

AN ABSTRACT OF THE THESIS OF

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Title: Evaluation of Pollinizers for Royal Ann Sweet Cherry
(Prunus avium L.) in the Willamette Valley

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Several pollinizers were evaluated for phenology, fruit set, pollen tube growth, comparative flower characteristics, and bee behavior for Royal Ann sweet cherry in the Willamette Valley during 1982 and 1983.

Fruit set of hand-pollinated flowers on uncaged limbs were used to test the fertility of the cultivars evaluated. Hand-pollination to evaluate pollen tube growth was made on stigmas and the branches were placed in controlled temperature rooms or on caged limbs in the field. Pollen tubes were scanned in the microscope under fluorescent light with aniline blue as a dye.

Field observations were used to determine the floral characteristics and bee behavior. Length of stamens and pistil, amount of pollen, sugar content in nectar, phenology of bloom period and bee attractiveness were determined. Number of bee

visits per flower, and the type of bee and their behavior when foraging the different pollinizers were also evaluated.

Among the cultivars evaluated, Bada was the most suitable pollinizer for Royal Ann due to its high fruit set, greatest pollen tube growth under low temperatures (5.9°C), compatibility with Royal Ann, and comparable bloom phenology, floral characteristics and bee behavior. Also, the floral characteristics of Corum, Rainier, and Vega, as well as Royal Ann, did not show any restrictions to either bee behavior or pollination.

Mean orchard temperatures of 12.6°C in 1982 and 5.9°C to 7.1°C in 1983 during the bloom period slightly reduced fruit set and pollen tube growth rate of several pollen sources, but these low temperatures had a greater reduction of growth for Corum pollen tubes. In 1982, the latter were more temperature dependent than those of Bada while growth of Black Republican pollen tubes seemed to be restricted at high temperatures of 16.4°C , at certain days during the whole period.

The low temperatures in 1983 were more restrictive to bee flight. However, pollen transfer was less limiting in these trials than the pollen source which was affected by the poor environmental conditions and by the partial genetic incompatibility. Pollen tube growth of Corum and Vega at low temperatures was also affected by low pollen germination and, consequently, reduced fruit set in Royal Ann. Rainier as pollen source could give low fruit set on Royal Ann because of the genetic incompatibility between the two.

Evaluation of Pollinizers for Royal Ann
Sweet Cherry (Prunus avium L.)
in the Willamette Valley

by

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Evaluation of Pollinizers for Royal Ann
Sweet Cherry (*Prunus avium* L.)
in the Willamette Valley

I. INTRODUCTION

Yield components of sweet cherry (*Prunus avium* L.) are bearing surface, bloom density, and fruit set (Lombard et al., 1983). Bearing surface depends on planting distance, age of tree and tree growth, while bloom density depends on floral initiation during the previous year which can be affected by solar radiation.

The principal limiting factor for sweet cherry fruit set is cool, rainy weather during, and which affects, pollination which is the pattern during blooming time in Western Oregon. Cool weather can also affect pollen tube growth (Brown, 1973) and embryo sac development (Howlett, 1938).

For auto-sterile cultivars such as Royal Ann sweetcherry, cross-pollination with compatible pollen is fundamental for a crop (Stebbins and Thompson, 1976; Stephen et al., 1977). Honey bees are the main pollinator for sweet cherry and other fruit crops (Lane, 1979), and bee activity will be restricted if the temperature is below 12.7°C (55°F) and if the wind is greater than 400 to 533 m.p.m. (15 to 20 m.p.h.) (Stephen et al., 1977).

Several cultural practices such as distribution and distance of pollinizers with respect to the main variety can influence pollination and fruit set (Free and Spencer-Booth, 1964). Summer application of nitrogen increased ovule longevity in apple

(Williams and Wilson, 1970), and boron sprayed during the fall and spring increased fruit set of prunes (Callan et al., 1978). Phosphorus had an influence on fruit set in young apple (Taylor and Goubran, 1975). Pesticides had a deleterious effect on bees (McGregor, 1976), and fungicides on reducing sweet cherry pollen germination (Eaton, 1961).

Growth regulators have increased fruit set in sweet cherry (Goldwin and Webster, 1978; Modlibowska and Wickenden, 1982). Viral diseases have had adverse effects on fruit set of sour cherry (Vertesy and Nyeki, 1974). Several pollutants such as hydrogen fluoride and sulphur dioxide at high concentrations have reduced pollen germination, pollen tube growth and fruit set of sweet cherry (Facteau et al., 1973; Facteau and Rowe, 1981).

Oregon is the second largest sweet cherry producer in the U.S.A., with Royal Ann as the main cultivar on approximately 6,000 ha., and this cultivar is principally used for maraschino cherries. Low crops in certain years have been due to poor cross-pollination and lack of sufficient fertilization. In assessing the lack of sufficient fruit set, several light color cherry pollinizers presently used in commercial orchards were evaluated and compared with Royal Ann as a pollinizer and pollen receptor, for bloom development and floral characteristics which could be important in pollination, bee attractiveness, bee behavior, pollen tube growth and fruit set. Ovary development of Royal Ann was examined microscopically to determine the probable cause of early fruit drop.

II. REVIEW OF LITERATURE

Pollination

Only in angiosperms is pollination typically developed in three phases: 1) release of pollen from the 'male' part of a flower, 2) transfer from the paternal to the maternal part, and 3) successful placing of pollen on the recipient surface, followed by germination of the pollen grain (Faegri and van der Pijl, 1979). Pollination in gymnosperms occupies an intermediate position between the simple micro-spore dispersal of lower plants and proper pollination. Unless the pollen grain reaches a micropyle in a compatible female blossom, it has no chance of germinating and of producing spermatozoids or male nuclei. In the absence of a stigma, the receptive surface of a 'female' gymnosperm ovule is the micropyle or an adjacent cone scale (Faegri and van der Pijl, 1979).

Optimal pollination in fruit orchards depends on several factors, each of which may be limiting if not met. These include adequate planning of proper pollen sources in the orchard prior to the introduction of pollinators, good bee management practices, and favorable weather conditions during bloom period (Stephen, Burgett, and Capizzi, 1977).

Pollinizer Requirements

Pollinizers. The requirements of a good pollinizer, in general, are: 1) it has to be a commercially desirable variety, 2)

it should come into bearing (flowering) at an early age, 3) it must produce an ample amount of good viable pollen, 4) it should bloom coinciding or substantially overlapping and consistently during the bloom period of the main variety, and 5) it should not be easily subject to the biennial habit (Murneek, 1937). Williams and Wilson (1970) stated that for apples, the need for the flowering times of donor and receptor to overlap is self-evident. Duggan (1961) stated that it is possible to state the minimum overlap of flowering periods required between two varieties for effective cross-pollination. Therefore, it would be prudent to have maximum overlap in order to allow for the most unfavorable circumstances likely to be encountered.

Anther dehiscence should start in the pollinizer before it starts in the main cultivar (Westwood, 1978a). Stebbins and Thompson (1976) stated that Royal Ann sweet cherry can be pollinized with Corum, Black Republican, and Van, although the first will usually bloom earlier than Royal Ann. Brooks and Griggs (1964) stated that Bada is cross-compatible with Bing, Royal Ann, and Black Tartarian. Bada blooms relatively late, but its bloom period sufficiently overlaps those of Van, Bing, and Royal Ann.

Distance from and Pollinizer Placement. Stebbins and Thompson (1976) mentioned that the number and placement of pollinizers required for optimum pollination is largely determined by the foraging habits of the honey bees. The effect of pollinizer distance from the main cultivar is influenced by weather (Westwood,

1978) and can be critical for pollination. Free and Spencer-Booth (1964) in a study on the effect of distance from pollinizer in cherry and other fruits, found that in a sweet cherry orchard containing a block of five rows of Early Rivers, spaced 9 x 10.8 m., with pollinizer rows on each side, the most fruit was set on the two outside rows and trees in the outside rows set more fruit on their sides facing the pollinizers than on their far sides. In another sweet cherry orchard (10.8 x 10.8 m.), they found that Frogmore trees set more fruit on their south sides compared with other tree sectors. Tukey (1925) found that fruit set decreased from 43 to 26 percent on Windsor cherry when the Black Tartarian pollinizer tree was 6 to 12 m. from the Windsor tree. Free and Spencer-Booth (1964), when working with Early Rivers cherry pollinized with Early Amber and Caroon, reported a decrease in fruit set from 12.8 to 8.9 percent when the distance from pollinizer tree to main variety increased from 9 to 27 m.

Stephen, Burgett, and Capizzi (1977) and Westwood (1978) stated that to insure consistent commercial yields of the self-unfruitful varieties, adequate numbers of compatible pollinizer trees must be interspersed with the variety to be pollinized. Both the number of pollinizers and their placement should be determined by weather conditions that normally prevail during the blooming period as well as by foraging behavior of honey bees. An optimal arrangement is to have every other tree in every row a pollinizer, especially in cherry which requires more pollinator insects than other fruits. This presents harvesting difficulties, however, and

is often impractical if the fruit of the pollinizer variety has relatively little commercial value. A recommended compromise is every third tree in every third row be planted to the pollinizer cultivar. This provides for each tree of the commercial cultivar to be adjacent to one pollinizer.

Pollinators

Pollen dispersal of Moss Early cherries and honey bee activity were studied by Langridge and Goodman (1973) in the laboratory and in a cherry orchard. They found that maximum airborne pollen concentration recorded in the open orchard was 42.5 grains per cubic meter. Honey bees averaged 7.3 per tree and the maximum number observed was 21 per tree during 38 counts on each of 8 uncaged trees. Honey bees comprised 97% of the total insect visits of the cherry flowers and they concluded that their importance as compared with other insects in the pollination of cherries is because of their predominance as visitors to cherry.

Wind is not a factor in cherry pollination, as was established by Roberts (1922a), Burtner (1923a), Murneek (1930a), Claypool et al. (1931a), Brown (1968a) as cited by McGregor (1976), and Stebbins and Thompson (1976). Free and Spencer-Booth (1964), Free (1970), McGregor (1976), Stebbins and Thompson (1976), and Stephen, Burgett, and Capizzi (1977) gave the primary credit for the pollination of cherries to honey bees because a high level of pollinators is needed and because flowering occurs too early in the year for other insects to be plentiful. Gardner (1913) was the

first to establish scientifically the need for pollination for fruitfulness and he stressed the importance of bees. Lane (1979), working with sweet cherry, Stella, showed that even though it is self-fertile, bee pollination improved final fruit set and number of fruits harvested, compared to pollination by wind and gravity. The treatments consisted of: 1) untreated, open-pollinated branches by insects (mainly bees), wind, and gravity; 2) Stella branches tied with bouquets of the pollinizer, Van, pollinated by insects, wind, and gravity; 3) branches enclosed in cloth bags to insure isolation from flying insects, but pollinated by wind and gravity; 4) several treated branches with combinations of self-, cross-, emasculated, and hand-pollinated, and wind and gravity. This study showed the advantage of bee pollination for Stella sweet cherry, but gave no evidence of the benefit of an additional pollen donor cultivar.

Weather Effects on Bees

Langridge and Goodman (1973) found that flight activity of bees was related to ambient temperatures, with virtually no flight activity below 13°C, a rapid increase up to 20°C, and a levelling off above this temperature.

