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This study was initiated to determine the incompatibility status of Bartlett and Seckel pears in Oregon. The investigations as to the cause and site of this reaction were directed at studying meiosis in Bartlett and Seckel pears, pollination experiments in three different geographical locations to determine the possible effect of environmental conditions on fruit setting, pollen tube growth studies in reciprocal cross-pollinations, and embryo development.

Meiosis in Bartlett and Seckel pears occurred normally with the exception of two per cent of pollen mother cells showing chromosome lagging and bridges at anaphase I in Seckel.

Cross-pollination of Bartlett with Seckel yielded no fruit in Corvallis, low in Medford and in Hood River. All the fruits from this cross in Medford were seedless. The reciprocal pollination yielded 1.3 per cent fruit set with an average of three seeds per fruit in Corvallis; 46.5 per cent fruit set in Medford, with 5 per cent of them containing one seed; 15.2 per cent fruit set in Hood River, with 1.8 per cent of these being seedless.

Bartlett pollen tubes grow considerably faster in Seckel styles than Seckel pollen tubes in Bartlett. No pollen tubes were found beyond the base of the style in the cross of Bartlett x Seckel; two out of 36 ovules were fertilized in the reciprocal cross. One 4-celled embryo was observed from the cross of Seckel x Bartlett, fifteen days after pollination.

It is concluded that Bartlett and Seckel pears, under the conditions of these experiments are at least partially, if not completely, inter-incompatible. Inter-incompatibility varies greatly in reciprocal crosses and in different environmental conditions. The incompatibility reaction takes place when the tubes are about half way down the style and increases in intensity through the base of the style.

# MEIOSIS, POLLINATION AND INCOMPATIBILITY IN BARTLETT AND SECKEL PEARS

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TABLE OF CONTENTS


| | Page |
|---|---|
| INTRODUCTION | 1 |
| LITERATURE REVIEW | 3 |
| Meiosis | 3 |
| Pollination | 6 |
| Incompatibility and Pollen Tube Growth | 9 |
| MATERIALS AND METHODS | 15 |
| Meiosis or Microspore Formation | 15 |
| Pollination | 16 |
| Pollen Viability and Germination | 18 |
| Pollen Tube Growth in the Style and Embryo Development | 19 |
| RESULTS | 23 |
| Meiosis in Bartlett and Seckel Pears | 23 |
| Pollination | 26 |
| Viability and Germination of Bartlett and Seckel Pollen | 31 |
| Pollen Tube Growth in Cross-pollinations and Embryo Development | 32 |
| DISCUSSION | 38 |
| SUMMARY | 41 |
| BIBLIOGRAPHY | 42 |

# MEIOSIS, POLLINATION AND INCOMPATIBILITY IN BARTLETT AND SECKEL PEARS

## INTRODUCTION

One of the main causes of unfruitfulness or a very light crop in pear orchards is lack of fertilization and subsequent seed development. With the exception of fruit setting parthenocarpically, fertilization is necessary for fruit development. Parthenocarpy, although common among pear varieties, is not a constant condition; parthenocarpic fruit setting shows wide varietal differences and the yield varies greatly in different years (29). The need for cross-pollination in pear orchards has been known for a long time.

Although no cases of complete inter-incompatibility have been found among the diploid pear varieties, very poor fruit sets have been shown in some areas from the reciprocal crossing of Bartlett (Williams Bon Chretian) and Seckel (6, 7, 30, 31, 44). The first report on the inter-incompatibility of Bartlett and Seckel was published by Marshall et al. (44) on pollination studies in Mighigan. These workers reported that Bartlett and Seckel are not only self-incompatible, but also commercially inter-incompatible. In Oregon, however, Bartlett and Seckel pears are

regarded as good pollinizers for each other by some of the growers, but no real investigation has been made on the inter-incompatibility of these two varieties.

This study was initiated to determine whether Bartlett and Seckel pears are inter-incompatible or inter-compatible; and, if they are inter-incompatible, the cause and how and when this reaction occurs. The investigations were directed at studying meiosis or microspore formation in these two varieties; actual pollination in three different areas to determine the possible effect of environmental conditions on fruit setting; at studying the pollen tube growth of Bartlett on Seckel and the reciprocal; and embryo development following fertilization. The investigations were carried out during the 1962 growing season.

# LITERATURE REVIEW

Abnormal breeding behavior and unfruitfulness in pear varieties may result from several factors acting either alone or collectively; some of these are:

1. Irregularities in the formation of microspores which result in the production of defective pollen grains (10, 16, 24, 47, 68).
2. Unfavorable environmental conditions during or after bloom such as too cold or too hot weather, or continuous rain (15, 27, 34).
3. Rate of growth of pollen tubes in the style, too slow to effect fertilization (2, 9, 10, 11, 12, 18, 19, 20, 40, 41, 42, 45).
4. Embryo abortion after fertilization (16).

## Meiosis in Pears

Meiosis in several varieties of cultivated pear has been studied previously by Florin (24), Moffett (47), Ito and Fukushima (37), Cummings et al. (12), Gorczynski (28), Crane and Thomas (8), Dowrick (16) and Uhlik (66). Darlington and Moffett (13),

and Moffett (46) have shown that there is a strong secondary chromosome pairing in cultivated apples and in the Pomoideae as a whole. Secondary pairing of chromosomes at meiosis demonstrates chromosome homology of a type more remote than that shown by primary or prophase pairing, and this has been interpreted as indicating that the group consists of complex polyploids with an original basic number of x = 7. In cultivated varieties of pears, however, the evidence of secondary pairing is not so obvious (47).

Formation of defective or unviable pollen grains as a result of irregularities during meiosis is seen mostly in the triploid varieties Beurré d'Amanlis, Beurré Diel, Catillac, Pitmaston Duchess (24, 47). Presence of more than two homologous chromosomes in the pollen mother cells of triploids and tetraploids results in the formation of trivalent and quadrivalent associations.

Bartlett and Seckel pears are both diploid, having the chromosome number of 2n = 34 (48). With few exceptions, chromosome behavior at meiosis of diploid pears is very regular, and 17 bivalents are formed in every cell (12, 16, 37, 47, 66). Observed irregularities in the diploid varieties of pears so far reported are:

1. Occurrence of binucleate pollen mother cells (8). Crane and Thomas (8) studying meiosis in diploid and tetraploid fertility, found certain abnormalities due to somatic instability rather than to any defect in the process of meiosis itself. The presence of pollen mother cells at pachytene with either two large normal-sized nuclei or one large and one small nucleus was reported.

2. Failure of pairing of some of the chromosomes (8, 28, 47). Occasional univalents were observed by Crane and Thomas (8) and by Gorczynski (28). Rarely a bridge and a fragment were seen at anaphase, indicating the presence of an inversion, and, since inversions often lead to difficulties in pairing at pachytene, it is probable that the occurrence of univalents was due to this cause (8). Moffett (47) observed two univalents in only two or three cells in Conference and Marguerite Marillat pears.

