

AN ABSTRACT OF THE THESIS OF

Ronald S. Bekey for the degree of Doctor of Philosophy in
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Title: Effects of Method and Timing of Pollination and
Temperature on Fruit Development in Red Raspberry

Abstract approved: _____
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The contribution of honey bees (Apis mellifera L.) to fruit set, length of the flower receptivity period, and effect of temperature on flowering and fruit set of red raspberry (Rubus idaeus L.) were investigated.

One hour of bee visitation over one day in 'Willamette', and two hours over each of two days in 'Meeker', were sufficient for good drupelet set. Drupelet set over a two-year period in 'Willamette' could be attributed 62.5 % to self-pollination, 22 % to bees, and 15.5 % to wind and/or small insects.

The frequency of flower visitation by bees was significantly correlated with nectar secretion, and also associated with the periods of petal retention, anther dehiscence and the condition of pistils and stamens. It was estimated that two colonies of bees would be required per ha for 'Willamette', and five colonies for 'Meeker', to thoroughly pollinate all of the flowers present.

The peak receptivity period of 8 cultivars of red raspberry lasted from 1 to 4 days. Most cultivars set as well with one day of hand pollination at the peak of the receptivity period as with three days of consecutive pollination. Autogamy was as effective as allogamy in flowers of 'Willamette', and 'Meeker'.

Development of raspberry flowers, pollen-tube growth and drupelet set were all highly affected by temperature. 'Willamette' flowers matured in 3 days at 22° C, but required 18 days at 6° C.

Nectar secretion in 'Willamette' occurred over a maximum of 3 days at 22° C, but was prolonged to 4 days at 6° C. Stigmas and anthers of 'Willamette' remained healthy for 3 - 16 days after anthesis, depending on the temperature.

Drupelet set of 'Willamette' was significantly lower and pollen tube growth of 'Scepter' was considerably slower at 6 and 10° C than at 14, 18 or 22° C. Cold temperatures may reduce pollen tube growth to the point where the ovules degenerate before fertilization can occur.

The results of this study strongly emphasize the importance of providing sufficient numbers of bees to adequately pollinate the flowers in the event of undesirable weather conditions.

EFFECTS OF METHOD AND TIMING OF POLLINATION AND TEMPERATURE
ON FRUIT DEVELOPMENT IN RED RASPBERRY

by

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RSB
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PREFACE

This study consists of three parts, presented here as three separate papers. The first, which will be submitted to the Journal of Apicultural Research, covers the work related to bees. This study was designed to fill in some of the gaps in our knowledge of bee visitation of raspberries by providing an estimate of how much bee activity is required to pollinate them.

The second and third papers will be submitted to the Journal of the American Society for Horticultural Science. The second paper deals with the receptivity period of raspberries, which has been studied in detail in many tree fruits but has not been documented in caneberries.

The third paper concerns temperature effects on flowering and fruit set in raspberries. The flower maturation process, drupelet set and pollen-tube growth were studied in detail in relation to temperature, and predictions were made regarding pollination under temperatures likely to occur in the field.

All of these papers are interrelated, and it was necessary to cite from each to the others fairly frequently. The major theme concerns pollination of flowers by honey bees, partly because of my interest in the subject and also because of my desire to emphasize the importance of providing adequate numbers of bees in any crop benefiting from bee visitation.

EFFECTS OF METHOD AND TIMING OF POLLINATION AND TEMPERATURE ON FRUIT DEVELOPMENT IN RED RASPBERRY

LITERATURE REVIEW

Introduction

The American red raspberry, Rubus idaeus L., produces flowers in the spring on laterals formed in the leaf axils of the previous year's canes. The red raspberry flower contains ca. 70 - 100 pistils surrounded by a similar number of stamens. Each pistil, if pollinated, will produce a drupelet containing a seed. If enough drupelets develop they will hold together (with the aid of tiny hairs on each drupelet) to form a berry. Commercially-grown red raspberry cultivars are largely self-fertile, although cross-pollination will increase drupelet set in some cultivars (Daubeny, 1971; Redalen, 1976). Only the outermost stigmas are likely to come into contact with the anthers, so an external pollinating agent is needed (Free, 1970). With no additional pollination a berry will frequently develop, although it will be relatively small and may have a tuft of unpollinated pistils at its center (Shanks, 1969). Insect activity helps to spread pollen over the pistils and to increase the set of drupelets. The extent of this increase seems to vary with the cultivar.

Importance of Bees

Of the insect visitors to red raspberry, honey bees (Apis mellifera L.) are the most conspicuous and probably the most

important (McGregor, 1976). Bees are strongly attracted to raspberry flowers, as their nectar is high in both volume and sugar concentration (Free, 1970).

Several workers have compared the set of enclosed flowers (bees excluded) with that of flowers exposed to bees. Couston (1963) found that the mean seed number in 'Malling Jewel' fruits isolated from bees by bagging was only 36 % of that in fruits resulting from open-pollinated flowers. Shanks (1969) found that drupelet set on caged 'Sumner' plants was only 59 % of that on exposed plants, and that wind was not a significant factor in pollen distribution. McCutcheon (1975) found that bagged flowers of 'Woolama' produced only 57 % of the number of drupelets of unbagged flowers. The weight, appearance and fruit set of the bagged flowers were also lower. Thus it is well documented that bee visitation can help to increase drupelet set. Few workers, however, have looked at the timing of this visitation or the frequency necessary to produce a good set of drupelets.

Factors in Pollination

Few studies exist on the relative importance of different factors in pollination of red raspberry. Early research by Wellington (1913) and Hardy (1931a,b) showed that the anthers begin to dehisce as soon as an enlarging bud splits and the petals begin to show. The first evidence of an insect contribution to set came from Johnston (1929), who found that fruit set and drupelet number decreased dramatically when pollinating insects were excluded. The contribution of insects seems to vary with the cultivar. Couston

(1963) found that the mean seed number in 'Malling Jewel' fruits excluded from bees by bagging was only 36 % of that in fruits resulting from open-pollinated flowers. Shanks (1969) found that drupelet set on caged 'Sumner' plants was only 59 % of that on exposed plants, and that a simulated wind did not increase drupelet set. McCutcheon (1976) compared drupelet set from flowers of 'Woolama' that were exposed to bees, covered with a plastic bag, or covered with cheesecloth. Flowers in plastic bags and cheesecloth produced berries with 57 and 66 % of the number of drupelets of open-pollinated flowers, respectively. This suggests that wind may also contribute to pollination.

Most commercially-grown cultivars of red raspberry are self-fertile (McGregor, 1976). Actual transfer of pollen between flowers may not be necessary if there is sufficient pollen within a flower to pollinate all or most of its pistils. Transfer of pollen between flowers and within a flower have been termed allogamy and autogamy, respectively (Percival, 1979). Daubeny (1971) found that flowers pollinated only with their own pollen or that from adjacent flowers set nearly as well (58 - 77 %) as open-pollinated flowers. Redalen (1976) and Daubeny (1971) have shown that cross-pollination with another cultivar will increase drupelet set in some cultivars.

Receptivity and the Effective Pollination Period

The effective pollination period (EPP) has been defined as the longevity of the ovules minus the time required for pollen-tube growth (Williams, 1966). The set of fruit after delayed pollination

is a good indication of the success of fertilization, and thus provides a suitable estimate of the EPP. EPP's obtained in this manner include 2 - 9 days for apple (Williams, 1966), 1 - 9 days for pear (Lombard et al., 1971), 4 - 7 days for cherry and 7 - 12 days for peaches (Toyama, 1980). No specific studies have been done to determine the EPP of raspberry or other cane fruits. Several workers have made observations relating to stigma receptivity, however, based on hand pollination of emasculated flowers.

Hardy (1931a) found that optimum hand pollination occurred two to five days after emasculation, when the stigmatic surfaces had become split into small "forks". Eaton et al. (1968) studied the receptivity period of 'Willamette' using hand pollination with 'Newburgh' pollen. Buds of various sizes were emasculated and then hand pollinated at various intervals from 0 - 4 days later. Optimum fruit and drupelet set of the largest buds occurred when they were pollinated once daily for four consecutive days after emasculation. However, drupelet number was not significantly lower when pollen was applied only on the second day. This suggests that optimum pollination for breeding experiments can be obtained by emasculating fully enlarged buds, then pollinating them two days later. In a similar experiment of longer duration, Redalen (1976) found that open pollination of emasculated 'Norna' flowers after isolation for three to six days decreased drupelet set by 36 and 58 %, respectively. Drupelet set was also higher in a year when the flowers were pollinated two days after emasculation than in a year when they were pollinated after three.

Temperature Effects on Flower Development and Fruit Set

Several studies exist relating to temperature effects on floral initiation, bud development, and time of flowering in red raspberry (e.g. Lockshin, 1981; Vasilakakis et al., 1979, 1980). Hill (1958) followed the weight, diameter and chemical changes of raspberry fruits at two day intervals from full bloom to maturity. Effects of temperature on development of the flowers and fruit, however, have not been documented.

Otterbacher et al. (1983) showed that high temperatures will result in rapid loss of viability of raspberry pollen. High temperatures have also been shown to shorten the period of stigma receptivity in pear (Lombard et al., 1972), and to hasten the rate of ovule degeneration in cherry (Eaton, 1959), though they may enhance the rate of pollen-tube growth in both apple (Child, 1966) and pear (Lombard et al., 1972). Temperatures below freezing, which can occur during flowering and fruit set of fruit crops in the Northwest, may result in freezing injury to flower tissues or young fruits of many species including cherry, apple and pear (Westwood, 1978). Less severe cold may also affect fruit set by reducing bee activity (Free, 1970) or decreasing pollen germination and the rate of pollen-tube growth so that the ovules degenerate before fertilization can occur (Lombard et al., 1983; Thompson and Liu, 1973). The optimum temperature for pollen-tube growth, fertilization and fruit set in tree fruits seems to be between 18 and 27° C (Free, 1970).

In addition to direct effects on fruit set and bee flight,

temperature may affect the attractiveness of flowers to bees by influencing pollen dehiscence, nectar secretion and petal retention (Percival, 1979). Nectar volume and concentration and the presence of petals are particularly important in bee attraction (Free, 1970).