Stephen, Burgett, and Capizzi (1977) described honey bees as temperature and light sensitive, rarely flying if the temperature is below 55°F (12.7°C) or during a wind of more than 400 to 533 m/min. (15 to 20 m.p.h.). Bees from a strong colony, with a minimum of 30,000 bees, will initiate flight at lower temperatures.

In poor weather when flight conditions are marginal, bees foraging at more distant locations will remain in the hive and only those bees that have been foraging nearby will be active. Nectar and pollen collectors are most active between the hours of 9 a.m. and 1 p.m.

Lerer, Bailey, Mills, and Pankiw (1982), working with Megachile rotundata (leaf-cutter bees), which are comparable to honey bees in behavior (Burgett, D.M., personal communication), found that they had a temperature threshold of 16-17°C for the initiation of pollination activity. Once this temperature threshold is surpassed, activity is dependent on solar irradiance. Cessation of activity occurs when the level of solar irradiance decreases to a critical value even though the temperature is still more than adequate to maintain activity.

Number and Strength of Honey Bee Colonies

Free (1970) indicated that the bee population required in an orchard depends on many factors, one of which is the necessary level of fruit set. Thus, a greater bee population is recommended for cherry orchards which need a high fruit set compared with other species (cherries: 20-75 percent, apples: 2-8 percent, pear: 3-11 percent (Chaplin and Westwood, 1980)).

Stephen, Burgett, and Capizzi (1977) defined an efficient pollination hive as having a minimum adult population of 30,000 bees. A colony of this size should have a foraging force of from 10,000 to 13,000 bees. As a rule of thumb, one good colony of

honey bees per acre is satisfactory for fruit tree pollination (Schuster, 1925 and Tuft and Philp, 1925a). Two to four colonies per acre are recommended for areas where inclement weather conditions are common during bloom (Brown, 1968a).

Eaton (1962a) stated that strong colonies should be brought into the sweet cherry orchard on or before the day the first flowers open due to the E.P.P. (Effective Pollination Period - duration of longevity of the egg apparatus minus the time required for pollen tubes to reach the egg sac), because placement in the orchard even one day late could result in a reduced crop.

Foraging Behavior of Bees on Flowers

Free (1970) working on apple, apricot, peach, pear, plum and sweet cherry, found that the proportions of nectar to pollen collecting honey bees on blossoms depends on the relative availability of nectar and pollen and on the food requirements of their colonies. He also found that the ratio of nectar-gatherers to pollen-gatherers varied greatly from day to day and during the same day. The rate at which bees visited blossoms depended on the amount of nectar and pollen present which varies with the type and the stage of the flower development, with climatic conditions, and with the number of foraging insects present. The average number of flowers, including those of cherry (Free, 1960a), that bees have been seen to visit were as follows: nectar-gatherers, 6.0 flowers per minute; and pollen-gatherers, 6.7 flowers per minute.

Parker (1926a) indicated that, on flowers of several fruit species, including cherry, pollen-collecting bees scrambled over the anthers and pulled them towards their bodies, frequently biting them. Vansell (1942a) observed that honey bees collecting nectar from cherry and peach pushed through the stamens and pistil to reach the nectary, and became covered with pollen in the process.

Free (1970) stated that the behavior of bees when visiting flowers determines their efficiency as pollinators and their efficiency depends where they stand on the anthers, where they push their tongue and the front part of their bodies toward the nectaries, or where they touch the stigmas and stamens to pollinate. When they stand on the petals and push between the stamens to reach the nectaries, thereby not touching the stigmas, pollen transfer cannot take place. The proportion of nectar-gatherers that approach the nectaries from the sides depended on the stamen structure of the variety concerned. If the stamens are short and comparatively flexible, bees prefer to approach the nectaries from the top and are thus able to effect pollination.

Robinson (1979) working on bee behavior on Delicious apple flowers, found that in flowers with gaps at the base of the stamens bees learn to collect nectar through these gaps, thereby avoiding the flower's sexual parts reducing pollination and fruit set by more than a half.

Floral Structure and Characteristics

Free (1970) described the flowers of the genus Prunus as having five petals, numerous stamens, a single style, and an ovary with a single carpel containing two ovules.

McGregor (1976) defined the sweet cherry flowers as being white, faintly fragrant, in clusters of two to five on short lateral spurs. The five petals of the flowers are oval, white and rather widely spread. There is a single upright pistil and about 30 loose stamens. The flower remains open seven to eight days. The stigma is receptive when the flower opens, but the anthers are still closed. Anthers begin opening shortly after petal opening and continue into the second day. Nectar is secreted on the inner surface of the receptacle. Pollen and nectar are both attractive to insects, particularly bees. He found that sweet cherry nectar is much richer in sugar (55 percent sugar) than the tart cherry nectar (28 percent sugar).

Anther Dehiscence

Luckwill (1960) noted that Rosaceous flowers have many stamens arranged in whorls which dehisce successively over a period of one to nine days, though pollen is normally liberated in greater abundance during the first half of this period. Srivastava and Singh (1970) stated that the dehiscence of anthers in several sweet cherry cultivars started from the inner to the outer whorl, dehiscence was completed in two days after anthesis; maximum

dehiscence took place from 9:00 to 15:00 hr and the rate of dehiscence increased with an increase in temperature.

Percival (1955) indicated that the temperature range for anthesis is 5 to 14°C, and was about the same for cherry and pear. The duration of anther dehiscence in single flowers was one to two days for cherry, pollen release was from 8:00 to 17:00 hr and peaking from 8:00 to 12:00 hr.

Langridge and Goodman (1973) mentioned that the ripening and dehiscence of Moss Early cherry anthers was at its maximum when the temperature reached 30°C and the relative humidity was 50 percent.

Percival (1955) stated that conditions limiting anther dehiscence appear to be: a) temperatures which were too low to permit the anthers to attain maturity, b) presence of free water on the anther, or c) 100 percent relative humidity.

Pollen Characteristics

Srivastava and Singh (1970) reported that the shape of sweet cherry pollen grains is mostly triangular, with 3 germinal pores.

Percival (1955) reported that the total amount of pollen per flower in mg for apple was 1.7; pear, 1.2; peach, 0.7; and sour cherry, 0.3. She also stated that there is some evidence that the bees will exercise a preference for a pollen of particular biological potency (nutritive value). Joppa, McNeal, and Berg (1968) reported that the mean number of pollen grains per anther in a group of wheat cultivars ranged from 2687 to 3867.

Luckwill (1960) found that pollen viability in fruit species may vary between different flower clusters, flowers on the same cluster, and stamens in the same flower. But these were of minor importance because he found season to season variations in pollen quality, in particular reduced viability which occurred following periods of cold, wet weather. Pollen quality was also influenced by nutritional factors, particularly the nitrogen status of the pollen parent tree (Luckwill, 1960). Good pollen should show a germination of 70 percent or higher when cultured in the appropriate concentration of sucrose. Many varieties normally produced pollen with a germination rate of less than 30 percent which he considered as poor pollinizers.

Nectar Characteristics

Percival (1955) concluded that in several species the amount of pollen produced per flower is of little consequence as an 'attractive' quality for the bees compared with the presence or absence of available nectar in the flower.

Brown (1951a) observed that varieties of plum with the greatest quantity of nectar attracted the largest number of bees and that these varietal preferences persisted even when foraging activity changed from nectar to pollen collection.

Way (1961) pointed out that, in general, the attractiveness of flowers, to honey and bumble bees, closely corresponds to the sugar concentration in the nectar, and this varies over a wide range. The concentration, and consequently, the attractiveness of nectar

in any one flower, varies with environment, particularly humidity, dew, and rain, also evaporation which makes the concentration higher. He reported that sweet cherry nectar had 55 percent sugar concentration; that of apple was 42 percent; that of sour cherry, 28 percent; that of plum, 21 percent, and that of pear, 15 percent.

Free (1970) reported that the sugar concentration of nectar for sweet cherry ranged from 21 to 60 percent. Nectar secretion occurred above a threshold temperature which varies with species. Wild cherry secreted nectar at temperatures of 8°C or above.

Fruit Set

After pollen transfer from the anther to the stigma surface, several factors influence fruit set. Some of the factors mentioned are pollen germination (Luckwill, 1960), pollen germination on the stigmatic surface (Lee, 1980), stigma receptivity period (Williams and Wilson, 1970), pollen tube growth (Brown, 1973), ovule longevity (Stösser and Anvari, 1982), and ovule abnormalities (Thompson and Liu, 1973).

Fruit set can be influenced by nutrition levels (Luckwill, 1960; Chaplin and Westwood, 1980), by growth regulators (Goldwin and Webster, 1978), by pesticides (Stephen, Burgett, and Capizzi, 1977; Vertesy and Nyeki, 1974), by cultural practices (Luckwill, 1960; Westwood and Stevens, 1979), by environmental conditions (Braak, 1978), and by certain pathogens (Vertesy and Nyeki, 1974).

Pollen Germination

Thompson and Batjer (1950) increased pollen germination and pollen tube growth when boron at 10 ppm was included in the germination media for plum, peach, apricot, cherry, pear and apple. They concluded that boron would increase fruit set in pears when sprayed at blooming time.

Eaton (1961) tested pollen germination of sweet cherry in vitro and observed that pollen germination was decreased by certain fungicides. The products tested were sulphur, dichlone, ferbam, and captan in the pollen germination media. Sulphur did not reduce the germination of pollen while dichlone and ferbam reduced germination from 53 to 47.1 percent and 40 percent, respectively. Captan sprayed at 0.2 pounds per 100 gallons or less did not reduce germination, but at the 2.0 pound rate captan almost entirely prevented pollen germination and arrested the elongation of pollen tubes.

Lee (1980b), when testing pollen germination, pollen tube growth, and fertilization behavior in Prunus domestica, found that pollen tube growth was positively correlated with the amount of pollen on the stigma. He also noted that cultivars with low pollen grain germination failed to have pollen tubes completely penetrating the styles.

Williams (1953a) and Faegri and van der Pijl (1979) found reduced pollen viability following periods of cold, wet weather.

Stigma Receptivity and Pollen Germination

Coutaud (1948a), working with apple pollen, suggested that stigmas secrete certain hormonal substances which reduce the germination of pollen grains of the same variety are comparatively ineffective against pollen of non-related varieties. Thus, it was found that the germination of pollen of Baumann's Reinette on an agar medium was reduced from 89 percent to 59 percent by the presence of Baumann's Reinette stigmas, but pollen germination was not affected by the presence of Reine des Reinettes stigmas.

Visser (1951a) showed that when apple pollen is grown in hanging drop cultures of 10 percent sucrose, the percentage germination varies directly as the number of grains per drop. He suggested that pollen grains appear to excrete substances (possibly auxins) which stimulate the germination of other pollen grains in the immediate vicinity.

Zaec and Sedov (1957a) maintained that in five out of seven apple varieties which had an addition of pear pollen to their own pollen, improved fruit set, that of the self-sterile pear Buerre Zimnaya could be made partially self-fertile by a mixture of apple pollen with its own.

Addicott (1943a), Beams and King (1947a), Beck and Joly (1941a), and Brink (1924a) showed that a larger mass of grains gave better pollen germination and tube growth than a small number of grains per drop. They termed this phenomenon the mutual stimulation of pollen grains in germination and they believe it can be assumed to be generally present in nature.