3. Failure of the formation of cell walls after meiosis (16, 61). Thomas (61) and Dowrick (16) found that the abnormal breeding behavior of Beurré Bedford pears is due to the failure of cell wall formation after meiosis in pollen mother cells, and young pollen grains had four nuclei. These nuclei were usually closely arranged toward one side of the cell. In

81.4 per cent of pollen grains the four spindles fused at the first pollen grain mitosis and gave rise to mature pollen grains with a single tetraploid generative nucleus and a similar vegetative nucleus; in the remainder of the pollen grains there were a variable number of haploid, diploid and triploid nuclei. Where no fusion of spindles occurred, the mature pollen grains contained four generative and four vegetative nuclei.

## Pollination

Most of the varieties of cultivated pears are self-incompatible (6, 10, 11, 12, 30, 31, 58), and the need for cross-pollination has been known over a long period of time. Waite (67), in 1894, differentiated self-incompatible from self-compatible varieties of pear and he attributed the failure of fruit setting when certain varieties were selfed to pollen sterility rather than to a mechanical cause. He believed that impotency was not an innate condition but was due to lack of affinity between pollen and ovules of the same variety. Fletcher (23) in 1910, working with Bartlett and Kieffer pears, found that self-incompatibility is caused by the inability of the pollen to fertilize the pistils of that variety. He also

considered that self-incompatibility is not a constant character with any variety, since the same variety may be self-fertile in one area and partly or completely self-sterile in another, according as soils, climates and cultural treatments vary from one area to another. Tufts (62) found that Bartlett pollen varied in viability from 18 to 80 per cent and 2 to 3 per cent of the blossoms set fruit under natural conditions.

Only a few examples of cross-incompatibility and inter-incompatibility among pear varieties have been reported. These can be summarized as follows:

Belle Lucrative x Louise Bonne de Jersey and the reciprocal (30).

Belle Lucrative x Seckel and the reciprocal (30).

Louise Bonne de Jersey x Seckel and the reciprocal (30).

Louise Bonne de Jersey x Bartlett and the reciprocal (30).

Bartlett x Seckel and the reciprocal (6, 30, 31, 44).

Beurré d'Amanlis (3x) x Conference (2x) (11).

Fertility (4x) x Fertility (2x) (11).

A survey of literature on pear pollination reveals that conclusions drawn by workers in different areas of the world are inconsistent with regard to behavior

of specific varieties. Bartlett pear has been classified as self-fertile by Weldon (71) and Rawes (53); partly to completely self-sterile by Tufts (62), Tufts and Philp (63), Marshall et al. (44), Cummings et al. (12); partly to completely self-fruitful by Hooper (36), Reinecke (54), Dwyer and Bowman (17); and self-incompatible by Wellington et al. (72). Most of these workers have agreed that cross-pollination will give increased fruit sets over self-pollination.

The seed content of the fruits as an indication of fertility has received a great deal of consideration. Kim (39), Reinecke (55), Cummings et al. (12), Tydeman (65) and Griggs and Iwakiri (32) reported that self-pollination in several varieties of pears resulted in fewer seeds than cross-pollination. Although the self-fruitful varieties will usually give commercial crops when planted in solid blocks, they will generally produce larger crops when cross-pollinated. This was demonstrated by the fact that in some areas of California yields from Bartlett trees would be increased if the orchards were provided with adequate facilities for cross-pollination (31).

Changes in the shape of pears due to foreign pollen were reported by Waite (67), Reinecke (55 and Kim (39). Cummings et al. (12) found no relation between size of

fruit and numbers of good seed in several varieties of pears. Griggs and Iwakiri (32) concluded that in California parthenocarpic Bartlett pears on the average have a more desirable and more uniform shape than those containing seeds; and seedless ones had significantly greater length/diameter ratios than seeded ones. Stephen (60) did not find any relation between fruit size of Bartlett pear and presence or absence of seed under Oregon conditions.

## Incompatibility and Pollen Tube Growth Studies

Incompatibility in plants can be classified according to whether the incompatibility reaction of the pollen is determined (a) by its gametic constitution or (b) by the constitution of the maternal tissue from which it arose (9, 10, 18, 19, 20, 41, 42). Brewbaker (5) observed a correlation relating pollen cytology, the type of incompatibility system and site of incompatibility inhibition in homomorphic plants. Gametophytic type incompatibility is observed primarily in species with binucleate pollen at the time of anthesis and with incompatibility inhibition occurring during pollen tube growth. Sporophytic incompatibility is linked to trinucleate grains and inhibition of germination or early tube growth. The most conspicuous exception to this correlation are members of Gramineae (5).

The gametic determination of incompatibility in its simplest case and most common form, is by a series of multiple allelomorphs designated by $S_1 \ldots\ldots S_k$. In this system, pollen germinates and penetrates into the style before it is inhibited. It cannot effect fertilization in a flower which has a style carrying the same incompatibility gene (9, 10, 18, 19, 20, 21, 41, 42). Sears (59) has shown that self-incompatibility in plants, irrespective of the genetic mechanism, is due either to the inhibition of growth of pollen tube or to inhibition of fertilization. The inhibition may take place at any stage between pollen germination and entry into the embryo sac. The nature of the inhibition of the incompatible pollen tubes was thought to be due to the immunity reaction of specific proteins or polysaccharides (41). The effect of temperature on pollen tube growth is very pronounced. The rate of incompatible pollen tube growth is slowed down by high temperatures of 30° C. or over (4, 40, 41, 45). This has been interpreted (41) as evidence for increased precipitation of the specific proteins at the higher temperatures.

The rate of pollen tube growth is always faster in compatible crosses than in incompatible crosses; and the retardation of the pollen tube growth is more marked in self-incompatibility than in

cross-incompatibility (40, 45). Lewis and Modlibowska (40) have found incompatible as well as compatible tubes in cross-pollination of Early Market with Fertility 2x, and also found that inhibition of pollen tube growth, due to incompatibility, took place when the tubes were one-third of the way down the style. The presence of incompatible and compatible pollen tubes in a cross between two diploid varieties of pears indicates the gametic determination of incompatibility.

In an extensive study of the pollen tube growth in apples and pears, Modlibowska (45) has distinguished three types of pollen tubes in the style: (a) incompatible tubes, these grow slowly and are more inhibited at an earlier stage at a temperature of 25° C to 30° C than at a lower temperature of 10° to 20° C. At the higher temperatures of 30° C or more they were completely arrested in the upper part of the style and a high proportion of them develop swollen ends; (b) semi-compatible tubes also grew slowly, but they did not become inhibited in the style. They continued to grow at all temperatures and reached the ovule, but seldom effected fertilization; and (c) compatible tubes in which the rate of growth at low temperatures was similar to (a) and (b), but was more accelerated by higher temperatures. Compatible

tubes reached the ovule earlier than (b) and effected fertilization.