Rates of pollen-tube growth have also been more extensively investigated in tree fruits than in caneberries. The time of growth of pollen tubes from the stigma to the ovary has been reported as 3 - 6 days for apple at 11 - 20° C (Child, 1966), 3 - 9 days for cherry at 7.5 - 15° C (Lombard et al., 1983) and 3 - 12 days for pear at 6 - 15° C (Lombard et al., 1972). Engelhardt and Stosser (1979) found that pollen tubes reached the base of the style of blackberry flowers in about 18 hours and the ovule in about 30 hours at field temperatures of 11 - 24° C. No data could be located on pollen tube growth rates in raspberry.

The periods of anther dehiscence, nectar secretion and petal retention in red raspberry have been studied to some degree, but this has not been related to temperature. Wellington (1913) and Hardy (1931) found that dehiscence began almost immediately after the flowers had opened. McGregor (1976) and Free (1970) stated that anther dehiscence in red raspberry occurred for 2 - 9 days, but gave no reference to temperature effects. Nectar secretion in raspberry ranges from 3.8 to 14 mg per day (Sazykin, 1952; Simidchiev, 1976) and a total production of 13 - 27 mg during the life of a flower (Petkov, 1963; Szklanowska, 1972). Again, these figures have not been related to temperature and no mention was given as to over how many days the nectar was secreted. McGregor (1976) stated that

raspberry petals are shed "a day or so after the flower opens", but no mention was made as to temperature.

I. BEE VISITATION AND ITS RELATION TO DRUPELET SET IN RED RASPBERRY

ABSTRACT

Exposure of 'Willamette' red raspberry flowers to bees for one hour on one day or 'Meeker' flowers for two hours on each of two days resulted in nearly as high a drupelet set as exposure for eight hours on each of three or four days. Data from time-lapse cinematography showed that this represented 27 and 136 bee visits per flower, respectively. Drupelet set in 'Willamette' could be attributed 62.5 % to self-pollination (within an individual flower), 22 % to bees, and 15.5 % to wind and/or small insects.

The frequency of flower visitation by honey bees was significantly correlated with nectar secretion, and also associated with the periods of petal retention, anther dehiscence and the condition of pistils and stamens. The duration of flower visits was nearly the same at all stages of flower opening, averaging 8.4 to 12.8 seconds, except for the first visit in the morning which averaged 80.8 seconds in length.

It was estimated that two colonies of bees would be required per ha for 'Willamette', and five colonies for 'Meeker', to thoroughly pollinate all of the flowers present. The higher number for 'Meeker' reflects the higher flower number, lower percentage of self-pollination, and higher bee visitation requirement in this variety.

Observations of bee behavior were made, and curves constructed of bee activity over a foraging day. Light intensity and

temperature were most important in regulating this activity. Surveys of flower-visiting bees showed that 18 to 58 % had pollen in their corbiculae, but none were observed deliberately collecting it. It was concluded that raspberry pollen is not a primary attractant to foraging honey bees.

Based on the results of this study, growers are advised to provide at least 2 strong colonies of bees per ha and retain them until the completion of bloom.

INTRODUCTION

The American red raspberry, Rubus idaeus L., produces flowers in the spring on laterals formed in the leaf axils of the previous year's canes. The red raspberry flower contains ca. 70 - 100 pistils surrounded by a similar number of stamens. Each pistil, if pollinated, will produce a drupelet containing a seed. If enough drupelets develop they will hold together, with the aid of tiny hairs on each drupelet, to form a berry. Commercially-grown red raspberry cultivars are largely self-fertile, although cross-pollination will increase drupelet set in some cultivars (Daubeny, 1971; Redalen, 1976). As only the outermost stigmas are likely to come into contact with the anthers, an external pollinating agent is needed to increase drupelet set (Free, 1970). With no additional pollination a berry will frequently develop, although it will be relatively small and may have a tuft of unpollinated pistils at its center (Shanks, 1969). Insect activity helps to spread pollen over the pistils and to increase the set of drupelets. The extent of this increase seems to vary with the cultivar. Of the insect visitors to red raspberry, honey bees (Apis mellifera L.) are the most conspicuous and probably the most important (McGregor, 1976). Bees are strongly attracted to raspberry flowers, as their nectar is high in both volume and sugar concentration (Free, 1970).

Several workers have compared the set of enclosed flowers (bees excluded) with that of flowers exposed to bees. Couston (1963) found that the mean seed number in 'Malling Jewel' fruits, isolated from bees by bagging, was only 36 % of that in fruits resulting from

open-pollinated flowers. Shanks (1969) found that drupelet set on caged 'Sumner' plants was only 59 % of that on exposed plants, and that wind was not a significant factor in pollen distribution. McCutcheon (1976) found that bagged flowers of 'Woolama' produced only 57 % of the number of drupelets of unbagged flowers. The weight, appearance and fruit set of the bagged flowers were also lower. Thus it is well documented that bee visitation can help to increase drupelet set. Few workers, however, have looked at the timing of this visitation or the frequency necessary to maximize drupelet set.

Eaton et al. (1968) studied the receptivity period of 'Willamette' using hand pollination with 'Newburgh' pollen. Buds of various sizes were emasculated and then hand pollinated at various intervals from 0 - 4 days later. Optimum fruit and drupelet set of the largest buds occurred when they were pollinated once daily for four consecutive days after emasculation. However, drupelet number was not significantly lower when pollen was applied only on the second day. This suggests that optimum pollination for breeding experiments can be obtained by emasculating fully enlarged buds, then pollinating them two days later. In a similar experiment of longer duration, Redalen (1976) found that open pollination of emasculated 'Norna' flowers, after isolation for three or six days, decreased drupelet set by 36 and 58 %, respectively.

One has to be careful when extrapolating results from hand pollination experiments to honey bee pollination in the field. Many bee visits may be required to bring in the same amount of pollen

that was applied by hand, and bees may frequently be carrying pollen from the same cultivar rather than a different one as in Eaton's study, as raspberries are usually planted in solid blocks. Furthermore, the use of emasculated flowers cannot be considered representative of typical bee visitation as they are considerably less attractive to bees and the absence of pollen does not allow for normal self-pollination within the flowers.

In the study reported here, unemasculated flowers were exposed to bees at various intervals after anthesis, and the effect on drupelet number of the resulting fruit was determined. This gave an indication of both the timing and frequency of bee visitation needed to pollinate the flowers. Drupelet number, rather than set, was considered the most likely indication of the level of pollination since set can occur without bee visitation. Bloom phenology data were taken and combined with the bee visitation data in order to estimate the number of bees required per hectare for optimum pollination. Finally, additional observations were made on the length of time flowers remain attractive to bees, frequency of bee visits to flowers over a foraging day, and the behavior of individual bees while visiting flowers.

MATERIALS AND METHODS

The studies reported here were conducted in Corvallis, Oregon, from 1980-83, utilizing the Oregon State University Botany and Lewis-Brown Horticulture research farms. Studies on the 'Willamette' variety were conducted at the Botany farm in 1981 and 1982 on plants which were 3 years of age in 1981. Studies on 'Willamette' in 1980 and 1983 and work with 'Meeker' and other varieties was conducted at the Lewis-Brown farm in a mixed planting approximately 6-8 years of age. Plants were trained on a horizontal, double-wire trellis and thinned to ca. five strong canes per plant each winter. At least one strong colony of bees was provided in each of the study areas each year, and additional colonies were present in the vicinity. These studies can be divided into three parts: 1) Frequency and timing of bee visitation required for optimum drupelet development, 2) Behavioral observations of bees, and 3) Number of bees required. Each of these areas will be discussed separately.

I. Frequency and Timing of Bee Visitation

Bee visitation was controlled by covering raspberry buds with size 1 or 2 paper bags, held in place with metal clips so that they could be easily removed and replaced with minimum disturbance to the flowers. Individual flowers were selected when in the swollen bud stage (a day before normal opening), marked with small paper tags, and covered with the bags. To minimize variation only primary flowers, the largest and first ones opening in a flower cluster,

were used. Although they may have contributed to pollination, neighboring flowers were not removed as it was felt that this would not represent the natural level of competition for food reserves. Thus an entire cluster of flowers (usually 5-7) was actually present in each bag, with the study flower tagged for identification. The bags were removed for a pre-determined number of bee visits or time interval to expose the flowers to bees, then replaced and left attached until the fruit had formed. This bagging process is commonly used by plant breeders and does not appear to affect development of the fruit (Lawrence, pers. comm.).

Berries were frozen immediately after picking to prevent rot. Drupelet number was then determined either by a direct count of drupelets or by counting the seeds after extraction in a blender.

A time-lapse movie camera was used to determine the approximate number of bee visits occurring during each time interval. The camera was aimed at a group of four to five flowers on several sunny days during the bloom period in 1980 - 1982. Data collected from an exposure once every 5 seconds agreed closely with the results of actual counts in the field.

Preliminary studies were conducted in 1980 on 'Willamette' and 'Meeker' by removing the bags from 10 flower clusters for 1 - 10 bee visits after the marked flower had been open for one day, then replacing them. A more detailed experiment was set up in 1980-82, using 16 different treatments consisting of various combinations of hours (1, 2, 4, 8) and/or days (1, 2, 3, 4) in which the flowers were exposed to bees. The treatments were randomly allocated to a

row of plants such that there were four flowers sampled for each treatment, each on a different plant. Any given plant had up to three treatments. Three different checks were used: no bag (open pollination), bagged with the not removed until fruit set (self pollination) and screen "bag" with 3 mm x 3 mm openings (pollination by wind and/or small insects). As in the previous experiment, the blossoms were bagged the day before opening, when in a swollen bud stage. The 1, 2, and 4 hour treatments were run between 1300 and 1700 hours (PDT) each day, when bee activity was the greatest. A preliminary run of this experiment (with no replication) was conducted on 'Meeker' in 1980. In 1981 and 1982, the entire experiment was replicated three times at intervals of two days during the peak bloom period (late May - early June). The sky was clear or partly cloudy, with only a trace of rain and only a few short periods unsuitable for bee foraging during these two pollination periods, with lows of 5 - 15 and highs of 15 - 30° C. This study was conducted with 'Willamette' in 1981, and both 'Willamette' and 'Meeker' in 1982.