Ter-Avanesian (1978), working with cotton, Vigna, and wheat, found that the number of pollen grains placed upon a stigma influenced both the development of pollen tubes and subsequently, the progeny which resulted from fertilization by gametes from these pollen tubes. The first effect demonstrated that there was reduced pollen tube growth rates when pollen grains were few in number.

Schemske and Fenster (1983) tested the effects of clump size and competitors of pollen grains in a neotropical herb (Costus guanaiensis). Pollen germination and pollen tube growth were greater for 16 as compared to 4 grain clumps, which indicated the consistency of the mass effect. There was a highly significant effect of pollen number on the probability of successful fertilization and seed production. Pollination with single grains produced no fruit in 79 crosses, while the frequency of fruit production increased successively to 68 percent for 64 grain pollinations. These data, in conjunction with the evidence for the positive effect of pollen number on germination and pollen tube growth in vitro, suggest that interactions among pollen grains may have a significant effect on fertilization.

Jennings and Topham (1971) compared fruit set of raspberry flowers pollinated with undiluted pollen, with pollen diluted with talc, or with nonviable pollen. Diluted pollen reduced set, but the reduction was not the same for all stigmatic cultivars. Observations on the number of pollen grains germinating on the stigmas revealed differences attributable to the maternal parent. The lowest pollen germination occurred on the parent whose pyrene

(fruitlet) set was reduced most by pollen dilution. They concluded that pollen germination was conditioned by growth substances provided partly by the pollen grains and partly by the stigmas of the seed parent, and also by the interactions between the two.

Brewbaker and Majumder (1961) when studying the pollen population effect and the self-compatibility inhibition in eight angiosperm genera, found a significant effect of decreasing population size on pollen germination in vitro.

McKenna and Mulcahy (1983) studying the ecological aspects of gametophytic competition in Dianthus chinensis, concluded that the advantage of rapid early growth rate and competitive ability, and gametophytic competition may be important to plants in natural situations, if the ecology of pollination is such that intense pollen competition is possible. It is interesting to consider the potential effects of pollinator behavior in this regard; certainly the timing, amount, and placement of pollen could have an important effect on the strength of pollen competition in natural communities.

Zamir, Tanksley, and Jones (1981) studying the effect of low temperature (5°C) on selective fertilization of wild and cultivated tomato species, found that a peruvian ecotype of Lycopersicum hirsutum originating from an altitude of 3200 m. contributed to hybrid zygote formation more than double when controlled fertilizations with pollen mixtures (L. esculentum and L. hirsutum) occurred at 12/6°C as compared to crosses with the same mixtures at

24/19°C. They suggested that differential selection at the gametophytic level occurs in response to low temperature regimes.

Stigma Receptivity

Free (1960a) mentioned that stigmas of all species and varieties of Prunus and Pyrus are receptive as soon as the flowers open. But Williams (1966) stated that it is sometimes assumed that flowers are fully receptive and capable of setting until there are signs of senescence of the stigmas or petal abscission occurs.

Dorsey (1929a) recognized that these external features (senescence of stigma or petal abscission) were less important than the longevity of the ovules.

Williams (1966) found an effective pollination period (E.P.P.) from two to nine days in several different apple cultivars, and an E.P.P. from one to ten days on pears. Lombard et al. (1971) found that the E.P.P. ranged from one to nine days in three different pear cultivars in Oregon.

Srivastava and Singh (1970) reported that the maximum receptivity of stigma in all the varieties of sweet cherry they tested was on the day of anthesis, because the highest percentage of fruit set was recorded on that day. There was good setting from one day before anthesis until two days after. McGregor (1976) reported that the sweet cherry flowers remain open seven to eight days and the stigma is receptive when the flower opens before anther dehiscence.

Toyama (1980) indicated the E.P.P. was four to seven days on sweet cherry and seven to twelve days for peach. Lombard et al. (1983) found an E.P.P. of four to five days for Royal Ann sweet cherry in Oregon.

Stösser and Anvari (1983) working on sweet cherry, concluded that even though it is assumed that the stigma has to be in a receptive phase to capture the pollen grains and to ensure their germination, it was found that in sweet cherry the condition of the stigma was not significant for the germination of pollen grains and the penetration of the tubes to the transmitting tissue. Even ten days after anthesis, when the papillae were collapsed completely, no inhibitory effect was found. Thus, it appears that the receptiveness of the stigma has been overemphasized.

Pollen Tube Growth

Temperature on Pollen Tube Growth

Goff (1901a) found that pollen of Moldavka and Wood plums, Dyehouse cherry, and *Prunus* apple germinated very poorly at 4.4°C. Pollen tube growth of the tree species was best at 18 to 21°C, while growth was appreciably retarded below 10.5°C

Lewis (1941a) found that the growth of incompatible pollen tubes at different temperatures in *Prunus avium* was similar to that in *Oenothera organensis* and other self-incompatible plants. That is, the difference between incompatible and compatible pollen tube growth at 15° to 20°C range was too similar to determine the incompatibility by pollen tube observation. Also, Crane (1942)

found no difference in tube growth of compatible and incompatible pollen in cherry at 15 to 20°C. However, at 30°C the difference was so marked that it afforded a reliable quick test for compatibility.

Lewis (1942) tested compatible pollen tube growth at different temperatures in the sweet cherry cross Guigne d'Annonay x Bedford Prolific. Just as in Oenothera organensis, the growth rate increased as the temperature increased until the lethal point was reached. However, the optimum temperature for pollen tube growth was 25°C in Prunus as compared with 33°C in Oenothera. This difference indicated that natural selection has been acting on the pollen tubes. P. avium, which is indigenous to temperate regions and blooms in the spring, evolved under lower temperatures ranging from 5 to 20°C, while Oenothera, which grew in Arizona, was subjected to much higher temperatures.

Child (1966) working on pollen tube growth in vivo at 10°, 14°, 17°, and 20°C, of the cider apple, concluded that the rate of growth was slower in the free styles than in the joint style and receptacle. This pattern was most marked at the lower temperatures. In the free style, the increase in rate of pollen tube growth between 14°C and 24°C was six times as great as the increase between 5° and 14°C.

Free (1970) stated that an optimum temperature for pollen tube growth and for fertilization occurs between 18° and 27°C, while growth was retarded or nearly stopped at temperatures below 16°C in Prunus and Pyrus flowers.

Lombard et al. (1972) working on pollen tube growth of Bartlett and Doyenne du Comice pears, observed at a range of temperatures from 5°C to 30°C that no tube growth occurred for selfed Bartlett below 15°C. But at higher temperatures, some selfed pollen tubes penetrated the incompatibility zone of the style. At 6°C, compatible pollen tubes in Bartlett styles grew at only a quarter the rate of tubes at 15°C. The periods required for the pollen tubes to reach the ovules increased from three days at 15°C to 15 days at 6°C. They suggested that mean post-bloom temperature below 9°C would reduce the growth rate and could reduce fruit set because of poor synchronization of the pollen tube reaching viable ovules.

Brown (1973) working with Royal Ann sweet cherry in Oregon, found that at 4.9°C it would take about five days for the pollen tubes of Corum or Bada to grow the length of the style (10 to 11 mm).

Socias i Company et al. (1976) studying the effect of temperature and genotype on pollen tube growth in self-incompatible and self-compatible almond cultivars, concluded that pollen tube behavior was similar to that described by Lewis (1942) for sweet cherry. In general, the optimum temperature of 25°C for pollen tube growth found in almond is similar to that in cherry (Lewis 1942).

Jefferies et al. (1982) studying the effect of temperature on Victoria plum pollen tube growth and fertilization, concluded that above the threshold of 2.5°C maximum growth rate of pollen tube was

0.34 mm per day-degree and that the tubes reached half their final length at 16.6 day-degrees above 2.5°C. The model they developed indicated that fertilization of plum flowers required 16 to 20 days at a constant temperature of 5°C after pollination, but only three to four days at 15°C.

Incompatibility on Pollen Tube Growth

Crane and Brown (1937) concluded from data from various self- and cross-pollination of a total of 236,000 flowers of sweet cherries that self-incompatibility was found to be the rule, while cross-incompatibility was common and always reciprocally expressed. They noted that incompatibility in the sweet cherry was expressed by the young fruits ceasing to grow and dropping at an early stage. Selfed cherry pollen tubes were arrested in their growth through the stylar tissue and hence, fertilization did not take place and the fruit failed to develop. Since cross-incompatibility is also common in the sweet cherry, the interplanting of suitable pollinizer varieties to provide for effective cross-pollination is of first importance.

Modlibowska (1945) stated that self-incompatibility in plants is a physiological mechanism to ensure cross-pollination, and it is brought about by inhibition of the growth of the pollen tubes due to a reaction between the haploid pollen tube and the diploid stylar and ovarian tissue.

Crane and Brown (1937) and Crane and Lawrence (1929a) demonstrated that cherry incompatibility was due to multiple

alleles of an S gene which prevent the normal growth of pollen tubes into floral styles. Incompatibility exists when the specific S allele that is carried by a pollen grain is the same as one of the two specific S alleles carried by the somatic tissue of the receptor pistil.

Stott (1972) working with several apple cultivars, found that compatible pollen tubes grew rapidly down the style, while incompatible tubes were slower growing, often stopping completely, and had heavy depositions of callose tissue along and at the end of the tube.

Lane (1979) mentioned several advantages of self-fertile sweet cherry cultivars such as their use as universal pollen donors, management of single cultivar orchards, and reliable cropping in years when insect pollinators are not active.

Pollutants on Pollen Tube Growth

Facteau et al. (1973) studying the effect of fluoride (F) on Royal Ann pollen germination and pollen tube growth, found that increased F fumigation levels resulted in a decrease of Royal Ann pollen germination and pollen tube growth. As the dose increased (hour x concentration in $\mu\text{g F}/\text{m}^3$), pollen tube growth of Van cherry in vivo decreased. A linear relationship between increased dose and F residue in the flowers was shown.

Facteau and Rowe (1981) in a study of Tilton apricot pollen tube growth, found that pollen tube growth was reduced by exposure to SO_2 . Response of Van pollen tube growth in Royal Ann sweet

cherry styles to SO_2 exposure was similar to that of apricot, but not as definitive because of greater differences within year variation and between years.

Ovule Longevity on Fruit Set

Cooper (1938a) found that tree vigor affected fruit set. Dorsey (1930) indicated that the greater the tree or spur vigor, the longer it takes for the organization of the embryo and, therefore, the longer the ovule persists.

Howlett (1936a) found that flowers from nitrogen deficient trees showed a high proportion of embryo sacs which had degenerated before reaching the egg cell stage. Williams (1965a) confirmed the value of nitrogen for increasing female fertility, and since summer applications were most effective, he suggested that the primary effect was on flower initiation and then later it was reflected in ovule longevity at flowering time.

Stösser and Anvari (1982) studying the senescence of sweet and sour cherries, found that applied growth regulators usually enhanced senescence of the ovules, especially GA_3 at 50 mg l^{-1} . Even those that delayed senescence of the external flower parts, e.g 2,4-D at 10 mg l^{-1} , benzyladenine at 50 mg l^{-1} , and kinetin, accelerated the aging of ovules in most instances. No growth substance was found to extend the longevity of ovules in cherries.

Dennis (1983) reported that aminoethoxyvinylglycine (AVG) on apple increased fruit set relative to the control as pollination

was delayed in McIntosh apple, suggesting that the chemical prolongs ovule longevity.

Ovule Abnormalities on Fruit Set

Abnormalities in the embryo sac development can reduce fertilization in sweet cherry (Eaton, 1959, 1962).