Several methods used to determine the rate of pollen tube growth have been reviewed by Boller (4). These are (a) growing pollen tubes in an artificial media which permits direct visual observation; (b) microscopic examination of pollinated pistils with the aid of selective stains; (c) cutting of the styles at various intervals after pollination; and (d) establishing correlation between pollen tube growth and pistil response. In addition to these, the use of various segments of an incompatible style or stigma grafted between the two portions of a compatible style might serve to show whether there is a difference in reaction at the various levels of the incompatible style (33). Hecht reported that the incompatibility reaction is much stronger in the stigma than in the style in Oenothera.

Swollen ends of pollen tubes in many cases indicate incompatibility (2, 35, 40, 70); but this condition alone cannot be taken as conclusive evidence for incompatibility in pears (40). In the Japanese pear, more swollen ends were present after incompatible pollinations of Ichiharawase x Meigetsu and Taihaku x Waseka, than after compatible

pollinations (2). Heilborn (35) found swollen tube ends after self-pollination in apples. In Tradescantia, pointed ends were associated with incompatible tubes and swollen ends with compatible tubes (1). Emerson (22) observed swollen ends only when certain plants were used as the female parent, indicating there are other factors besides the S genes which affect incompatibility or pollen tube growth.

Linskens and Esser (43) found some correlation between callose formation in pollen tubes and incompatibility. They ascertained that incompatible pollen tubes showed nearly twice as many callose plugs as in compatible tubes, but the size of the plug was the same in both cases. It can be assumed that increased accumulation of callose in incompatible pollen tubes is not a primary result of incompatibility, but is caused secondarily by inhibition of their growth (64). Tupy (64) also found at least twice the amount of callose relative to their length in incompatible pollen tubes as there was in normally growing compatible tubes; in apples this was due to the greater length of the callose plugs, but in tobacco to their greater density.

The attraction between the pollen tubes and certain pistil parts in vitro has also received some attention (56). With further investigations, this

phenomenon might serve as an indication of cross-incompatibility or cross-compatibility among various varieties of fruits.

# MATERIALS AND METHODS

Pollination studies were conducted in three different locations; (a) Lewis-Brown Horticultural Farm, Corvallis, Oregon; (b) Southern Oregon Branch Experiment Station, Medford, Oregon; and (c) Mid-Columbia Branch Experiment Station, Hood River, Oregon. The trees used in these investigations varied in age at the three locations, but all were of uniform, moderate vigor and over ten years of age.

## Meiosis

A cytological study of microspore formation was made to determine whether there were irregularities in the meiotic processes of Bartlett and Seckel. For this purpose, small branches were cut from trees at the Lewis-Brown Horticultural Farm. The cut branches were placed in water in beakers for a few days in the laboratory at temperatures ranging from 22° C to 25° C. When the flower buds began to swell, they were cut from the branches at different time intervals and used either fresh or fixed for 6 to 24 hours in three parts absolute alcohol and one part saturated solution of ferric acetate in glacial acetic acid as suggested by previous workers (16, 38). The buds were stored in 70 per cent alcohol. After five to six months in

70 per cent alcohol, the anthers were still useful in studying meiosis although the stainability of the chromosomes was not as clear as fresh or newly stored material. Anthers were smeared on clean slides and stained with iron-acetocarmine, as recommended (16, 38, 57). Additional iron was added by teasing with steel needles immediately before staining. The slides were slightly heated over an alcohol flame, but the slides from stored material required additional heating in order to obtain a sharp differentiation of the chromosomes. Most of the slides were examined immediately after staining and some were preserved temporarily by sealing the edges of the cover slip with Clearcol mounting medium.

Drawings of the various stages of meiosis were made with the aid of an Abbé Camera lucida and 15x oculars. Photomicrographs were taken with an attached Makam camera.

## Pollination

One hundred to two hundred blossoms from each variety were emasculated at the Lewis-Brown Horticultural Farm, Corvallis, Oregon for studies of cross-pollination and parthenocarpic fruit set. Emasculation was accomplished by cutting the calyx

cup with the nails, lifting the entire corolla with its stamens and leaving the pistils undisturbed. Blossoms which were in an expanded balloon stage, but not yet open at the tips, were selected for emasculation and pollination; all others were removed from the branches. Three to four blossoms were left per spur. Emasculated blossoms for studying vegetative parthenocarpy were left uncovered. The flowers which were self-pollinated in Corvallis, and those which were self and cross-pollinated at the Southern Oregon and Mid-Columbia Branch Experiment Stations were not emasculated. They were bagged just prior to bloom to prevent contamination by foreign pollen. Pollination in all cases was accomplished by applying the appropriate pollen by means of camel's hair brushes. Pollen was collected from buds at bloom time just before anthesis. All the bags were removed from the branches one or two days after petal fall, when the styles started to dry out. Counts of the flowers in the bags and on exposed branches were made at the time of pollination. The flowers on a branch bearing 200 to 300 flowers of each variety were also counted and tagged to check and compare fruit set under natural pollination. Counts of fruit set were made at approximately 30 day

intervals after pollination and final counts were made three months after pollination. At the end of this period, each lot of fruit was picked separately and cut to determine the number of seed per fruit as an index of the degree of fertility.

Daily mean temperatures and temperature-hours before and after pollination at Hood River and Corvallis were obtained from thermographs which were located in the orchard in a standard shelter at Lewis-Brown Horticultural Farm, Corvallis and inside a tree 100 feet away from the pollinated trees at Mid-Columbia Branch Experiment Station, Hood River, Oregon.

## Pollen Viability and Germination

The extent of normal pollen in both varieties was determined from mounts in aceto-carmine diluted with 45 per cent acetic acid. Plump, regular appearing grains that were filled with cytoplasm were counted as normal. The term "normal" is used here as the antonym of "aborted" and vice versa. Slides were manipulated by a mechanical stage so as to avoid duplicate counts and insure random sampling.

The medium used for all germination counts consisted of the following: 100 cc of distilled water, 25 gm sucrose and 1.5 gm shredded agar. Recommendations

of several workers (3, 12, 25, 50, 51, 52) for the artificial culture of pollen were considered as the basis for the above nutrient formula. Final counts on germinating pollen grains were made approximately 24 hours after the cultures were started. All pollen germinations were carried out on slide plates held in horizontal staining dishes containing wet paper towelling for moisture control. Only pollen grains which produce tubes equal to or greater than the diameter of the pollen grains were recorded as having germinated. The tests were carried out at room temperature ranging from 22° C to 25° C. Data were recorded and the germination percentage was determined as the mean from observations of at least five fields.

## Pollen Tube Growth in the Style

Pollen tube growth studies were made on flowers from cut branches. Branches were cut from the trees just before bloom and kept in water. The effect of branch cutting on pollen tube growth was examined earlier by Lewis (41) who found no significant difference. Flowers on these cut branches were emasculated and pollinated when they were receptive. The styles were excised with a razor blade, at intervals ranging from 6 to 72 hours after pollination. Specimens

of entire pistils, for each cross, were taken from the orchard at 6, 10 and 15 days after pollination.