II. Behavioral Observations

Throughout the study period, behavioral notes were made of the bees' activities on the flowers. The portion of each flower visit that had potential for pollination, defined as contact with the stigmas and anthers, was noted as well as the duration of visits to flowers at different stages of bloom. Individual flowers that had been covered with bags since the bud stage were observed for 15-minute periods to note the frequency of bee visitation. Time-lapse

cinematography was used to supplement these observations as well as to develop curves of bee visitation over a foraging day. These data were compared with weather data collected at a nearby site (Rao et al., 1981, 1982, 1983) in order to relate bee activity to light intensity and temperature. Light intensity data reported in this paper were measured as direct normal (total) irradiation with an Eppley Normal Incidence Pyrheliometer.

Nectar secretion data, measured by capillary action with 10 μ l micro-pipettes were compared with data on flower condition (appearance of pistils, stamens and petals) and correlated with attractiveness to bees, based on the frequency and duration of flower visitation. Finally, individual bees were examined while foraging to see whether they were actively collecting pollen in their corbiculae.

III. Number of Bees Required

Many authors have attempted to estimate the number of bees required to pollinate a crop (e.g. Free, 1970; McGregor, 1976), and many assumptions are always required in such a calculation. For this reason these numbers cannot be considered precise, but because they can provide at least a rough guideline for growers these calculations can be considered a valuable exercise.

In this study, a section of cane containing 10 strong laterals near the base of the cane was marked off with flagging tape and data were collected on the number of new flowers opening each day in this section. Ten canes of 'Willamette' were marked in this way in 1981,

and five canes each of 'Willamette' and 'Meeker' in 1982. The total number of flowers open was also recorded for each section in 1981. These phenology data were combined with bee visitation and receptivity data from other workers and data on the length of time individual raspberry flowers must be visited by bees (from Part I) to calculate the number of bees required per ha.

RESULTS AND DISCUSSION

Frequency and Timing of Bee Visitation

In preliminary (unreplicated) trials in 1980 on 'Willamette' and 'Meeker' exposed to a specific number of bee visits, it appeared that as few as two to four bee visits to 'Willamette' or 'Meeker' were as effective as ten visits, and nearly as effective as open pollination (Table 1). While this at first seems remarkable, it can be explained by examining the data on duration of flower visits in Table 11. The first visit to 'Meeker' flowers, uncovered on the first, second or third day open, averaged 3 - 12 times longer than successive visits. This would be comparable to the first visit made to a flower in the morning, after nectar had accumulated during the night. After alighting a bee would circle the flower methodically, probing the nectaries frequently and contacting both the stigmas and anthers in the process. On later visits it was more common for a bee to simply probe in one place on the flower, then fly off. This probably resulted in considerably less pollination.

In the unreplicated trial with 'Meeker' flowers uncovered for hourly intervals, one to two hours of bee visitation appeared as effective as eight hours and nearly as effective as open pollination (Table 2). Some benefit also appeared to be derived from exposing the flowers for two days rather than one day. This is reasonable, based on the observations of previous workers that the pistils are not all receptive at the same time (McGregor, 1976; McCutcheon, 1976). No obvious benefit of additional exposure beyond two days' duration was seen.

In the replicated trials in 1981 and 1982, similar results were obtained and verified. 'Meeker' flowers exposed to bees for two days produced a significantly higher number of drupelets than those exposed for only one day, and as many drupelets as open pollination (Table 3). Four hours of visitation per day produced significantly more drupelets than one hour but not two, and as many drupelets as open pollination. This suggests that two hours per day of bee visitation over two days may be required for the 'Meeker' cultivar.

In 'Willamette', one hour of bee visitation was as effective as eight hours, and one day of visitation as effective as two, three or four days, in both 1981 and 1982 (Tables 4 - 5). Thus one hour of bee visitation over one day may be sufficient for good drupelet set in this variety.

Screened blossoms of 'Willamette', which were exposed only to wind and small insects, produced significantly fewer drupelets than flowers exposed to bees, and significantly more drupelets than bagged blossoms (Tables 4-5). Since very few insects other than bees were seen, this suggests that wind may have had a substantial effect. This result is in contrast to that of Shanks (1969), who was unable to show a contribution of wind to pollination in his work with 'Sumner'. McCutcheon (1976), however, obtained a higher set in cheesecloth-bagged flowers of 'Woolama' than in plastic-bagged flowers and did not rule out the possibility of a wind contribution to set. Wind may be more important for spreading pollen within a flower or for moving the pistils and stamens into contact with one another rather than for carrying pollen over long distances, as we

will see later in this paper. A "shaking" action of wind is also possible, though this has not resulted in an increase in set in previous studies (Daubeny, 1969).

The bagged treatments of 'Willamette' showed remarkably high drupelet set, with an average of 59.3 drupelets in 1981 and 51.2 drupelets in 1982. If we consider the number of drupelets obtained in open pollination to be the maximum possible set, this represents a mean of 62.5 % set for the two years (Fig. 1). This is considerably higher than the 38 % reported by Couston (1963) for isolated flowers of 'Malling Jewel', but approximately equal to the values of 59 % reported by Shanks (1969) and 57 % reported by McCutcheon (1976) for isolated flowers of 'Sumner' and 'Woolama'. This suggests that 'Willamette', like 'Sumner' and 'Woolama', is capable of a high degree of self-pollination. Daubeny (1971) found that 'Willamette' and 'Meeker' flowers pollinated only with their own pollen or that from adjacent flowers set nearly as well (58 - 77 %) as open-pollinated flowers. This suggests that there is sufficient pollen on an individual flower to pollinate all or most of its pistils. All that is required, then, is for insect activity and/or wind to spread the pollen onto the stigmas. Natural contact between the pistils and stamens as the flower opens undoubtedly results in some pollination, as the outermost stigmas may come into contact with the innermost anthers (Free, 1970). There is also likely some pollen spread by wind which passes over the flowers.

In 'Meeker', drupelet set in all of the treatments visited by bees for two hours or more was significantly greater than in the

screen controls (Table 3). Set in the screened controls did not differ significantly from the bagged controls, however. This seems to be due to a higher relative contribution of bees to set than in 'Willamette'. It can be seen from Tables 3 - 5 that the presence of bees increased set by 48 % in 'Meeker' but only 22 % in 'Willamette'. The fact that the contribution of wind and small insects was lower for this variety than in 'Willamette' points to the sporadic nature of these factors and suggests that they cannot be relied upon for consistent pollination.

Self-pollination in 'Meeker' was less than in 'Willamette' but still quite substantial. 43 % drupelet set (compared to open pollination) occurred in the bags, as compared to a mean of 62.5 % in 'Willamette' (Tables 3 - 5). This is close to the 36 % reported by Couston (1963) for 'Malling Jewel', and suggests that these two varieties are less capable of self-pollination, and more dependent on bees for pollination than 'Willamette'. As in 'Willamette', autogamy is nearly as effective as allogamy (Daubeny, 1971). Thus, like 'Willamette', each 'Meeker' flower probably contains sufficient pollen to pollinate all or most of its stigmas.

Hand-pollination experiments conducted by Eaton et al. (1968) and Redalen (1976) showed that 'Willamette' flowers are receptive for at least three days under field conditions. Hand pollination for only one day at the peak of the receptivity period, however, was as effective as pollination for more than one day (Eaton et al., 1968). Thus the requirement of only one or two days of bee visitation seems reasonable. Eaton et al. (1968) concluded that the time of

application of pollen was more important than the number of pollinations or the amount of pollen applied. The results of this study strongly support that conclusion. Considering that the stigmas do not all become receptive at the same time, however, there must be a mechanism for spreading out the effect of bee visitation for several days. It seems likely that either the first stigmas to become receptive remain so for several days while the others become receptive, or that pollen deposited on the flowers (or present within a flower) the first day that they are open remains viable until the stigmas they are in contact with become receptive, or both. Pollen collected from 'Fallred' remains viable up to one week at 23° C (Otterbacher et al., 1983).

Behavioral Observations

When a honey bee landed on a raspberry flower which had not yet been visited, she first sat stationary on it and inserted her proboscis at one side into the nectaries. After 30-60 seconds or so, she began to circle the flower rapidly, following the ring of nectar-secreting tissue with her tongue. This latter behavior probably resulted in the most pollination, as both the anthers and pistils were contacted. Free (1968) also reported this circling activity, and noted that one side of a bee's body touches the stigmas in the process. On later visits, bees usually probed only a few times, then flew off.

Bee visitation was closely associated with each aspect of flower development including petal retention, nectar secretion,

anther dehiscence and the condition of pistils and stamens. These data were difficult to obtain, as air temperatures and therefore also the rate of flower development varied widely from day to day. Average daily high temperatures varied from 14 - 35° C, and mean daily temperatures from 9 to 24° C, in the study period from May 20 to June 15 in 1980-83.

Analysis of phenology plot data in 1981 (Fig. 2) showed that petal retention in the field on 'Willamette' varied from 1 to 5 days, with 76.3 % of the flowers retaining their petals for two days and 97.5 % for one to three days. The petals seem to be an important attractant to the bees, as most of the bee visitation occurred when they were all attached and visible. Small white tags used for labeling flowers were also frequently explored by bees. Visitation also occurred to flowers which had recently lost their petals, suggesting that bees can recognize flowers without petals, at least at short range. Although there was very little nectar at this stage, that which remained was most likely of a higher sugar concentration (Percival, 1979). Flowers which were only barely open were also visited, though no nectar was ever recovered at this stage. Wellington (1913) also noted that insects visited raspberry flowers at this stage.

The frequency of bee visitation on each day after flower opening was significantly correlated with nectar secretion in 'Meeker' ($r=.98$, $p=.05$). In both 'Willamette' and 'Meeker', over 95 % of the nectar secretion occurred within two days after the flowers opened, and only minor amounts were collected on the third

day (Tables 6 - 7). Similarly, bee visitation was most frequent on the first two days (Table 8). Simidchiev (1976) also noted a decrease in nectar exudation of raspberry flowers with increasing flower age.

It is unknown how long it takes for the flowers to replenish their supply of nectar, but frequent bee visitation, as high as 37 visits per hour, suggested that the process is very rapid (Table 9). The mean duration of bee visits to 'Willamette' was 11.1 seconds, and 9.6 seconds for 'Meeker' (Table 10). These are only slightly greater than the mean visitation times of 7.9 and 11.0 seconds recorded by Free (1968) for nectar- and pollen-collecting bees on raspberry, respectively.

