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INVESTIGATIONS INTO THE CAUSES OF INFERTILITY OF
PELARGONIUM X DOMESTICUM

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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* * * * *

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1995

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Figure 1. *Pelargonium x domesticum* selection number 91-6-49, a regal pelargonium.
To my parents Julia and Charlton Stuart
ACKNOWLEDGEMENTS

It's nice to know that there really is an end to this process. This dissertation has benefitted from the support and guidance of many people. I have had two advisors during my time here at Ohio State, Dr. Pablo Jourdan and Dr. Glenn Hanniford. Dr. Hanniford got me started and Dr. Jourdan saw me through to the end. Both of them made strong contributions to this dissertation and to guiding me through the Ph.D. process in general. I also want to express my gratitude to the rest of my committee, Drs. Dan Struve, Tim Rhodus and Rich Pratt for their guidance in this process. Dr. Richard Craig at Pennsylvania State University was very helpful in providing me with literature and needed information.

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Studies in: plant breeding and genetics, floriculture, fertility barriers, pollen-pistil relationships, self-incompatibility
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INTRODUCTION

The genus *Pelargonium* contains three groups of commercially important floricultural crops. The most important is *P. x hortorum*, the common garden geranium; it is produced as either vegetatively or seed propagated bedding plants. *P. peltatum*, the ivy geranium, is valued for its vining habit; it is vegetatively propagated and plants are grown in hanging baskets and window boxes. The third floricultural *Pelargonium* crop is *P. x domesticum*, also known as the regal *Pelargonium* or the 'Martha Washington' geranium. *P. x domesticum* is produced exclusively as vegetatively propagated flowering pot plants. Large, colorful blossoms make the plant very attractive to consumers. *P. x domesticum* has the potential to become a popular floricultural crop. However, because it requires cool temperatures (10° C) for flower bud initiation and night temperatures of no more than 14° C for optimum flower bud development (Hanniford and Riseman, 1993), plants taken into the comparatively warm homes of consumers often cease flowering in a disappointingly short time period.

Deneke et al (1992) have demonstrated that there is heritable genetic variability in *P. x domesticum* for post
production quality, indicating that longer post harvest life could be a selection criteria for plant breeders. Unfortunately, a barrier to rapid improvement of *P. x domesticum* is low fertility. Crosses often produce no more than 10 to 20% fruit set with each fruit averaging one seed instead of the five that are possible (Hanniford, et al., 1980 and personal communication).

This dissertation presents studies that examined various aspects of the *Pelargonium x domesticum* reproductive system to help elucidate the factors contributing to low fertility of this crop.
CHAPTER I
LITERATURE REVIEW

The Ancestry of Pelargoniums

The Geraniaceae is divided into eleven genera, one of which is Pelargonium. This genus has been divided, depending on the taxonomist, into as many as 16 sections (or sub-genera) one of which is also named Pelargonium (Webb, 1984). Among these sections, Ciconium is thought to be one of the more primitive (Van der Walt and Vorster, 1983). P. x hortorum, the garden geranium, is probably derived mostly from crosses between P. zonale and P. inquinans which belong to Ciconium. Since P. x hortorum is the most economically important of the Pelargoniums, most of the genetic research on Pelargoniums to date has been conducted with it, using diploid and occasionally tetraploid material. This group, although closely related to P. x domesticum, has a different chromosome number and will not successfully hybridize with P. x domesticum (Knicely, 1964; Coffin and Harney, 1978).

The sub-genus Dibrachya includes P. peltatum which is commercially known as the ivy geranium (Clifford, 1970). P. peltatum will successfully hybridize with P. x hortorum; both have the same chromosome number (Yu and Horn, 1984).
Oglevee Floral Co. markets a series of vegetatively propagated cultivars called the Floribunda series which are derived from crosses between *P. x hortorum* and *P. peltatum* (personal communication). DeMarie (1991) states that further evaluation will probably result in merging this section with *Ciconium*.

The ancestry of *P. x domesticum* is more complex and less well understood. This hybrid is hypothesized to be derived from as many as seven species (Clifford, 1970). The putative ancestors (Table 1.1) were introduced into Europe during the 1700's at which time gardeners and hybridizers began intermating them. *P. x domesticum* was developed into a recognizable class by the 1830's.

Five of the putative ancestral species are from the *Pelargonium* sub-genus. *P. angulosum*, *P. cuculatum*, *P. fulgidum* and *P. grandiflorum* are thought to be the primary contributors to *P. x domesticum*’s ancestry. Van der Walt and Vorster (1981, as referenced by Webb, 1984) have classified *P. angulosum* as not being distinct from *P. cuculatum*. The *Pelargonium* sub-genus, considered the most primitive section of *Pelargonium*, includes most of the species that are grown commercially for their essential oils (Van der Walt and Vorster, 1983). It has a relatively small distribution range, occurring mostly in the S.W. Cape Province of South Africa, the probable center of origin of the *Pelargonium* genus (Albers and van der Walt, 1984).
Table 1.1. Putative ancestors of *P. x domesticum*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sub-genus</th>
<th>n</th>
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<tr>
<td><em>P. angulosum</em></td>
<td>Pelargonium</td>
<td>11y</td>
</tr>
<tr>
<td><em>P. cuculatum</em></td>
<td>Pelargonium</td>
<td>11z,w,y</td>
</tr>
<tr>
<td><em>P. fulgidum</em></td>
<td>Polyactium</td>
<td>11w</td>
</tr>
<tr>
<td><em>P. grandiflorum</em></td>
<td>Eumorpha</td>
<td>11x</td>
</tr>
<tr>
<td><em>P. betulinum</em></td>
<td>Pelargonium</td>
<td>11z,v</td>
</tr>
<tr>
<td><em>P. capitatum</em></td>
<td>Pelargonium</td>
<td>27w,33z,y</td>
</tr>
<tr>
<td><em>P. cordifolium</em></td>
<td>Pelargonium</td>
<td>11z,v</td>
</tr>
</tbody>
</table>

*Albers and van der Walt, 1984
*Daker, 1969
*Gibby and Westfold, 1986
*Knicely, 1964
*Yu and Horn, 1988
The putative ancestor, *P. grandiflorum*, (n=11) is currently classified in the *Eumorpha* sub-genus. However, Gibby and Westfold (1986) found that *Eumorpha* contains species which are x=4, 8, 9 and 11. They report that the three species which were determined to be x=11 seem to be more related to other groups and may be misclassified. Van der Walt (1985) lists natural hybrids between the *Pelargonium* and *Eumorpha* sub-genera that have been identified. Some of these natural hybrids have *P. grandiflorum* as a parent. Yu and Horn (1984) found that crosses between members of the same genomic chromosome number and ploidy in the genus *Pelargonium* were fairly successful. They also suggested that some of the current classifications within the genus should be reviewed in light of the variation in base chromosome numbers within sub-genera and their crossability data. These findings indicate that it is reasonable for *P. grandiflorum* to have successfully combined with species from the *Pelargonium* sub-genus and to be one of the putative ancestors of *P. x domesticum*. If *P. grandiflorum* is eventually reclassified to the *Pelargonium* section, all but one of *P. x domesticum*'s putative ancestors will then be included in the same section making them closely related. This would make *P. x domesticum*'s ancestry a bit less complex.

Other than attempts at interspecific hybridization and chromosome counts, there has been little systematic research
conducted on the genetics of \textit{P. x domesticum}.

\textbf{Possible Causes of Poor Fertility}

\textbf{Interspecific ancestry}

The interspecific nature of \textit{P. x domesticum} probably contributes to its low fertility. In artificial hybrids, such as \textit{P. x domesticum}, evolutionary divergence between the progenitor genomes may exist. The genetic information for coordination between various plant processes may have diverged sufficiently to result in lack of coordination of the activities involved. These processes include those associated with reproduction such as pollen and pistil relationships and fertilization and embryo development. This lack of proper physiological coordination due to non-matching of genetic information regulating the interaction is known as incongruity (Hogenboom, 1984).

With the large number of species that are thought to have contributed to the ancestry of \textit{P. x domesticum}, it is quite possible that incongruity is a factor in its reduced fertility. \textit{P. x domesticum} has existed as a distinct group for little more than 160 years. The poor fertility of the group suggests that there has not been a large number of sexual cycles during this time. The lack of sexual recombination means that there have not been many opportunities for selection to decrease incongruity in the species.
Incongruity during embryo and seed development may be one of the causes of embryo abortion which has been observed in *Pelargonium*. Philippi (1961) found embryo abortion in self and cross pollinations of diploid and especially tetraploid *P. x hortorum*. Tilney-Bassett (1963) found that as high as 50 percent of the self fertilized ovules of diploid *P. x hortorum* did not develop into seeds. Five days after self pollination he found both empty ovules which appeared to be normal, but were not fertilized as well as ovules which were shriveled. The ovules included cases of developing endosperm with no embryos, embryos with no endosperm and embryos whose expansion was restricted because the ovule walls were pinched together (Tilney-Basset, 1965). In later research, Tilney-Basset (1970) indicated that some embryos did not develop cotyledons and by 2-3 weeks were dying. Cross pollinations had better embryo survival than self pollinations. Kubba and Tilney-Bassett (1981b) indicate that the female used had a very significant influence in embryo survival, but the male had no effect.

*P. x domesticum's* polyploid nature

Meiotic irregularities are one of the most important causes of infertility in polyploids. Improved fertility as the result of selection tends to be associated with improved meiotic pairing. However, this success is not always the result of increased bivalent pairing at meiosis, but more
the result of increased percentages of cytologically balanced gametes (Gillies, 1989). Meiotic irregularities resulting from polyploidy very likely contribute to poor seed set in *P. x domesticum*.

There has been some confusion about the chromosome number of *P. x domesticum*. Reports of diploid chromosome numbers range from 17 to 45 (Darlington and Ammal, 1945; Saito, 1970; Daker, 1969; Kato and Tokumasu, 1983; Chanon and Hanniford, 1989; Coffin and Harney, 1978). One of the reasons for this is that it is difficult to achieve good chromosome spreads with *P. x domesticum* (Chanon, personal communication). Since all of the putative ancestors of *P. x domesticum* are *x*=11, (see Table 1.1) it is reasonable to assume that *P. x domesticum* is *x*=11 also. Daker (1969), Coffin and Harney (1978) and Tokumasu et. al (1974) have all come to this conclusion. Most reports indicate chromosome counts of more than 40, which suggests that *P. x domesticum* is a tetraploid with plants that are not multiples of 11 being aneuploids. Aneuploidy is a common characteristic of polyploids because of meiotic irregularities. This is particularly true in early generations (Evans and Davies, 1983). Both Daker (1969) and Kato and Tokumasu (1983) reported plants of *P. x domesticum* that were 2n=22. Pan (1991) determined that the cultivar 'Tiny Tot' has 2n=22 chromosomes. As its name implies, 'Tiny Tot' is a diminutive cultivar with small leaves and flowers. It is
possible that the 2n=22 plants a) are haploids, b) have been misclassified or c) that P. x domesticum is typically autotetraploid rather than allotetraploid with the 2n=22 plants being diploid forms. Whether P. x domesticum is an allopolyploid or autopolyploid has not yet been determined.

Badr and Horn (1971a) determined that older cultivars of vegetatively propagated P. x hortorum are mostly diploid while newer ones are mostly tetraploid. High numbers of multivalents during meiosis indicated autoploidy for the tetraploids. The segregation ratios of progenies from the tetraploids also indicated that they are autotetraploids (Badr and Horn, 1971b). They believe that breeders have been unconsciously selecting the tetraploid plants which tend to be more vigorous and have larger flowers (Badr and Horn, 1971a).

Tetraploid cultivars of P. x hortorum have irregular meiosis whereas diploid cultivars have fairly regular meiosis (Badr and Horn, 1971a; Glicenstein, 1986; and Philippi, 1961). Tetraploid P. x hortorum cultivars also have lower seed set than diploids (Badr and Horn, 1971c; Philippi, 1961).

Many of the varieties of P. x hortorum that are commercially available today are seed propagated. In order to be commercially feasible, seed propagated species require parents with high fertility. Seed propagated P. x hortorum cultivars are primarily diploids (Badr and Horn, 1971a).
Researchers have experimented with increasing and decreasing the ploidy levels of *Pelargoniums* in order to see the subsequent effect on fertility. Tamai et al (1958, 1964) doubled the chromosome number of *P. roseum* Bourbon (2n=77) and *P. denticulatum* (2n=88) with colchicine. Both of these plants had been male, and presumably female, sterile before the chromosome numbers were doubled. They were both fertile after chromosome doubling. Although they exhibited some meiotic irregularities, hybrids between these plants were fertile and could be successfully backcrossed to the parents (Tamai et al, 1969). Both *P. denticulatum* and *P. roseum* are from the *Pelargonium* sub-genus (Webb, 1984). *P. denticulatum* has been reported by Yu and Horn (1988) to have 2n=44. It is possible that the *P. denticulatum* which Tamai et al. used was an interspecific hybrid with lack of pairing between the genomes at meiosis. Doubling the chromosome number would have produced an allopolyploid with successful meiosis.

Since tetraploid *P. x hortorums* are autopolyploids, decreasing their chromosome numbers to the diploid level could result in fertile plants. Abo El-Nil and Hildebrandt (1973) successfully produced diploid plants from 3 tetraploid *P. x hortorum* cultivars through anther culture. The original tetraploid cultivars were not fertile, but the diploids were.
If *P. x domesticum* is an autoploid species, reducing its chromosome number to the diploid level could conceivably produce fertile plants. If the species contains interspecific genomes with poor meiosis, doubling the chromosome number could produce fertile allopolyploids. To date, no one has reported successful anther culture or colchicine doubling of *P. x domesticum* plants.

Biparental inheritance of plastids

*Pelargonium* pollen transmits plastids as well as nuclear DNA; the progeny may thus contain plastids from both parents. Baur (referenced by Tilney-Bassett, 1963) first discovered the non-Mendelian inheritance of chloroplasts in *Pelargonium* in 1909.

Biparental inheritance research has involved crossing plants having green chloroplasts with ones that contain white plastids. The chloroplast color frequencies of developing embryos are then observed (Kirk and Tilney-Bassett, 1967). The white chloroplasts used in biparental research have a defect at the transcriptional level resulting in plastid ribosome deficiencies (Steiner et al., 1988).

Chloroplast variegation can also occur in *Pelargonium* as a result of cytoplasmic incompatibility. Metzlaff et al. (1982) performed reciprocal crosses with *P. zonale* and *P. x hortorum* both of which contained entirely green
chloroplasts. The progenies produced plants which were variegated and bleached or completely green. Comparisons of the chloroplast DNA restriction patterns of the white and green leaves with those of the parents established that the white areas contained \textit{P. x hortorum} plastids and the green areas contained \textit{P. zonale} plastids. The percentage of variegated plants in the progeny was higher when \textit{P. x hortorum} was the female. Metzlaff et al. (1982) called this characteristic hybrid variegation. Apparently hybrid variegation occurs when there is incompatibility between the chloroplasts and the hybrid nucleus. Pohlheim (1986) reciprocally crossed solid green plants of \textit{P. zonale} and \textit{P. inquinans} which are the putative ancestors of \textit{P. x hortorum}. He found variegation in the progenies with increased percentages when \textit{P. inquinans} was the female. However, other researchers reporting crosses between \textit{P. zonale} and \textit{P. inquinans} did not indicate the occurrence of hybrid variegation (Harney and Chow, 1971). Both labs used just one source of each of these species. The presence of hybrid variegation may be genotype specific.

The interspecific ancestry of \textit{P. x domesticum} and biparental inheritance of plastids in \textit{Pelargonium} means that it is theoretically possible for \textit{P. x domesticum} to contain mitochondrial and chloroplast DNA from different sources both of which could differ from the primary makeup of the nuclear DNA. Biparental inheritance could produce
cytoplasmic incompatibility which might contribute to lack of fertility.

**Accumulation of mutations**

Sexual recombination can be thought of as a meiotic sieve. Through sexual recombination, mutations which constantly occur in plant populations are recombined and sieved out. Gametes that have successfully passed through meiosis tend to have normal chromosome pairing and segregation (Richards, 1986). Species that are primarily maintained through vegetative propagation, as is *P. x domesticum*, rarely go through sexual recombination. These species have limited opportunities to screen out accumulating mutations. The propagator would make selections if the mutations or sports are obvious. Desirable mutations would be maintained, as in the case of a new flower color. Mutations would be eliminated if they were perceived as detrimental. However, mutations affecting the sexual function of a species would likely not be identified under a vegetative propagation regime and would tend to accumulate.