Three different solutions were used in fixing the styles and ovaries; 1. formalin-acetic-alcohol (glacial acetic acid 5 ml., formalin 5 ml., 50 per cent alcohol 90 ml.). Material can be preserved in this solution indefinitely (38, 69). 2. Gilson's fluid, as described by Cummings et al. (12), material may be left in the fluid until dissected, for a year or so; and 3. Navashin's fluid for 24 hours and then transferred to 70 per cent alcohol (38, 57).

The styles preserved in F.A.A. solution were handled as described by Watson (69). They were bleached overnight in a 20 per cent solution of chlorox, washed for ten minutes in running water and placed in 70 per cent ethyl alcohol for three minutes. The styles were then transferred on the end of a camel's hair brush and placed in a small glass container of distilled water containing a few drops of safranin O in 50 per cent alcohol. After the desired bright, but pale cerise color appeared in the material, the styles were crushed on a slide by using even pressure on the cover slip through a blotter pad. The cover slip was removed and the styles were stained with either lacmoid or lacmoid-martius yellow (49). The

pollen tubes are more easily followed through the style with this technique than from serial sections, although some of the pollen tubes are displaced.

The material fixed in Navashin's fluid and preserved in 70 per cent alcohol was used for microtome sectioning. Imbedding was done in Tissuemat paraffin; excessive hardening of the tissues was avoided by the use of butyl alcohol as a solvent of paraffin as described by Sass (57) and Johansen (38). Sections were cut 10 to 15 microns in thickness and stained with a saturated solution of lacmoid in 30 per cent alcohol. The lacmoid stain is highly selective for callose. The complete schedule used in staining the pollen tubes in styles and in ovaries is given below:

1. Remove paraffin in xylol and take the slides through the alcohol series to 25 per cent.

2. One per cent $NaHCO_3$ (baking soda) in 25 per cent alcohol for half an hour.

3. Saturated solution of lacmoid in 30 per cent alcohol, with three cc of one per cent $NaHCO_3$ in 25 per cent alcohol added, 12 - 18 hours (0.15 gm lacmoid, 100 cc 30 per cent alcohol, 3 cc solution of $NaHCO_3$ in 25 per cent alcohol).

4. One per cent solution of $NaHCO_3$ in 25 per cent alcohol for a few seconds or minutes, depending on how rapidly the lacmoid leaches out.

5. Eighty per cent alcohol, two to three minutes.

6. Ninety-five per cent alcohol, two to three minutes.

7. Two changes of 100 per cent alcohol, two to three minutes in each.

8. Equal parts of 100 per cent alcohol, clove oil and xylol, two to three minutes.

9. Two changes of xylol, two to three minutes in each.

10. Mount in balsam.

The ovaries of specimens taken 10 and 15 days after pollination, were sectioned at 10 to 15 microns. They were stained with safranin O and counterstained with fast green to show either fertilization or embryo development.

The measurements of the length of pollen tubes were made with the aid of an ocular micrometer and the scale on the mechanical stage of the microscope. In all the cases only the length of the longest tubes in the style was measured. Some difficulty was encountered in measuring the length of the tubes, because of the displacement of the tubes in smeared material and the curling position of the styles in paraffin. The data were recorded only for those in which accurate measurements could be made.

# RESULTS

## Meiosis in Bartlett and Seckel Pears

Approximately 1000 anthers of each variety were examined. The behavior of the chromosomes in meiosis in both varieties appeared so similar, therefore it is not necessary to deal with them separately.

Various stages of meiosis in Bartlett are shown in Figure 1 and in Seckel in Figure 2. Pairing of the chromosomes in both varieties appears to be normal and complete, and 17 bivalents can be counted at diakinesis and at metaphase I. In Seckel, lagging of some of the chromosomes and bridges were observed in approximately two per cent of the pollen mother cells studied. The cause of these abnormalities in Seckel pear is not known at present. Disjunction of the chromosomes in both varieties is normal otherwise. There is no cell division at the end of first meiotic division.

The second meiotic division is also normal, with a consequent formation of four daughter nuclei of similar size and form. The four microspores are then formed by quadripartition without previous cell plate formation.

The microspores mature into normal pollen grains which have one vegetative and one generative nucleus at the time of anthesis.