The first bee visit to a flower in the morning was always the longest, regardless of the age of the flower (Table 11). This was probably due to a pooling of nectar during the night. Although nectar secretion is generally higher when air temperatures are high, it may continue for some time after temperatures drop (Percival, 1979). Flowers which had been bagged overnight were always glistening with nectar when the bags were removed the following morning. This supports the results of Simidchiev (1976) who noted that nectar secretion of raspberry was highest in the early morning and evening, and Szklanowska (1972) who found the heaviest secretion of raspberry nectar following cold nights. 'Meeker' flowers observed for five consecutive visits in 1980 lasted an average of 80.8 seconds on the first visit, but only an average of 10.8 seconds on successive visits (Table 11). Visits to 'Willamette' were of a

similar length, averaging 12.3 seconds on the first day the flowers were open (Table 10). Visitation time was remarkably consistent on consecutive visits, once the initial pool of nectar was exhausted (Table 11).

Amounts of nectar collected were highly variable between flowers, replicates and varieties. Some of this variation may be due to the open nectaries of raspberry, which are exposed to the air and are highly affected by the relative humidity of the surrounding air (Percival, 1979). Nectar was most easily removed on cool days, when it was presumably less concentrated.

No comparable data on nectar volume could be found in the literature, since previous workers have all measured nectar secretion in mg of nectar rather than μl . Sazykin (1952) found differences in nectar secretion of raspberries by variety, soil and climatic conditions, with an average of 4-6 mg of nectar per flower per day. Petkov (1963) and Szklanowska (1972) obtained mean total weights of 13 mg and 27 mg of nectar per raspberry flower, respectively, and Simidchiev (1976) found a mean secretion of 3.8 to 14 mg of nectar per day.

Anther dehiscence and condition of pistils and stamens were also associated with bee visitation. A detailed study of these parameters in 'Meeker' in 1983 (Bekey and Lawrence, 1985b) showed that anther dehiscence began in a few of the anthers of a flower almost immediately after the split of the calyx. At field temperatures dehiscence was heaviest on the first three days, and nearly ceased by the fourth day the flowers were open. Thus pollen

is available during the first two days, the time of maximum bee visitation of the flowers. The peak period of receptivity of raspberry flowers lasts from 2 - 3 days after anthesis (Bekey and Lawrence, 1985a), which corresponds closely with the periods of petal retention, nectar secretion and bee visitation.

Data from the time-lapse movie camera were used to construct curves of bee activity over a foraging day. These are plotted with corresponding data on light intensity and temperature in Figs. 4 - 6. Bee activity seemed to be most highly associated with air temperature, and secondarily with light intensity. Morning activity appeared to be limited by air temperature, which increased more slowly than light intensity. This was also observed by Lerer et al. (1982) for foraging alfalfa leafcutting bees (Megachile rotundata) on alfalfa (Medicago spp.) In contrast with the results of Lerer et al., however, activity in the evening also seemed to be related more closely to temperature than light intensity. Foraging was observed as late as 2000 hours (PDT), when light intensity was quite low, on warmer days. Free (1968) observed foraging honey bees on raspberries as late as 1700 hours, and Szabo (1980) recorded outgoing from hives as late as 2100 hours during a nectar flow. Evidently if temperatures remain high enough and there is little wind or overcast the bees will continue to forage until dark.

The major effect of light intensity in this study was seen as a reduction in bee activity whenever cloud cover appeared. Activity quickly increased soon after the sun reappeared from behind the clouds. No obvious effects of wind were seen in this study, though

at least a light breeze was present on most of the study days and the velocity reached as high as 4.1 m/sec on some afternoons. Wind has been shown to effect bee activity in previous studies, but it is difficult to set a threshold as factors such as wind direction, air temperature and light intensity interact (Szabo, 1980).

Three common patterns of weather and bee activity are summarized in Figs. 4 - 6. The first, as exemplified by 19 June 1981 (Fig. 4) was that of heavy morning overcast burning off to afternoon sunshine. On this particular day the cloudiness returned in the late afternoon, resulting in a smooth increase in activity in the morning and a leveling or decrease in activity in the afternoon. The increase in bee activity at 1800 is clearly associated with the brief period of higher light intensity at this time.

The second major pattern was that on clear days, shown on 18 June 1980 (Fig. 5). The general pattern was for rapidly increasing activity until early afternoon, then a gradual decline into the evening. This clearly follows the increase and decrease in both light intensity and temperature.

The third pattern was that on cool days, exemplified by 26 May 1982 (Fig. 6). On these days activity seemed to be related much more to temperature than to light intensity, and bee activity was very low. It can be seen from this figure that periods of partial clearing at 1300 and 1500 hours did not result in an increase in bee activity at these temperatures.

These data compare favorably with those of other workers. Free (1968) showed increasing bee activity on raspberries during the

entire period from 1000 - 1700 hours, and Szabo (1980) showed that outgoing flights from colonies occurred from 0900 to 2100, with a maximum between 1400 and 1600.

A survey of bees on five different occasions, conducted by walking down the rows and examining 50 bees, showed that between 18 and 58 % had pollen in their corbiculae. Similar observations were made by Free (1968), who noted that between 21 and 66 % of foraging bees were carrying pollen. Discarding of pollen, which was described by Free, was also observed. No deliberate pollen-collecting activity was seen, though the bees nearly always brushed against the anthers in the process of flower visitation. Similarly, Free (1968) noted that only two bees out of 387 observed were deliberately scrabbling for pollen, and then only on two or three flower visits. Apparently raspberry pollen is not very attractive to bees, despite the fact that it is produced throughout the day (Percival, 1979) and that it is available during most of the period from calyx split to petal fall (Bekey and Lawrence, 1985b).

Number of Bees Required - Hypothetical Model

In the replicated trials of the bee visitation study described earlier, it was found that one hour of bee visitation over one day on 'Willamette' and two hours of bee visitation over two days on 'Meeker' were sufficient for good drupelet set. Based on the time-lapse data, these represented approximately 27 visits on 'Willamette' and 136 visits on 'Meeker' (Table 9). The phenology data showed that an average of 4.6 flowers opened per day during the 2 weeks of most intense bloom in 'Willamette', and 5.1 flowers per

day in 'Meeker', on a 10-lateral distance on the cane (Fig. 3). Orkney and Martin (1980) showed that 'Willamette' produces about 15 laterals per cane, and Waister and Barritt (1980) reported a mean of 17 laterals per cane in 'Meeker'. If we assume a typical planting density of 3663 plants/ha (0.9 m X 3.0 m spacing) and 10 strong canes/plant (Scheer et al., 1982), using 5 flowers opening/10 laterals/day we obtain a figure of 274,725 flowers opening/day/ha in 'Willamette', and 311,355 flowers/day/ha in 'Meeker'.

In 'Meeker', the flowers must be visited for two 2-hour days in order to produce a well-formed berry. On any given day, therefore, bees must visit 311,355 new flowers, plus 311,355 day-old flowers, so we need enough bees to pollinate 622,710 flowers each day. Free (1968) has shown that honey bees visit an average of 49 flowers on a foraging trip on red raspberry. No data could be found on the number of foraging trips a bee makes per day on red raspberry; however on apple, honey bees make about seven foraging trips on a favorable day (Karmo and Vickery, 1954). If we assume this is similar for raspberry then an individual bee can visit 343 flowers in one day. A colony of 40,000 bees, which would be typical for the western Oregon area at the time of raspberry bloom, should produce about 60 % foragers, or approximately 24,000 bees. If each of these foragers visits 343 flowers/day and each flower is visited 68 times, this colony could pollinate 121,059 flowers/day. Thus in order to thoroughly pollinate a hectare of 'Meeker' flowers we would need approximately 5 colonies of bees/ha/day.

Although 'Willamette' required bee visitation on only one day,

because the flowers are attractive for two days, half of the bees will be visiting flowers already pollinated. Thus it is necessary to have enough bees to pollinate 549,450 flowers/ha/day. If a colony provides 24,000 foragers, each forager visits 343 flowers/day, and each flower is visited 27 times, it can pollinate 304,889 flowers/day. This means that approximately 2 colonies of bees/ha/day would be required to thoroughly pollinate a hectare of 'Willamette' flowers.

The figures of 2 - 5 colonies of bees/ha seem reasonable, since recommendations for most crops that require bees for pollination fall into the range of 2.5 - 5 colonies/ha (Free, 1970; McGregor, 1976). The higher figure for 'Meeker' seems reasonable considering the higher flower number, lower percentage of self-pollination, and higher bee visitation requirement of this variety. The fact that 'Meeker' may out-yield 'Willamette' by 59 %, and produces up to 75 % more fruit per lateral (Daubeny, 1978) also emphasizes the importance of additional bees.

It must be emphasized that these numbers are only estimates, based on a series of assumptions which may need further research. Factors such as the distribution of varieties, age and size of plants, weather, and competition from other floral sources could significantly alter these figures (Gary et al., 1976). The presence of native wild bees and/or other pollinators in the vicinity would also be a significant factor. It is also important to recognize that not all colonies of honey bees are sufficiently strong to provide a good pollinating unit. A colony used for pollination

of fruit crops should consist of at least 2 hive bodies, with at least 6 combs well filled with brood and 10 combs covered with adult bees (Burgett et al., 1984).

The bee density supplied in this study, which was used as a basis for calculation, may have been well above the required level considering the high number of visits each flower received (up to 48 per hour). But considering the volatile nature of the weather at the time of raspberry bloom and the reluctance of bees to fly very far from their hives when it is cool or overcast, the provision of more bees than necessary should be considered good pollination management. It should also be pointed out that the larger the field, the more difficult it will be to provide good pollination toward its center. Murrell and McCutcheon (1977) have studied this situation in raspberries and made recommendations for colony placement. Similarly, Smith et al. (1973) showed a decrease in fruit and drupelet set of raspberry with increasing distance from the hives.

Since raspberry flowers can retain their petals for as long as five days, and they will continue to be visited by bees for that period, growers are advised to leave the bees in the field until five days after the last flowers one expects to produce fruit are open.