Accumulating mutations may play a role in the lack of fertility of *P. x domesticum* cultivars which are maintained through vegetative propagation. Some cultivars have arisen as bud sports, indicating the occurrence of somatic variation (mutations?). Cassells and Carney (1987) found
that 16% of *P. x domesticum* 'Grand Slam' plants from tissue culture were variants. Nord (1989) produced plants from tissue cultures which had been stored at cool temperatures for one or three months. She found that 'Grand Slam' had the highest percent phenotypic stability (99 and 97%) of the four cultivars tested. Color mutations or sports are occasionally identified and released as new commercial cultivars. For example, 'Grand Slam' has produced 'Lavender Grand Slam' and 'Country Girl' has produced 'White Country Girl'.

Mutations occur in *P. x hortorum* (Craig, 1982) and a build up of small mutations rather than obvious sports may be selected. One case describes a grower who had, for years, been selecting the next season's stock plants from ones that remained after the current season's sales. The remaining plants were unsold because they were the last to flower. The grower had been unknowingly selecting for any mutations causing lateness in flowering resulting in unsalable plants.

Harney and Kung (1967) examined fertile and partially male sterile plants from *P. x hortorum* cultivar 'Jacqueline'. Differences in anther development between two clones were characterized. They made no observations as to how the fertility difference between them occurred. However, there must have been a genetic mutation to create the difference.
Daker (1967) discovered that the miniature *P. x hortorum* cultivar 'Kleine Liebling' was haploid having only 9 chromosomes. The roots were haploid, diploid or a mixture. Its microsporocytes produced a few bivalents at meiosis. The cultivar was very floriferous but sterile. Colchicine induced diploids were also sterile and displayed erratic chromosome pairing at meiosis. 'Kleine Liebling' had been released into the trade about 1925. Daker suggested that the lack of fertility in the colchiploid was the result of mutations affecting meiosis that had accumulated over many years of vegetative propagation.

However, there are examples of *Pelargonium* cultivars remaining fertile and stable over many years. The vegetatively propagated diploid *P. x hortorum* cultivars that Tilney-Bassett uses for biparental plastid inheritance research were largely introduced in the 1800's. Tilney-Bassett's first publication appeared in 1963 and there have been many more since. By 1989 his lab had screened 194,008 embryos from crosses using the cultivars and their progenies (Tilney-Bassett and Almouslem, 1989). These old cultivars would not have been successfully used for so many experiments if they were not reasonably fertile.

**Nuclear male sterility**

Nuclear sterility resulting in no viable gametes occurs in *Pelargonium*. Dale and Rogers (1971) found two
different recessive genes, and evidence of more, for male sterility in *P. x hortorum*. All male sterile lines became fertile in certain environments. Tokumasu (1976) hybridized male fertile and male sterile plants of *P. crispum*, a member of the *Pelargonium* sub-genus. The progeny, with one exception, had normal meiosis. The plants exhibited a wide range of fertility that varied from flower to flower and with the season. He concluded that the sterility was genetic, but its inheritance was complicated and, as most of the plants were fertile, fertility was dominant over sterility. Both of these authors state that the environment affected fertility, but neither provided any information as to what environmental variables were important.

Possible self-incompatibility

Self-incompatibility is a genetically inherited outcrossing mechanism which prevents a plant's ovules from being fertilized with its own pollen thereby avoiding inbreeding depression. If there is an active self-incompatibility system operating, depending on the system and the number of S alleles present, the percentage of successful crosses can be very low (Liedl and Anderson, 1993). This is because, in addition to not setting seed with itself, a plant will not set seed with any other plant having the same S alleles. For example, chrysanthemums
have a sporophytic self-incompatibility system; approximately 1 out of 3 combinations between unrelated plants are successful (Anderson and Ascher, 1992).

When crops are domesticated, incompatibility systems are often broken down or circumvented by the domesticators. The accumulation of Pseudo-self-compatibility (PSC) genes is one way in which this occurs. PSC is usually a quantitatively inherited trait which allows varying degrees of self seed set in a species with a self-incompatibility system. It is possible to obtain seed set equivalent to that of compatible crosses in incompatible crosses when PSC is present (Liedl and Anderson, 1993).

There are suggestions in the literature of self-incompatibility in *Pelargonium*, but there is very little formal research to prove its existence one way or the other. Sears (1936) self pollinated several varieties of *P. x hortorum*, found three that were "self-sterile" and performed some cytological preparations of self pollinated styles from them. Pollen germination was inhibited at the stigma surface which is indicative of sporophytic self-incompatibility. However, Sears did not state if the plants were fertile when hybridized to other plants. East (1940) examined "perhaps a dozen" cultivated forms of *Pelargonium* and found them all "weakly self-sterile". Partial self-sterility would suggest the presence of pseudo-self-compatibility (PSC) genes.
Dale and Rogers (1971) claim that there is evidence for incompatibility among their lines, but offer no data. Harney and Chow (1971), while investigating the crossability of *P. x hortorum* and its putative ancestors, found that *P. stenopetalum* was completely self-sterile. They found varying degrees of seed set from self pollinations in the other ancestral species and inferred that they may have PSC. Tilney-Bassett (1963) found very low self pollinated seed set in three cultivars which suggested that they were self-incompatible and speculated that self-fertility in *P. x hortorum* arose by breakdown of the self-incompatibility system through hybridization.

Glicenstein (1986) is the only researcher to date to offer data that tests for differences between self cross and outcross seed set. He found equal fruit set but decreased seed set for self pollinated crosses of tetraploid *P. x hortorum*. He argues that the decreased seed set in self pollinations is the result of higher embryo abortion resulting from inbreeding depression rather than self-incompatibility.

Though there is very little hard data as to the existence of self-incompatibility in *Pelargonium*, there are a number of suggestions in the literature that it may exist. If there is an active self-incompatibility system in *Pelargoniums* it is likely to be a sporophytic system. *Pelargoniums* have trinucleate pollen (Brewbaker, 1959)
which is typical of sporophytic self-incompatibility systems. The system may have been mostly broken down in *P. x hortorum* after many years of hybridization, but could still be active in *P. x domesticum*.

Natural selection for low seed set

There appears to have been natural selection in *Pelargoniums* over the millennia for fewer, but larger seeds. Sauer (1933, referenced by Glicenstein, 1986) found evidence of a vestigial ovary on top of the existing one in *P. x hortorum* flowers. This means that at one time there were more ovules per flower than the current 10.

Though there are two ovules per carpel giving 10 functional ovules per flower, usually only one ovule per carpel is fertilized. It is primarily the upper ovule that is fertilized, but the percentage of upper to lower varies with the cultivar indicating genetic control (Kubba and Tilney-Bassett, 1981a; Tsai et. al., 1973; Yano et. al., 1975). This control is maternal and polygenic (Kubba and Tilney-Bassett, 1981b). When both ovules are fertilized there is an increased percentage of embryo abortion especially of the lower ovule. If both mature, the seeds are misshapen because of lack of room in the carpel. The frequency of twins is also under genetic control (Kubba and Tilney-Bassett 1980).
There is some debate in the literature relative to the progress of pollen tubes through *Pelargonium* styles. The number of pollen tubes would affect the number of ovules that are fertilized. Philippi (1961) observed poor seed set with self-pollinations of a diploid and cross pollinations between 3 tetraploid *P. x hortorum* clones. He believed that this was the result of the low numbers of pollen tubes observed growing down their styles. Tilney-Bassett (1963) believed that the fertilization of one ovule per carpel is the result of pollen tube inhibition. With self pollinations, though numerous pollen grains germinated, a limited number grew all the way down the style. Later Kubba and Tilney-Bassett (1981c) found the limiting pollen tube growth both with self and cross pollination. Yano et. al. (1975) in examining crosses between two members of the *Pelargonium* section, *P. roseum* and *P. crispum*, found that most of the pollen tubes stop half way down the style and also proposed that there is some mechanism in the pistil to prevent more than one tube from entering the carpel. Cross pollinations involving *P. crispum* exhibited 2 to 5 times more pollen tubes than self-pollinations of *P. roseum*. Kubba and Tilney-Bassett (1981c) speculated that the proposed mechanism for fertilization control moves laterally between carpels and inhibits fertilization of neighboring carpels. Glicenstein (1986), working with tetraploid *P. x hortorum*, also
mentions that some pollen tubes stop growing and others burst in the style. The amount of inhibited pollen tubes varied by cultivar. He observed, however, that sufficient tubes grew down the style to effect fertilization of at least one ovule per carpel. Tsai et. al. (1973) observed more than enough pollen tubes growing down self-pollinated styles of P. x hortorum to effect fertilization of all ovules.

Inhibited pollen tube growth in the style is typical of gametophytic self-incompatibility systems. It is difficult to know where self-incompatibility ends and selection to regulate the amount of seed set per flower begins. Hessing (1985), working with Geranium caespitosum which is from a different genus in the Geraniaceae family, found that pollen tube growth was slower following self pollination than following cross pollination. Thus a plant's own pollen does not compete well with pollen from a different genotype resulting in a higher percentage of out cross seed set. He classed G. caespitosum as cryptically self-incompatible (Hessing 1988).

There does appear to be some kind of mechanism in the Pelargonium gynoecium which limits the number of ovules that are fertilized. This allows the plant to commit its resources to developing fewer, but more vigorous seeds which would be better able to survive conditions encountered in nature. It is possible that the mechanism
involves regulating the number of pollen tubes arriving at the ovaries thereby lowering seed set. Selection for increased fertility could involve selecting against this limiting mechanism.

Though a mechanism limiting pollen tube growth may exist, there are additional possibilities. For instance gamete competition could result in only a few tubes surviving in the race down the style. Yu (1985) made attempts at interspecific and inter sub-generic crosses in the *Pelargonium* group. She felt that the speed of pollen tube growth was related to the success of the cross. Mulcahy et al (1983) found that successful pollen tubes of *Geranium maculatum* grew 34-41% faster than the average growth rate. In other words they were simply more competitive. Successful selection for fertility in *Pelargonium* may involve selection for more competitive gametes.

**Pollen quality**

Proposing a mechanism which limits pollen tube growth in the style presumes the existence of viable pollen which would grow through the style if it were not inhibited. At present, there are no correlations of seed set in the *Pelargonium* with an accurate assessment of pollen viability. A complication in this type of research is that *Pelargonium* pollen, as is typical of trinucleate pollen,
does not germinate well in vitro (Philippi, 1961; Chanon, 1989). Another concern is that the various pollen staining techniques used to test pollen viability vary in what they stain and may produce different estimates of viability.

Philippi (1961) compared a peroxidase stain which indicates activity of peroxidase enzymes with acetocarmine staining which stains cytoplasm. Both stains are presumed to indicate pollen viability by staining grains that are viable. There were differences in percentage of stained pollen between the 2 techniques with the peroxidase stain producing lower percentages. Grains that did not stain with acetocarmine were smaller than those that did stain. Philippi believed that the peroxidase stain which indicates metabolic activity more accurately represented pollen viability. Pollen grains that will stain with acetocarmine but not with peroxidase stain have developed far enough to have some stainable cytoplasm, but are probably not truly viable. Pollen viability varied from season to season and even from anther to anther within the same flower.

Chanon (1989) compared cotton blue, acetocarmine and potassium iodide staining to 2,3,5-triphenyl tetrazolium chloride (TTC) using 9 cultivars of P. x domesticum. The first three stains gave comparable staining percentages. TTC which changes from colorless to red as a result of reduction by a proton indicating active respiration gave staining percentages that were more than 50% lower than the
other 3 stains. Hauser and Morrison (1964) used *P. x hortorum* pollen to compare staining percentages of nitro blue tetrazolium and cotton blue. They found the two techniques gave comparable estimates of viability.

The only data available comparing pollen staining and seed set in the *Pelargonium* has been provided by Glicenstein (1986) with tetraploid *P. x hortorum*. Cotton blue staining produced pollen viabilities mostly in the 40-60% range. There was a correlation (*r*=.91) between percent viability and percent germination. However, there was no correlation between percent viability and seed set.

An additional concern relative to the success of the male gamete is the competence of the pistil in supporting it. The pistil is not simply a passive medium through which the pollen tube passes. The relationship between the pollen and the style is a complex one (Heslop-Harrison, 1987). It is possible for genotypes to vary in the pistil's abilities to support pollen germination and development. There have been few studies of female differences in any species. However, there is evidence that genotypes can vary. For example, Pfahler (1967) and Mulcahy (1971) found differences in females while conducting gamete competition studies in *Zea mays*.
Cultural and environmental factors

There has been very little research relative to environmental and cultural effects on *Pelargonium* seed set. Disease can affect fertility (Scarborough and Smith 1977). Tomato ringspot virus infection reduced flower production as well as seed set in *P. x hortorum* plants. Chemicals being used in the greenhouse such as pesticides and growth regulators are also potential candidates for factors that affect fertility. Cohan and Craig (1966) found that the growth retardant, Phosphon-S, reduced male and female fertility of *P. x hortorum*.

Greenhouse temperatures and both the quantity and quality of light have large effects on plant growth and flowering in general. Pollinations performed during the cooler months of the year resulted in higher seed set for Glicenstein (1986). Tilney-Basset (1963) working at Oxford, England reported seasonal variation in pollen production. Three of the cultivars used produced pollen during parts of June to August and were male sterile during the rest of the April to October flowering season. Philippi (1961) refers to difficulty with pollen shed in cases where greenhouse humidity was either too high or too low.

Since *P. x domesticum* is sensitive to light and especially temperature in its flowering response (Hanniford and Riseman, 1993) it is reasonable to suspect that
environmental factors will affect fruit set and seed development. Determining the optimum environment for seed set would probably decrease problems with fertility in *P. x domesticum*.

**Dissertation Objectives**

In summary, there are many possible causes of sterility of *P. x domesticum*. These include problems connected to interspecific hybridization and polyploidy; genes for sterility including possible self-incompatibility; natural selection for fewer, but larger seeds; biparental inheritance, and cultural and environmental conditions.

The goal of this research was to examine various aspects of the *P. x domesticum* reproductive system with the intent of illuminating which are the more important causes of sterility. The final measurement of fertility is the number of viable seeds that are obtained. However, this final measurement is the sum total of many processes including the ability to produce competent ovules and pollen, the ability of the pistil and pollen to interact with each other and the ability to mature viable seed. Once it is known what parts of the *P. x domesticum* fertility system are not functioning properly, future research could be focused on those particular areas. This knowledge will also be useful to aid the breeder in knowing
what to select for to increase \( P. \times domesticum \) fertility. Understanding causes of infertility in the interspecific polyploid \( P. \times domesticum \) could also be useful in dealing with infertility in other species. Species that are polyploids and/or result from interspecific crosses are common in horticulture. Also attempts to move genes for such things as insect and disease resistance from undomesticated material into domesticated crops often involves wide crosses with related species. Lack of fertility is a common problem in the resulting progeny.

There were 5 objectives for this research. They were:

1. To investigate the effects of supplemental lighting applied to the female clone after the cool temperature floral initiation period on fruit and seed set of \( P. \times domesticum \).

2. To investigate any influences of bulk pollen on fruit and seed set.

3. To examine the causes of differences in the ability of male gametes to effect fertilization by testing for pollen viability, pollen germination and development, and subsequent seed set.

4. To examine genotypic variation in pistil support for the development of the male gametophyte.

5. To determine if self-incompatibility is a contributing factor to poor fertility.
Literature Cited


Chapter II

THE EFFECTS OF SUPPLEMENTAL LIGHT TO THE FEMALE CLONE
AND BULK POLLINATION ON FRUIT AND SEED SET OF

PELARGONIUM X DOMESTICUM

With its large, colorful blossoms, the vegetatively propagated florist's pot crop, Pelargonium x domesticum (regal pelargonium) is very attractive to consumers. However, poor seed set is a barrier to the production of improved cultivars. Crosses often produce no more than 10 to 20% fruit set with fruits usually containing 1 seed instead of the 6 that are possible (Hanniford et al. 1980 and personal communication). The species is a complex interspecific hybrid which is thought to be derived from as many as seven species (Clifford, 1970). With 2n=44 chromosomes, the species is tetraploid (Coffin and Harney, 1978, Daker, 1969). However, there is no information available whether P. x domesticum is a polysomic or disomic polyploid. Fertility problems, both as a result of meiotic irregularities and of incongruity, are common in interspecific hybrids and polyploids (Evans and Davies, 1983; Gillies, 1989; Jenkins, 1989). P. x domesticum has been recognized as a species for only about 160 years.
(Hanniford and Riseman. 1993). Because it is successfully propagated asexually, there has not been sufficient sexual recombination through breeding in this short amount of time to provide selection for significant increase in fertility.

