vegetation libre du pollen et de l'ovule et sur la fecondation directe des plantes. Ann. Sci. Nat. Bot. V - 12: 312-28.

87. Vasil, I.K.
1958 a. The cultivation of excised anthers and the culture and storage of pollen grains. Ph.D. Thesis, Delhi Univ. India.

88. _____
1958 b. Studies on pollen germination. Proc. Delhi Univ. Seminar "Modern Developments in Plant Physiology". Maheshwari, P. (Ed.) 123-6.

89. _____
1960 a. Studies on pollen germination of certain Cucurbitaceae. Amer. Jour. Bot. 47: 239-47.

90. _____, and N. Bose.
1959. Cultivation of excised anthers and pollen grains. Indian Bot. Soc. Mem. 2: 11-15.

91. Vieitzev, E.
1952. Use of 2,3,5 - triphenyl tetrazolium chloride for testing pollen viability. An. Edafol. y Fisiol. Veg. 11: 297-308.

92. Visser, T.
1955. Germination and storage of pollen. Mededeel. Landbouwhoogeschool (Wageningen) 55: 1-68.

93. Wanscher, J.H.
1941. Partial pollen sterility as a somatic character of the peach. K. Vet. — og Landbohojskole Aarsskr (Copenhagen) 91-105.

94. Went, F.W.
1945. Plant growth under controlled conditions. V. The relation between age, light, variety and thermoperiodicity of tomatoes. Amer. Jour. Bot. 32: 469-79.

95. _____, and L. Cosper.
1945. Plant growth under controlled conditions. VI. Comparisons between field and air conditioned greenhouse culture of tomatoes. Amer. Jour. Bot. 32: 643-54.

96. Winkler, A.J.
1926. The influence of pruning on the germinability of pollen and the set of berries in Vitis vinfera. Hilgardia 2: 107-24.

97. Wittwer, S.H., and F.G. Teubner.
1956. Cold exposure of tomato seedlings and flower formation. Proc. Amer. Soc. Hort. Sci. 67: 369-76.

98. Yeager, A.F.
1927. Determinate growth in tomato. Jour. Heredity 6: 263-5.