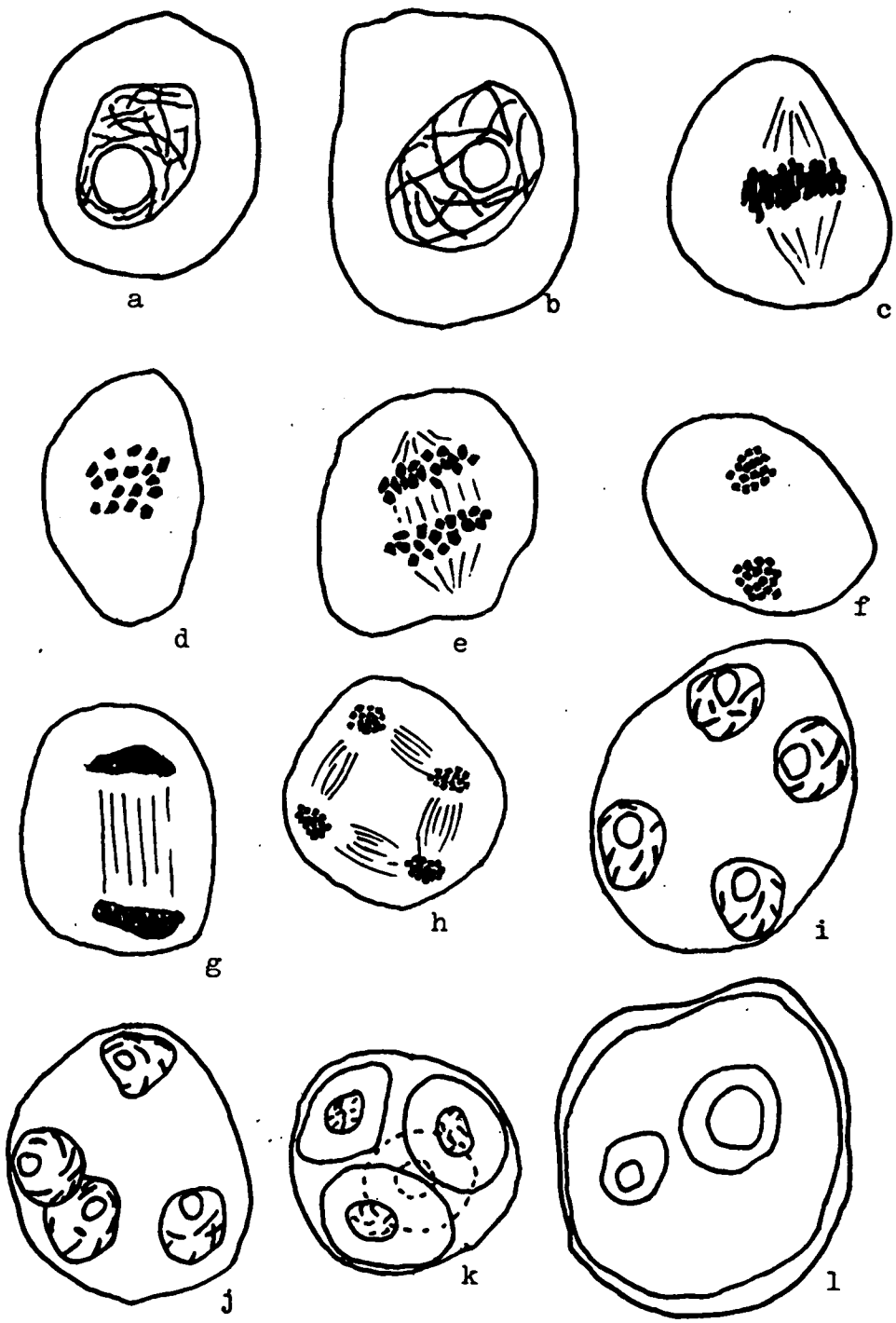


Fig.1 Meiosis in Bartlett

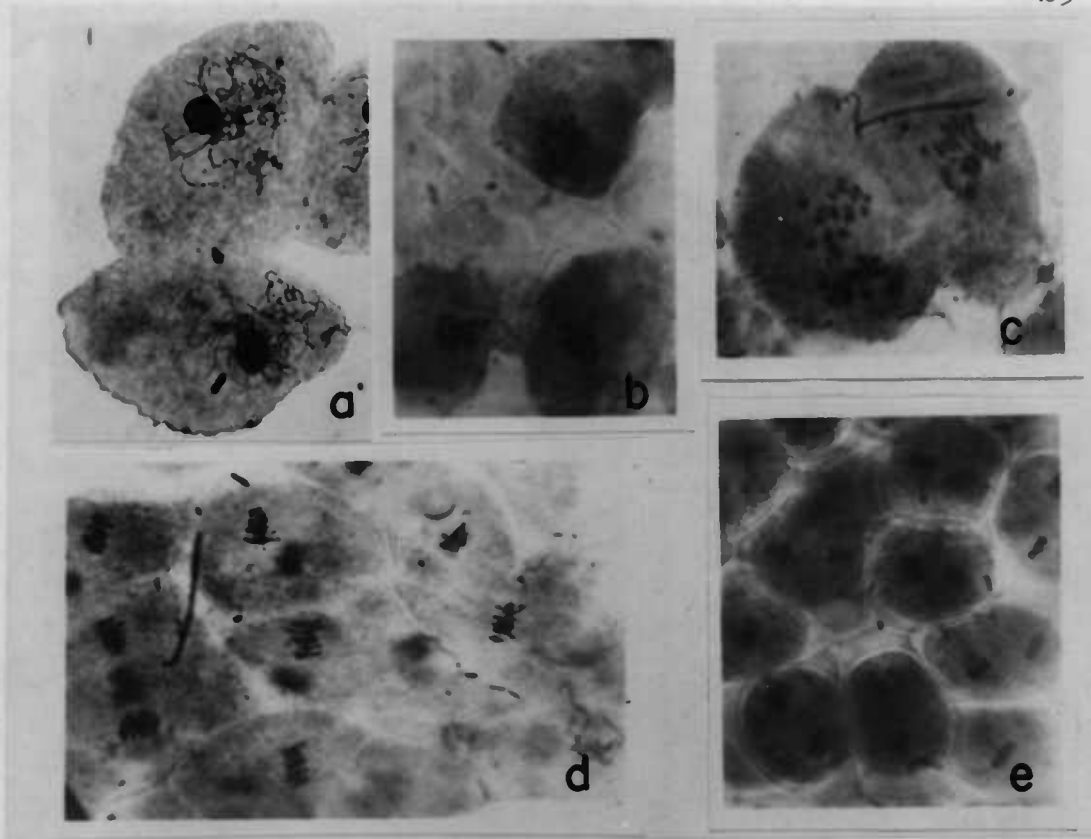


Fig.1 Meiosis in Bartlett pear a-b. Early prophase I; c.Metaphase I; d.Metaphase I, polar view; e.Anaphase I; f.Late anaphase I; g.Telophase I; h.Late anaphase II; i-j.End of meiosis with four nuclei; k.Tetrad of microspores; l.Mature pollen grain with one generative and one tube nucleus ( X 1350 )

Fig.2 Meiosis in Seckel pear a.Early stage of prophase I, Pachytene; b.Metaphase I and early anaphase I (upper cell); c.Mid-anaphase I showing abnormal disjunction of chromosomes; d.Metaphase I; e.Metaphase II ( X 900 )

## Pollination

The results of pollination studies with Bartlett and Seckel pears in three different locations are summarized in Table 1. All the pollinations set fruit at the end of 30 days after pollination, although some more than the other.

At Corvallis, no fruit was present on Bartlett trees 60 days after pollination from selfed, emasculated, or emasculated and pollinated with Seckel pollen of high viability. The two fruits growing on an open pollinated branch contained an average of eight apparently normal seeds. One fruit from an unbagged emasculated Seckel flower without any pollination contained seven seeds. This points out the necessity of bagging even after emasculation of the flowers (26), because the emasculated flowers, may be rarely visited by insects. Very light set of fruit was obtained from self-pollination of Seckel and cross-pollination with Bartlett on Seckel. Two fruits resulted from the Seckel x Bartlett combination, each having three apparently normal seeds in each.

In Medford, a commercial set of fruit resulted when Seckel was pollinated with Bartlett, from the reciprocal pollination, and from selfing Seckel; but 89.3, 100 and 96.8 per cent of the fruits were

Table 1. THE RESULTS OF POLLINATION STUDIES IN THREE LOCATIONS

| Location | Pollination | No. of Flowers | No. of Fruits Set 30 d. | 60 d. | 90 d. | Seeds/ Fruit | Per Cent Fruit Set Seeded | Seedless |
|---|---|---|---|---|---|---|---|---|
| Corvallis, Oregon | Bartlett selfed | 72 | 15 | 0 | 0 | - | - | - |
| | Bartlett-emasculated | 100 | 1 | 0 | 0 | - | - | - |
| | Bartlett-open pol. | 100 | 17 | 2 | 2 | 8.0 | 2.0 | - |
| | Bartlett x Seckel | 131 | 1 | 0 | 0 | - | - | - |
| | Seckel selfed | 248 | 13 | 11 | 5 | 0.2 | 0.4 | 1.6 |
| | Seckel-emasculated | 100 | 1 | 1 | 1 | 7.0 | 1.0 | - |
| | Seckel-open pol. | 167 | 44 | 42 | 42 | 3.2 | 25.2 | - |
| | Seckel x Bartlett | 154 | 4 | 2 | 2 | 3.0 | 1.3 | - |
| Medford, Oregon | Seckel-open pol. | 250 | -- | -- | 68 | 0.4 | 7.2 | 20.0 |
| | Bartlett x Seckel | 200 | -- | 23 | 15 | 0.0 | - | 7.5 |
| | Bartlett-open pol. | 250 | -- | -- | 100 | 3.8 | 37.6 | 2.4 |
| | Seckel selfed | 250 | -- | 69 | 63 | 0.0 | 0.8 | 24.4 |
| | Seckel x Bartlett | 200 | -- | 97 | 93 | 0.1 | 5.0 | 41.5 |
| Hood River, Oregon | Bartlett-open pol. | 162 | -- | 29 | 27 | 2.4 | 13.0 | 3.7 |
| | Bartlett x Seckel | 104 | 11 | 11 | 11 | 1.0 | 6.7 | 3.8 |
| | Seckel-open pol. | 126 | 21 | 18 | 14 | 4.0 | 11.0 | - |
| | Seckel x Bartlett | 164 | 34 | 28 | 25 | 2.7 | 13.4 | 1.8 |

seedless respectively. Ten fruits from the cross of Seckel x Bartlett contained an average of one apparently normal seed.