The physiology of the flowering response of *P. x domesticum* is influenced by the environment. These influences include light as well as temperature. It is possible that an environment which provides optimum conditions for flowering may also be beneficial for fruit set and seed development.

The flowering response of *P. x domesticum* is affected by light, both by duration and the total amount of irradiance. Total irradiance can partly substitute for the low temperature requirement for floral initiation (Hackett and Kister, 1974; Nielson, 1975). Oglevee and Craig (1990, referenced by Hanniford and Riseman, 1993) have a process patent proposing the use of high irradiance and warmer temperatures to produce *P. x domesticum* on a year-round basis. High irradiance also increases the rate of *P. x domesticum* floral development resulting in faster flowering (Powell and Bunt, 1978).

Day length does not affect the time required for floral initiation (Hackett and Kister, 1974; Nielson, 1975). However, long days hasten floral development (Hackett and Kister, 1974; Hanniford et al., 1985).
Because high temperatures inhibit *P. x domesticum* fruit and seed set, crossing is typically conducted in the cooler months of the year. The cooler months of the year also experience lower levels of irradiance and shorter photoperiods. Since the physiology of flowering of *P. x domesticum* is influenced by both high irradiance and long day length, it is possible that fruit and seed set is also influenced by light. The objective of this study was to investigate the effects of supplemental lighting applied after the cool temperature floral initiation period on fruit and seed set of *P. x domesticum*.

The supplemental lighting study focused on female fertility. An issue in such a study is what will be the source of male gametes. Little was known about the male or female fertility of the clones that were available. It was decided to use a bulk of pollen containing the anthers of at least 20 clones which were unrelated to the female clones in the experiment. An additional study was conducted to investigate if the bulk pollinating technique itself had an influence on fruit and seed set.

The hypotheses for the experiments were:

Hypothesis 1. Do female clones treated with supplemental lighting after the cool temperature floral initiation period exhibit differences in plant morphology as indicated by differences in time to flower, number of flower clusters and plant height.

Hypothesis 2. Do female clones treated with supplemental lighting after the cool temperature floral initiation
period exhibit higher fruit and seed set.

Hypothesis 3. Do female clones pollinated with a bulk of pollen exhibit a higher percent seed per pollination than when they are pollinated with the pollen of a single male.

Materials and Methods

Supplemental lighting Table 2.1 lists the 16 selected clones used as females for this study. The clones were mostly of unknown fertility, but were chosen to originate from a variety of sources including some from our breeding program. Table 2.2 gives the 3 supplemental lighting treatments for the experiment. Treatment 2 increased the total irradiance over the control, but did not affect the photoperiod. Treatment 3 provided both increased irradiance and a long day photoperiod. Supplemental lighting was provided by 1,000 watt high pressure sodium lamps (HID) hung 1.8 m above the surface of the bench. The photosynthetically active radiation (PAR) of the lights was measured using a filtered selenium photocell in a Parkinson leaf chamber (Analytical Development Company, Herts, England). The lights provided an average photosynthetically active radiation (PAR) of 202 microeinsteins/m/sec² (s = 47) to the top of the foliage (20 cm above the bench).

The experimental design was a split-plot with lighting treatments as the whole plots. Two greenhouse benches were divided into 3 whole plots using 2 black poly curtains. The middle plots were 38% wider than the end plots to
reduce the extra shading resulting from having curtains on both sides of the plot.

Cuttings for the experiment were taken on November 10 and placed under intermittent mist for 5 weeks to root. After rooting, they were grown in the greenhouse for 4 weeks at which time the temperature was decreased to a constant 10° C for 5 weeks for floral initiation. After floral initiation, the plants were potted into 16.5 X 12.5 cm plastic pots and placed in their respective treatments. The greenhouse temperatures were 13° C nights and 20° C days. Fertilizers and pesticides were applied as needed. No pesticides were applied while pollinations were made. The plants were fumigated once with Resmethrin® during pollination. Shading was applied to the greenhouse on April 25 to help prevent high daytime temperatures.

Starting March 17, the first 14-18 flowers to open were emasculated and pollinated. Pollination was accomplished with a bulk of pollen from 20 or more clones unrelated to the female clones which had been collected in another greenhouse section. After the appropriate number of pollinations had been conducted, any new flower clusters were removed.

**Bulk genotype effects** Figure 2.1 depicts the crosses that were performed for the study. The 10 clones used as females and the 5 clones used as males were chosen, based
on previous crossing records, to represent a range of female and male fertility with none being completely sterile. Figure 2.2 gives the pollen viability of the males determined with fluorescein diacetate (FDA) in 61% sucrose (Heslop-Harrison, et al., 1984).

Emasculating began 12 weeks after the end of the cool temperature floral initiation period when all plants were well in flower. Ten emasculations were performed per female per day with the emasculations being spread evenly over the plants available. No more than 2 flowers per cluster were emasculated. Pollinations began 5 days after emasculating started using flowers that had become receptive that day. One replication per cross was performed per day for 8 days. The orders of the females and males were randomized for pollinating each day. Unused emasculated flowers were tagged and left on the plants as a check for pollen contamination. Unused blossoms were removed from female clone plants every other day. After pollinating was completed, all new flower clusters were removed. The number of locules and fully developed seed were counted for each fruit harvested.

Cuttings for the experiment were taken on October 6, dipped in Hormodin 1® and placed under intermittent mist for 3.5 weeks to root. One week later, the cuttings were pinched to 4 nodes. A week after pinching they were moved into a greenhouse section kept at a constant 10°C for 5
weeks to initiate flowering. After floral initiation, 5 plants for each female clone and 16 for each of the 5 male clones were potted into 16.5 X 12.5 cm plastic pots and put under 16 hr HID supplemental lighting with 16° C nights and 18° C days. HID lighting was discontinued in early April and protective shading was applied to the greenhouse roof and sides in the later part of May. The plants were given 2 applications of 1,500 ppm Cycocel® with the first 1 week after potting and the second a month later. Fertilizer and pesticides were applied as needed.

Pesticides were not sprayed while pollinations were being conducted to prevent disturbing the newly pollinated flowers. During this time, the white fly population was managed by weekly releases of the parasite, *Encarsia formosa*.

Data for both experiments were analyzed using SPSS for Windows®. Residuals were examined for lack of normality and heteroscedasticity.

Results

**Hypothesis 1** The plants with supplemental lighting were noticeably more compact than the unlighted control plants and flowered earlier. The degree of difference in height and flowering varied by clone. Although the female clones varied significantly for the size and flowering characteristics measured, the plants from the different
supplemental lighting treatments, for the most part, did not. Tables 2.3 and 2.4 give treatment averages for number of days to flower from the end of the floral initiation period, number of flower clusters at first flower and plant height (cm) for the 3 treatments and 16 female clones respectively (the treatment by clone interactions were not significant). The average time to flower for the female clones ranged from a low of 33 days for 83-11-3 to a high of 52 days for 80-18-6. The number of flower clusters varied by a factor of 4 from 7.7 for 80-10-2 to 33.6 for 'Virginia'. Plant height varied from 15.8 cm for 'Elegant Rapture' to 25.7 cm for 'L. C. Hickman. There does appear to be a trend for increasing light to result in faster flowering, more flowers and more compact plants. However, these differences are much smaller than the range of differences between the clones and, except for Treatment 3 height, none of the variables are significantly different from the control.

Hypothesis 2 Tables 2.3 and 2.5 provide, respectively, the percent fruit set and number of seeds per fruit for the supplemental lighting treatments and female clones. Five of the sixteen clones (80-18-6, 80-20-1, 82-44-6, Elegant Rapture and Grand Slam) were deleted from the data because their overall seed set was too low for meaningful analysis. The 11 female clones that were included in the analysis
vary for percent fruit set by a factor of 4 from 21.5% for 'Olga' to a high of 88.7% for 'Dolly'. The number of seeds per fruit vary by a factor of 3 from 1.1 to a high of 3 for 83-11-3. Figure 2.3 compares percent fruit set and number of seeds per fruit for 11 clones. The correlation for the plot is $r = .78$ indicating there is a trend for clones producing higher fruit set to also produce more seeds per fruit. Although the differences are smaller than those between the selections, there does appear to be a trend for supplemental lighting to produce higher percent fruit and seed set. However, the F test for treatments resulted in $p = .72$ which is far from significantly different.

**Hypothesis 3** Figures 2.4 and 2.5 present the percent seed per pollination (\((# \text{ fully developed seed} / # \text{ locules})\times 100\)) for the females and the crosses respectively (Female x Cross interaction was non significant, $p = .29$). The 2 female clones, 78-4-1 and Virginia produced such low seed set that they did not provide a good test of the effects of the crosses and were, therefore, deleted from the analysis. The female clones (Figure 2.4) varied in % seed set by a factor of 2.5 from a low of 12% for 'Chelvey' to 30% for 83-11-3. Among the 7 crosses there was only 1 statistically significant difference in percent seed per pollination which occurred between 83-52-1 and 'Grandma Fischer'. Since 83-52-1 has the lowest initial pollen
viability of the 5 males (Figure 2.2), it is not surprising that it produced a lower percent seed set. The 2 crosses using bulk pollen produced the same overall percent seed set as pollen from a single male. The residual plot for the analysis (Figure 2.6) indicates heteroscedasticity (nonconstant variance). The residual plot shows a megaphone shape in that the spread in the residuals increases as the predicted value increases. SPSS provides the Lilliefors test for normality of data. The test is based on a modification of the Kolmogorov-Smirnov test for when means and variances are not known and must be estimated from the data (Norusis, 1990). This test of the residuals shows they are not normally distributed (p = .0001). The residuals from a square root data transformation (Figure 2.7) indicate improvement relative to the heteroscedasticity though they are still not normally distributed (p = .0001). The consequence of a lack of normality is that the actual probability of the statistical test used is lower or higher than the tabular probability that is given (Cochran, 1947). Though the square root transformation improves the heteroscedasticity, it produces the same interpretation of the results. Therefore, the untransformed analysis is presented here.
Discussion

Figure 2.8 reveals the most important lesson learned from the supplemental lighting experiment. This figure depicts the percent fruit set of all 16 clones used as females for the experiment. A few of the clones produced fruit set as low as 10 to 20% which was what had been expected based on previous research. However, the overall fruit set for the experiment was 49%. Fruit set for the clones ranged from 10% to as high as 90%. Though there is plenty of room for improvement, there is more fertility available in *P. x domesticum* than had been expected. Considerable gain in fruit and seed set can be made by screening existing material for male and female fertility.

The time of the year that the experiment took place probably affected the lack of improvement in fruit and seed set with supplemental lighting. The plants did not begin flowering until the end of March with most of the pollinating taking place in April. By April the natural day length is over 12 hours which is apparently enough light for setting and developing fruits. At this point supplementing the ambient light is not beneficial to fruit or seed set. In fact, at this time of year the amount of heat produced by the HID lights is a concern as high temperatures are known to cause flower bud abortion (Hanniford and Riseman, 1993).
Though the experiment did not produce firm conclusions about the effects of supplemental light on *P. x domesticum* fertility, the study did establish that there is significant fertility in the population. At this point the author believes that it is more profitable to focus research efforts on fertility aspects of the genotypes themselves rather than to continue to pursue effects of environmental factors.

The bulk pollinating technique was used in the supplemental lighting experiment because it provided a random sample of a number of male genotypes and was a fast, efficient way to conduct large numbers of pollinations. Bulked pollen from many males essentially permits conducting many crosses at once. A bulk of pollen increases the probability of fertile pollen in the cross, and, provided the female has some degree of fertility, increases the likelihood of fruit and seed set. However, a bulk of pollen is no more effective at producing seed set than a fertile male, if the fertility of the male is known.

Because of the ease in conducting bulk pollination, it is a useful technique for screening for female fertility as well as a good method for conducting large numbers of pollinations in a season. Bulk pollination would be a useful technique in a recurrent selection program for improvement of fertility in *P. x domesticum*. The simplest recurrent selection program would follow the procedures for
the mass selection method. The advantage of mass selection is that it is the least labor intensive of the various selections schemes and takes only one season per cycle. A mass selection scheme keeps the seed from the desirable females to produce the plants for the next generation. As the females are typically evaluated after pollination, the pollen for the seed for the next generation comes from a random mating among the plants and derives from both the desirable and undesirable plants of that generation. Bulk pollination provides a random sample of pollen of the male genotypes available. This would make the random mating part of the selection process very simple to conduct. One of the drawbacks of mass selection is that there is control in the quality of the female producing seed for the next generation, but the selection scheme does not control the quality of the genotype of the male. This decreases the amount of improvement for the desired trait in each generation (Fehr, 1987). However, during the random mating with bulk pollen, the pollen from fertile genotypes would produce seed for the next generation with pollen from infertile genotypes being selected out by default. This results in control of both the male and female genotypes which would yield faster improvement of fertility.
Table 2.1. Clones used as female parents in the supplemental lighting study.

<table>
<thead>
<tr>
<th>Selection</th>
<th>Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>80-10-2</td>
<td>'Dolly'</td>
</tr>
<tr>
<td>80-18-6</td>
<td>'Elegant Rapture'</td>
</tr>
<tr>
<td>80-20-1</td>
<td>'L. C. Hickman'</td>
</tr>
<tr>
<td>82-44-6</td>
<td>'Grand Slam'</td>
</tr>
<tr>
<td>83-11-3</td>
<td>'Honey'</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>'Inez'</td>
</tr>
<tr>
<td>'Chelvey'</td>
<td>'Olga'</td>
</tr>
<tr>
<td>'Country Girl'</td>
<td>'Virginia'</td>
</tr>
</tbody>
</table>
Table 2.2. Treatments for the supplemental lighting study.

<table>
<thead>
<tr>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control—natural daylight</td>
</tr>
<tr>
<td>2. HID Natural Day Length—duration adjusted weekly to match natural day length</td>
</tr>
<tr>
<td>3. HID 16 Hour Day Length</td>
</tr>
</tbody>
</table>
Table 2.3. Treatment averages for number of days to flower from the end of the cool temperature period, number of flower clusters at first flower and plant height (cm) for the supplemental lighting study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days to Flower</th>
<th># Flr Clusters</th>
<th>Height</th>
<th>% Fruit Set</th>
<th># Seeds Per Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (natural light)</td>
<td>44.2</td>
<td>16.2</td>
<td>22.8</td>
<td>54.8</td>
<td>1.6</td>
</tr>
<tr>
<td>2. HID natural day length</td>
<td>43.1</td>
<td>16.0</td>
<td>21.4</td>
<td>60.8</td>
<td>1.6</td>
</tr>
<tr>
<td>3. HID 16 hour day</td>
<td>40.4</td>
<td>20.2</td>
<td>20.5</td>
<td>57.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*LSD_{0.05} = 6.6
*LSD_{0.01} = 5.1
*LSD_{0.05} = 1.4
*LSD_{0.01} = 29.4
*LSD_{0.01} = 1.2
Table 2.4. Clone averages for number of days to flower from the end of the cool temperature period, number of flower clusters at first flower and plant height (cm) for the supplemental lighting study.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Days to Flower</th>
<th>Number of Clusters</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>80-10-2</td>
<td>44.0</td>
<td>7.7</td>
<td>20.5</td>
</tr>
<tr>
<td>80-18-6</td>
<td>52.1</td>
<td>19.1</td>
<td>19.4</td>
</tr>
<tr>
<td>80-20-1</td>
<td>39.7</td>
<td>11.9</td>
<td>21.5</td>
</tr>
<tr>
<td>82-44-6</td>
<td>43.3</td>
<td>12.9</td>
<td>24.0</td>
</tr>
<tr>
<td>83-11-3</td>
<td>33.1</td>
<td>21.1</td>
<td>23.5</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>45.5</td>
<td>22.9</td>
<td>24.7</td>
</tr>
<tr>
<td>'Chelvey'</td>
<td>45.2</td>
<td>16.8</td>
<td>19.7</td>
</tr>
<tr>
<td>'Country Girl'</td>
<td>39.2</td>
<td>16.4</td>
<td>20.5</td>
</tr>
<tr>
<td>'Dolly'</td>
<td>39.1</td>
<td>15.6</td>
<td>19.1</td>
</tr>
<tr>
<td>'Elegant Rapture'</td>
<td>48.3</td>
<td>10.7</td>
<td>15.8</td>
</tr>
<tr>
<td>'L. C. Hickman'</td>
<td>36.6</td>
<td>20.1</td>
<td>25.7</td>
</tr>
<tr>
<td>'Grand Slam'</td>
<td>46.8</td>
<td>14.6</td>
<td>22.5</td>
</tr>
<tr>
<td>'Honey'</td>
<td>38.2</td>
<td>20.3</td>
<td>16.5</td>
</tr>
<tr>
<td>'Inez'</td>
<td>41.9</td>
<td>13.0</td>
<td>25.3</td>
</tr>
<tr>
<td>'Olga'</td>
<td>41.0</td>
<td>23.0</td>
<td>21.1</td>
</tr>
<tr>
<td>'Virginia'</td>
<td>47.6</td>
<td>33.6</td>
<td>25.4</td>
</tr>
</tbody>
</table>

\(^2\text{LSD}_{.01} = 3.2\)
\(^7\text{LSD}_{.01} = 4.2\)
\(^8\text{LSD}_{.01} = 1.9\)
Table 2.5. Clone averages for percent fruit set and number of seeds per fruit for the supplemental lighting study.