CONCLUSION

One or two hours' flower visitation over one or two days does not seem like a difficult requirement to meet until one considers that the conditions limiting bee flight, including rainfall and low air temperature, occur frequently during the bloom period in the major raspberry production areas of western Oregon, Washington and British Columbia. The fact that one or two hours of visitation per day is enough, however, means that even brief periods of sunshine or warmer temperatures may be sufficient for good pollination. Low temperatures, though restricting bee flight, also prolong the life of the flowers and their receptivity period so that effective pollination can still occur (Bekey and Lawrence, 1985b). Growers are advised to provide at least 2 strong colonies of bees per ha in their raspberry fields and retain them until the completion of bloom.

Table 1. Drupelet set in red raspberry by no. of bee visits, 1980.

No. of Visits	Mean No. of Drupelets (S.E.) ¹	
	Willamette	Meeker
open	75.1 (3.6)	91.2 (3.3)
9-10	66.5 (4.9)	61.9 (9.6)
7-8	69.9 (6.5)	50.8 (7.4)
5-6	74.1 (5.2)	58.4 (8.4)
3-4	68.2 (4.7)	72.0 (7.0)
1-2	66.9 (4.9)	49.9 (9.6)
0	20.2 (2.4)	23.3 (3.9)

¹10 flowers per treatment.

Table 2. Drupelet set of 'Meeker' red raspberry by hours and days of bee visitation, 1980.

Exposure Period	Mean no. drupelets (S.E.) ¹
open	91.4 (1.8)
4 days	79.9 (4.4)
3 days	85.9 (6.0)
2 days	86.6 (3.3)
1 day	65.7 (2.1)
8 hours	79.7 (6.1)
4 hours	87.1 (8.1)
2 hours	77.9 (3.4)
1 hours	73.4 (4.3)
bagged	23.3 (3.9)

¹10 flowers per treatment.

Table 3. Drupelet set of 'Meeker' red raspberry by hours and days of bee visitation, 1982¹

Hours	Mean no. drupelets	Days	Mean no. drupelets
open	94.7 a	open	94.7 a
8	79.2 a	3	71.7 a
4	69.0 ab	2	71.4 a
2	58.2 bc	1	52.2 b
1	53.9 cd	screen	49.7 c
screen	49.7 d	bagged	41.2 c
bagged	41.2 d		

¹ 3 replications, 4 flowers per replication. Values in left column are pooled means of days 1, 2 and 3; values in right column are pooled means of 1, 2, 4 and 8 hours. Mean separation within columns by Duncan's Multiple Range Test, 5% level.

Table 4. Drupelet set of 'Willamette' red raspberry
by hours and days of bee visitation, 1981¹

Hours	Mean no. drupelets	Days	Mean no. drupelets
open	87.2 a	open	87.2 a
8	80.2 ab	4	79.4 ab
4	79.5 b	3	81.7 ab
2	83.3 b	2	82.7 b
1	79.4 b	1	78.5 b
screen	70.3 c	screen	70.3 c
bagged	59.3 d	bagged	59.3 d

¹ 3 replications, 4 flowers per replication. Values in left column are pooled means of days 1, 2 and 3; values in right column are pooled means of 1, 2, 4 and 8 hours. Mean separation within columns by Duncan's Multiple Range Test, 5% level.

Table 5. Drupelet set of 'Willamette' red raspberry
by hours and days of bee visitation, 1982¹

Hours	Mean no. drupelets	Days	Mean no. drupelets
open	89.3 a	open	89.3 a
8	85.6 a	3	84.6 a
4	82.6 a	2	85.7 a
2	85.0 a	1	83.7 a
1	85.3 a	screen	66.7 b
screen	66.7 b	bagged	51.2 c
bagged	51.2 c		

¹3 replications, 4 flowers per replication. Values in left column are pooled means of days 1, 2 and 3; values in right column are pooled means of 1, 2, 4 and 8 hours. Mean separation within columns by Duncan's Multiple Range Test, 5% level.

Table 6. Nectar secretion in 'Willamette' red raspberry, 1982-83.

	Nectar volume (μ l) per flower (S.E.) ¹			
	Day 1	Day 2	Day 3	Total
Field	9.9 (4.7)	7.7 (4.5)	0.3 (0.2)	17.9
Potted plants, greenhouse	12.0 (0.9)	7.4 (1.1)	0.0 (0.0)	19.3
Potted plants, lab (21° C)	12.2 (1.6)	8.7 (1.5)	0.8 (0.6)	21.6

¹3 replications, 4 flowers per replication.

Mean of 10 flowers.

Table 7. Nectar secretion in 'Meeker' red raspberry, 1982-83.

	Nectar volume (μ l) per flower (S.E.)			
	Day 1	Day 2	Day 3	Total
Field ¹	7.8 (4.1)	5.4 (4.4)	trace	13.2
Potted plants, greenhouse ²	12.8 (0.9)	9.8 (1.9)	1.7 (0.6)	22.4
Potted plants, lab ² (21° C)	6.2 (0.9)	6.0 (0.6)	0.1 (0.1)	12.3

¹ 3 replications, 4 flowers per replication.

² Mean of 10 flowers.

Table 8. Frequency of bee visitation on 'Meeker' red raspberry, based on 15-minute counts, 1980.

Flower Age (Days open)	Number of bee visits observed/15 min. (S.E.) ¹
1	6.8 (0.1)
2	7.8 (0.1)
3	2.8 (0.2)
1-3	5.8

¹5 flowers per treatment.

Table 9. Frequency of bee visitation compared with exposure times, based on time-lapse cinematography, 1980-82.

Variety	Year	N ¹	Flower Age ²	Number of bee visits (S.E.)			
				1 hour (1400-1500)	2 hours (1400-1600)	4 hours (1400-1800)	8 hours (1000-1800)
Meeker	1980	2	1,2	37.5 (1.9)	64.0 (2.9)	118.5 (4.1)	218.0 (3.7)
Meeker	1981	2	1,1	36.5 (0.9)	72.0 (0.0)	129.5 (1.8)	190.0 (0.0)
Willamette	1980	3	1,1,1	29.7 (0.9)	52.3 (1.3)	88.0 (1.5)	176.0 ³
Willamette	1982	1	1	20.0	49.0	83.0	139.0
Meeker	ALL	4	1-2	37.0	68.0	124.0	204.0
Willamette	ALL	4	1	27.3	51.5	86.8	166.8 ⁴

¹Number of flowers photographed.

²Days open.

³Extrapolated from 4-hour value.

⁴Mean includes extrapolated value.

Table 10. Duration of bee visits to open-pollinated flowers of 2 cultivars of red raspberry.

Variety	Year	N ¹	Mean visitation time in seconds (S.E.)			
			Day 1	Day 2	Day 3	Mean
Meeker	1980	8-32 ²	12.8 (0.1)	8.4 (0.2)	11.4 (0.1)	10.9
Meeker	1982	30	9.7 (0.1)	6.9 (0.1)	----	8.3
Willamette	1981	50	11.3 (0.1)	9.1 (0.1)	----	10.2
Willamette	1982	30	13.3 (0.1)	10.7 (0.1)	----	12.0
Meeker	ALL	38-62	11.3	7.7	11.4	9.6
Willamette	ALL	80	12.3	9.9	----	11.1

¹Number of flowers per treatment.

²N=32 for days 1 and 3; N=8 for day 2. First visit after bag was removed not included.

Table 11. Duration of consecutive bee visits to recently unbagged flowers of 'Meeker' red raspberry, 1980.

		Mean visitation time in seconds (S.E.)						
		Visit no.						
Days Open	N ¹	1	2	3	4	5	Mean (2-5)	
1	2	43.0 (0.7)	11.1 (0.4)	14.6 (0.5)	11.8 (0.3)	13.6 (0.4)	12.8	
2	8	106.0 (4.1)	6.5 (0.7)	7.5 (0.4)	14.0 (0.8)	5.5 (0.4)	8.4	
3	8	93.4 (0.9)	12.8 (0.6)	19.1 (0.6)	4.6 (0.2)	9.1 (0.3)	11.4	
Mean	18	80.8	10.1	13.7	10.1	9.4	10.8	

¹Number of flowers per treatment.

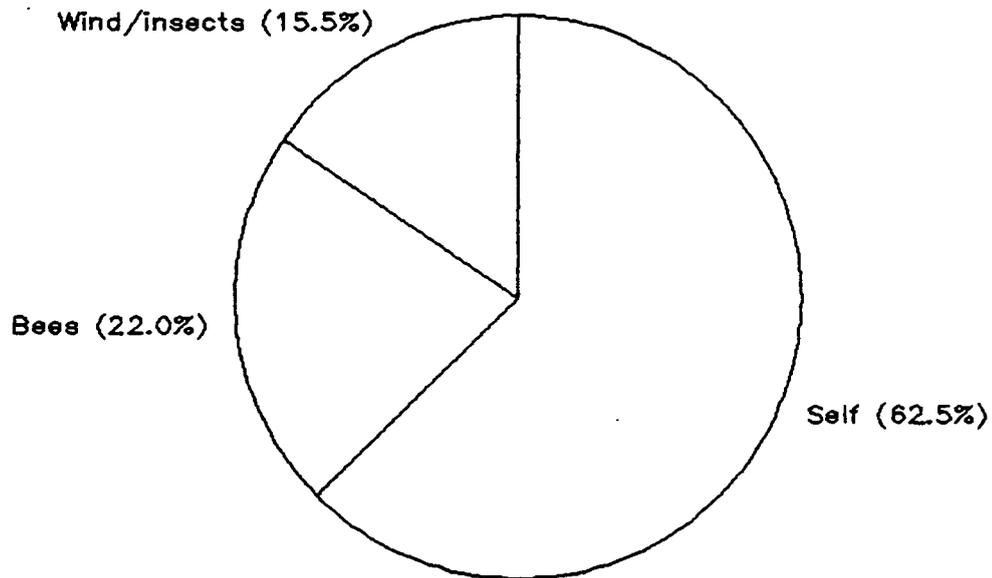


Figure 1. Factors affecting drupelet set in 'Willamette' red raspberry, 1981-82. Full circle represents set in open-pollinated flowers.