In Hood River, the results were somewhat different from the other two areas. Although the percentage fruit set was less than in Medford, 88.0 per cent of fruits from the cross of ~~Bartlett~~ Seckel x ~~Seckel~~ Bartlett and 63.6 per cent of fruits from the reciprocal cross were seeded. This may possibly be explained on the basis of the cooler weather existed at Hood River (Table 2) which is more favorable for the growth of incompatible pollen tubes.

The frequency distribution of fruits in relation to their seed content from all pollinations is given in Table 3. These data indicate that 105 out of 148 fruits obtained from the reciprocal crosses of Bartlett and Seckel are seedless. The cross-pollination of Seckel with Bartlett at the three locations yielded more fruit with one or more good seeds than the reciprocal cross. The percentage fruit set including seedless fruits was also higher in the cross of Seckel x Bartlett than in the reciprocal.

Bartlett and Seckel pears, under the conditions of these experiments were found to be at least partially self-incompatible, although they were self-fruitful in the Medford area. In cross-pollinations, Seckel appeared

Table 2.

DAILY MEAN TEMPERATURES AND TEMPERATURE-HOURS AT MID-COLUMBIA BRANCH EXPERIMENT STATION, HOOD RIVER AND LEWIS-BROWN HORTICULTURAL FARM, CORVALLIS, OREGON, FROM APRIL 10 TO APRIL 29.

| | CORVALLIS | | | HOOD RIVER | | |
|---|---|---|---|---|---|---|
| Date | Mean Temp. F | Hours Below 60° F | Hours Below 50° F | Mean Temp. F | Hours Below 60° F | Hours Below 50° F |
| April 10 | 47 | 24 | 14 | 50 | 22 | 14 |
| April 11 | 54.5 | 17 | 11 | 55 | 15 | 11 |
| April 12 | 59 | 13 | 8 | 60 | 13 | 9 |
| April 13 | 62.5 | 11 | 6 | 66 | 17 | 10 |
| April 14 1/ | 61.5 | 14 | 2 | 63 | 12 | 5 |
| April 15 | 55 | 19 | 12 | 61.5 | 19 | 12 |
| April 16 2/ | 54 | 14 | 8 | 59 | 14 | 11 |
| April 17 | 58.5 | 13 | 7 | 61 | 13 | 9 |
| April 18 | 60.5 | 14 | 7 | 66 | 9 | 6 |
| April 19 | 52.5 | 23 | 4 | 55 | 20 | 6 |
| April 20 | 47.5 | 24 | 8 | 51.5 | 17 | 15 |
| April 21 | 58 | 14 | 2 | 62 | 14 | 8 |
| April 22 | 58 | 15 | 8 | 66 | 11 | 8 |
| April 23 | 59 | 12 | 8 | 61 | 10 | 6 |
| April 24 | 54 | 20 | 5 | 51.5 | 23 | 12 |
| April 25 | 49 | 20 | 12 | 51 | 21 | 13 |
| April 26 | 50 | 24 | 7 | 48.5 | 24 | 17 |
| April 27 | 48 | 24 | 8 | 51.5 | 24 | 22 |
| April 28 | 46.5 | 24 | 18 | 51.5 | 24 | 19 |
| April 29 | 48 | 24 | 12 | 50.5 | 23 | 14 |
| Total | | 363 | 167 | | 345 | 227 |

1/ Date of pollination at Corvallis

2/ Date of pollination at Hood River

Table 3. FREQUENCY DISTRIBUTION OF FRUITS IN RESPECT TO THEIR SEED CONTENT

| Location | Pollination | NUMBER OF SEEDS PER FRUIT | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Corvallis | Seckel-open pol. | - | 4 | 11 | 12 | 7 | 6 | - | 1 | 1 | - | - |
| | Seckel-selfed | 4 | 1 | - | - | - | - | - | - | - | - | - |
| | Seckel-emasculated | - | - | - | - | - | - | - | 1 | - | - | - |
| | Seckel x Bartlett | - | - | - | 2 | - | - | - | - | - | - | - |
| | Bartlett-open pol. | - | - | - | - | - | - | - | 1 | - | 1 | - |
| Hood River | Seckel-open pol. | - | - | 3 | 4 | 3 | 1 | 2 | - | - | - | 1 |
| | Seckel x Bartlett | 3 | 6 | 4 | 4 | 5 | 1 | - | 1 | - | - | 1 |
| | Bartlett-open pol. | 6 | 7 | 4 | 3 | 2 | 1 | 1 | 3 | - | - | - |
| | Bartlett x Seckel | 4 | 4 | 2 | 1 | - | - | - | - | - | - | - |
| Medford | Seckel-open pol. | 50 | 15 | 1 | 1 | - | - | - | - | 1 | - | - |
| | Seckel-selfed | 61 | 2 | - | - | - | - | - | - | - | - | - |
| | Seckel x Bartlett | 83 | 10 | - | - | - | - | - | - | - | - | - |
| | Bartlett-open pol. | 6 | 24 | 14 | 11 | 10 | 8 | 6 | 9 | 7 | 4 | 1 |
| | Bartlett x Seckel | 15 | - | - | - | - | - | - | - | - | - | - |
| Del Rio Orchard, Medford | Seckel-open pol. | 18 | 9 | 11 | 7 | 3 | 1 | 3 | 2 | - | 2 | - |

to be more fertile, that is, yielded more apparently normal seed, when used as the female parent than as the male parent. From the commercial standpoint, these two varieties must be regarded as partially, if not completely, inter-incompatible. The degree of inter-incompatibility varies greatly in different locations. This is probably related to environmental conditions of which the temperature may be the most important.

## Viability and Germination of Bartlett and Seckel Pollen

The pollen grains of both varieties are similar in size and the variation among individual grains is slight (Table 4). Ten and three-tenths per cent of the Bartlett and 6.7 per cent of Seckel pollen grains was aborted.

Table 4.

VIABILITY OF BARTLETT AND SECKEL POLLEN

| Variety | Diameter of Pollen Grains 1/ Micron | NUMBER OF POLLEN GRAINS Counted | | | Per Cent Normal |
|---|---|---|---|---|---|
| | | Normal | Aborted | Total | |
| Bartlett | 42.64 ± 2.08 * | 1004 | 115 | 1119 | 89.7 |
| Seckel | 42.95 ± 2.42 * | 829 | 59 | 888 | 93.3 |

1/ Average of 20 pollen grains

* Standard error

Germination tests were usually made soon after collection and with pollen from the same vial from which the abortion counts were made. Although the percentage of normal pollen is little higher in Seckel than in Bartlett (Table 4), the percentage of germinated pollen is considerably lower (Table 5).