<table>
<thead>
<tr>
<th>Clone</th>
<th>% Fruit Set</th>
<th># Seeds/Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>80-10-2</td>
<td>59.6</td>
<td>1.4</td>
</tr>
<tr>
<td>83-11-3</td>
<td>85.8</td>
<td>3.0</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>60.8</td>
<td>1.1</td>
</tr>
<tr>
<td>'Chelvey'</td>
<td>66.3</td>
<td>1.8</td>
</tr>
<tr>
<td>'Country Girl'</td>
<td>41.6</td>
<td>1.3</td>
</tr>
<tr>
<td>'Dolly'</td>
<td>88.7</td>
<td>2.3</td>
</tr>
<tr>
<td>'L. C. Hickman'</td>
<td>50.3</td>
<td>1.5</td>
</tr>
<tr>
<td>'Honey'</td>
<td>24.2</td>
<td>1.1</td>
</tr>
<tr>
<td>'Inez'</td>
<td>79.7</td>
<td>1.6</td>
</tr>
<tr>
<td>'Olga'</td>
<td>21.5</td>
<td>1.1</td>
</tr>
<tr>
<td>'Virginia'</td>
<td>56.6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

^LSD_{0.01} = 12.7
\gamma{LSD}_{0.01} = 0.54
Figure 2.1. Crosses used to compare effects of single parent pollen vs bulk pollen.

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>78-4-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
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<td>81-29-1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81-59-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82-53-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>83-11-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelvey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. C. Hickman</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flora Pie</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inez</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Figure 2.2. Average percent of pollen staining with FDA of the 5 clones used as males for the bulk genotype effects study. (LSD.01 = 13.8)
Figure 2.3. Plot of average percent fruit set and number of seeds per fruit of 11 female clones from the supplemental lighting study. (r = .78)
Figure 2.4. Average percent seed per pollination for clones used as females in the bulk genotypes study. Values are averaged over the 7 single male and bulk pollen crosses. (LSD.01 = 5.8)
Figure 2.5. Average percent seed per pollination for single male and bulk pollen crosses. Values are averaged over the 8 female clones. (LSD$_{0.01} = 5.8$)
Figure 2.6. Residual plot of untransformed data for percent seed per pollination. (Lilliefors, p = .0001)

Figure 2.7. Residual plot for a square root data transformation of percent seed per pollination. (Lilliefors, p = .0001)
Figure 2.8. Percent fruit set of all 15 female clones in the supplemental lighting study.
Literature Cited


CHAPTER III

VARIATION IN MALE FERTILITY OF PELARGONIUM X DOMESTICUM

Poor fruit set is a challenge to breeding P. x domesticum with fruit set often no more than 10 to 20% with fruits usually containing 1 seed instead of the 6 that are possible (Hanniford et al 1980). Previous research has shown that a highly fertile female crossed with a highly fertile male will yield 90% or more fruit set (Chapter II).

P. x domesticum is a complex interspecific hybrid which is thought to be derived from as many as seven species (Clifford, 1970). All but one of the putative ancestors have chromosome numbers of n=11 (Daker, 1969; Gibby and Westfold, 1986; Knicely, 1964; Yu and Horn, 1988). With n=22, P. x domesticum is a tetraploid species (Daker, 1969). There is no information to date as to whether the species is a disomic or polysomic polyploid.

There is some debate in the literature about the success rate of pollen tube progression through Pelargonium styles. The number of pollen tubes reaching the ovary would affect the number of ovules fertilized. Philippi (1961) attributed poor seed set following self-pollinations of a diploid and cross pollinations of 3 tetraploid P. x
hortorum clones to the low numbers of pollen tubes he observed growing down the styles. *P. x hortorum* flowers have five locules with two ovules per locule. However, usually only one ovule per locule becomes fertilized. Tilney-Bassett (1963) proposed this is the result of pollen tube inhibition. He found that, though numerous pollen grains germinated with self-pollinations of diploid *P. x hortorum*, a limited number produced pollen tubes that grew all the way down the style. Later, Kubba and Tilney-Bassett (1981), found the limited pollen tube growth with both self and cross-pollinations. They speculated on the existence of a hormonal mechanism for fertilization control which prevents a pollen tube from entering the second ovule in a carpel after the first one has been fertilized. As less than half of the ovules are fertilized, they suggested that the hormonal mechanism moves laterally inhibiting fertilization of neighboring carpels. Yano et. al. (1975), with *P. roseum* and *P. crispum*, found that many pollen grains germinated on the stigma, but most of the pollen tubes stopped half way down the style. They proposed the existence of some mechanism to prevent more than one tube from entering the carpel. Cross pollinations of *P. crispum* exhibited 2 to 5 times more pollen tubes than self-pollinations of *P. roseum*.

However, other researchers have disagreed with concerns relating to insufficient numbers of pollen tubes.
Glicenstein (1986), working with tetraploid *P. x hortorum*, observed that some pollen tubes stopped growing whereas others burst in the style. The amount of inhibited pollen tubes varied by cultivar. He observed up to 26 tubes at the ends of the styles and believed that there were sufficient tubes to effect fertilization of at least one ovule per carpel. Tsai et. al. (1973) observed an average of 27 pollen tubes per self-pollinated style of *P. x hortorum* and believed this to be more than enough pollen tubes to effect fertilization of all ovules.

A possible explanation for poor seed set may be male sterility due to inviable pollen resulting from meiotic problems associated with interspecific hybridization and polyploidy. It is also possible that interactions between the pollen and the style in some way limit the number of pollen tubes arriving at the ovary. This experiment is part of an effort to determine which components of the *P. x domesticum* reproductive system contribute to reduced fertility. The ability of *P. x domesticum* male gametes to effect fertilization was examined by crossing 8 clones as males to 2 common females. The males were tested for pollen viability, pollen germination and development, and subsequent seed set.

The hypotheses for the experiment were:

**Hypothesis 1.** Do the male clones differ in pollen viability as indicated by differences in pollen staining.
Hypothesis 2. Do the male clones crossed to two common female clones differ in pollen germination, pollen tube elongation and seed set. Also, do the female clones pollinated by 8 different males differ for the same parameters.

Hypothesis 3. Are there relationships between percent pollen staining and the number of pollen tubes and between the number of pollen tubes and seed set for the male clones when crossed to a common female.

Materials and Methods

The 8 clones used as males for the experiment (Table 3.1) vary in male fertility as determined by previous crossing studies. The 2 clones used as females were 'Virginia' which had previously produced up to 50% fruit set when pollinated with bulk pollen (Chapter II) and 'Flora Fie' which had produced 94% fruit set with bulk pollen in the previous crossing season (data not shown).

Cuttings were taken on October 27 and placed under intermittent mist. After rooting, the cuttings were removed from the mist bench and grown under 15.5° C nights and 18° C days. The cuttings were pinched 9 days after removal from the mist. Two weeks after pinching, the cuttings were moved to a greenhouse section where the temperature was maintained at a constant 10° C to initiate flowering. Thirty seven days after the start of cooling the cuttings were transferred into 16.5 X 12.5 cm pots and placed under 16 hour HID lighting with 15.5° C nights and 18° C days. HID lighting was discontinued in early April.
and protective shading was applied to the greenhouse roof and sides in the latter part of May. Fertilization was based on results from soil tests. This included leaching the soil when tests indicated unacceptably high amounts of nutrients present. Insecticidal spraying for white fly occurred as needed.

Emasculating began on March 30 when all plants were well in flower with pollinating beginning 6 days later. Each male clone was crossed to both 'Virginia' and 'Flora Fie' using emasculated flowers that had become receptive that day and freshly dehisced pollen. A replication for a particular cross combination involved pollinating 2 flowers. One style was harvested 2 hours later to be examined for pollen germination and development while the other was allowed to mature on the plant to determine seed set. The experiment consisted of a total of 9 replications with a replication—for all 8 males taking 2 days to complete. The order of the males was randomized for each replication. No more than 2 flowers per cluster were pollinated and pollinations were evenly distributed over the plants available for both female clones. There were 8 plants available of 'Virginia' and 18 of 'Flora Fie' with 6 plants for each male clone. Open flowers were removed from the plants every other day to insure that the pollen available was fresh. Insecticide spraying was discontinued during pollination so that newly pollinated flowers would
not be disturbed. During this time, the white fly population was managed through weekly releases of the parasite, *Encarsia formosa*.

Styles were harvested 2 hours after pollination because preliminary trials indicated that, pollen tubes reach the end of the style between 1 to 2 hours after pollination. Harvested styles were processed using a modification of the technique of Martin (1959). After fixing overnight in FAA (37% formaldehyde:ethanol:glacial acetic acid, 1:8:1), the styles were softened in 8N NaOH for 24 hours. They were then washed twice in distilled water and placed in a solution containing 0.1% methyl blue and 0.7% K₃PO₄. Methyl blue, which fluoresces under ultraviolet light, stains the callose in pollen tube walls (Kho and Baër, 1968). The styles were kept refrigerated until they could be squashed under a cover slip and viewed on a Zeiss IM light microscope with a G365 exciter filter, an FT395 dichroic mirror and an LP420 barrier filter. Data taken included number of pollen grains on the stigmatic surface, number of pollen tubes in the style just under the stigma and number of pollen tubes at the end of the style.

On the day that a particular male was used for pollination, anthers were collected for viability staining using fluorescein diacetate (FDA). FDA tests for the presence of an active esterase and the integrity of the plasmalemma of the pollen grain's vegetative cell (Heslop-
Harrison et al. 1984). The FDA freely enters the cell where it is hydrolyzed to produce fluorescein which does not readily move across an intact membrane. Thus in cells with intact membranes fluorescein builds up in the cell and fluoresces brightly enabling it to be viewed using fluorescence microscopy. Staining was accomplished by placing 3 drops of FDA in a 61% sucrose solution on a slide and dipping 1 to 3 anthers into the solution. After covering with a cover slip, the percent of fluorescing grains was counted using a Zeiss IM light microscope with a BP450-490 exciter filter, an FT510 dichroic mirror and an LP520 barrier filter.

Data were analyzed using both analysis of variance and regression with SPSS for Windows®. Residuals were examined for heteroscedasticity and nonnormality and, where necessary, variance stabilizing transformations were used.

**Results**

**Hypothesis 1.** The 8 male clones differ in pollen staining by a factor of almost 4 from 18% for 'Purple Firedancer' to 69% for 83-11-3 indicating that they vary in initial pollen viability (Figure 3.1). The intensity of staining obtained with FDA varies from grain to grain making classification more difficult than with other stains. However, the gradation in fertility could actually be a more realistic assessment of *P. x domesticum* pollen viability than the
clear +/- results produced with other stains. Mature pollen may contain both normal and abnormal grains resulting in varying degrees of viability.

**Hypothesis 2.** The residual plot for the analysis of variance of the number of pollen grains clinging to the stigma (Figure 3.2) indicates heteroscedasticity (nonconstant variance). The residual plot shows a megaphone shape in that the spread in the residuals increases as the predicted value increases. SPSS provides the Lilliefors test for normality of data. The test is based on a modification of the Kolmogorov-Smirnov test for when means and variances are not known and must be estimated from the data (Norusis, 1990). The Lilliefors test of the residuals shows they are not normally distributed (p = .0000). The residuals from a square root data transformation (Figure 3.3) show improvement relative to the heteroscedasticity and the Lilliefors test indicates that they are normally distributed (p > .2). Because of the improvement, the square root data transformation is used for the analysis of the number of grains clinging to the stigma.

All the stigmas in the experiment were pollinated with excess pollen. The number of grains remaining on the prepared styles varied from cross to cross (Figure 3.4). It appears that the viable grains adhere to the stigma and
begin to germinate while most of the nonviable grains wash off as the styles are being fixed and softened. Pollen was observed at the bottom of the vials that were used for preparing the styles.

When crossed to the female, 'Flora Fie', the 8 males varied in number of grains on the stigma from 59 grains (7.6) for the male 'Purple Firedancer' to 297 grains (17.2) for 83-11-3. The number of grains remaining on stigmas of 'Virginia' varied from a low of 43 grains (6.6) for 'Purple Firedancer' to a high of 154 grains (12.4) for 83-11-3. Comparing the performance of each male between the 2 females reveals that 3 of the 4 most fertile male clones; 83-11-3, 'Dolly' and 'Bredon' had more grains left on the stigma when crossed to 'Flora Fie'.

Figure 3.5 presents the residual plot for the analysis of variance of the number of pollen tubes at the top of the style. The residual plot indicates heteroscedasticity of the residuals which are also not normally distributed (Lilliefors p = .0000). The residuals from a log(x +1) data transformation (Figure 3.6) show improvement relative to the heteroscedasticity and the Lilliefors test indicates that they are normally distributed (p > .2). Because of the improvement with the transformation, the log(x +1) data transformation is used for the analysis.

The male clones that produced more pollen grains adhering to the stigma also tended to produce more pollen
tubes at the top of the style (Figure 3.7). When crossed to the female, 'Flora Fie', the number of pollen tubes at the top of the stigma varied from a low of 4 tubes (0.65) for the male 'Purple Firedancer' to 35 tubes (1.56) for 83-11-3. The number of tubes at the top of the style for the males when crossed to 'Virginia' varied from 1 tube (0.36) for 'Bredon' to a high of 8 tubes (0.95) for 83-11-3. The difference in the performances of the male clones between the 2 females is more pronounced for the number of tubes at the top of the style than it was for the number of grains on the stigma. All of the 8 male clones produced more tubes when crossed to 'Flora Fie'. The difference between 'Flora Fie' and 'Virginia' was significant for the 4 most fertile males; 83-11-3, 'Inez', 'Dolly' and 'Bredon'.

The residual plot for the analysis of variance of the number of pollen tubes at the end of the style is presented in Figure 3.8. The plot indicates heteroscedasticity of the residuals which are also not normally distributed (Lilliefors p = .0000). A log(x +1) data transformation (Figure 3.9) shows improvement in the residuals relative to the heteroscedasticity with the Lilliefors test indicating that they are normally distributed (p > .2). The log(x +1) data transformation is used for the analysis because of the improvement in the residuals with the transformation.

By the time that the pollen tubes arrive at the end of the style the difference between the 2 females is very
pronounced (Figure 3.10). Of the 8 male clones all but 1, 'Chelvey' produced significantly more tubes at the end of the style when crossed to the female, 'Flora Fie'. With 'Flora Fie' as a female, the males varied in the number of pollen tubes at the end of the style from 1 tube (0.36) for the male, 'Chelvey' to 18 tubes (1.2) for 83-11-3. When crossed to the female, 'Virginia', the overall pollen tube production of the males was so poor that none of the differences between them were significant. With an average of 0.5 tube (0.167), the male 83-11-3 was the most successful at producing tubes at the end of the style when crossed to 'Virginia'. In addition to the fact that the males differ in how successful they are at producing pollen tubes, the data suggest that the 2 females differ in their ability to support the pollen tubes of the males.