Fruit drop and reduced fruit set in sour cherry was due to nutritional factors which aborted the embryo causing fruit drop (Bradbury, 1925, 1929).

Nutrition on Fruit Set

Taylor (1969) mentioned that, although the literature has been mainly concerned with carbohydrate reserves, there is evidence to show that a reserve of mineral elements play an important role in fruit set and early fruit growth. Fruit set can be reduced by faulty mineral nutrition. Poor fruit set is one of the symptoms of deficiencies of nitrogen (Harris and Boynton, 1952a), potassium (Ballinger, 1965a), phosphorus (Kobayashi et al., 1961a), magnesium (Ford, 1966a), boron (Batjer et al., 1953), zinc (Cooke, 1966a), copper (Cooke, 1966a), and iron (Udris, 1965a). Both boron and nitrogen deficiencies give rise to reduced pollen viability (Bamzai and Randhawa, 1967a; Winkler, 1926a, respectively). There is evidence to show that nitrogen deficiency has a more pronounced effect on fruit set than deficiencies of potassium and phosphorus (Zvara, 1967a).

Superphosphate applied to Jonathan apples in pots increased fruit set proportional to the phosphorus rate over part of the

content range in two years (Taylor and Goubran, 1975). However, fruit set was not significantly influenced in 1974, due to a low and variable level of flowering. The rates of phosphorus were from 0 to 9.5 Kg in the total mixture per pot.

A pre- or postharvest foliar boron application was found to increase fruit set of Italian prune (Callan et al., 1978a), while prebloom boron spray failed to increase set. Neither fall nor spring applications influenced the amount of fruit drop in the midsummer or 'blue' drop.

Chaplin and Westwood (1980) in a review of organic and inorganic nutrients affecting fruit set, concluded that some of the essential elements (N,P,K) are directly required for a short transitory time while others are required on a continuing basis from work by Singh (1974a), Taylor and Goubran (1975), Vang-Petersen (1975a). Fruitfulness is not associated with highest intensity nutrition nor the highest carbohydrates, but with a balance between the two (Kraus and Kraybill, 1918a).

Luckwill (1960) mentioned that nitrogen is one of the most important factors influencing the growth and cropping of fruit trees. In apple, pollen from nitrogen-deficient trees was found to be markedly inferior in its capacity to bring about fertilization and fruit set, although it germinated well in vitro as compared with pollen from high-nitrogen sources.

Luckwill (1960) stated that there was some evidence that the boron requirement of pollen grains from the first flowers to open on a tree was greater than that of the later flowers. He also

mentioned Greenham and White (1959a) worked in which they increased apple yield by increasing final set when sprays of two percent of Epsom salt were applied for preventing leaf scorch from K deficiency.

Growth Regulators on Fruit Set

Crane (1964) in a review on hormone activity related with fruit set and growth, concluded that the most intriguing problem in connection with fruit set and growth resides in the characterization of the ovarian stimulus resulting from only pollination in some fruits, from pollination and fertilization in others, or from exogenous growth substance application in a few.

Goldwin and Webster (1978) tested various combinations of GA₃, NOXA (naphthoxyacetic acid), and DPU (NN¹-diphenylurea) as fruit-set agents for Early Rivers sweet cherry. A mixture of GA₃ and NOXA increased set and final yield, while the inclusion of DPU did not result in a significant set improvement. Flowering in the year after treatment was suppressed only when GA₃, alone or mixed, had been used. Lowering the GA₃ level reduced the adverse effect on blossom formation but decreased effectiveness in fruit set.

Facteau and Rowe (1979) sprayed young Royal Ann sweet cherry trees for three consecutive years with ethephon (2-chloroethyl-phosphonic acid) at 50 and 100 ppm, and with daminozide (acid-2,2-dimethyl hydrazide) at 1000, 2000, and 4000 ppm, and found no antagonistic or synergistic effects on either growth or flowering.

Daminozide at 4000 ppm reduced fruit set of clusters of wood of all ages.

Single-year experiments on Merton Glory cherry showed that the application of GA₃ alone did not increase fruit yield. Treatment with mixtures of GA₃ + 2,4,5-T or GA₃ + 2,4,5-TP, with or without DPU, led to significant yield increases (Modlibowska and Wickenden, 1982). A long-term experiment was undertaken with annual applications of low-concentration gibberellin-auxin mixtures. Experimental results suggested that 2,4,5-TP on Van and Merton Glory cherry trees was more effective than similar concentrations of NAA with applications at the cot-split stage (when the growing ovary splits the calyx outward with stamens still present) being more effective than at either petal-fall or two weeks after cot-split.

Edgerton (1983) reported the applications of the sterol inhibitor "Vanguard" in a seasonal program at recommended fungicidal rates increased the fruit set of several strains of Delicious apple.

Cultural Practices on Fruit Set

Varieties on Fruit Set

The natural tendency to set fruit varies greatly with variety. With cherries, Corum and Bada tend to set much heavier than Royal Ann (Westwood and Stevens, 1979) especially on young trees. A realistic target yield for orchards in Oregon for sweet cherry is

eight to ten tons per acre, and a fruit set of 20 to 60 percent (Chaplin and Westwood, 1980).

Royal Ann, Corum, Bada, and Rainier are considered highly productive (Brooks and Griggs, 1964; Way, 1967; Stebbins and Walheim, 1981), while Vega is known by its low genetic ability for fruit set (Thompson, M.M., personal communication).

Rootstocks on Fruit Set

Westwood and Stevens (1979) stated that rootstocks not only control tree size, but they also affect both flower initiation and fruit set. Fruit set of sweet cherries has been good on young trees grown on OCR-2 and MXM clones 2,60, and 97. Flowering and set with F 12/1 and mazzard seedling roots are satisfactory when the trees reach five to eight years, provided that pollination and nutrition are not limiting.

Pruning on Fruit Set

Luckwill (1960) reported that more lightly pruned trees have more blossoms open during the latter part of the blossoming period than do spur pruned apple trees, and that this provide a valuable safeguard against poor setting conditions during any particular portion of the period. Very heavy winter pruning or dehorning of apples and pears may greatly increase fruit set because the large carbohydrate reserves stored in the trunk and roots are then shared among a smaller number of fruitlets the following spring (Luckwill, 1960).

Westwood and Stevens (1979) concluded that pruning on young sweet cherry and prune trees will reduce both bloom and yield. This is particularly true for sweet cherry which tends to be late in bearing. Thus, any pruning done in training the young tree should be as light as possible to avoid delaying the first crop.

Pathogens on Fruit Set

Luckwill (1960) indicated that almost any fungus or insect pest of fruit such as apple sucker (Psylla mali S.), apple blossom weevil (Anthonomus pomorum L.), apple sawfly (Hoplocampa testudinea K.), pear midge (Contarina pyrivora R.), apple mildew (Podosphaera leucotricha S.), blossom wilt in all top fruits (Sclerotinia laxa A.), grey mold (Botrytis cinerea P.), and mildew (Sphaeroteca humuli B.) in strawberry, will have an adverse effect on cropping.

Westwood and Stevens (1979) stated that damage during the previous season by mites or insects, which destroy or damage the leaf system or its capacity to produce organic food, may reduce fruit set the following spring. The most important fungal disease affecting fruit set in cherries is Brown Rot (Monilinia fruticola) (Westwood and Stevens, 1979).

Vertesy and Nyeki (1974) studied the effect of different ringspot viruses in sour cherry. The flowering period of the infected Montmorency trees was longer than that of the virus-infected Pandy-48 clone which blooming period took place three to five days earlier. The effect on the fertilization (pollination) of Montmorency trees appeared to have an effect in reducing fruit

set depending on the source of the infecting ringspot viruses involved, and not on the Pandy-48 clone, which was affected by the pollen source and not by the ringspot virus involved.

Environmental Conditions on Fruit Set

Luckwill (1960) stated that the dependence of pollination and fruit set on the weather prevailing at blossom time is universally recognized, such as is frost damage which can reduce fruit set by killing flowers at temperatures below -2.2°C . Even when the freezing point is not reached, persistent low temperatures at blossom time can have a very drastic effect on fruit set. Cold weather prolongs the blossoming season and severely restricts the activity of pollinating insects. Probably of equal importance is the retardation of pollen germination and tube growth. Apple pollen fails to germinate below 4.4°C , and pollen tubes grow slowly in the style unless the temperature exceeds 10.5°C . Rain may have a deleterious effect on pollination as pollen can be washed from the stigmas.

Braak (1978) found that both a delay in the flowering date and low temperature during embryo growth had an adverse effect on sweet cherry embryo development in the Netherlands. He concluded that early flowering and high temperature have a favorable effect on embryo growth while late flowering and low temperature have an adverse effect.

Meland (1982) reported that the tonnage of sweet cherries in Norway for a 10-year period (1970-1979) was positively correlated

with May bloom temperature and negatively correlated to number of days with 1 mm precipitation or more in May. Each day with rainfall reduced the tonnage by 7.5 percent. The correlations were significant at the 0.01 level. No significant correlation was found between tonnage and precipitation in July during the harvest season of cherries.

Fruit Drop

Physiological Fruit Drop

Luckwill (1960) reported that flowers in which fertilization of one or more ovules have taken place, soon begin to swell and develop into small fruitlets. But in the tree fruits, only a small proportion of these normally develop to maturity, the remainder being shed at varying stages of development. Thus, in the apple in a good blossom year, a full crop of fruit may represent only five percent of the total number of flowers on the tree, the remaining 95 percent having been shed in a series of drops known as the 'first' drop, the second or 'June' drop, and the 'preharvest' drop. The first and second drops are basically a manifestation of the intense competition for food materials which exists between the developing fruitlets, but their occurrence is controlled by a specific hormone emanating from the endosperm of the developing seeds. The first drop normally contains a large number of small fruitlets which, due to defective pollination or to inherent genetical factors, have a low complement of developing seeds.

Ovule Abortion

Bradbury (1925, 1929) stated that early fruit drop is a very well known phenomenon in sour cherry. A study to find out the factor or factors responsible for this drop was made. She described three drops with embryo degeneration or abortion in all of them, but it was concluded that unfavorable nutritional conditions may be largely responsible for the abortion of the fruits in all three drops.

Tukey (1933) in a study of early-ripening varieties of sweet cherry found abortive embryos were frequently associated with dropped fruits, but fruit drop was associated also with poor pollination, sterility, incompatibilities or nutrition. There was evidence to show that the problem was nutritional by successfully artificial culturing the abortive embryos.

Jaumien (1968) in a study of the low fertility in Comice pear, found that the main cause of excessive fruit drop was the lack of seed formation which is a consequence of degeneration of the embryo sacs and ovules.

Callan and Lombard (1978) found that the percentage of malformed or aborted sacs of Comice pear was very low and concluded that early degeneration of embryo sacs in Comice pear was not the cause of low female fertility, but was due to incomplete cross-pollination.

Thompson and Liu (1973) in a study of Italian prune, determined that the cause of erratic fruit set was attributed to its genetically determined sensitivity to cool weather (7.7° to

10.7°C) in the post-bloom period. Cool temperatures delayed pollen tube growth and fertilization so long that the ovule began to degenerate before fertilization.

Pimienta and Polito (1982) stated that ovule abortion is a generally neglected aspect of flowering plant reproduction, but is a significant parameter in almond production and variety improvement. Ovule abortion was accompanied by blockage in the metabolitic supply, although it was uncertain if this blockage was the primary cause or a consequence of ovule abortion.