Table 5.

POLLEN GERMINATION IN BARTLETT AND SECKEL PEARS

| | POLLEN COUNT | | | |
|---|---|---|---|---|
| Variety | No. of Grains Counted | No. of Grains Germinated | Per Cent Germination | Per Cent Normal* |
| Bartlett | 547 | 456 | 83.4 | 89.7 |
| Seckel | 578 | 431 | 74.6 | 93.3 |

* Data taken from Table 4.

## Pollen Tube Growth in Cross-pollinations

Measurements of the length of pollen tubes in cross-pollinations at different time intervals after pollination are summarized in Table 6 and Figure 8. Bartlett pollen tubes grew considerably faster in Seckel styles than the pollen tubes of Seckel in Bartlett styles. The rapid rate of growth of Bartlett pollen tubes was very obvious up to 24 hours after pollination. After 24 hours, the difference between the rates of pollen tube growth in both crosses was

Table 6.

POLLEN TUBE GROWTH MEASUREMENTS IN RECIPROCAL CROSSES OF BARTLETT AND SECKEL

| Time After Pollination | No. of Pistils Examined | Source of Measurements | Ave. Length of Longest Tubes (mm) |
|---|---|---|---|
| | | Bartlett x Seckel | |
| 6 hours | 3 | smear | 1.28 |
| 12 hours | 2 | smear | 1.92 |
| 24 hours | 3 | smear | 2.44 |
| 32 hours | 3 | smear | 3.28 |
| 48 hours | 3 | smear & sect. | 4.58 |
| 60 hours | 5 | smear & sect. | 4.82 |
| 72 hours | 3 | smear & sect. | 5.12 |
| 6 days | 3 | section | near the base of the style |
| 10 days | 6 | section | no further growth |
| 15 days | 5 | section | no further growth |
| | | Seckel x Bartlett | |
| 6 hours | 2 | smear | 1.80 |
| 12 hours | 4 | smear | 2.65 |
| 24 hours | 2 | smear | 3.26 |
| 32 hours | 3 | smear | 3.58 |
| 48 hours | 6 | smear & sect. | 4.70 |
| 60 hours | 6 | smear & sect. | 5.19 |
| 72 hours | 3 | smear & sect. | 5.36 |
| 6 days | 3 | section | in the locule |
| 10 days | 5 | section | embryo sac |
| 15 days | 5 | section | 4-celled embryo |

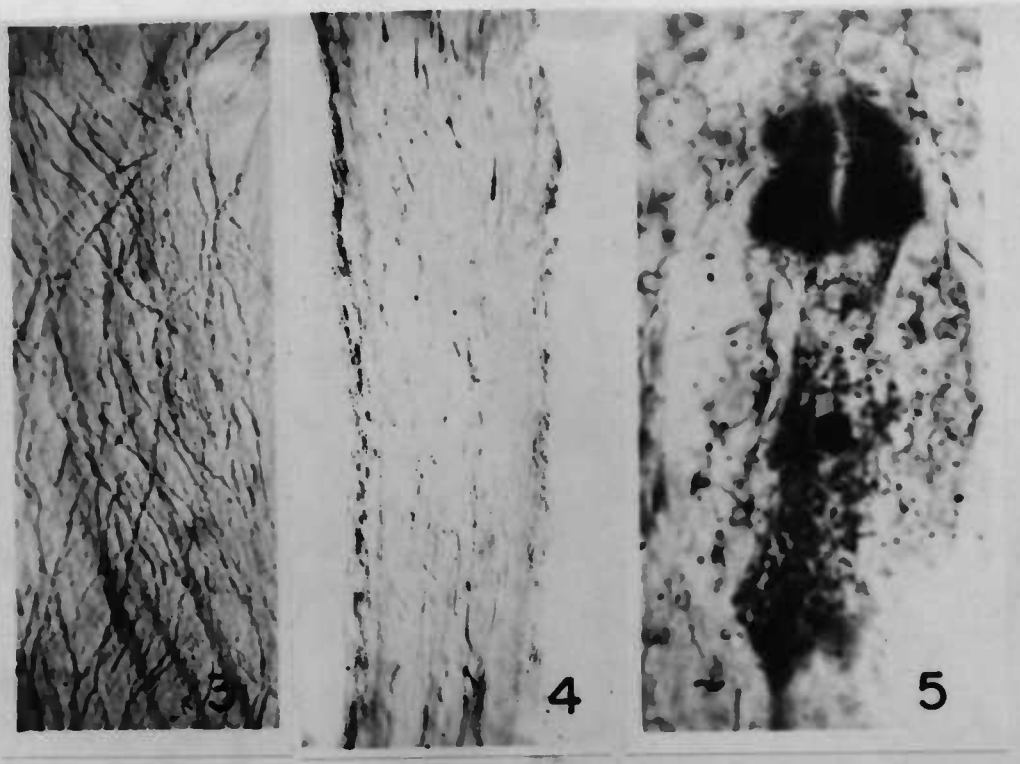


Fig.3 Seckel pollen tubes in Bartlett style, from smeared material showing upper part of the style ( X 430 )

Fig.4 Bartlett pollen tubes in Seckel style, from sectioned material. Dark spots are the callose plugs in the pollen tubes ( X 100 )

Fig.5 Mature female gametophyte of Bartlett showing the egg apparatus and endosperm nucleus, ten days after pollination ( X 430 )

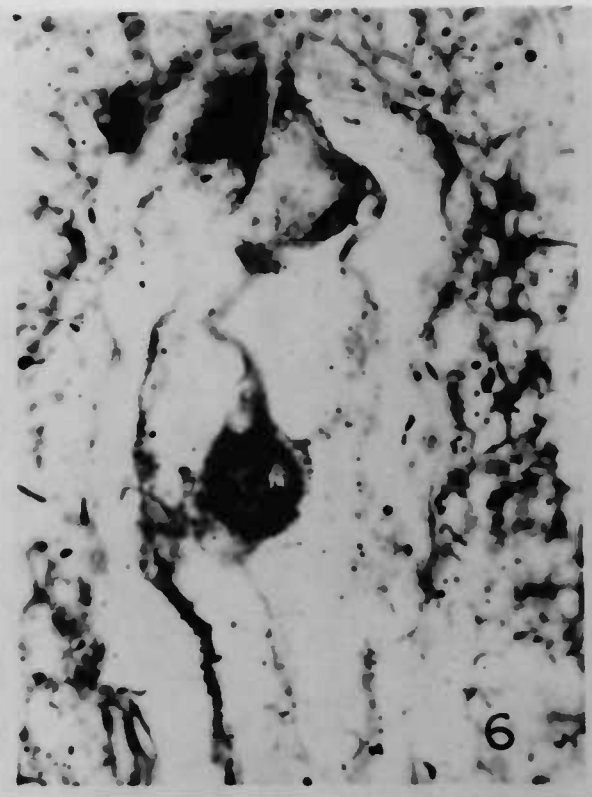



Fig.6 An unfertilized ovule from the cross of Bartlett X Seckel, 15 days after pollination, only the endosperm nucleus is dividing ( X 430 )