The overall amount of fruit set for 'Virginia' was 7% which was too low for meaningful analysis. The number of seeds per 6 locules was analyzed for 'Flora Fie' alone. Although, the Geraniaceae family is characterized as having 5 locules per flower (Bosenwinkel and Been, 1979), 'Flora Fie' has 6. The residual plot for the analysis of the number of seed per 6 locules (Figure 3.11) indicates some heteroscedasticity. The Lilliefors test of the residuals showed they are not normally distributed (p = .0006). The arcsin transformation residuals indicate some improvement relative to the heteroscedasticity (Figure 3.12) though
they are still not normally distributed \( (p = .0049) \). The consequence of a lack of normality is that the actual probability of the statistical test used is lower or higher than the tabular probability that is given (Cochran, 1947). Since the arcsin data transformation provides some improvement, it is used for the analysis.

Seed set (Figure 3.13) ranged from 0.3 seed/6 locules (0.58) for 83-11-3 to 0.094 seed/6 locules (.094) for 83-52-1. 83-11-3 is the most fertile clone identified to date. The seed obtained in the cross between 'Flora Fie' and 83-11-3 for this experiment was a total of 18 out of a possible 54. This is in the high range of what is typically obtained in \( P. x \) domesticum crosses. Although there is significant fertility in the species, there is obviously room for improvement.

**Hypothesis 3.** Relationships between percent pollen staining and the number of pollen tubes and between the number of pollen tubes and seed set are examined in Figures 3.16 through 3.18. The data were regarded as supporting a relationship between the variables if the t-test for the slope of the line had a probability of .05 or lower and the \( R^2 \) was .7 or higher. During the course of this experiment, 3 clones from another study and 'Virginia' were tested for male fertility by crossing to 'Flora Fie' and by pollen staining with FDA. This allowed for comparisons using 12
males instead of 8 for the female, 'Flora Fie'.

The residual plot for the regression analysis using the percent pollen staining to predict the number of pollen tubes at the end of the style for the female 'Flora Fie' is presented in Figure 3.14. Residuals for the same analysis using a natural log(x +1) transformation are in Figure 3.15. The residuals in the plot of the untransformed data increase in spread as the predicted value increases indicating nonconstant variance. The natural log(x +1) transformation shows improvement in the residuals. The Lilliefors test of the residuals for both plots indicates that both are normally distributed (p > .2). The natural log(x +1) data transformation is used for the analysis because of the improvement in the residuals with the transformation.

The percent stained pollen of the male appears to be a good predictor of the number of pollen tubes that are produced at the end of the style when comparing the 12 males crossed to 'Flora Fie' (Figure 3.16). The model for the regression is: \( y = -1.4 + .98(x) + e \). The \( R^2 \) for the equation is .81 with a t-test probability of .0001. Within the scope of this model, the males react predictably within the common female environment in that the initial pollen viability is a strong predictor of the number of pollen tubes that arrive at the end of the style.
When crossed to the female, 'Virginia', the 8 male clones had produced so few pollen tubes at the end of the style that it was necessary to compare percent pollen staining with the number of tubes at the top rather than the end of the style (Figure 3.17). The model for the equation is: \( y = -0.7 + 0.14(x) + e \), with a t-test probability of 0.01. The \( R^2 \) for the analysis was 0.65 which is lower than the critical value that used for a relationship between the variables for this research. The low \( R^2 \) for this analysis indicates a poorer fit than the analysis using the percent pollen staining to predict the number of tubes at the end of the style for 'Flora Fie'. However, the analysis suggests that the percent stained pollen of the males is a predictor of the number of pollen tubes in a common female environment for 'Virginia' as it is for 'Flora Fie'. One point at \( x=69 \) does have a strong influence on the existence of a significant relationship. This point represents data for the clone, 83-11-3, which is the most fertile plant identified to date and is valid data.

Since the percent stained pollen appears to be a good predictor of the number of pollen tubes of different males in a common female environment, the next question that was asked was are the number of pollen tubes at the end of the style a good predictor of the amount of seed set per 6 locules for 'Flora Fie' (Figure 3.18). The model for the
regression is:  \( y = 0.0 + 0.02(x) + e \), with the t-test \( p = 0.0007 \) and the \( R^2 = 0.7 \). The regression equation indicates that it takes approximately 8.33 pollen tubes to ensure pollination of enough ovules to produce 1 seed in 6 locules or 1 seed in a pollinated 'Flora Fie' flower. Within the scope of the model, more pollen tubes result in more seed.

Each data point in Figures 3.16 through 3.18 represents an average of the 9 replications for a given male crossed to that particular female. The data were not broken down into separate points for each replication. Breaking the data into individual replications assumes that the measurement for the independent variable is correlated with the measurement for the dependent variable of that replication. Correlations between percent pollen staining and number of pollen tubes at the end of the style for the 9 replications within each male were not significant for 9 out of 12 males (Table 3.2). For the 3 males that the correlations were significant, 1 was a negative correlation and 2 were positive. This means that, for a given male, percent pollen staining on day 1 is no more related to the number of pollen tubes on day 1 than it is to the number of pollen tubes for any other day. It was, therefore, deemed more appropriate to use averages rather than individual replications for the regressions.
Discussion

The 8 male clones for the experiment varied in fertility based on previous seed set data. This experiment demonstrates that they also varied in percent pollen staining, pollen germination and development, and resulting seed set. Since percent stained pollen is a good predictor of number of tubes which is a good predictor of amount of seed in a common female environment, the variation in the fertility of the male clones appears to be mostly the result of differences in initial pollen viability rather than differences in the ability to develop tubes and effect fertilization. The lack of optimum pollen viability is probably primarily because of P. x domesticum's interspecific ancestry and polyploid condition. Meiotic irregularities are one of the most important causes of infertility in polyploids (Gillies, 1989). The P. x domesticum species developed into a recognizable group as the result of hybridizers' efforts by the 1830's (Clifford, 1970) which means it is a young species. With the recent development of this species and its high degree of sterility, there have not been many generations of sexual recombination to select for improvement in such problems as meiotic irregularities. Tetraploid clones of P. x hortorum are polysomic (Badr and Horn, 1971b) and have irregular meiosis whereas diploid clones have fairly regular meiosis (Badr and Horn, 1971a; Glicenstein, 1986; and Philippi,
1961). There is no information available as to whether *P. x domesticum* is a polysomic or disomic polyploid.

Previous discussion about seed set problems with other *Pelargonium* species has involved concerns as to whether adequate numbers of pollen tubes reach the end of the style. Two authors who disagreed with this concern found up to 26 (Glicenstein, 1986) and 27 (Tsai, 1973) tubes at the ends of *P. x hortorum* styles and believed those numbers sufficient to effect fertilization of all ovules. A regression using number of pollen tubes at the end of the style to predict seed set for 'Flora Fie' (Figure 3.18), within the scope of the model, indicated approximately 16.7 tubes were necessary for production of 2 seeds per 6 locules. Since three or less of the possible 6 seeds per flower were produced in any given pollination, it remains to be seen if adding more pollen tubes would eventually result in full seed set. It is not known, at present, if all the ovules in the flower are competent. However, if the relationship between the number of pollen tubes and seed set were to be true for other *Pelargonium* species, it is suggestive that numbers of pollen tubes in the mid 20's may not be sufficient to fertilize all of the ovules present in the flower.

The average number of grains on the stigma for the more fertile female, 'Flora Fie', was 219 while the average number of pollen tubes arriving at the end of the style was
5. In other words, only a little over 2 percent of the pollen grains that adhered to the stigma produced tubes that successfully grew to the bottom of the style. This has also been observed in several other species. Sanders and Lord (1989) state that a subset of the pollen tubes on the stigma reach the ovary for *Hemerocallis flava*, *Raphanus raphanistrum* and *Vicia faba*. The number of pollen tubes decreased linearly with the distance from the stigma in styles of *Nicotiana glauca* (Cruzan 1986). Herro (1992) found that 2 percent of the pollen grains on the stigma of *Prunus persica* produced tubes that reached the base of the style. The biggest reduction in number of pollen tubes occurs when the tube penetrates the stigma and enters the transmitting tissue which was associated with a change from autotrophic to heterotrophic growth. She related the further decrease in number of tubes in the style to a decrease in the width of the transmitting tissue as well as a decrease in carbohydrates along the length of the stylar transmitting tissue. The avocado (*Persea americana* Mill.) germinates an average of 66 pollen grains on the stigma, but a single pollen tube reaches the ovary containing a single embryo sac (Sedgley, 1976). Control by the embryo sac over pollen tube growth in the style has been suggested.

The two females in this experiment were very different in the number of pollen tubes that a given male produced in
the style. It appears that the genotype of the female affects the success of pollen germination and development. The style provides both nutrition (Knox, 1984) and guidance (Heslop-Harrison, 1987) to the developing pollen tubes. It is possible that clones vary in their ability to support pollen tubes. An additional experiment has been conducted to examine female effects on pollen tube development (Chapter IV).

Within a common female environment, FDA appears to be a good predictor of pollen tube production and subsequent seed set. This is advantageous as pollen staining with FDA is a simpler and faster technique than preparing styles and counting pollen tubes.
Table 3.1. Clones used as males in the male fertility study.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>83-11-3</td>
<td>'Dolly'</td>
</tr>
<tr>
<td>83-52-1</td>
<td>'L. C. Hickman'</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>'Inez'</td>
</tr>
<tr>
<td>'Chelvey'</td>
<td>'Purple Firedancer'</td>
</tr>
</tbody>
</table>
Table 3.2. Correlation of number of pollen tubes at the end of the style with percent stained pollen for the 9 replications.

<table>
<thead>
<tr>
<th>Clone</th>
<th>r</th>
<th>t²</th>
<th>Clone</th>
<th>r</th>
<th>t²</th>
</tr>
</thead>
<tbody>
<tr>
<td>83-11-3</td>
<td>.02</td>
<td>0.0</td>
<td>'Inez'</td>
<td>.90</td>
<td>5.5</td>
</tr>
<tr>
<td>83-52-1</td>
<td>-.11</td>
<td>0.0</td>
<td>'P. Firedancer'</td>
<td>.07</td>
<td>0.0</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>-.19</td>
<td>0.1</td>
<td>'Grandma Fischer'</td>
<td>.00</td>
<td>0.0</td>
</tr>
<tr>
<td>'Chelvey'</td>
<td>-.43</td>
<td>1.2</td>
<td>'Pink Gard. Joy'</td>
<td>-.15</td>
<td>0.4</td>
</tr>
<tr>
<td>'Dolly'</td>
<td>-.64</td>
<td>2.2</td>
<td>'Vendig'</td>
<td>.53</td>
<td>1.7</td>
</tr>
<tr>
<td>'L. C. Hickman'</td>
<td>-.29</td>
<td>0.1</td>
<td>'Virginia'</td>
<td>.60</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*P(t ≥ 1.9) = .1; P(t ≥ 2.4) = .05
Figure 3.1. Average percent stained pollen of the 8 clones used as males for the experiment. (LSD.01 = 13.85)
Figure 3.2. Residual plot of untransformed data for number of pollen grains on the stigma. (Lilliefors, p = .0000)

Figure 3.3. Residual plot of a square root data transformation for number of pollen grains on the stigma. (Lilliefors, p > .2)
Figure 3.4. Average number of pollen grains on the stigma of 'Flora Fie' or 'Virginia' for 8 different clones used as males using a square root transformation. (LSD,0.01 = 3.02)
Figure 3.5. Residual plot of untransformed data for number of tubes at the top of the style. (Lilliefors, $p = .0000$)

Figure 3.6. Residual plot of log($x + 1$) data transformation for the number of pollen tubes at the top of the style. (Lilliefors, $p > .2$)
Figure 3.7. Average number of pollen tubes at the top of the style under the stigma for 8 clones crossed as males to either 'Flora Fie' or 'Virginia' using a log(x+1) transformation. (LSD.0.05 = 0.421)
Figure 3.8. Residual plot of untransformed data for number of tubes at the end of the style. (Lilliefors, p = .0000)

Figure 3.9. Residual plot of log(x +1) data transformation for the number of pollen tubes at the end of the style. (Lilliefors, p > .2)
Figure 3.10. Average number of pollen tubes at the end of the style for 8 clones crossed as males to 'Flora Fie' or Virginia using a log(x+1) transformation. (LSD.01 = 0.321)
Figure 3.11. Residual plot of untransformed data for number of seeds per 6 locules. (Lilliefors, p = .0006)

Figure 3.12. Residual plot of arcsin data transformation for the number of seeds per 6 locules. (Lilliefors, p = .0049)
Figure 3.13. Average number of fully developed seed per 6 locules for 8 clones crossed as males to 'Flora Fie' using an arcsin transformation. (LSD_{₀.₀₁} = 0.329)
Figure 3.14. Residual plot of untransformed data for percent stained pollen as a predictor of number of pollen tubes at the end of the style with 'Flora Fie' as the female. (Lilliefors, p > .2)

Figure 3.15. Residual plot of natural log(x +1) data transformation for the percent stained pollen as a predictor of the number of pollen tubes at the end of the style with 'Flora Fie' as the female. (Lilliefors, p > .2)
Figure 3.16. 'Flora Pie' as female; average percent stained pollen as predictor of the average number of pollen tubes at the end of the style using an ln(x+1) transformation.

Figure 3.17. 'Virginia' as female; average percent stained pollen grains as predictor of the average number of pollen tubes at the top of the style.
Figure 3.18. 'Flora Fie' as female; the average number of tubes at the end of the style as predictor of the average number of seed per 6 locules.

\[ y = 0.0 + 0.02(x) + e \]

\[ R^2 = 0.7 \]
Literature Cited


An intimate relationship exists between the angiosperm pistil and pollen. The stigma provides a site of attachment for pollen grains, supporting their hydration and subsequent germination (Heslop-Harrison, 1987). The style secretes carbohydrates and proteins which support the growth of the pollen tubes (Knox, 1984) and its transmitting tissue guides the elongating tubes from the stigma to the ovary (Heslop-Harrison, 1987). The style is thought by some researchers to provide a medium in which the male gametes compete, with the pollen tubes of the most vigorous gametes arriving at the ovary first resulting in a more 'fit' sporophytic generation (Ottaviano and Mulcahy, 1989). Mulcahy (1979) suggests that pollen competition may be one of the primary reasons the angiosperms have been so successful in the plant kingdom.

Interactions occur between the pistil and the developing pollen tubes resulting in structural and physiological changes in the pistil; some of which occur in areas of the pistil that pollen tubes have not yet entered
(Shivanna and Johri, 1985). Among these are changes in source-sink relationships in *Petunia x hybrida* (Linskens, 1974), changes in levels of free amino acids in *Nicotiana alata* (Tupy, 1961), degeneration of the synergid that the pollen tube is to enter in many species (Jensen et al, 1983), and in *Cymbidium, Quercus,* and *Corylus* the megaspore is immature until pollen is deposited on the stigma (Knox, 1984).

Given the complex interactions between pistil and pollen, it is likely that different genotypes within a species could vary in their ability to interact with, and support the development of, the male gametophyte. A previous experiment examining the germination and development of *P. x domesticum* pollen from various clones crossed onto 2 common females revealed that the females differed in their ability to support the pollen tubes of the males (Chapter III). The objective of this study was to further examine genotypic variation in pistil support for the development of the male gametophyte. Two clones were crossed as males to 10 different females. Assessments were made of differences in germination frequency and growth and development of the pollen in the styles of the 10 females when pollinated with a common male. Differences between the 2 males were also assessed.
Hypotheses for the experiment were:

Hypothesis 1. Do the female clones in the experiment vary in stigmatic area or style length. If they do vary, is there a relationship between either and the number of pollen tubes at the end of the style.

Hypothesis 2. Do the female clones pollinated by two common male clones differ in pollen germination, pollen tube elongation and seed set. Also, do the male clones crossed to 9 different females differ for the same parameters.

Hypothesis 3. Are there relationships between the number of pollen grains clinging to the stigma and the number of pollen tubes at the top of the style or between the number of pollen tubes at the top of the style and the number of pollen tubes that arrive at the bottom of the style for the female clones when pollinated by a common female.