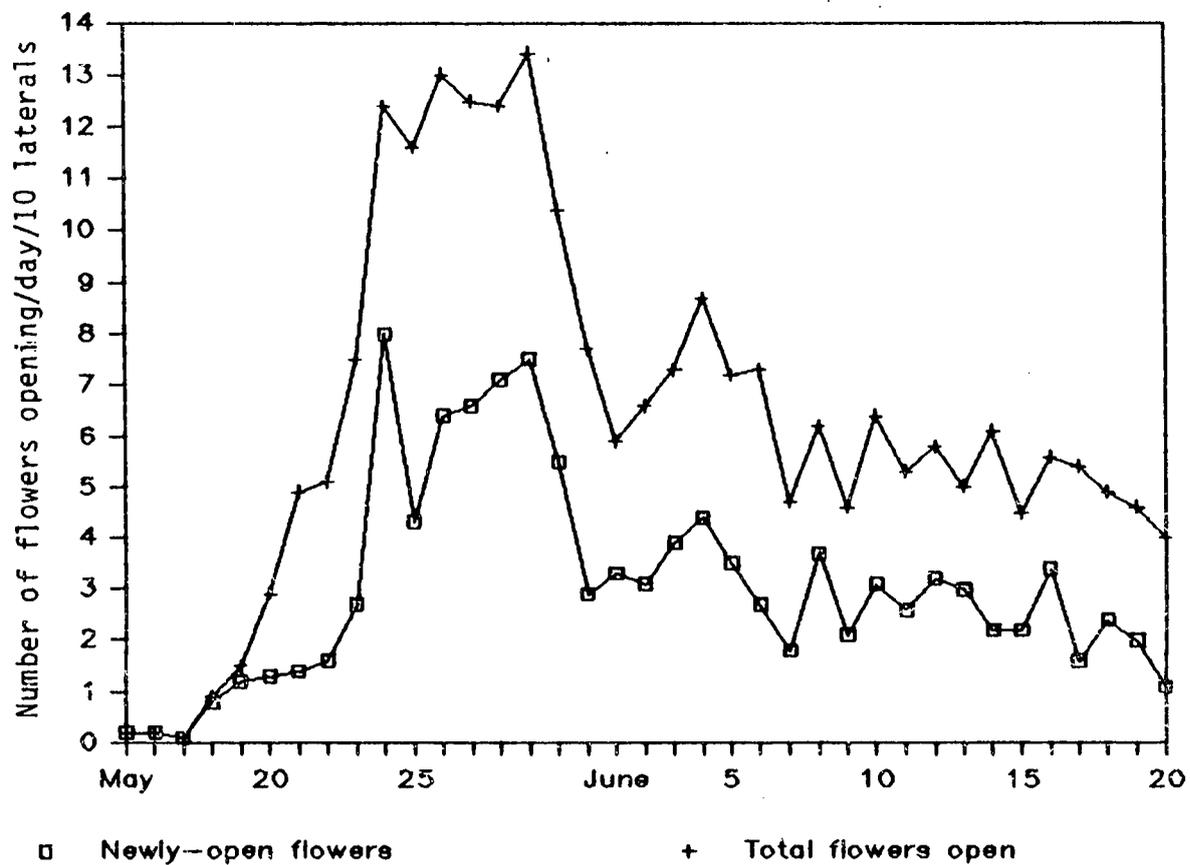


Figure 2. Bloom phenology in 'Willamette' red raspberry, 1981. Each point represents the mean number of flowers opening in a 10-lateral section on 10 canes.

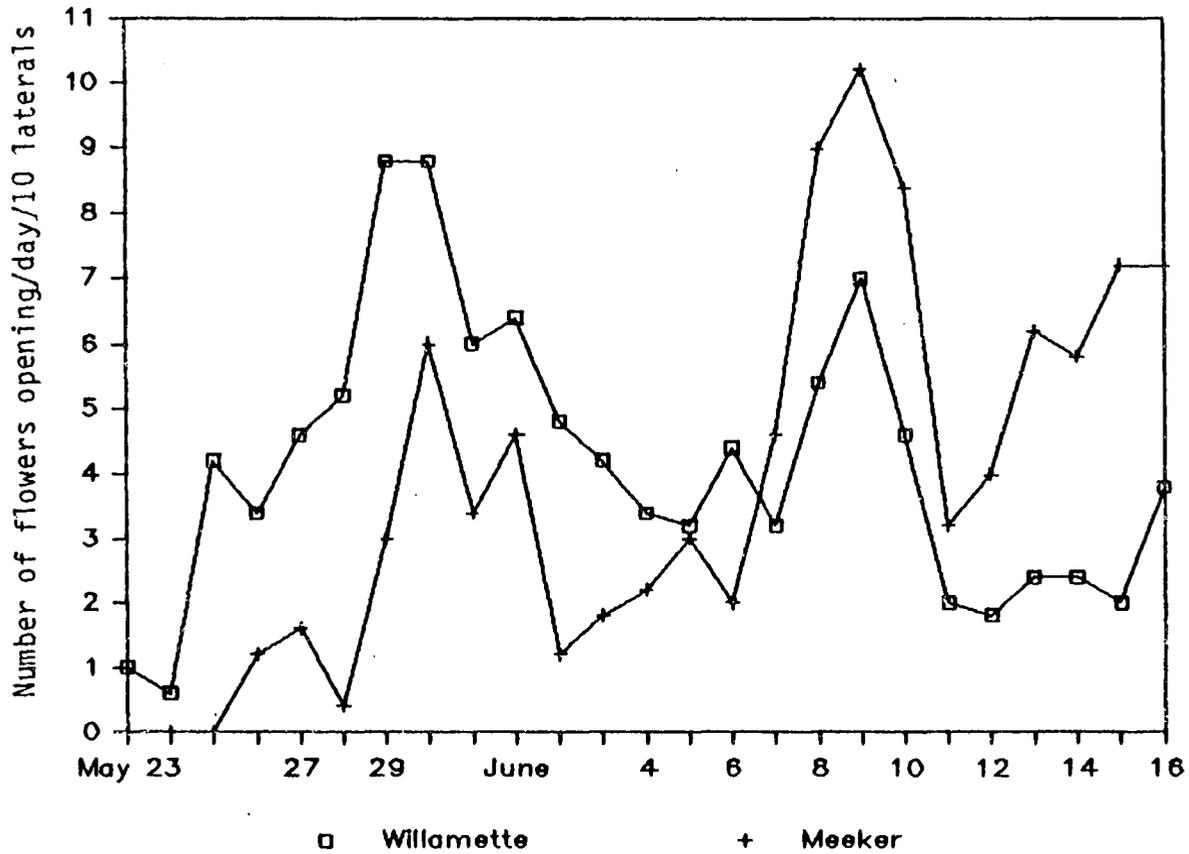


Figure 3. Bloom phenology in 'Willamette' and 'Meeker' red raspberry, 1982. Each point represents the mean number of flowers opening in a 10-lateral section on 5 canes.

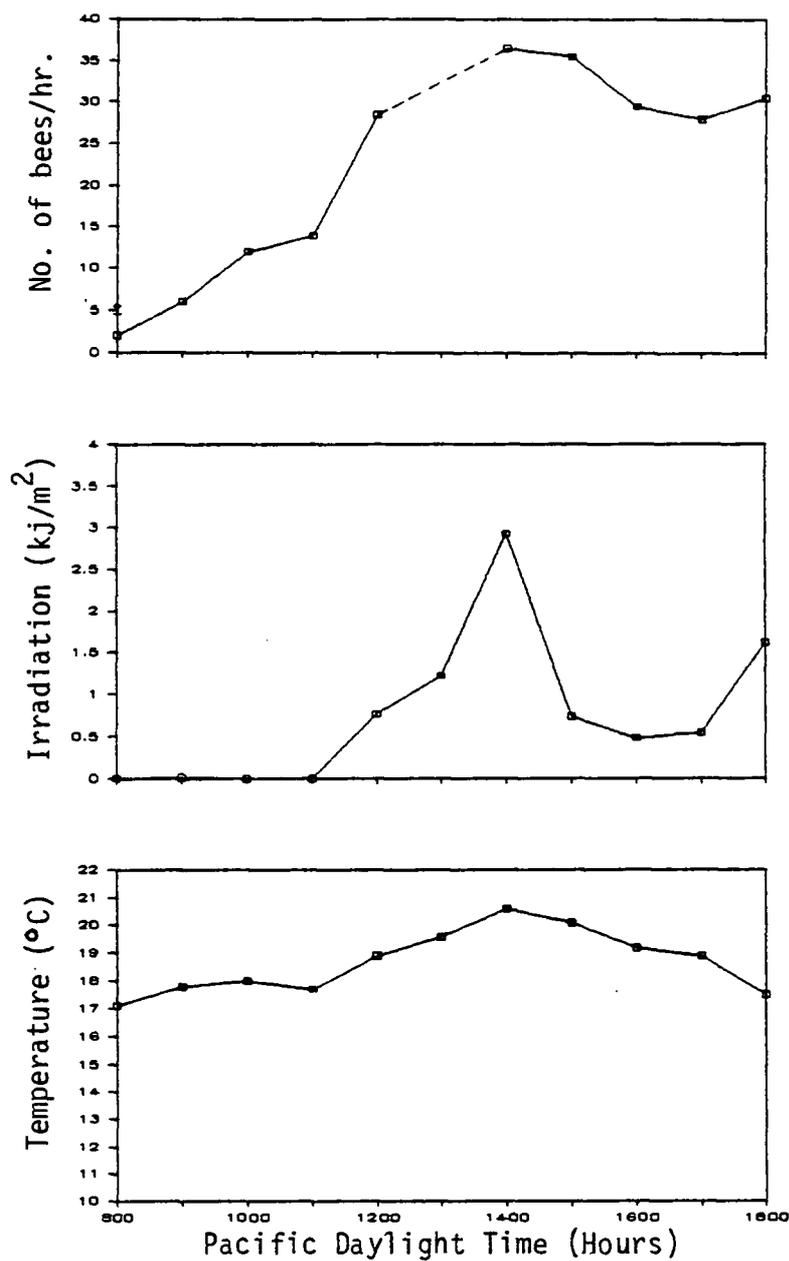


Fig. 4. Typical bee visitation and associated weather data on a day with morning overcast. Data for 'Meeker' red raspberry flowers, based on time-lapse cinematography, 19 June 1981. Each point is a mean of 2 flowers.