Fig.7 A fertilized ovule from the cross of Seckel X Bartlett showing the developing 4-celled embryo, 15 days after pollination ( X 430 )

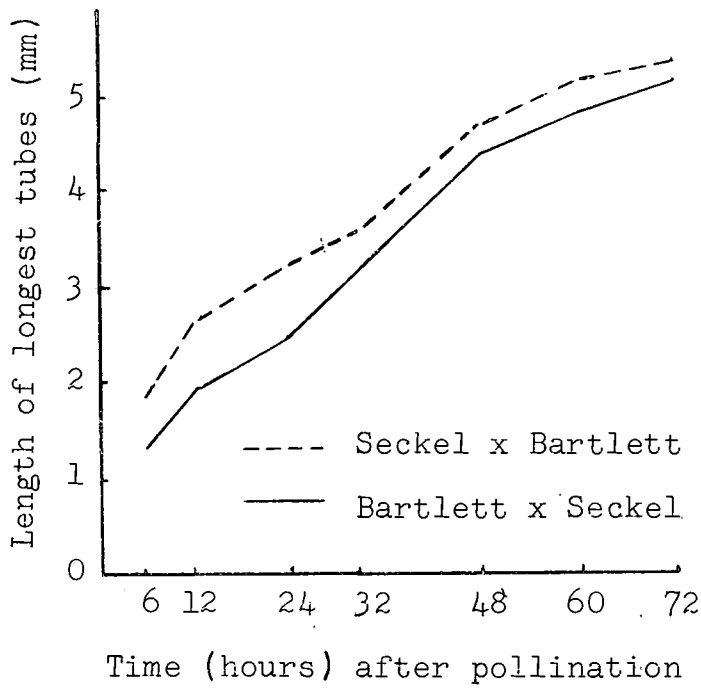


Figure 8. Pollen Tube Length at Different Time Intervals after Pollination at Temperatures Ranging from 22° C to 25° C.

greatly reduced. At the end of 72 hours the difference between the lengths of the longest pollen tubes was .24 mm. In both crosses, there was no recognizeable increase in length during the third day.

Although the data were recorded only for the longest pollen tubes, the differences in length among the individual pollen tubes in a given cross were .5 mm or less during the first three days after pollination. Some, but not all, of the pollen tubes had swollen ends. Swollen ends were observed during the third day when the growth rate of the pollen tubes

was greatly reduced. These observations indicate that the growth of pollen tubes had almost ceased when the ends started to swell. None of the tubes had swollen ends during the first day of growth.

Six days after pollination, the pollen tubes in the cross of Bartlett x Seckel had reached to the base of the style. The ovaries were sectioned ten and fifteen days after pollination, and no pollen tubes were observed either in the locule or in the micropyle. There was no indication of fertilization or of a developing zygote (Figure 6).

In the cross of Seckel x Bartlett, pollen tubes were found growing into the locule in one out of three pistils examined six days after pollination. Ten days after pollination, from a total of 34 ovules examined, only one showed any indication that fertilization had occurred. Fifteen days after pollination, only two out of 36 ovules examined contained embryos; one was four-celled and developing normally (Figure 7) but the other was abortive.

# DISCUSSION

Bartlett and Seckel pears, under the conditions of these experiments, were found to be partially, if not completely, inter-incompatible. The degree of inter-incompatibility varies greatly between reciprocal crosses and in different locations. Variations between areas are probably related to environmental factors, of which the temperature may be the most important.

Cytological studies on microspore formation in both varieties revealed no appreciable irregularities in meiosis, although approximately two per cent of the pollen mother cells in Seckel showed an abnormal anaphase I, that is, lagging of chromosomes in some cells and bridges in others. The cause of these irregularities at anaphase I is not known at present. Darlington (14) suggests that "in organisms having complete terminalization, if an occasional bivalent has an interstitial chiasma, it is sharply distinguished at anaphase by its lagging." The presence of bridges in some pollen mother cells may result from inversions; fragments might be lost in cytoplasm so that none were observed. Whatever the cause of these irregularities at anaphase I in Seckel, they have little or no importance with respect to pollen fertility, because of their low incidence.

Pollen viability and germination tests indicate that Bartlett and Seckel pears produce a high percentage of normal grains and they germinate readily on sugar-agar medium. Pollen of each variety germinate on the stigma of the other and grow normally through the style during the first 48 hours. Bartlett pollen tubes grow considerably faster in Seckel styles than Seckel pollen tubes in Bartlett styles. The growth rate of pollen tubes in both crosses slows down after 48 hours following pollination, when they reach half way down the style. At this time some of the tubes show swollen ends, but the exact number could not be determined in these slides. The incompatibility reaction is initiated when the tubes reach approximately half way down the style, and increases in intensity near the base of the style, as evidenced by the observations that most of the tubes cease growth near the base of the style. No pollen tubes were found beyond the full length of the style in the cross of Bartlett x Seckel and only two tubes in the reciprocal cross reached the ovule and affected fertilization. Therefore, the slow rate of growth of pollen tubes in the base of the style, due to genetic incompatibility, is postulated as being responsible for failure of fruit and seed set.

These results slightly differ from the findings of Lewis and Modlibowska (40), who concluded that the

inhibition of pollen tube growth due to incompatibility in pears took place when the tubes were about one third of the way down the style.

The results of the study of pollen tube growth in the style agree with the results of the pollination experiments in Corvallis, since no fruit was obtained from the cross of Bartlett x Seckel and only two fruits from the reciprocal cross. The same agreement applies to the results of pollination in Medford. In Hood River, however, a comparably high percentage of seeded fruits was obtained. The cooler temperatures which are more favorable for the growth of incompatible pollen tubes in that area may be responsible for this result.

The length of pollen tubes at different time intervals recorded, is considerably longer than in self-pollinations, but shorter than in cross-pollinations reported by other workers (12, 40, 41, 45).

# SUMMARY

1. Meiosis in Bartlett and Seckel pears is regular, with the exception of approximately two per cent of the pollen mother cells showing lagging chromosomes and bridges in anaphase I in Seckel.
2. A high percentage of the pollen grains are normal in appearance and germinate in artificial medium.
3. Failure of fertilization is due to slow rate of growth of pollen tubes following cross-pollination. Bartlett and Seckel pears, therefore, must be regarded as at least partially, if not completely, inter-incompatible.
4. The degree of inter-incompatibility varies in reciprocal crosses and in different locations, probably related to environmental factors of which the temperature may be the most important.
5. The slow rate of growth of pollen tubes due to incompatibility is much more pronounced in the cross of Bartlett x Seckel than in the reciprocal cross. No explanation for this relationship is presently evident.

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