III. MATERIALS AND METHODS

1982 Trials

Fruit Set Field Trials

Mature Royal Ann sweet cherry trees on F12-1 rootstocks at the Lewis-Brown Research Farm in Corvallis were hand-pollinated to compare the fruit set effectiveness of supplemental cross-pollination with Bada, Corum, and Black Republican pollen.

Four uncaged limbs, three hand-pollinated limbs of each pollinizer and a nonhand-pollinated limb (control) were used on each of ten trees. A pair of limbs were chosen alternatively on the N and S sector of the trees so that all treatment limbs were equally placed in each sector five times for the ten trees.

Pollen was collected from depetaled flowers at popcorn stage by brushing anthers against a wire mesh to let the non-dehiscing anthers gather in a petri dish. Anthers were dehisced at room temperature for two to three days, and the pollen was stored at 0°C until the day of pollination. Hand-pollination was achieved by brushing the pollen onto the stigma with a camel-hair brush. Self-pollination was not avoided.

Hand-pollination was carried out twice, April 21 and 22, 1982, at 70 to 90 percent full bloom, while anthers were dehiscing and pistils were in good condition.

The limbs used for the trials had a minimum of 3500 flowers per treatment. Two total fruit counts were made (May 21, and June 10, 1982). Percent fruit set was calculated from the second count

and this data was analyzed as a randomized block design, applying the LSD (.05) test for multiple comparison when significant difference was shown.

Pollen Tube Growth

Royal Ann bouquets from trees at the Lewis-Brown Research Farm at Corvallis were hand-pollinated with Bada, Corum, and Black Republican, and the pollen tube extension was followed for each pollinizer at four temperatures. Flower buds just prior to anthesis were emasculated, and 300 flowers were hand-pollinated with one of three pollinizers and placed in one of four controlled-temperature rooms which averaged 16.4°, 12.3°, 9.9°, and 7.3°C as recorded with thermo-couples every three hours during the pollen tube growth period. The methods of pollen collection and application were as those used for fruit set. The cut ends of each bouquet were kept under water to prevent wilting.

Thirty flowers were sampled daily, from each treatment-temperature combination. Flowers at 16.4°C and 12.3°C were sampled one to ten days after hand-pollination, and flowers at 9.9°C and 7.3°C were sampled three to ten days and four to ten days after hand-pollination, respectively. In the field, 350 depetaled and emasculated flowers of Royal Ann were hand-pollinated with Bada pollen on April 22, 1982 (one day after flower preparation). Thirty flowers each day were sampled daily two to ten days after hand-pollination. The average temperature was 12.6°C during the period from hand-pollination until the tenth day of sampling,

although the variation from day to day was considerable (ranging from 1° to 27°C).

The entire pistils were killed and fixed in F.A.A. (Formalin, Acetic Acid, 70% ETOH) after sampling, and then vacuumed and stored in 70% ETOH until preparation for squashing. Before squashing, the styles were softened in NaOH 8N solution for 24 hr., rinsed in water, and then squashed in a 0.1 percent aniline blue, buffered with K₃PO₄ on a slide under a cover slip and finally observed under a fluorescent light microscope to measure the pollen tube growth (Martin, 1959).

The pollen tube length was defined by progressively scanning across the style until the furthest point could be detected, the distance from this point to the stigmatic surface was then estimated using a vernier scale on the microscope stage. The length of the styles and pollen tube growth were averaged for each individual day-treatment sample. Pollen tube length was reported as percentage in relation to style length.

The observations for pollen tube length were made daily until at least 80 percent of the styles had pollen tubes at the base of the style. The number of replications for each treatment varied from 10 to 20 styles. The absolute value of the pollen tube length was converted into percentage and then the arc sin $\sqrt{\%}$ transformation was applied, and these figures were used for the ANOVA, using a completely randomized and randomized block design, when an equal number of replications were available. Treatments were compared during the same period following pollination. When

statistical significance was detected, the LSD (.05) test for different number of replications was applied.

Regression analysis (linear) was applied to each cultivar-temperature combination per day, using as many treatments as possible before pollen tubes reached the stylar base. The formula used was: $\hat{Y} = a + b(x)$.

After being killed and fixed in FAA, ovules from the same samples used for pollen tube growth were sent through a TBA series, embedded in paraplast, sectioned at 8 μm and stained with Hematoxylin or Hematoxylin-Fast Green for light microscope to determine time of fertilization for each cultivar-temperature combination.

Fruit Drop Study

Samples of normal and dropping Royal Ann cherries were taken twice a week before bloom to two weeks before harvest from a commercial orchard in Lewisburg, which had a ratio of one pollinizer to six Royal Ann trees and three hives per acre. The study was established to look for possible anatomical reasons for the early drop, such as malformation of the embryo sac prior to fertilization, etc.

The tissue was killed and fixed in C.R.A.F. (Randolph, 1935), vacuumed and dehydrated through a TBA series, embedded in paraplast, sectioned at 8, 10 or 13 μm , mounted and stained with Hematoxylin for oil-immersion microscopy. The embryo sac stages of

development, embryo and endosperm development or abortion were reported in number of ovules observed.

The sampling dates included: popcorn, older bloom, early petal fall, petal fall (four sampling dates), and post petal fall (three sampling dates). Samples included normal fruits and those that had dropped. The results are reported in the Appendix section.

1983 Trials

Fruit Set Field Trials

Evaluation of four pollinizers on the fruit set of Royal Ann and of Royal Ann as a pollinizer on the fruit set of the same four sweet cherry cultivars were made in a commercial orchard in the Eola Hills of West Salem. Each pollinizer treatment consisted of 32 uncaged limbs on eight trees; half were hand-pollinated and the other half were nonhand-pollinated.

A pair of limbs, one of which was hand-pollinated, were placed in both the N and S sector of each of the eight trees. Each limb consisted of a minimum of 100 clusters with an average of 2.5 flowers per cluster.

The eight receptor trees were adjacent to the pollinizer source trees for each treatment. The pollen sources used were: Bada, Corum, Rainier, and Vega on Royal Ann pistils, and Royal Ann pollen on Bada, Corum, Rainier, and Vega pistils. The hand-pollination dates varied for each treatment due to the different

date of blooming of each pollinizer-cultivar used. The pollen was used directly from fresh flowers of the pollinizer being used, brushing their anthers against the stigma of each flower (4000 flowers per treatment).

Fruit counts were made on June 11, 1983. The percentage of fruit set was calculated using the total number of flowers and the total number of fruits per limb. The fruit set data was analyzed as a completely randomized design with an equal number of replications; if significance was detected, the LSD (.05) was applied.

Pollen Tube Growth Field Trials

About 150 flowers of each pollinizer treatment used in the fruit set study were prepared for pollen tube growth observations. After the flowers were emasculated and depetaled prior to anthesis, they were enclosed in cages, and hand-pollinated with the appropriate pollen. Pollen from fresh flowers, as in fruit set trials, was hand-brushed on the prepared flowers.

Samples were taken daily beginning on the fourth day after pollination and ending on the tenth day. Sample size varied with each treatment based on the total flowers prepared and pollinated.

The pistils were fixed, squashed, stained and observed in the same manner as in 1982, autoclaving the pistils in Na_2SO_3 at 5% for 30 minutes at 90°C , was used as the tissue-softening technique (Lombard et al., 1972).

The pollen tube length was measured as in 1982, with daily measurements until 72 percent of the styles had pollen tubes reaching the base. The length of the pollen tube was recorded as absolute value and this and the length of the style were averaged separately each day of sampling.

The absolute value was transformed to percent of length and this was tranformed by arc sin $\sqrt{\%}$. Transformed lengths were analyzed by ANOVA, as a completely randomized design with 4 to 22 replications. The LSD (.05) test, for different number of replicatons (sample size), was applied when statistical significance was detected. The ANOVA was conducted for treatments at the same number of days after pollination.

Phenology and Floral Characteristics

The blooming period for each of the five cultivars: Bada, Corum, Rainier, Vega, and Royal Ann, was recorded by visual observation to compare blooming periods. These were first and full bloom stages and petal fall stage.

Floral characteristics were measured to observe possible pollination differences or deficiencies of each of the five pollinizers. Pistil and stamen length was measured using a sample size of five flowers for each pollinizer. The anther dehiscence period was also observed.

Pollen amount per anther and/or per flower was determined by using a hemocytometer for the pollen grain counts (Joppa et al., 1968). The pollen was collected as for fruit set trials. Aniline

blue as a stain mixed with 45% acetic acid and 100% ETOH (1:1:1) was used to release the pollen grains from the anthers. After the anthers were in aniline blue, they were shaken for five minutes.

The soluble solids ($^{\circ}$ Brix) in the nectar were determined by catching bees (sample size: 10 bees) that had been working on each pollinizer. The bees were killed with dry ice and dissected for their honey-stomaches. The honey-stomachs were smeared on a hand-refractometer (Atago 0-90 $^{\circ}$) (Butler, 1945). Bees were not active on Rainier when samples for $^{\circ}$ Brix were taken.

Bee Behavior when Foraging

Several observations of short duration were made of foraging bees in the four pollinizer cultivars plus Royal Ann to characterize bee behavior.

Observations were made at temperatures above 14.4 $^{\circ}$ C for Corum, Rainier, and Vega, and above 9.9 $^{\circ}$ C for Royal Ann and Bada, and were effectuated from noon to 16:00 hr at several bloom development stages.

Pollen Germination Test

The hanging drop method (Lewis, 1942) was used in both years using a 15% saccharose solution plus 2.5 ppm boric acid (Thompson and Batjer, 1950), as a germination media.

After about six hours at room temperature, the pollen grains which germinated were then counted under a light microscope to compute the percentage of germination.

The pollen germination trials were conducted at each pollination time.

IV. RESULTS AND DISCUSSION

Pollen Germination

One of the characteristics required for a good pollinizer is a sufficient percent of pollen germination. Pollen germination was near or above 70 percent for most cultivars in 1982 and 1983 in artificial media in vitro (Table 1) which was considered to be a sufficient level for effective pollination by Luckwill (1960). However, Corum had only 27 percent germination in 1982 (Table 1).

During the microscopy work, Corum pollen germination in vivo was lower on the stigmatic surface and consequently had lower number of pollen tubes than the other cultivars in both years. Even though the percent pollen germination in vitro was in or close to the normal range (70 percent), except for Corum in 1982, it cannot be assumed that the pollen germinates at the same percentage on the stigmatic surface of the style. Pollen germination on the stigma can be reduced by low stigma receptivity (Williams and Wilson, 1970), by low number of pollen grains in the surface (Brewbaker and Majumder, 1961; Ter-Avanesian, 1978), by pollen-stigma incompatibility (Heslop-Harrison, 1975, 1976), or by low pollen viability due to high humidity or due to high metabolic rate (Faegri and van der Pijl, 1979). Therefore, pollen germination on the stigma under field conditions of this study might have been reduced by one or several of the factors previously mentioned.

Fruit Set

High fruit set is one of the more important yield components of sweet cherry (Lombard et al., 1983), and is another characteristic of a good pollinizer. The effect of supplementary pollination from three different pollinizers on the fruit set of Royal Ann was compared with open pollination in 1982. There was no statistical differences among the fruit set from the various pollen sources, but fruit set from supplementary pollination with Bada was significantly greater than that of open pollination treatment (Table 2).