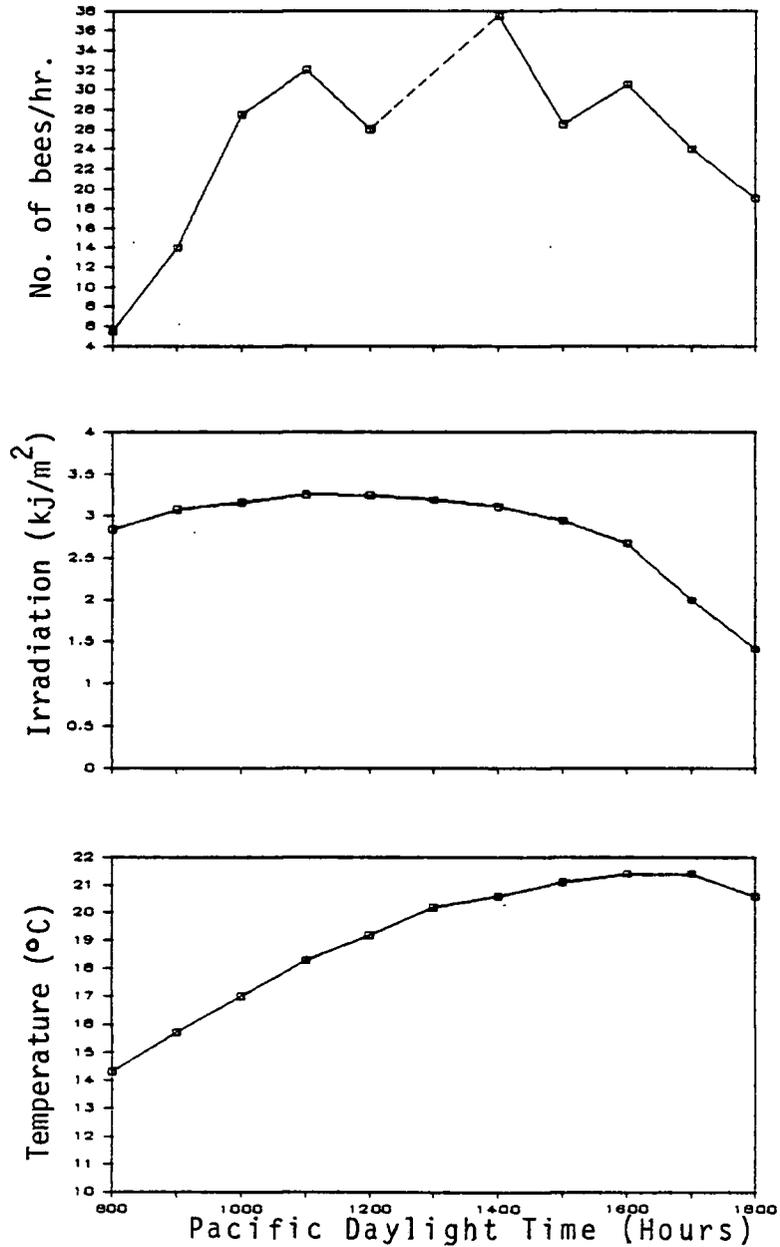


Fig. 5. Typical bee visitation and associated weather data on a clear day. Data for 'Meeker' red raspberry flowers, based on time-lapse cinematography, 18 June 1980. Each point is a mean of 2 flowers.

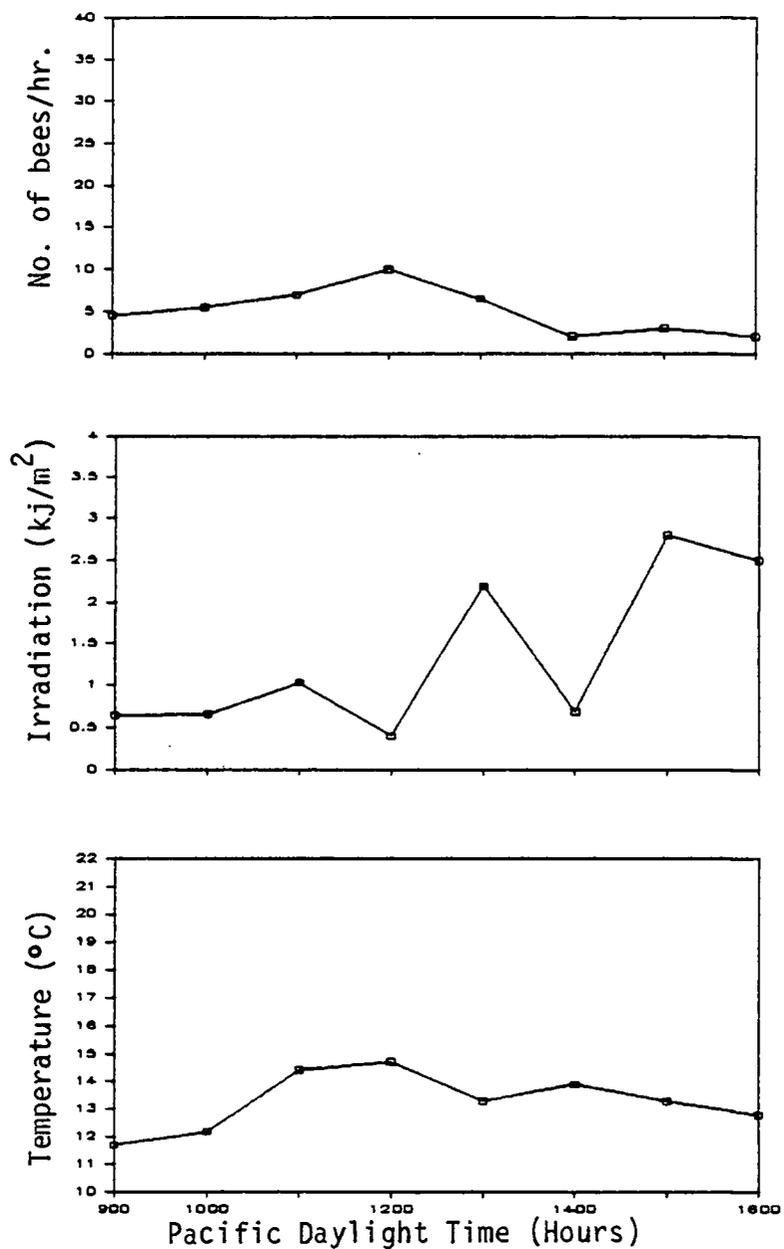


Fig. 6. Typical bee visitation and associated weather data on a cold, cloudy day. Data for 'Willamette' red raspberry flowers, based on time-lapse cinematography, 26 May 1982.

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II. EFFECTS OF METHOD AND TIMING OF POLLINATION ON DRUPELET NUMBER IN RED RASPBERRY

ABSTRACT

Five summer-fruiting ('Willamette', 'Meeker', 'Nootka', 'Skeena', 'Chilcotin') and three primocane-fruiting cultivars ('Fallred', 'Heritage', 'Scepter') of red raspberry were emasculated and hand-pollinated at daily intervals to determine their effective pollination period. The period of maximum receptivity lasted from 1 to 4 days after emasculation, depending on the variety, with a peak most often on the first or second day. Primocane-fruiting cultivars had a shorter receptivity period than summer-fruiting cultivars. All varieties except 'Willamette' and 'Scepter' set as well with one day of hand pollination at the peak of the receptivity period as with three days of consecutive pollination. This may have been related partly to a difference in flower morphology in 'Chilcotin'.

An additional study showed that autogamy was as effective as allogamy in 'Willamette' and 'Meeker'. This indicates that bees or wind are needed only to transfer pollen within flowers, and not between them. Drupelet number was 1.5 - 2.8 times higher in open-pollinated flowers exposed to bees than in flowers covered with a fine screen "bag", depending on the variety. This indicates the importance of providing adequate numbers of bees for pollination.

INTRODUCTION

Few studies exist on the relative importance of different factors in pollination of red raspberry (Rubus idaeus L.). Early research by Wellington (1913) and Hardy (1931a, 1931b) showed that the anthers begin to dehisce as soon as an enlarging bud splits and the petals begin to show. The first evidence of an insect contribution to set came from Johnston (1929), who found that fruit set and drupelet number decreased dramatically when pollinating insects were excluded. The contribution of insects seems to vary with the cultivar. Couston (1963) found that mean seed number in 'Malling Jewel' fruits excluded from bees by bagging was only 36 % of that in fruits resulting from open-pollinated flowers. Shanks (1969) found that drupelet set on caged 'Sumner' plants was only 59 % of that on exposed plants, and that a simulated wind did not increase drupelet set. McCutcheon (1976) compared drupelet set from flowers of 'Woolama' that were exposed to bees, covered with a plastic bag, or covered with cheesecloth. Flowers in plastic bags and cheesecloth produced berries with 57 and 66 % of the number of drupelets of open-pollinated flowers, respectively. This suggests that wind may also contribute to pollination. Of the insect visitors to red raspberry, honey bees (Apis mellifera L.) are the most conspicuous and probably the most important (McGregor, 1976).

Most commercially-grown cultivars of red raspberry are self-fertile (McGregor, 1976). Actual transfer of pollen between flowers may not be necessary if there is sufficient pollen within a flower to pollinate all of most of its pistils. Transfer of pollen between

flowers and within a flower have been termed allogamy and autogamy, respectively (Percival, 1979). Daubeny (1971) found that flowers pollinated only with their own pollen or that from adjacent flowers set nearly as well (58 - 77 %) as open-pollinated flowers. Redalen (1976) and Daubeny (1971) have shown that cross-pollination with another cultivar will increase drupelet set in some cultivars.

Several workers have made recommendations for hand pollination following emasculation of raspberry flowers. Hardy (1931a) found that optimum hand pollination occurred two to five days after emasculation, when the stigmatic surfaces had become split into small "forks". Eaton et al. (1968) studied the receptivity period of 'Willamette' using hand pollination with 'Newburgh' pollen. Buds of various sizes were emasculated and then hand pollinated at various intervals from 0 - 4 days later. Optimum fruit and drupelet set of the largest buds occurred when they were pollinated once daily for four consecutive days after emasculation. However, drupelet number was not significantly lower when pollen was applied only on the second day. This suggests that optimum pollination for breeding experiments can be obtained by emasculating fully enlarged buds, then pollinating them two days later. In a similar experiment of longer duration, Redalen (1976) found that open pollination of emasculated 'Norna' flowers after isolation for three to six days decreased drupelet set by 36 and 58 %, respectively. Drupelet set was also higher in a year when the flowers were pollinated two days after emasculation than in a year when they were pollinated after three.