Materials and Methods

Ten clones of varying female fertility were chosen to be used as females for the experiment based on previous seed set data (Table 4.1). The 2 clones used as males were 83-11-3, the most fertile clone identified to date, and 'L. C. Hickman' which had produced an average number of pollen tubes as a male the previous season. The pollen viability of the males as determined by staining with fluorescein diacetate (Heslop-Harrison et al, 1984) was 81% (s = 5) for 83-11-3 and 24% (s = 13) for 'L. C. Hickman'.

Cuttings were taken on September 22 and placed under intermittent mist. After rooting, the cuttings were removed from the mist and placed under HID lighting at 15.5°C nights and 18°C days. The largest clones were pinched
after 8 days and the smaller ones 6 days later. Six days after the second group of cuttings had been pinched, the temperature was lowered to a constant 10° C to initiate flowering. After 6 weeks of cooling, the cuttings were transferred to 16.5 X 12.5 cm pots and the temperature was increased to 15.5° C nights and 18° C days to initiate floral development. A foliar spray of 1500 ppm Cycocel® was applied 6 days after transfer. Fertilization was based on soil test results. This included providing additional fertilizer when tests indicated unacceptably low amounts of nutrients present. Plants were sprayed with pesticides for white fly as needed. The HID lights were turned off in the beginning of April and protective shade was sprayed on the greenhouse roof and sides in early May.

Emasculations started on March 17 after all plants were well in flower with pollinations beginning 1 week later. The females were pollinated with each of the 2 males using emasculated flowers that had become receptive that day and freshly dehisced pollen. One replication was conducted per day for 10 days. The order of the females was randomized for each replication. A replication for a particular cross combination involved pollinating 2 flowers. The style of one flower was harvested 2 hours later for examination of pollen germination and development. The other flower was allowed to mature so that seed set could be obtained. Styles were prepared as
described previously (Chapter III). Data taken included the length of the style, number of stigmatic lobes, number of germinated pollen grains clinging to the stigma, number of pollen tubes in the style just under the stigma and number of pollen tubes at the end of the style. Opened blossoms were removed from the plants every 2 days to help ensure the availability of fresh pollen. No more than 3 flowers per cluster were pollinated. Pollinations were evenly distributed over the 4 to 6 plants available for each female. Sixteen plants of each male were available for pollen. The plants were not sprayed with insecticides while crosses were being made. The weather was cloudy and cool during most of the experiment which caused the emasculated flowers to mature slower than usual. In cases where there were not sufficient receptive flowers to conduct all of the planned pollinations, effort was focused on completing the pollinations to obtain styles to examine for pollen germination and development. 'Chelvey' matured so few emasculations that it was dropped from the study reducing the number of females from 10 to 9.

After the crosses were completed, unpollinated receptive styles were harvested from each female and preserved in FAA. The average stigmatic lobe area for each female was determined by measuring length and width under a Zeiss IM microscope of one stigmatic lobe from each of 10 styles. The stigmatic area of each style was obtained by
multiplying the average stigmatic lobe area by the number of stigmatic lobes for that style.

Data were analyzed using both Analysis of variance and regression with SPSS for Windows®. Residuals were examined for heteroscedasticity and nonnormality and, where necessary, variance stabilizing transformations were used.

Results

Hypothesis 1. The stigmatic area was calculated with the idea of possibly using it as a covariate since a larger stigma is capable of holding more pollen grains. The 9 females do vary significantly in average stigmatic area (Table 4.2) with the largest, 'Flora Fie' being nearly 70% larger than the smallest, 83-52-1. However, using the stigmatic area to predict the number of tubes at the end of the style was unsuccessful (Figures 4.1 and 4.2). Data is regarded as supporting a relationship between the variables if the t-test for the slope of the line had a probability of .05 or lower and the $R^2$ was .7 or higher. The model for the regression for the male, 83-11-3, is: $y = -9.7 + 1.8(x) + e$, with $R^2 = .3$ and t-test = .14. The model for the regression equation for the male, 'L. C. Hickman', is: $y = 2.6 + .18(x) + e$, with $R^2 = .01$ and t-test = .80. Both figures seem to have outliers in the data set. Deleting the data point at $y = 33.4$ tubes from the analysis for the male, 83-11-3, still does not result in a significant
regression ($t = .13, R^2 = .34$). Deleting the 2 data points at $y = 7.7$ and $y = 9.4$ tubes from the analysis for the male, 'L. C. Hickman', does produce a good regression ($t = .01, R^2 = .75$). However, achieving the regression involves removing close to 25% of the data set. Further work could show that there is a relationship between stigmatic surface size and the number of pollen tubes at the end of the style, but the data currently available indicates that there is no relationship.

The length of each style was recorded to determine if there was a relationship between style length and the number of pollen tubes at the end of the style. The female clones in the experiment vary significantly for style length by a factor of 2 from .53 cm for 83-52-1 to 1.11 cm for 'Virginia' (Table 4.2). However, graphs comparing the style length and number of tubes at the end of the style for each male showed so little likelihood of a relationship that statistical analysis was not even attempted (Figures 4.2 and 4.3). The lack of a relationship indicates that, by 2 hours after pollination, enough time had passed for the pollen tubes to arrive at the ends of all styles.

**Hypothesis 2.** Differences in pollen germination and development for the 9 female clones and 2 common pollen sources beginning with the number of pollen grains adhering to the stigma and ending with the number of pollen tubes
that arrived at the end of the style are represented in Figures 4.7, 4.10, 4.13 and 4.14.

The residual plot for the analysis of variance of the number of pollen grains clinging to the stigma (Figure 4.5) indicates heteroscedasticity (nonconstant variance). The residual plot shows a megaphone shape in that the spread in the residuals increases as the predicted value increases. SPSS provides the Lilliefors test for normality of data. The test is based on a modification of the Kolmogorov-Smirnov test for when means and variances are not known and must be estimated from the data (Norusis, 1990). The Lilliefors test of the residuals shows they are not normally distributed (p = .0098). The residuals from a square root data transformation (Figure 4.6) show improvement relative to the heteroscedasticity and the Lilliefors test indicates that they are normally distributed (p > .2). Because of the improvement, the square root data transformation was used for the analysis of the number of pollen grains clinging to the stigma.

When receptive stigmas of the 9 females were pollinated by the 2 males, wide differences in the number of grains that attached to the stigma and began to germinate were noticed (Figure 4.7). Though every stigma was pollinated with excess pollen, the grains that did not adhere and begin to germinate likely washed off during preparation of the styles for microscopic evaluation.
Pollinating with L. C. Hickman resulted in consistently and significantly less pollen grains on the stigma for all 9 female clones. In fact, the highest number of pollen grains found for this male, 11.5 (132 grains) with the female 'Flora Fie', was significantly lower than all but one of the females crossed to 83-11-3. When pollinated with the male, 83-11-3, the number of pollen grains adhering to the stigma varied from a low of 13.0 (170 grains) for 81-29-1 to a high of 22.5 (506 grains) for 'Pink Gardener's Joy'. Pollinating with 'L. C. Hickman' produced a range of pollen grains from 6.8 (46 grains) for 81-29-1 to 11.5 (178 grains) for 'Flora Fie'.

The residual plot for the analysis of variance of the number of pollen tubes at the top of the style (Figure 4.8) indicates heteroscedasticity. The Lilliefors test of the residuals shows they are not normally distributed (p = .0056). The residuals from a square root data transformation (Figure 4.9) show improvement relative to the heteroscedasticity with the Lilliefors test indicating that they are normally distributed (p > .2). Because of the improvement, the square root data transformation was used for the analysis of the number of pollen tubes at the top of the style.

The difference in number of pollen tubes at the top of the style just under the stigma that the females supported between the 2 males is less pronounced than it was for the
number of pollen grains on the stigma (Figure 4.10). Seven of the nine females supported significantly more pollen tubes at the top of the style when pollinated with 83-11-3 than with L. C. Hickman. The 2 females 83-52-1 and 'Pink Gardener's Joy' did produce more pollen tubes with 83-11-3 as the male than with 'L. C. Hickman', but the differences were not large enough to be significant. The number of pollen tubes produced at the top of the style when pollinated with the male, 83-11-3, varied from a low of 2.6 (7 tubes) for 78-4-1 to a high of 8.1 (66 tubes) for 'Dolly'. The number of pollen tubes when pollinated with 'L. C. Hickman' varied from 1.5 (2 tubes) for 78-4-1 to 3.8 (14 tubes) for 'Dolly'.

The residuals for the analysis of variance of the number of pollen tubes at the end of the style demonstrate heteroscedasticity (Figure 4.11). They are also not normally distributed (Lilliefors, p = .0000). There is improvement in the distribution of the residuals with a square root data transformation (Figure 4.12). The Lilliefors test for normality indicates that they are also normally distributed (p > .2). The square root data transformation was used for the analysis of the number of pollen tubes at the top of the style because of the improvement.

By the time the pollen tubes arrive at the end of the style, the females still supported more tubes overall when
pollinated with 83-11-3 (Figure 4.13). However, the difference is even less pronounced than it was for the number of pollen tubes at the top of the style. For 5 of the 9 female clones; 81-29-1, 81-59-1, 'Bredon', 'Dolly' and 'Flora Fie' significantly more pollen tubes are produced with the male 83-11-3. Three of the female clones, 78-4-1, 'Pink Gardener's Joy' and 'Virginia' did not produce significantly more pollen when pollinated with 83-11-3. In fact, 'Pink Gardener's Joy' produced slightly, though not significantly, more pollen tubes when pollinated with 'L. C. Hickman' (4.6 tubes vs 4.3 tubes). One female, 83-52-1, supported significantly more pollen tubes at the end of the style when pollinated with L. C. Hickman than with 83-11-3 (6.9 vs 2.0 tubes). Graphing the average number of tubes at the end of the style of the 2 males against each other (Figure 4.14) demonstrates that, in general, a female was equally effective in supporting the pollen tubes of either male ($r = .67$). With 2 exceptions, pollinating with 83-11-3 consistently produced more pollen tubes than 'L. C. Hickman' reflecting its higher initial pollen viability.

Seed production was very low when the females were pollinated with 'L. C. Hickman' as a male. In 90 pollinations with 'L. C. Hickman' only 3 seeds were produced; 2 seeds with 'Dolly' and 1 with 'Flora Fie'. The total number of fully developed seed for the females
pollinated with 83-11-3 is given in Table 4.3. The number of pollen tubes within a given style is a reflection of both the female's ability to support pollen tubes and the male's viability. The number of seed produced in the pollinated pistil includes additional characteristics that relate primarily to the fecundity of the female such as the female's ability to produce competent ovules and mature seed as well as the viability of the developing embryo. As an example, 'Pink Gardener's Joy' supports an average number of pollen tubes (Figure 4.13), but 43 pollinations in 2 years has produced no fully developed seed.

**Hypothesis 3.** The relationship between the number of pollen grains on the stigma and the number of pollen tubes at the top of the style is illustrated in Figures 4.15 and 4.16. The relationship between the number of pollen tubes at the top of the style and the number at the bottom of the style is illustrated in Figures 4.19 and 4.20. The figures examine the performances of the female clones within a common male environment.

The number of pollen grains on the stigma was not a successful predictor of the number of tubes at the top of the style when the females were pollinated with either 83-11-3 or 'L. C. Hickman' (Figures 4.15 and 4.16). The best regression fit in each case was obtained using a square root transformation. The model for the regression for the
male, 83-11-3, is: \( y = 2.4 + 0.14(x) + e \), with \( t = .03 \) and \( R^2 = .06 \). The model for the regression for the male, 'L. C. Hickman', is: \( y = 0.9 + 0.24(x) + e \), with \( t = .0000 \) and \( R^2 = .32 \). The lack of a statistically significant analysis is probably the result of lack of precision in determining which grains have successfully germinated resulting in poor precision in the analysis.

The residuals for the regression using the number of pollen tubes at the top of the style to predict the number of pollen tubes at the end of the style for the common male, 83-11-3 (Figure 4.17) are heteroscedastic as well as not normally distributed (Lilliefors, \( p = .0104 \)). A square root data transformation improves the heteroscedasticity while producing normally distributed residuals (Lilliefors, \( p > .2 \)). Because of the improvement, the square root data transformation was used for the regression.

The number of pollen tubes at the top of the style is a good predictor of the pollen tube number at the end of the style when pollinated with either male (Figures 4.19 and 4.20). The model for the regression for the male, 83-11-3, is: \( y = -1.02 + 0.77(x) + e \), with the \( t = .0000 \) and \( R^2 = .73 \). The model for the regression for the male, 'L. C. Hickman', is: \( y = -1.43 + 0.58(x) + e \), with \( t = .0000 \) and \( R^2 = .72 \). This indicates that, although the number of tubes declines from the top to the bottom of the style, the percentage of tubes that arrive at the bottom of the style
is consistent for the females pollinated with a common male.

Discussion

When pollinated with a common male, the *P. x domesticum* female clones in this study varied in how many pollen grains adhered to the stigma and in the number of pollen tubes produced in the style. The impact of the different female environments on the success of the male gamete was pronounced. By the time the pollen tubes arrived at the end of the style their numbers varied from 33 to .8 tubes for the more fertile male, 83-11-3, and from 9 to .5 tubes for the less fertile male, 'L. C. Hickman' (Figure 4.13). As Figures 4.13 and 4.14 demonstrate, the relationship between the females and males is not necessarily predictable in that they can interact with one another. Since there are not clear cut classes in the ability to support pollen germination and development, this trait appears to be quantitative in nature.

Differences in female support of the male gametophyte have been documented in other species. Pfahler (1967) and Mulcahy (1971) found female differences in pollen competition studies using *Zea mays*. Jennings and Topham (1971) investigating raspberries and Schlichting and Devlin (1989) using compatible crosses of *Phlox drummondii* discovered female differences in percent pollen
germination. Murdy and Carter (1987) found populations of *Talinum mengesii* that delayed pollen germination for up to 2 hours. They thought that the trait promoted an accumulation of pollen on the stigma thereby enhancing gamete competition. Sayers and Murphy found that more fertile *Medicago falcata* clones supported more pollen tubes no matter what clone was used as the pollen source. Sarr et al (1983) found female variation in speed of pollen germination and development in *Pennisetum typhoides*.

Fertilization is the result of a complex series of physiological and biochemical interactions that occur after the arrival of the pollen on the stigma (Hogenboom, 1984). The final measurement of female fertility is the number of seeds produced by the plant. However, this final measurement is the sum total of many processes including the ability to produce competent ovules, the ability to interact with and support the male gametophyte and the ability to mature viable seed. This experiment has helped illustrate that it is possible for the genotypes within a species to differ in their ability to accomplish various aspects of these stages. An awareness of the various components of *P. x domesticum* female fertility such as the genotypic variation in ability to support pollen tubes investigated in this experiment could aid in selecting for improved female fertility.
Table 4.1. Clones used as female parents for the experiment.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>78-4-1</td>
<td>'Chelvey'</td>
</tr>
<tr>
<td>81-29-1</td>
<td>'Dolly'</td>
</tr>
<tr>
<td>81-59-1</td>
<td>'Flora Fie'</td>
</tr>
<tr>
<td>83-52-1</td>
<td>'Pink Gardener's Joy'</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>'Virginia'</td>
</tr>
</tbody>
</table>
Table 4.2. Average stigmatic lobe area and style length of the female clones.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Stigmatic Area mm²</th>
<th>Style Length cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>78-4-1</td>
<td>1.62</td>
<td>0.82</td>
</tr>
<tr>
<td>81-29-1</td>
<td>1.73</td>
<td>0.85</td>
</tr>
<tr>
<td>81-59-1</td>
<td>1.73</td>
<td>1.04</td>
</tr>
<tr>
<td>83-52-1</td>
<td>1.23</td>
<td>0.53</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>1.34</td>
<td>1.06</td>
</tr>
<tr>
<td>'Dolly'</td>
<td>1.57</td>
<td>0.80</td>
</tr>
<tr>
<td>'Flora Fie'</td>
<td>2.05</td>
<td>0.73</td>
</tr>
<tr>
<td>'Pink Gardener's Joy'</td>
<td>1.92</td>
<td>0.70</td>
</tr>
<tr>
<td>'Virginia'</td>
<td>1.53</td>
<td>1.11</td>
</tr>
</tbody>
</table>

\(^a\text{LSD}_{0.01}=0.15\)
\(^b\text{LSD}_{0.01}=0.04\)
Table 4.3. The total number of fully developed seed per 10 pollinations using 83-11-3 as the male.