The percent of fruit set, including all treatments and open pollination, was in the range of 36 to 52 percent, which was considered sufficient for a commercial crop (Chaplin and Westwood, 1980). Therefore, pollen transfer was not a significantly limiting factor in the crop level of Royal Ann. However, there was a fruit set increase from supplementary cross-pollination under a mean temperature of 12.6°C. Fruit set response was slightly better with Bada as a pollen source than Corum or Black Republican, possibly due to a better compatibility between the two (Brooks and Griggs, 1964). Therefore, although not significantly, pollen source can influence fruit set. No significant difference occurred between tree sides nor were there any significant interactions of cultivar-side.

In 1983, four pollinizers were used on Royal Ann and Royal Ann was used as a pollinizer on the four cultivars to evaluate the pollen source on fertility. Fruit set data indicate a high

fertility level for Royal Ann and Bada trees as compared to the other three cultivars (Table 3). There were no statistical differences in fruit set among three pollen sources (Bada, Corum, and Rainier) for Royal Ann but those flowers pollinated with Vega pollen gave markedly lower set. As in 1982, Bada pollinizer gave the highest fruit set for Royal Ann, and Corum, Rainier, and Vega pollen gave lower, although sufficient, fruit set on Royal Ann.

Royal Ann used as a pollinizer showed the best compatibility with Bada, as indicated by a high fruit set. The fruit set on Corum and Vega from Royal Ann pollinizer was lower than that considered as sufficient for a commercial crop, less than 20 percent (Chaplin and Westwood, 1980). Bada and Corum are reported as setting a heavier crop than Royal Ann (Westwood and Stevens, 1979), while Stebbins and Walheim (1981) reported Rainier as a very productive cultivar. However, these reports were not confirmed in our study, which indicated Bada had similar fertility as Royal Ann while Rainier and Corum were much lower. A possible explanation for the low fruit set on Rainier and Corum could be the low compatibility with Royal Ann or the difference in blooming time between them, while Vega may have a low genetic fertility level (Thompson, M.M., personal communication).

The insignificant difference between supplementary and open pollination treatments indicated again that pollen transfer was not a limiting factor for fruit set, even though the average temperature was low (5.9° to 7.1°C), and much lower than in 1982 (12.6°C). The low temperatures (5.9° to 7.1°C) would affect bee flight and

evidently, has less effect on fruit set. However, during 1983 pollen source was a limiting factor for fruit set. The low fruit set for Royal Ann with Vega pollinizer makes it doubtful about its efficiency as a pollinizer, at least under the low temperatures prevailing in 1983, even though pollen germination in vitro was sufficient (Table 1).

Pollen Tube Growth

A good pollinizer should have fast and strong pollen tube growth, especially under temperatures such as 10°C or lower. Pollen tubes of sweet cherries grow in mass that combine into a single strand about half-way through the style to form a mass of tightly woven tubes through the remainder of the style. In contrast, the pollen tubes of pear grow individually and randomly throughout the style (Lombard, P.B., personal communication).

The pollen tube growth period required to reach the stylar base of 80 percent of the styles in 1982 was dependent on temperature and pollen source. Bada and Black Republican pollen tubes reached the stylar base within 4.0 days at 7.3°C, while Corum pollen tubes required 9.4 days (Table 4). Bada, under field conditions with an average temperature of 12.6°C, took 4.8 days to reach the stylar base.

ANOVA was applied to the 1982 and 1983 absolute values data of the pollen tube growth, and to the same values after arc sin $\sqrt{\%}$ transformation. Since the ANOVA showed no difference in results between absolute or transformed values, the data is reported in

percent after transformation analysis (Tables 5, 6, 7). The rate of pollen tubes extension in two days after pollination at 16.4° and 12.3°C (Table 5) indicated a significant cultivar and cultivar-temperature interaction. At two days after pollination, with temperatures at 12.3° and 16.4°C, Bada showed a slightly larger pollen tube extension than Black Republican and Corum (Table 5). The lack of a statistical difference between the average rate of growth at the two temperatures may be due to a reduced rate of Bada and Black Republican pollen tubes at 16.4°C, and/or low Corum rate at the same temperature.

At three days after pollination, pollen tube growth was influenced significantly by temperature and by pollen source (Table 6). Significant interactions indicated that Corum had the lowest pollen tube extension at 12.3° and 9.9°C, while those of Black Republican had the lowest extension at 16.4°C. Pollen tube growth of Bada and Black Republican pollen was less temperature dependent than that of Corum which had reduced growth at 9.9°C.

Similarly, by the fourth day, pollen tube extension was dependent on temperature and pollen source although most tubes had extended to the stylar base (Table 7). As was found at day 3, Corum pollen tubes had reduced growth at 9.9°C and 7.3°C while Black Republican tubes were reduced only at 7.3°C. Therefore, pollen tube growth of both Black Republican and Corum were particularly temperature dependent at the lowest temperatures. Throughout the three day period, Corum pollen tube growth showed a high

temperature-dependent behavior, mainly at the low temperatures of 9.9° and 7.3°C.

A linear regression analysis was applied to the percent of pollen tubes extension data in 1982 only on the fourth day with the four temperatures as the independent variable and the pollen tube growth of the pollinizers as dependent variables. Although most tubes had reached the stylar base at the higher temperatures, the fourth day was analyzed because it was the only day that all four temperatures could be compared (Table 8).

Pollen tube growth of Bada and Black Republican can be considered similar in response to temperature effects, particularly above 9.9°C. Corum as a pollinizer would not be as suitable as Bada and Black Republican at temperatures below 9.9°C, which is more typical of the temperature pattern during Royal Ann bloom period in western Oregon.

The mean temperatures in the field during the bloom periods in 1983 ranged from 5.9°C to 7.1°C and were much lower than those in 1982 (12.6°C). Bada and Vega pollen tubes required 4.5 and 4.8 days, respectively, to reach the stylar base in 72 percent of the Royal Ann flowers while Rainier and Corum tubes required 6.7 and 8.6 days, respectively (Table 9). Royal Ann pollen tubes required 4.9 to 6.9 days to reach the stylar end of various pistillate cultivars. Field results of 1983 were similar to 1982 where Bada pollen tube extension reached the stylar base in less time than Corum. Pollen tube growth of Royal Ann was more rapid in Bada and Vega styles than in those of Corum and Rainier.

Analysis of variance applied on the 1983 data at day 5 after pollination indicated that there was statistical differences between pollen tube extension between the various pistillate-pollinizer combinations. Corum and Rainier pollen on Royal Ann styles was less than Bada or Vega. Royal Ann pollen tube extension was less in Rainier styles than in other cultivars tested (Table 10). The slow pollen tube growth of Royal Ann (S_3S_4) and Rainier (S_1S_4), either as pollinizer or receptor cultivars can be due to the possession of a common gene (S_4) in the pollen grains which make them low in compatibility (Way, 1968). The explanation for the slow pollen tube growth of Corum in Royal Ann and of the reciprocal pollination is probably due to the low pollen germination on the stigmatic surface at low temperatures which was observed during the microscopy work in both 1982 and 1983 trials. The lack of pollen germination could influence the slow pollen tube growth. Zamir et al. (1981) and McKenna and Mulcahy (1983) indicated differences in pollen tube growth due to gametic pollen competition or differences in pollen tube growth from different species in the same stigma, respectively.

Low germination of Vega pollen was also noted on Royal Ann stigmas, only one to three pollen tubes per stigma were found. However, Vega pollen tube growth in Royal Ann styles was as rapid as Bada which had a mass of pollen tubes. But the fruit set of Royal Ann and of Vega were low in both reciprocal pollinations (Table 3). This could be an effect of Vega low number of pollen tubes growing (Thompson, M.M., personal communication).

Assessing the period required for fertilization after pollination was not possible to determine because the ovule collapsed in the samples. The method was suitable only for pollen tube growth studies (Jefferies et al., 1982).

Phenology and Floral Characteristics

Evaluation of pollinizers should include phenology as well as several floral characteristics. Such characteristics can be sugar content in nectar, bee attractiveness, etc. In 1983 the blooming period of Bada corresponded closely with Royal Ann. Bada bloomed less than one day earlier, while in the other pollinizers bloomed three to seven days earlier (Table 11). The blooming period of Bada coincided best with Royal Ann which met one of the criteria of a pollinizer which is the overlap of the flowering period (Way, 1961). Corum bloom occurred too early for Royal Ann in 1983 since it had reached petal fall prior to full bloom on Royal Ann.

Similarity of the flower parts of pollinizer and pistillate cultivar could be important in cross pollination, especially when their arrangement may modify bee behavior. The pistil and stamen lengths varied for each cultivar, but there was little difference in relative length between pistil and stamen among the cultivars (Table 11). This structural arrangement of the flowers would not interfere with honey bee pollination among any of five cultivars because the stigmas were located slightly above the level of the stamens, favoring adequate pollen transfer by insects (Luckwill, 1960).

Anther dehiscence of individual cherry flowers occurred prior to anthesis, usually at popcorn stage, and began in the inner whorl of stamens as Srivastava and Singh (1970) found on sweet cherry, and continued until petal fall on all five sweet cherry cultivars. Rosaceous flowers have many stamens arranged in whorls which dehisce successively over a period of one to nine days (Luckwill, 1960). Anther dehiscence was observed to start at 10°C and 80 percent relative humidity, with its optimum at 30°C and 50% relative humidity by Langridge and Goodman, 1973. During the 1983 bloom period in the orchard plot, there were two to four hours daily with relative humidity around 50 percent and with temperatures above 15.5°C with a maximum of only 21°C during the blooming period of the five cultivars. Although the conditions during the bloom period were not optimum, there were several hours every day which triggered anther dehiscence.

Pollen quantity is related to dehiscence of the anthers and to stage of development (Luckwill, 1960). Consequently, the pollen quantities reported in Table 11 were the potential level per flower for each cultivar during bloom. Bada had the greatest available amount of pollen, 5.1 million grains per flower, while Rainier had the least amount, 1.4 million grains. Corum flowers had the greatest mass of anthers plus pollen with 3.4 mg per flower and Rainier had the least, 2.2 mg per flower. The amount of pollen included both pollen grains and anthers; therefore, these amounts should be considered as relative amounts. This is the reason for the high amounts given here in comparison with those

reported by Percival (1955) which are 1.7 mg for apple, 1.2 mg for pear, and 0.3 mg for wild cherry reported as pollen per flower.

Soluble solids of the nectar from the honey stomach of bees differed little among four of the cultivars and would not indicate a cultivar preference because of nectar content (Table 11). Bees working Bada had slightly higher °Brix than the other three pollinizer flowers. °Brix from bees working Rainier was not reported because bees were not foraging Rainier flowers after late petal fall. Free (1970) mentioned ranges of sugar content in nectar from 21 to 60 percent for sweet cherry, 15 to 40 percent for sour cherry, and 2 to 37 percent for pear.

Luckwill (1960) quoted two examples of the effect of sugar concentration in nectar as related to bee behavior. He reported that bees visited sweet cherry flowers with a nectar content of 55 percent in preference to sour cherry flowers containing nectar of 20 percent in the same orchard. He also noted that mustard flowers with a nectar content of 44 to 60 percent attracted bees from plum and pear flowers which contained nectar of only 12 percent sugars. Flowers of all four cherry cultivars appeared to be equally attractive to bees from the standpoint of nectar content and none should be much less attractive compared to competing wild flower species, such as maple trees or ornamental apple.