<table>
<thead>
<tr>
<th>Clone</th>
<th># Seed</th>
<th>Clone</th>
<th># Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>78-4-1(^z)</td>
<td>0</td>
<td>'Dolly'(^z)</td>
<td>11</td>
</tr>
<tr>
<td>81-29-1(^z)</td>
<td>0</td>
<td>'Flora Fie'</td>
<td>5</td>
</tr>
<tr>
<td>81-59-1</td>
<td>7</td>
<td>'P. Gardener's Joy'</td>
<td>0</td>
</tr>
<tr>
<td>83-52-1</td>
<td>0</td>
<td>'Virginia'</td>
<td>3</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^z\)9 pollinations
Figure 4.1. 83-11-3; a comparison between the number of tubes at the end of the style and the stigmatic surface area.

Figure 4.2. 'L. C. Hickman'; a comparison between the number of tubes at the end of the style and the stigmatic surface area.
Figure 4.3. 83-11-3; a comparison between the number of tubes at the end of the style 2 hours after pollination and the style length.

Figure 4.4. 'L. C. Hickman'; a comparison between the number of pollen tubes at the end of the style 2 hours after pollination and the length of the style.
Figure 4.5. Residual plot of untransformed data for number of pollen grains on the stigma. (Lilliefors, p = .0098)

Figure 4.6. Residual plot of a square root data transformation for number of pollen grains on the stigma. (Lilliefors, p > .2)
Figure 4.7. The average number of pollen grains on the stigmas of 9 clones pollinated by 83-11-3 or L. C. Hickman using a square root transformation. (LSD$_{0.01}$ = 2.9)
Figure 4.8. Residual plot of untransformed data for the number of pollen tubes at the top of the style. (Lilliefors, $p = .0056$)

Figure 4.9. Residual plot of a square root data transformation for the number of pollen tubes at the top of the style. (Lilliefors, $p > .2$)
Figure 4.10. The average number of pollen tubes at the top of the style just under the stigma of 9 females pollinated with 83-11-3 or L. C. Hickman using a square root transformation. (LSD_{0.01} = .40)
Figure 4.11. Residual plot of untransformed data for the number of pollen tubes at the end of the style. (Lilliefors, $p = .0000$)

Figure 4.12. Residual plot of a square root data transformation for the number of pollen tubes at the end of the style. (Lilliefors, $p > .2$)
Figure 4.13. The average number of pollen tubes at the end of the style of 9 females pollinated with 83-11-3 or L. C. Hickman using a square root transformation. (LSD$_{0.01}$ = 1.0)

Figure 4.14. A comparison of the average number of pollen tubes at the end of the style for the male clones 83-11-3 and L. C. Hickman for each female using a square root transformation. ($r = .67$)
Figure 4.15. 83-11-3; the number of pollen tubes at the top of the style vs the number of pollen grains clinging to the stigma.

Figure 4.16. 'L. C. Hickman'; the number of pollen tubes at the top of the style vs the number of pollen grains clinging to the stigma.
Figure 4.17. Residual plot of untransformed data for the number of pollen tubes at the top of the style as a predictor of the number of pollen tubes at the end of the style for the male, 83-11-3. (Lilliefors, p = .0104)

Figure 4.18. Residual plot of a square root transformation of the number of pollen tubes at the top of the style as a predictor of the number of pollen tubes at the end of the style for the male, 83-11-3. (Lilliefors, p > .2)
Figure 4.19. 83-11-3; the number of tubes at the top of the style as predictor of the number of tubes at the end of the style using a square root transformation.

\[ Y = -1.02 + .77(x) + \epsilon \]

\[ R^2 = .73 \]

Figure 4.20. L. C. Hickman; the number of tubes at the top of the style as predictor of the number of tubes at the end of the style.


CHAPTER V
THE POSSIBILITY OF SELF-INCOMPATIBILITY IN
PELARGONIUM X DOMESTICUM

*Pelargonium x domesticum* (regal pelargonium) is produced commercially as a vegetatively propagated flowering pot crop. Efforts by plant breeders to produce improved cultivars in this interspecific tetraploid are hindered by poor seed set. Crossing often produces no more than 10 to 20% overall fruit set with the fruits usually containing 1 or 2 seeds instead of the 6 that are possible (Hanniford et al. 1980 and personal communication). A previous experiment with 16 genotypes produced an overall fruit set of 49% which was much higher than had been expected (Chapter II). This experiment involved pollinating clones with a mixture of pollen from 20 or more unrelated clones. One possible explanation for the unexpectedly high seed set could be the existence of an active self-incompatibility system in the *P. x domesticum* species.

Self-incompatibility is a genetically inherited mechanism which prevents a plant's ovules from being fertilized with its own pollen. This results in seed that is produced only through outcrossing to other genotypes.
Self-incompatibility is an efficient mechanism of preventing loss of reproductive effort by avoiding the problems associated with inbreeding (Charlesworth and Charlesworth, 1979). Whitehouse (1950) believed that the evolutionary advantages of self-incompatibility which is found only in the Angiosperms is the primary reason for their dominance of the plant kingdom.

The self-incompatibility response is usually controlled by multiple alleles at 1 locus called the S locus. There are 2 different systems of self-incompatibility: gametophytic and sporophytic. The 2 systems are differentiated by the mode of self-recognition.

Gametophytic self-incompatibility involves recognition of self pollen based on the S allele of the haploid pollen grain. Self pollen typically germinates normally and sends pollen tubes through the stigma and into the style. The pollen tubes become arrested within the style and do not progress to the ovary. Families that exhibit gametophytic self-incompatibility usually have binucleate pollen and include Solanaceae, Rosaceae and Leguminosae with the former being the most studied.

Sporophytic self-incompatibility involves recognition of self pollen based on both of the S alleles of the pollen parent. The self-incompatibility response takes place at the stigmatic surface with the pollen either not germinating or the tubes unable to penetrate the stigma.
Since it is the diploid parental genotype which determines specificity, it is possible to have dominance between the alleles. Families that exhibit sporophytic self-incompatibility usually have trinucleate pollen and include *Brassicaceae* and *Asteraceae* with the former being the most studied (de Nettancourt 1977).

Researchers are beginning to elucidate the molecular mechanisms of self-incompatibility. Solanaceous plants with gametophytic self-incompatibility have an S locus gene coding for a glycoprotein that is expressed in the style and has RNase activity (Newbigin, et al. 1993). *Brassica* species with sporophytic self-incompatibility have 2 genes at the S locus, one codes for a glycoprotein that does not have RNase activity and the other for a membrane associated kinase. Both of the genes are expressed in the pollen and at the stigmatic surface (Nasrallah and Nasrallah, 1993). Researchers have yet to determine how either self-incompatibility system recognizes the difference between self and outcross pollen.

The purpose of self-incompatibility is to prevent self pollination. However, a plant will not set seed with any other that has the same S alleles whether or not the two are related. Both types of self-incompatibility systems tend to have large numbers of S alleles. Depending on the number of S alleles in a population, the percentage of successful crosses can be low. This is particularly true
of the sporophytic system which prevents a successful cross if either of the 2 S alleles in the pollen match those of the pistil (Liedl and Anderson, 1993). For example, chrysanthemum (Dendranthema grandiflora) has a sporophytic self-incompatibility system; approximately one out of three combinations between unrelated plants are successful (Anderson and Ascher, 1993).

When species are domesticated, incompatibility systems are often broken down or circumvented over time. The accumulation of Pseudo-self-compatibility (PSC) genes is one way in which this occurs. PSC is usually a quantitatively inherited trait which allows varying degrees of self seed set in a self-incompatible species. With PSC, it is possible to obtain self seed set as high as that of compatible crosses (Liedl and Anderson, 1993).

There are suggestions in the literature for the existence of self-incompatibility in Pelargonium, but these suggestions include very little data to support its existence one way or the other. Sears (1936) self pollinated several cultivars of P. x hortorum, found three that were "self-sterile" and performed cytological preparations of self pollinated styles from them. Pollen germination was inhibited at the stigmatic surface suggesting sporophytic self-incompatibility. However, Sears did not state whether the self-sterile plants' pollen germinated when applied to other plants which would have
documented that the pollen was viable. East (1940) examined "perhaps a dozen" cultivated forms of *Pelargonium* and found them all "weakly self-sterile". Partial self-sterility would suggest the presence of PSC genes.

Dale and Rogers (1971) claim there is evidence for self-incompatibility among their *P. x hortorum* lines, but offer no data. Harney and Chow (1971), while investigating the crossability of *P. x hortorum* and its putative ancestors, found that *P. stenopetalum* was completely self-sterile. They found varying degrees of self seed set with the other ancestor species and suggested that this meant that they may have PSC. Tilney-Bassett (1963) found very low self seed set in three cultivars of *P. x hortorum* suggesting that they were self-incompatible, and speculates that self-fertility in this species has arisen by breakdown of the self-incompatibility system through hybridization.

Glicenstein (1986) is the only researcher to date that offers actual data comparing self cross and outcross seed set. He obtained equal fruit set but lower seed set with self pollinated crosses of tetraploid *P. x hortorum*. He argues that the lower seed set after self pollination is from higher embryo abortion due to inbreeding depression rather than self-incompatibility.

Though there is very little hard data about self-incompatibility in *Pelargonium*, there are a number of suggestions in the literature that it does exist.
Pelargoniums have trinucleate pollen (Brewbaker, 1959); if there is self-incompatibility, it is likely to be of a sporophytic type. The system may have been mostly broken down in P. x hortorum after many years of hybridization, but could still be active in P. x domesticum. The presence of an active self-incompatibility system in P. x domesticum could explain why plants pollinated with a bulk of pollen from many different clones produced an unexpectedly high percentage of fruit set. Pollen from many clones would contain a variety of S alleles some of which would be different from the pistil parent. The mix of S alleles would ensure that there would be pollen grains able to germinate and fertilize the ovules.

The objective of this research was to determine if self-incompatibility contributes to poor fertility in P. × domesticum. This was accomplished by comparing self and outcross pollen tube production and seed set.

The hypotheses for this research were:

Hypothesis 1. In examining the number of pollen tubes produced at the end of the style, is there a difference between pollinating the stigma with the plant's own pollen or an outcross pollen source. This hypothesis was addressed in Experiment 1.

Hypothesis 2. Is there a difference in the number of pollen tubes produced at the end of the style when a clone's pollen is applied to its own stigma or to the stigma of another clone. This hypothesis was addressed in Experiment 2.

Hypothesis 3. Is there a difference in seed set when the clone is pollinated with its own pollen or outcross pollen sources. This hypothesis was addressed in Experiment 3.
Materials and Methods

Experiment 1  This experiment examined pollen tube production in the styles of 9 different females (Table 5.1) pollinated with self or outcross pollen. The females were chosen, based on previous seed set data, to represent a range of female fertility. They were pollinated with 3 different pollen sources; a) self pollen, b) 83-11-3, and c) 'L. C. Hickman'. The male, 83-11-3, (81% s=5 pollen viability) is the most fertile clone identified to date. 'L. C. Hickman' (24% s=13 pollen viability) is significantly less fertile than 83-11-3.

Styles were harvested 2 hours after pollination and, after preparation, were examined for pollen tube production using fluorescent microscopy. Ten replications of each cross were conducted with one replication performed per day for a total of ten days. Details of this experiment have been described previously in Chapter IV.

Experiment 2  This experiment compared the pollen tube production of 8 clones (Table 5.2) used as males for either a) outcross or b) self pollinations. The outcross pollinations involved crossing the males to 'Flora Fie' which is a highly fertile clone and provided a common female environment in which to compare the performances of the 8 males. The 8 male clones were chosen based on previous crossing performance to represent a range of
As in the previous experiment, styles were harvested 2 hours after pollination and examined using fluorescent microscopy. Nine replications of each cross were conducted with one replication of the experiment taking two days to complete. Further details have been described previously in Chapter III.

Experiment 3 This experiment compared seed set of 8 female clones of varying fertility when pollinated with 5 different males of varying fertility or self pollen. Tables 5.3 and 5.4 list the 8 female clones and 6 pollen sources respectively. There were 8 replications with 1 conducted per day for 8 days. The specifics of how this experiment was conducted were described in Chapter II.

The data from all 3 experiments were analyzed using analysis of variance with SPSS for Windows®. The residuals were examined for heteroscedasticity and nonnormality and, where necessary, variance stabilizing transformations were used.

Results

Hypothesis 1 The residual plot for the analysis of variance of the number of pollen tubes at the end of the style for Experiment 1 indicates heteroscedasticity (Figure 5.1) in that the spread in the residuals increases as the
predicted value increases. SPSS provides the Lilliefors test for normality of data which is based on a modification of the Kolmogorov-Smirnov test when means and variances are not known and must be estimated from the data (Norusis, 1990). The Lilliefors test of the residuals shows they are not normally distributed \( p = .0000 \). A square root data transformation shows improvement in the residuals relative to the heteroscedasticity with the Lilliefors test indicating normal distribution \( p > .2 \). Because of the improvement, the square root data transformation was used for the analysis of the number of pollen tubes at the end of the style.

The number of pollen tubes produced at the end of the style when the 9 females were pollinated with a common male varied from .8 to 33 tubes (0.9-5.7) for the male, 83-11-3, and from .5 to 9 tubes (0.7-8.7) for the male 'L. C. Hickman' (Figure 5.3). Though the tube numbers covered a wide range, there are not the two clear classes of tubes and no tubes that would have occurred if the differences were due to self-incompatibility.

Whereas the data for either male represents a consistent male genotype matched with 9 female genotypes, each self cross represents a different male and female combination. For the self cross, 5 of the 9 female clones; 'Pink Gardener's Joy', 81-29-1, 81-59-1, 78-4-1 and 'Virginia'; produced pollen tube numbers equivalent to the
better of the 2 outcross males. For the remaining 4 clones: 'Dolly', 'Flora Fie', 83-52-1 and 'Bredon' the self cross is equal to whichever outcross male is poorest. The self cross did not produce the lowest number of pollen tubes for any of the 9 female clones.

Hypothesis 2. The residual plot for the analysis of variance of the number of pollen tubes at the end of the style is presented in Figure 5.4. The plot indicates heteroscedasticity of the residuals which are also not normally distributed (Lilliefors p = .0000). Figure 5.5 shows the residuals from a square root transformation of the data. There is improvement in the residuals relative to the heteroscedasticity with the Lilliefors test indicating that they are normally distributed (p > .2). The square root data transformation is used for the analysis because of the improvement in the residuals.

The common female used for the experiment, Flora Fie, was one of the better females at supporting pollen tube production in Experiment 1. The male clones, when crossed to the common female, vary in pollen tube production from 4.2 (18 tubes) for 83-11-3 to 1.4 (2 tubes) for 'Purple Firedancer' (Figure 5.6). However, as for the previous experiment, there are not classes of high and low numbers as would have occurred with the existence of an active self-incompatibility system. For all 8 male clones the
number of pollen tubes at the end of the style for the self cross was not statistically different from the outcross with 'Flora Fie'.

**Hypothesis 3** As the female times cross interaction for Experiment 3 was not significant \((p = .29)\), the percent seed per pollination for each cross has been averaged over the 8 female clones (Figure 5.7). For the 6 crosses (self cross and 5 different outcrosses) the only significant difference is between 83-52-1 and 'Grandma Fischer'. However, of the 5 clones used as males, 83-52-1 had the lowest percent pollen staining indicating the lowest initial pollen viability (Figure 5.8). The self cross resulted in the same percent seed set as the outcrosses indicating that self-incompatibility was not affecting the outcome.

**Discussion**

The Experiment 1 females displayed considerable variation in how well they supported pollen tube production. The males from the experiment varied both in initial pollen viability which affected the number of pollen tubes produced and in how well they responded to a given female. There is genetic variability for most phenotypic characteristics of any organism. Since the relationship between the pollen and pistil is a complex
one, it is reasonable that there is variation other than self-incompatibility in pollen tube production among the clones. If self-incompatibility did exist in the species, pollen tube production would be about the same for both self and outcross for any cross combination in which the outcross pollen had the same S alleles as the pistil. In this case the, self cross pollen tube production would be equal to that of the poorer of the two males which would be the one incompatible with the female, and whatever pollen tube production occurred would be the result of PSC. However, if this was the case in this experiment, the odds would be high that at least 1 of the female clones would not have had matching S alleles with either male in which case the self cross would have produced the lowest number of pollen tubes which did not occur.