Bee Behavior

Bee attractiveness and bee behavior should be evaluated when cultivars are being compared for their performance (Robinson,

1979). Bee behavior was observed in trees of the five cultivars to indicate if there were any pollen transfer problems of any of the five cultivars. Only nectar collector bees were foraging in all cultivars (Table 12). Most bees were top workers. Bada, Corum and Royal Ann trees had higher number of bee visits per flower than those of Vega and Rainier. There was little difference in bee activity between N and S side of the tree. The lack of much difference between north and south sides in bee activity was due to a lack of foliage which made for good light penetration through the trees. Rainier had a lower number of bee visits because of the later stage at which it was evaluated. The Rainier flowers were possibly less attractive due to less petals and pollen quantity and with greater nectar concentration (Ewert, 1940a). There were no pollen collector bees, possibly due to the stages at which bee flight was evaluated for each cultivar, and the time of the day at which pollen and nectar collectors forage (Free, 1970).

The efficiency of nectar collectors as pollinizers was corroborated by the way in which top-worker bees worked the flowers. They touched the stigmatic surface with their legs when working the nectaries which is an important pollinating technique (Robinson, 1979). The few side worker bees found on Corum flowers were due to fewer petals which made it easier for bees to work the sides. The side worker bees stood on the sepals and inserted their tongue (*glossa*) into the nectaries without touching the anthers, therefore making little or no pollen transfer at this late stage.

In summary, flowers of Royal Ann, Bada, and Corum appeared to be almost equally attractive to bees from the standpoint of bee visits, while Vega and Rainier were less attractive.

Low temperature (5.9°C) and rainfall were not a limiting factor for fruit set and pollen tube growth during 1982 and 1983 except for the poor Corum pollen tube growth. Although the poor conditions during 1983 would appear to be a limiting factor to bee flight, pollen transfer was not limiting in these trials. The source of pollen was limiting in relationship to poor environmental conditions and compatibility. Low pollen germination and pollen tube growth at low temperatures could affect fruit set in Royal Ann when pollen of Corum and Vega are used due to their poor pollen germination and low number of pollen tubes growing. Rainier as a pollen source gave high fruit set on Royal Ann because the flowers could be bee-pollinized with other pollen sources. Bada as a pollinizer was most suitable in overall performance and characteristics for Royal Ann, based mainly on bloom overlapping period and pollen tube growth.

Several possible future trials could study the effect of low temperature on the fruit setting ability of Corum pollen on Royal Ann. The germination of pollen, both in vivo and in vitro, should be studied under a different range of temperatures (5° to 20°C), and the relationship of pollen number on the stigmatic surface and on pollen tube growth and fruit set when using pollen sources of Corum and Bada.

The effect of humidity on pollen viability during bloom period and anther dehiscence should be studied on the pollinizer cultivars. Also, the effect of high temperatures on pollen viability and pollen tube growth and their effect on fruit set could be considered for a study.

Table 1. Pollen germination of several sweet cherry cultivars.

| <u>Cultivar</u> | <u>Percent Pollen Germination</u> | |
|------------------|-----------------------------------|-------------------|
| | <u>1982</u> | <u>1983</u> |
| Bada | 52 (± 3.2) ^z | 74 (± 16.4) |
| Corum | 27 (± 7.5) | 76 (± 11.5) |
| Black Republican | 52 (± 10.3) | --- |
| Rainier | --- | 74 (± 17.5) |
| Vega | --- | 65 (± 22.5) |
| Royal Ann | --- | 91 (± 1.51) |

^z Standard deviation in parenthesis

Table 2. Fruit set of Royal Ann sweet cherry with three pollinizers, comparing hand and non-hand pollinated uncaged flowers, 1982.

| <u>Cultivar</u> | <u>Percent of Fruit Set^y</u> |
|----------------------------------|---|
| Bada hand pollinated | 52 a ^z |
| Corum hand pollinated | 48 ab |
| Black Republican hand pollinated | 43 ab |
| Non-hand pollinated | 36 b |

^y Mean temperatures of 12.6°C

^z Numbers with separate sub-letters are significant at the 5% level.

Table 3. Effect of four sweet cherry pollinizers on fruit set of Royal Ann and Royal Ann as pollinizer on fruit set of four pistillate cultivars, using uncaged limbs, 1983.

| <u>Pistillate cv.</u> | <u>Pollinizer cv.</u> | <u>Percent Fruit Set^y</u> | |
|-----------------------|-----------------------|--------------------------------------|--------------------------------|
| | | <u>Hand Pollinated</u> | <u>Non-hand Pollinated</u> |
| Royal Ann | Bada | 59 a | 51 a ^z |
| Royal Ann | Corum | 54 a | 49 a |
| Royal Ann | Rainier | 53 a | 50 a |
| Royal Ann | Vega | 30 b | 37 b |
| Bada | Royal Ann | 56 a | 55 a |
| Rainier | Royal Ann | 27 b | 29 b |
| Corum | Royal Ann | 18 c | 14 c |
| Vega | Royal Ann | 17 c | 19 c |

^y Mean temperatures from 5.9° to 7.1°C.

^z Numbers with different letters are significant at the 5% level.

Table 4. Effect of sweet cherry pollinizer and temperature on pollen tube extension period in Royal Ann, 1982.

| <u>Pollinizer</u> | Number of days to reach the styilar base (80% of the styles) ^y at: | | | | |
|---------------------|--|---------------|---------------|---------------|---------------------|
| | 7.3°C | 9.9°C | 12.3°C | 16.4°C | 12.6°C ^z |
| Bada | 4.3 (±1.6) ^x | 3.7 (±1.0) | 3.4 (±0.8) | 3.0 (±1.3) | 4.8 (±2.2) |
| Corum | 9.4 (±0.9) | 8.5 (±3.3) | 3.9 (±2.0) | 2.9 (±2.6) | ---- |
| Black Republican | 4.9 (±1.5) | 3.8 (±1.1) | 2.8 (±0.7) | 3.6 (±2.0) | ---- |

^x Standard deviation of pollen tube extension for day 4 after pollination is in parenthesis.

^y Data in tenth of day was calculated by interpolation.

^z Field conditions.

Table 5. Effect of pollinizer source and temperature on pollen tube length in percent of stylar length two days after pollination of Royal Ann cherry.

| <u>Cultivar</u> | <u>Pollen Tube Length (% of style)^z</u> | | <u>Cultivar</u> |
|--------------------------------|--|---------|---------------------|
| | <u>Temperature</u> | | |
| | 16.4 °C | 12.3 °C | |
| Bada | 39.9 b ^y | 47.1 a | 43.5 A ^x |
| Black Republican | 33.8 c | 40.9 b | 37.3 B |
| Corum | 34.6 c | 28.0 d | 31.3 C |
| Temperature (N.S) ^w | 36.1 | 38.6 | |

^w Temperature averages are not significantly different at the 5% level.

^x Cultivar averages with separate letters are significantly different at the 5% level.

^y Treatment averages with separate letters are significantly different at the 5% level.

^z ANOVA was performed on arc sin $\sqrt{\%}$ transformed data.

Table 6. Effect of pollinizer source and temperature on pollen tube length in percent of stylar length, three days after pollination of Royal Ann cherry.

| <u>Cultivar</u> | <u>Pollen tube length (% of style)^z</u> | | | <u>Cultivar</u> |
|------------------|--|---------|---------|---------------------|
| | 16.4°C | 12.3°C | 9.9°C | |
| Bada | 92.1 a ^y | 60.4 c | 56.5 cd | 69.6 A ^x |
| Black Republican | 71.7 b | 96.1 a | 44.1 d | 70.6 A |
| Corum | 96.2 a | 45.1 cd | 24.6 e | 55.3 B |
| Temperature | 86.6 p ^w | 67.2 q | 41.7 r | |

^w Temperature averages with separate letters are significantly different at the 5% level.

^x Cultivar averages with separate letters are significantly different at the 5% level.

^y Treatment averages with separate letters are significantly different at the 5% level.

^z ANOVA was performed on arc sin $\sqrt{\%}$ transformed data.

Table 7. Effect of pollinizer source and temperature on pollen tube length in percent of stylar length four days after pollination of Royal Ann cherry.

| <u>Cultivar</u> | <u>Pollen tube length (% of style)</u> ^z | | | | <u>Cultivar</u> |
|---------------------|--|---------|---------|--------|---------------------|
| | 16.4°C | 12.3°C | 9.9°C | 7.3°C | |
| Bada | 100.0 a ^y | 100.0 a | 100.0 a | 71.7 b | 92.9 A ^x |
| Black Republican | 96.9 a | 100.0 a | 98.6 a | 39.2 c | 83.6 B |
| Corum | 92.9 a | 97.6 a | 44.6 c | 18.0 d | 63.2 C |
| Temperature | 96.6 p ^w | 99.2 p | 81.0 q | 42.9 r | |

^w Temperature averages with separate letters are significantly different at the 5% level.

^x Cultivar averages with separate letters are significantly different at the 5% level.

^y Treatment averages with separate letters are significantly different at the 5% level.

^z ANOVA was performed on arc sin $\sqrt{\%}$ transformed data.

Table 8. Intercept, slope, and correlation coefficient of pollen tube length in percent of stylar length four days after pollination on Royal Ann cherry, at four temperatures. ($\hat{Y} = a + b(x)$).

| Cultivar | <u>Intercept (a)</u> | <u>Slope (b)</u> | <u>Correlation Coefficient (r)</u> |
|---------------------|----------------------|------------------|------------------------------------|
| Bada | 62.0 | 2.7 | 0.71 |
| Black Republican | 20.4 | 5.7 | 0.69 |
| Corum | -43.1 | 9.5 | 0.90 |

Table 9. Effect of sweet cherry pollinizers on pollen tube extension period to reach the stylar base in five pistillate cultivars under field conditions.

| <u>Pistillate cv.</u> | <u>Pollinizer cv.</u> | Mean <u>Temp.</u> °C | No. of days to reach stylar base (72% of styles) ^y |
|-----------------------|-----------------------|----------------------------|---|
| Royal Ann | Bada | 5.9 | 4.5 (± 0.9) ^z |
| Royal Ann | Vega | 6.6 | 4.8 (± 1.8) |
| Vega | Royal Ann | 6.8 | 4.9 (± 2.7) |
| Corum | Royal Ann | 7.0 | 5.0 (± 4.09) |
| Bada | Royal Ann | 6.8 | 5.2 (± 0.8) |
| Royal Ann | Rainier | 7.1 | 6.7 (± 3.8) |
| Rainier | Royal Ann | 7.0 | 6.9 (± 4.6) |
| Royal Ann | Corum | 6.8 | 8.6 (± 3.9) |

^y Data in tenth of day was calculated by interpolation.

^z Standard deviation of pollen tube extension for day 5 after pollination is in parenthesis.

Table 10. Effect of pollinizer source on pollen tube length in percent of stylar length five days after pollination in five pistillate sweet cherry cultivars under field conditions.

| <u>Pistillate cv.</u> | <u>Pollinizer cv.</u> | Pollen tube length (% of style) ^z | Mean Temp °C |
|-----------------------|-----------------------|--|--------------------|
| Royal Ann | Bada | 100.0 a ^y | 5.9 |
| Royal Ann | Vega | 95.8 a | 6.6 |
| Bada | Royal Ann | 95.7 a | 6.8 |
| Vega | Royal Ann | 89.7 a | 6.8 |
| Corum | Royal Ann | 81.1 ab | 7.0 |
| Royal Ann | Corum | 70.5 b | 6.8 |
| Royal Ann | Rainier | 62.1 b | 7.1 |
| Rainier | Royal Ann | 26.0 c | 7.0 |

^y Treatment averages with separate letter are significant at the 5% level.

^z ANOVA was performed on arc sin $\sqrt{\%}$ transformed data.

Table 11. Phenologic and floral characteristics of five sweet cherry cultivars.

| <u>Observation</u> | <u>Cultivars Observed</u> | | | | |
|---|----------------------------|------------------|------------------|------------------|------------------|
| | <u>Corum</u> | <u>Rainier</u> | <u>Vega</u> | <u>Bada</u> | <u>Royal Ann</u> |
| Phenology: (number of days after Corum bloom development) | | | | | |
| First open bloom | 0 (3/22) | 1 | 4 | 7 | 7 |
| Full bloom (80%) | 0 (3/30) | -2 | 5 | 6 | 6 |
| Petal fall | 0 (4/4) | 2 | 10 | 10 | 10 |
| Floral Characteristics: | | | | | |
| Pistil length (mm) ^z | 16.0 | 11.0 | 17.0 | 15.5 | 17.0 |
| Stamen length (mm) ^z | 17.0 | 10.5 | 18.0 | 17.0 | 16.0 |
| Pollen grains per flower: | | | | | |
| x million grains | 2.0 (±3.8) ^x | 1.4 (±2.5) | 2.8 (±3.2) | 5.1 (±5.5) | 3.9 (±4.2) |
| Pollen grains in mg/flower | 3.4 (±0.0018) | 2.2 (±0.0046) | 2.7 (±0.0015) | 2.7 (±0.0016) | 2.8 (±0.0005) |
| Nectar °Brix from bee honey stomachs ^y | 56° (±3.5) | --- | 56° (±2.1) | 58° (±5.1) | 49° (±8.1) |

^x Standard deviation in parenthesis

^y n = two samples with 10 bees each

^z ranges from five flowers

Table 12. Bee behavior in five sweet cherry cultivars.

| <u>Behavior</u> | Total number for 20 minute period | | | | |
|----------------------|-----------------------------------|----------------------------|-------------------------|-------------------------|------------------------------|
| | <u>Cultivar</u> | | | | |
| | <u>Corum^z</u> | <u>Rainier^z</u> | <u>Vega^z</u> | <u>Bada^y</u> | <u>Royal Ann^y</u> |
| Pollen collector | 0 | 0 | 0 | 0 | 0 |
| Nectar collector | 8 | 3 | 4 | 8 | 7 |
| Top worker | 6 | 3 | 4 | 7 | 7 |
| Side worker | 2 | 0 | 0 | 1 | 0 |
| Bees/ 25 flowers: | | | | | |
| North side | 5 | 2 | 1 | 2 | 4 |
| South side | 3 | 1 | 3 | 6 | 3 |
| Total bees/cv. | 8 | 3 | 4 | 8 | 7 |

^y Two observations at full and late bloom stages at temperatures above 9.9°C.

^z Two observations, late bloom stage at temperatures above 14.4°C.

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APPENDIX

FRUIT DROP

Fruit drop is an important factor in final yield. Although being a physiological process, it can be a serious problem when excessive drop affects yield (Bradbury, 1925, 1929).

The 1982 fruit drop study included 18 dates of sampling (normal and abscissed fruit) with ten dates observed under the oil-immersion light microscope objective. The phenology stages were reported along with the embryo sac and embryo stages (Appendix Tables 1 and 2).

Ovule development at the early stages prior to anthesis and through petal fall stage did not show any abnormality in its development. We can say that up to the polar nuclei fusion stage, the embryo sac development was normal and ready for fertilization. At petal fall (4/27) ovules from dropping fruits showed either signs of lack of fertilization or abortion.

The stages from late petal fall (5/13) to preharvest fruit drop (6/11) showed normal development in persisting fruits with a high number of ovules showing signs of fertilization containing an embryo plus endosperm. But in the abscised fruits, there was an indication of lack of fertilization from the disintegrating embryo sacs or in a collapsing ovule present at post-petal fall stage (5/10).

Also, dropped fruits collected at this stage on May 10 showed embryo and endosperm development by either fertilization or a hormonal stimulus (Luckwill, 1960). However, several embryo and

endosperms collapsed during the later development with the highest incidence observed during the beginning of pit hardening stage (5/20). Some ovules showed a complete collapse (misshapen and shrunken ovules with embryo sac present, but disintegrated) with a peak level at the beginning of stage II (May 17) and into stage III (May 31 and June 11).

These results indicate that there was very little or no abnormal embryo sac development at the fusion of the polar nuclei stage. Because of the high number of collapsed endosperms (Appendix Tables 1 and 2), it appeared that late fruit drop was due to embryo abortion because of the lack of developing endosperm. Endosperm abortion has been reported to occur because of a sudden arrest of development from a nutritional blockage or other causes not yet known (Detjen, 1926; Tukey, 1933). Embryo abortion has been attributed to nutritional blockage also (Bradbury, 1926; Detjen, 1926; Tukey, 1933; Pimienta and Polito, 1982).

The lack of full embryo development could be from a low pollen germination on the stigmatic surface due to low pollen viability or incompatibility (Heslop-Harrison, 1976), causing a few incomplete pollen tubes growing down the style which would not fertilize. These few may stimulate initial embryo development, but not for sufficient endosperm growth. Luckwill (1960) found similarly that embryo abortion would occur because of the lack of an endosperm and this would cause fruit abscission.

Therefore, fruit drop occurring in late May and early June could be due to endosperm and embryo abortion from nutritional

factors, possibly either from a blockage at ovular level (Bradbury, 1926; Detjen, 1926; Tukey, 1933) or a lack of complete fertilization, or a lack of endosperm.

Appendix Table 1. Embryo sac development from popcorn to early petal fall stages in Royal Ann sweet cherry, 1982.

| Embryo Sac Development | | | | | | | | | | | | | |
|------------------------|------------------|---------------------------------|---------------------------|---------------------|--------------|---|-------------------|--------------|---|---------------------------|-----------------------------|----------------------------|--|
| Date | Stage | Number of Days After Anesthesia | Number of Ovules Observed | Normal Development | | | | | After Fertilization | | | | |
| | | | | Immature Embryo Sac | | | Mature Embryo Sac | | Normal Development | | Abnormal Development | | |
| | | | | Nuclei Stage | | | | | Nuclei Stage | | | | |
| | | | | 2-Cell Stage | 4-Cell Stage | Sinergids, Antipodal, Egg or Polar Nuclei | Polar Nuclei | Polar Nuclei | Polar nuclei plus egg + deintegrating sinergida | Desintegrating embryo sac | Ovule collapsed & misshapen | Observations | |
| 4/13/82 | Popcorn | -6 | 40 | 5 | 1 | 6 | 13 | 15 | - | - | - | | |
| 4/20/82 | Older Bloom | 1 | 34 | - | - | - | 4 | 30 | - | - | - | | |
| 4/22/82 | Early Petal Fall | 3 | - | - | - | - | - | - | - | - | - | | |
| 4/27/82 | Petal Fall | 8 | 24 | - | - | - | 0 | 1 | 13 | 8 | 2 | 80% swollen, green ovaries | |
| 4/30/82 | Petal Fall | 11 | - | - | - | - | - | - | - | - | - | 86% swollen, green ovaries | |

Appendix Table 2. Embryo sac and embryo development from late petal fall stage to preharvest fruit drop in Royal Ann sweet cherry, 1982.

| Date | Stage | Number of Days After Anthesis | Average of Fruit Diameter Normal (mm) Aberrated (mm) | Average of Nect Longue (mm) | No. of Ovules Observed | Embryo Sac-Embryo Development | | | | | | | | | | Observations | |
|---------|--|-------------------------------|--|-----------------------------|------------------------|-------------------------------|----|---------------------|---|---|----------------------|---|---|--------------------|----|------------------|---|
| | | | | | | Parthenocarpic Fruits | | | | | Aberrated Fruits | | | | | | |
| | | | | | | Normal Development | | Unusual Development | | | Abnormal Development | | | Normal Development | | Aberrated Fruits | |
| | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | |
| 5/3/82 | Late Petal Fall | 14 | - | - | - | 13 | 6 | 6 | - | 1 | - | - | - | - | - | - | BLT swollen, green ovary |
| 5/7/82 | Late Petal Fall | 18 | 5-11 | 2, 3-10 | - | 14 | - | - | - | - | - | - | - | - | - | - | vegetatized embryo; cell off neatly |
| 5/10/82 | Stage I | 21 | 6-15 | 4-5 | - | 3 | - | 2 | - | 1 | - | - | - | - | - | - | first post- pollination drop |
| 5/10/82 | First Drop Period | 21 | 4-10 | - | - | 14 | 3 | - | - | 5 | - | - | - | - | - | 7 | subsolidarable fruit drop observed |
| 5/13/82 | End of Stage I | 24 | 7-17 | - | - | - | - | - | - | - | - | - | - | - | - | - | 10% swollen, green ovary |
| 5/17/82 | Begin Stage II; Slow Fruit Growth | 28 | 7-17 | 5-11 | - | 22 | 10 | - | 2 | 1 | 3 | - | - | - | - | 4 | old ovule, green ovary |
| 5/20/82 | Stage II, Fruit Pit Hardening | 31 | 17 | 5-10 | 5-10-5 | 16 | - | - | 1 | - | - | 1 | - | 1 | 7 | 3 | first signs of pit hard- ening |
| 5/24/82 | Stage II, Slow Fruit Growth | 35 | - | 5-12 | 3-10 | 11 | - | - | - | - | 10 | - | 1 | - | - | - | hard ovary |
| 5/27/82 | Stage II, Slow Fruit Growth | 38 | 13-17.5 | 5-14 | 2.5-10 | - | - | - | - | - | - | - | - | - | - | - | pit tender than before |
| 5/31/82 | Beginning of Stage III; Rapid Fruit Growth | 42 | 16.5-17.5 | 7-15 | 3-10 | 6 | - | - | - | - | - | - | - | - | - | 6 | fruit show- ing signs of color change |
| 6/3/82 | Stage III | 45 | 16.5-19 | 10-18 | 8-10.5 | - | - | - | - | - | - | - | - | - | - | - | catkins 5-6 mm long |
| 6/8/82 | Stage III | 50 | 19-20 | 11-19 | 0-10.5 | - | - | - | - | - | - | - | - | - | - | - | embryos com- pletely filled in 95% of ovules |
| 6/11/82 | Stage III | 53 | 10-21 | 10-20 | 3-10 | 6 | - | - | - | - | - | - | 2 | - | - | 6 | rosy hue beginning to show |

Key:

- 1. Polar nucellus and egg
- 2. Embryo and free nuclear endosperma
- 3. Embryo and cellular endosperma
- 4. Polar nucellus only
- 5. Disintegrating embryo sac
- 6. No embryo with endosperma present
- 7. Embryo with no endosperma present
- 8. Embryo collapsed with endosperma present
- 9. Endosperm collapsed with embryo present
- 10. Embryo collapsed with no endosperma