As was discussed in Chapter III, percent pollen viability of the male is a good predictor of the number of tubes for a common female in Experiment 2. Therefore the differences in pollen tube production with the common female were mostly the result of differences in initial pollen viability rather than self-incompatibility. Here again, if self-incompatibility did exist, it is possible that there could be matching S alleles between 'Flora Fie' and the clones resulting in approximately the same number of pollen tubes produced for both self and out cross pollinations with the tubes that were produced the result
of PSC. However, the likelihood that all 8 clones chosen for the experiment had the same S alleles as 'Flora Fie' would be very low.

A clear cut self-incompatibility response of no pollen tubes and no seed in self crosses is easy to spot and obviously does not exist in *P. x domesticum*. Clones do vary in both pollen viability and the pistil's ability to support pollen tubes. This cross to cross variation makes it difficult to completely eliminate the possibility of self-incompatibility mitigated by large amounts of PSC. However, as long as there are no large differences between self and outcross, whether this is the result of no self-incompatibility or a self-incompatibility system that has been mostly circumvented because of PSC doesn't really matter relative to breeding *P. x domesticum*. In either case the conclusion that self-incompatibility is not a consideration in the fertility problems of *P. x domesticum* would still stand. This means that self pollination is a viable option in a *P. x domesticum* breeding program.
Table 5.1. Clones used as females in Experiment 1 to compare pollen tube production of self vs outcross pollinated styles.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Clone</th>
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</thead>
<tbody>
<tr>
<td>78-4-1</td>
<td>'Dolly'</td>
</tr>
<tr>
<td>81-29-1</td>
<td>'Flora Fie'</td>
</tr>
<tr>
<td>81-59-1</td>
<td>'Pink Gardener's Joy'</td>
</tr>
<tr>
<td>83-52-1</td>
<td>'Virginia'</td>
</tr>
<tr>
<td>'Bredon'</td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2. Clones used as males in Experiment 2 to compare pollen tube production of self vs outcross pollinated styles.

<table>
<thead>
<tr>
<th>Clone</th>
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</tr>
</thead>
<tbody>
<tr>
<td>83-11-3</td>
<td>'Dolly'</td>
</tr>
<tr>
<td>83-52-1</td>
<td>'L. C. Hickman'</td>
</tr>
<tr>
<td>'Bredon'</td>
<td>'Inez'</td>
</tr>
<tr>
<td>'Chelvey'</td>
<td>'Purple Firedancer'</td>
</tr>
</tbody>
</table>
Table 5.3. Clones used as females in Experiment 3 to compare seed set of self vs outcross pollination.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>81-29-1</td>
<td>'Chelvey'</td>
</tr>
<tr>
<td>81-59-1</td>
<td>'L. C. Hickman'</td>
</tr>
<tr>
<td>82-53-7</td>
<td>'Flora Fie'</td>
</tr>
<tr>
<td>83-11-3</td>
<td>'Inez'</td>
</tr>
</tbody>
</table>
Table 5.4. Crosses used to compare effects of self vs outcross pollen in Experiment 3.

<table>
<thead>
<tr>
<th>Cross</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self Cross</td>
</tr>
<tr>
<td>83-52-1</td>
</tr>
<tr>
<td>'Bredon'</td>
</tr>
<tr>
<td>'Grandma Fischer'</td>
</tr>
<tr>
<td>'Pink Gardener's Joy'</td>
</tr>
<tr>
<td>'Vendig'</td>
</tr>
</tbody>
</table>
Figure 5.1. Residual plot of untransformed data for the number of pollen tubes at the end of the style. (Lilliefors, \( p = .0000 \))

Figure 5.2. Residual plot of a square root data transformation for the number of pollen tubes at the end of the style. (Lilliefors, \( p > .2 \))
Figure 5.3. The average number of pollen tubes at the end of the style of 9 female clones pollinated with self or outcross pollen. (LSD₁₀ = .98)
Figure 5.4. Residual plot of untransformed data for the number of pollen tubes at the end of the style. (Lilliefors, p = .0026)

Figure 5.5. Residual plot of a square root data transformation for the number of pollen tubes at the end of the style. (Lilliefors, p > .2)
Figure 5.6. The average number of pollen tubes at the end of the style for 8 clones used as males for self and outcross pollinations. (LSD$_{0.01} = 1.25$)
Figure 5.7. The average percent seed per pollination of self vs outcross averaged over 8 female clones. (LSD.01 = 5.8)
Figure 5.8. The average percent pollen staining of the 5 male clones used in comparing self and outcross seed set. (LSD,₀₁ = 14)
Literature Cited


CHAPTER VI
CONCLUDING REMARKS

There were five objectives proposed at the beginning of this research. The first objective was to investigate the effects of supplemental lighting applied to the female after the cool temperature floral initiation period on fruit and seed set of *P. x domesticum*. This was done by treating 16 different clones with HID light for a duration of either the normal day length or for 16 hours per day and pollinating the females with a bulk of pollen containing the anthers of at least 20 unrelated clones. The treatments did not increase seed or fruit set when compared to the unlighted control. However, as pollinating for the experiment did not take place until the natural day length was over 12 hours, this experiment was probably not a true test of the effect of irradiance on fertility.

A further experiment conducted during the time of the year when day lengths are significantly below 12 hours would provide a more definitive answer for this objective. All subsequent experiments had supplemental HID light applied during the short day months of the year as a precaution because the author believes that short day
lengths probably do impact fertility.

The most important lesson learned from this experiment did not relate to the supplemental lighting treatments. The overall fruit set for the experiment was 49% which is considerably higher than the 10-20% that had been expected based on previous research. Though there is plenty of room for improvement in the fertility of *P. x domesticum*, simply choosing more fertile clones could significantly increase seed set.

The second objective of the research involved investigating any influences of bulk pollen on fruit and seed set. Since the first experiment produced far more fruit and seed set than had been expected, it was deemed prudent to document any influence of the bulk pollinating technique that had been used. There were 7 different crosses for the experiment. Eight clones used as females were pollinated with five male clones one at a time. These single male crosses were compared against 2 bulk pollen crosses. One bulk pollen cross used a mix of pollen from the 5 males while the second was a mix of many males. The bulk pollen crosses set no more seed than the single male crosses. A bulk of pollen increases the probability of fertile pollen in the cross, and, provided the female has some degree of fertility, increases the likelihood of fruit and seed set. However, a bulk of pollen is no more effective at producing seed set than a single fertile male,
if the fertility of the male is good.

The bulk pollinating technique was used in the supplemental lighting experiment because it provided a random sample of the genotypes available and was a fast, efficient way to conduct large numbers of pollinations. Because of the ease in conducting bulk pollination, it is a useful technique to screen for female fertility. It is also a good method for conducting large numbers of pollinations in a season which would make it useful in a recurrent selection program for fertility improvement.

The third objective was to examine the causes of differences in the ability of male gametes to effect fertilization by testing for pollen viability, pollen germination and development, and subsequent seed set. This was accomplished by crossing 8 clones of varying male fertility to 2 common female clones, 1 with high female fertility and 1 with poor female fertility, and testing the males for pollen staining with fluorescein diacetate (FDA). The males in the experiment did vary in pollen germination and development. However, most of the variation in pollen tube numbers at the end of the style within a common female environment was explained by initial pollen staining. Also, most of the variation in seed set with the common female, 'Flora Fie' was explained by the number of pollen tubes at the end of the style. In other words, within the scope of the regression models, higher pollen staining
produces more pollen tubes and more pollen tubes produce more seed set.

Because of the amount of labor involved, the number of female environments in this experiment was limited to 2. Testing male effects in more female environments would help to solidify the ability to infer the results to the entire species.

The fact that pollen staining with FDA appears to be a good predictor of male fertility would be useful in a breeding program that was selecting for fertility. Mature seed is not obtained until a month after pollinating, whereas FDA staining produces immediate results. Staining with FDA requires about the same amount of effort that conducting crosses to test for male fertility would, but allows the researcher to choose appropriate males immediately.

The fourth objective of this research was to examine genotypic variation in pistil support for the development of the male gametophyte. Two common males, one with high pollen viability and one with poor pollen viability, were crossed to nine clones of varying female fertility. Pollen germination and development in the style were examined. There was considerable variation in the number of pollen tubes produced when a given male was crossed to different females. Genotypes vary in the ability of their pistils to support the germination and development of the male. For
the most part, the male with the higher pollen viability produced more pollen tubes. However, there was one female in which the poorer male produced significantly more pollen tubes than the more fertile male.

The number of males in this experiment was limited to 2. It would be useful to explore pistil differences with additional males, particularly since there was an interaction between females and males in this experiment. How consistent the differences are between the males when crossed to different females would affect decisions made on the kinds of female environments to use when selecting for male fertility.

The final objective of this research was to determine if self-incompatibility is a contributing factor to poor fertility. This was done by comparing the amount of self seed set to outcross seed set and the number of self cross pollen tubes to the number of outcross pollen tubes. One truism in research is that it is easier to prove that something does exist than to prove that it does not (absence of proof is not proof of absence). The differences between clones in initial pollen viability and in pistil support of the male confuse the issue of the existence of self-incompatibility. Since the males vary in viability, comparisons between pollen tube production of a clones' own pollen verses that of another pollen source are not as clear cut as one would like. However, comparing
self pollination and outcross pollination in 3 different experiments does provide a lot of evidence that self-incompatibility does not contribute to fertility problems in P. x domesticum.

The fact that self pollination is an option in breeding P. x domesticum is useful in that a self pollination tests for both male and female fertility simultaneously. The production of inbred lines is also an option, both as a research tool in studying inheritance of characters as well as a breeding tool in possibly future production of seed propagated lines.

The research presented here could be useful in future attempts to improve fertility in P. x domesticum. A good next step would be a selection scheme for fruit and seed set. Since this research has shown that the clones vary in both male and female fertility, testing for both characteristics in each clone may prove to be important. The plants that are used as testers will affect the outcome. Questions that would be asked include:

1. Is a known genotype important or is an unknown genotype such as a bulk of pollen in the case of testing for female fertility acceptable?

2. If a known genotype is important is a good male or female the best tester or would a poorer one provide a better test environment?

3. Considering the variation in male and female fertility and the possibility of interactions between males and females, how many testers are needed?
The most interesting outcome of this research was the genotypic differences in pistil support of pollen germination and development. A careful search of the literature has revealed a few other species in which this has been noted. However, except for the effects of self-incompatibility, genotypic pistil effects have been largely ignored. Part of the reason for this is that studying pollen is easier to do than studying pistils. The pollen is a simpler organ than that of the entire pistil and is also easier to access than parts of the pistil such as the ovules. The result of this is that, in studies investigating such things as mentor pollen effects and pollen competition, researchers often ignore whether or not the particular female they have chosen could affect the outcome of the experiment. Researchers would do well to pay more attention to female effects on the success of the male in future research.

Environmental effects on fertility of *P. x domesticum* is an area of research that is largely unexplored. Future research identifying optimal crossing environments would be helpful. Soil tests revealed that the plants in the experiment dealing with male fertility were found to have an excess of soil nutrients while the plants in the experiment dealing with pistil differences had too low soil nutrients. Steps were taken to correct these situations as soon as they were identified, but I feel that the overall
fertility in the experiments was probably decreased. Further research on environmental parameters such as nutrition, light and temperature would be helpful.

As a precaution for this research, the number of pollinations per plant was limited to 15 and the number of pollinated flowers per cluster, except for the first experiment, was limited to 3 or less. However, at the present time, the pollination loads that clones are able to support is not known. Further research in this area would be helpful.
BIBLIOGRAPHY


APPENDIX A

Fertility of Genotypes
The following is a summary of all of the clones that have been used in crossing during the production of this dissertation. Included is a rating of male and female fertility and, where it is known, the parentage.

Fertility ratings are:
- No fertility
+ 25% fruit set
++ 50% Fruit set
+++ 75% or higher fruit set

<table>
<thead>
<tr>
<th>Clone</th>
<th>Parentage</th>
<th>Fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>78-4-1</td>
<td>Parisian X Grand Slam</td>
<td>Male Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ +</td>
</tr>
<tr>
<td>80-10-2</td>
<td>Inez X Inez</td>
<td>Male Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>80-18-6</td>
<td>Virginia X Virginia</td>
<td>Male Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>80-20-1</td>
<td>W. Country Girl X W. Count. Girl</td>
<td>Male Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>81-29-1</td>
<td>L. C. Hickman X Dark Mabel</td>
<td>Male Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>++ ++</td>
</tr>
<tr>
<td>81-59-1</td>
<td>White Glory X Melissa</td>
<td>Male Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>++ ++</td>
</tr>
</tbody>
</table>

Comments:
- Susceptible to white fly.
- Like its parent, it is late to flower and not particularly floriferous.
- Resistant to white fly.
- Has over 90% fruit set, but fruits rarely have seed in them.
<table>
<thead>
<tr>
<th>Clone</th>
<th>Parentage</th>
<th>Fertility</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>82-44-6</td>
<td>Male Female</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>82-53-7</td>
<td>Male Female</td>
<td>+ ++</td>
<td>Miss Cherryvale X Grand Slam</td>
</tr>
<tr>
<td>83-11-3</td>
<td>Male Female</td>
<td>+++ +++</td>
<td>Highest male and female fertility found to date. Somewhat white fly susceptible</td>
</tr>
<tr>
<td>83-52-1</td>
<td>Male Female</td>
<td>++ +</td>
<td>Grand Slam X Virginia</td>
</tr>
<tr>
<td>91-6-29</td>
<td>Male Female</td>
<td>0 out of 20</td>
<td>Bredon X Bulk pollination Susceptible to white fly.</td>
</tr>
<tr>
<td>91-6-47</td>
<td>Male Female</td>
<td>3 out of 15</td>
<td>Bredon X Bulk pollination</td>
</tr>
<tr>
<td>91-7-37</td>
<td>Male Female</td>
<td>2 out of 14</td>
<td>Chelvey X Bulk pollination Flowering is resistant to high temperatures.</td>
</tr>
<tr>
<td>91-7-58</td>
<td>Male Female</td>
<td>8 out of 16</td>
<td>Chelvey X Bulk pollination</td>
</tr>
<tr>
<td>Clone:</td>
<td>Parentage:</td>
<td>Fertility</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>91-8-45</td>
<td>Country Girl X Bulk pollination</td>
<td>17 out of 17</td>
<td></td>
</tr>
<tr>
<td>Bredon</td>
<td>Male Female</td>
<td>++ ++</td>
<td></td>
</tr>
<tr>
<td>Chelvey</td>
<td>Male Female</td>
<td>+ ++</td>
<td></td>
</tr>
<tr>
<td>Country Girl</td>
<td>Male Female</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Dolly</td>
<td>Male Female</td>
<td>+++ +++</td>
<td></td>
</tr>
<tr>
<td>Elegant Rapture</td>
<td>Male Female</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Flora Fie</td>
<td>Male Female</td>
<td>+++ +++</td>
<td></td>
</tr>
<tr>
<td>Grand Slam</td>
<td>Male Female</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Clone:</td>
<td>Parentage:</td>
<td>Comments:</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Grandma Fischer</td>
<td></td>
<td>Very late to flower.</td>
<td></td>
</tr>
<tr>
<td>Josie</td>
<td></td>
<td>According to Bob Oglevee this has + good male fertility.</td>
<td></td>
</tr>
<tr>
<td>Honey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inez</td>
<td></td>
<td>Late to flower. Not highly floriferous. Progeny have these traits as well.</td>
<td></td>
</tr>
<tr>
<td>L. C. Hickman</td>
<td></td>
<td>Resistance to white fly.</td>
<td></td>
</tr>
<tr>
<td>Olga</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pink Gardener's Joy</td>
<td></td>
<td>White fly susceptibility.</td>
<td></td>
</tr>
<tr>
<td>Purple Firedancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vendig</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone:</td>
<td>Virginia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parentage:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comments:</td>
<td>White fly susceptibility. It's + ++ fruit set is particularly sensitive to lack of optimum soil fertility.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Rating is the number of self pollinations to set fruit. A self pollination will not set fruit unless both male and female fertility is present.*