It is difficult to determine the relationship between these data and the effective pollination period, since it is unknown when the flowers would have opened if they had not been emasculated and since their set was not followed daily. In addition, pollination with pollen from a different cultivar as in Eaton's study (Eaton et al., 1968), while common in breeding work, does not represent the usual field situation in which raspberries are planted in solid blocks. The purpose of this study was to better document the effective pollination period (EPP) of red raspberry flowers, by pollinating a sampling of both summer-fruiting and primocane-fruiting cultivars at daily intervals after anthesis. In addition the relative importance of bees, wind and self-pollination was determined for three summer-fruiting cultivars grown in the Northwest.

MATERIALS AND METHODS

Five summer-fruiting ('Willamette', 'Meeker', 'Nootka', 'Skeena', 'Chilcotin') and three primocane-fruiting ('Fallred', 'Heritage', 'Scepter') cultivars of red raspberry were chosen for this study. The plants were located at the Lewis-Brown Horticulture Farm of Oregon State University, and ranged from 6-8 years of age. All plants were tied to a double-wire trellis and pruned to about five strong canes per plant each winter. The tips of the canes were woven on the upper trellis wire and not topped. Flowers in the swollen bud stage, one day before normal opening, were chosen at random from both sides of a 12-foot planting of each cultivar. Each bud was emasculated, then covered with a paper bag. Four buds of each variety were then subjected to each of the following treatments: pollinated once on the first, second, third or fourth day after emasculation; pollinated three times on the first, second and third day after emasculation; and not pollinated (check).

In all treatments except the check, the bag was removed and the flower pollinated by brushing the pistils with the anthers of four other flowers of the same cultivar. Laterals containing flowers to be used as pollen sources were collected 1-2 days prior to opening and placed in water in the laboratory. In each case the bag was replaced immediately after pollination and not removed until the fruit had formed. In the check, the flowers were emasculated but no external pollen was applied. The single-pollination treatments were designed to determine the effective pollination period (EPP) of each cultivar. The treatment pollinated three times showed how much

drupelet set was possible under ideal pollinating conditions. The unpollinated check tested for experimental error caused by the introduction of pollen in the emasculation and/or bagging processes.

A second experiment was set up in a similar way to test the effects of several methods of pollination on drupelet set. Four treatments were used: open pollination, self-pollination days 1, 2 and 3 after bagging, cross-pollination days 1, 2 and 3 after bagging, and screen "bag". Pollination was conducted as in the first experiment, but none of the flowers were emasculated. The self- and cross-pollination treatments tested whether the pollen present on one flower was sufficient to pollinate all of its pistils, or whether external pollen must be brought in by insects and/or wind. The selfed flowers were rubbed with a cotton swab to distribute the pollen, and no external pollen was applied. The cross-pollinated flowers were brushed with pollen from other flowers as in the earlier experiment. The screen "bags" were constructed of a fine nylon mesh with 1 mm x 1 mm openings, which provided minimal obstruction to air flow. This treatment was designed to be compared with open pollination to determine the benefit derived from exposure to bees and other insects.

Both experiments were replicated three times at intervals of two days. The weather was sunny or partly cloudy during each of the study periods, with very little wind or rain (Table 12).

All resulting fruit from both studies were harvested and frozen immediately to prevent rot. Drupelet number was determined either by a direct count of drupelets or by counting the seeds after

extraction in a blender. The analysis of variance was used to determine differences between treatments.

At least one strong colony of bees was present in the study area during each of the experimental periods.

RESULTS AND DISCUSSION

The results of the EPP study are summarized in Table 13. It can be seen that both the peak day of receptivity and the length of the EPP varied between cultivars. Part of this variation may be due to differences in weather (primarily temperature) between the various study periods (Table 12). Cultivar differences in receptivity are common in other fruits such as apples, however (Williams, 1966). Unemasculated flowers passed through the stages from calyx split to petal fall in as short as one day or as long as five days, depending on the temperature. Laboratory studies showed that this period was closely associated with that when the stigmas appeared healthy under the microscope. This period lasted from 3 days at 22° C to 16 days at 6° C in 'Willamette' (Bekey and Lawrence, 1985).

All of the varieties studied retained their receptivity for two days except 'Willamette' and 'Skeena', which were receptive for three days each, and 'Chilcotin', which was receptive for four days. No particular difference was seen in the receptivity period of flowers from summer-fruiting and primocane-fruiting varieties, though none of the latter were receptive for more than two days. By far the most common pattern was for the greatest receptivity during the two days following emasculation. This would most likely have coincided with the first two days the flowers would have been open, since they were emasculated just before normal opening. The peak period of receptivity occurred 1-2 days after emasculation in all varieties except 'Chilcotin', which showed a peak on the third day. The later

peak in 'Chilcotin' may have been due to an earlier emasculation rather than a varietal difference, as the shape of these flowers was more conical than in the other varieties and it was difficult to tell when they would open.

In all varieties tested except 'Willamette' and 'Scepter', pollination on only one day at the peak of the receptivity period was as effective as pollination for 3 consecutive days. Since all of the stigmas do not become receptive at the same time (McCutcheon, 1976; McGregor, 1976), there must be a mechanism to spread out the effect of pollinating activity over several days. One possibility is that pollen deposited on flowers soon after they open remains viable long enough to pollinate later-ripening stigmas. Pollen of 'Fallred' remains viable as long as one week at 23° C (Otterbacher et al., 1983). Another possibility is that the first stigmas to become receptive remain so for several days while the others become receptive. This does not seem to be the case for 'Willamette' or 'Scepter', which set significantly fewer drupelets with one day of pollination than with three days.

The results of this study agree favorably with those of other workers, and with the results of bee-pollination studies. Eaton et al. (1968) found that the peak period of receptivity for 'Willamette' was two days after emasculation of the largest bud size. Redalen (1976) found that receptivity of 'Norna' was higher when it was pollinated after three days rather than six, and showed evidence that pollination after two days was more effective than three.

Bekey and Burgett (1985) found that bee visitation to 'Willamette' flowers for one day or 'Meeker' flowers for two days produced as high a drupelet set as exposure for 3-4 days. This supports the observation in this study that the peak receptivity period lasts only a few days.

The mean number of pistils per flower was remarkably similar in all varieties tested (Table 14). All ranged between 87 and 91 except 'Fallred', with a mean of 98.1 pistils and 'Nootka', with a mean of only 73.2 pistils. The set by hand pollination as a fraction of pistil number was among the highest for 'Nootka' and among the lowest for 'Fallred'. As a result the number of drupelets produced by pollination at the peak of the receptivity period was nearly the same (62.8 and 58.1, respectively). Potential drupelet set, based on hand pollination for 3 consecutive days was remarkably high, above 86 % of the mean pistil number in all cultivars except 'Fallred' (Table 13). This showed that little or no damage was caused by the emasculation process. Daubeny and co-workers also obtained very high drupelet sets (>90 %) from open-pollinated, unemasculated 'Willamette' and 'Meeker' flowers (Daubeny *et al.*, 1970, 1975; Daubeny, 1971).

Of the primocane-fruiting cultivars, only 'Heritage' set as well as the summer-fruiting cultivars. Although the number of drupelets set at the peak of the receptivity period in 'Fallred' was close to that of 'Willamette' and 'Nootka', the set on the preceding and following days was considerably lower. Drupelet set in 'Scepter' was lower on all days than the other cultivars. Lawrence

(1980) also noted that 'Scepter' fruit does not have the quality of 'Heritage', and Ourecky (1973) noted that fruit of 'Fallred' is "small, very soft, coarse and poor quality". Heritage may produce 6 - 7 times more fruit per hectare than 'Scepter' (Stiles, 1980). It is possible that 'Scepter' and 'Fallred' would benefit from cross-pollination with another cultivar. Daubeny (1971) has shown that there is considerable variation in self-fertility among commercially-grown cultivars.

The term "effective pollination period", or "EPP" has been defined as the longevity of the ovules minus the time required for pollen tube growth (Williams, 1966). The drupelet set after delayed pollination is a good indication of the success of fertilization, and thus provides a suitable estimate of the EPP. Although the receptivity period of the stigmas may be longer than the EPP, pollination after this period is of no significance as later-germinating pollen tubes will not be able to fertilize the ovules. No data could be found on the longevity of the ovules in red raspberry, but Engelhardt and Stosser (1979) found that blackberry pollen tubes reached the base of the style in 18 hours and the ovule in 30 hours at field temperatures. If we assume this to be similar in red raspberry, then the ovule longevity must be at least 30 hours longer than the EPP, or at least three days in all cultivars examined in this study.

Thus fertilization must occur within about 4 days in 'Willamette', 'Skeena' and 'Chilcotin', and within 3 days in all of the other varieties studied. Pollination must occur at least 30

hours ahead of this, so we are left with a 1-3 day "window" during which pollinator density must be sufficient and weather must be suitable for pollinator visitation and pollen-tube growth. Studies by Bekey and Burgett (1985) showed that bee visitation to flowers occurred most heavily during this period, when the petals were still attached and nectar secretion was highest.

Although drupelet set dropped significantly after the third day in all cultivars studied, the set on the fourth day was 44 % or more in all of the summer-fruiting cultivars. The lowest average number of drupelets on this day was 39.3 for 'Willamette', which would probably be an acceptable berry, or at least complete enough to hold together. This means that bee visitation could be delayed until the fourth day by poor weather or low pollinator numbers and still be at least partially effective. In contrast, all three primocane-fruiting cultivars showed a sharp drop in set on the fourth day, with a mean of only 8.3 - 16.6 drupelets per berry. These berries would be unacceptable for harvest as the drupelets would not hold together, or "crumble". This may be due partly to accelerated flower development as a result of the high air temperatures experienced during this period, but this cannot be the entire explanation as set of the summer-fruiting cultivars was higher on the fourth day even during the warm spring season of 1983 (Table 12). Thus the primocane-fruiting varieties would have to be pollinated by the third day.

Set that occurred in the checks (up to 17.8 drupelets in 'Nootka') could be attributed either to pollen on the hands of the

investigator during the emasculation process or to early-dehiscing anthers. The first anthers begin to dehisce at about the time of calyx split, which was only shortly after the time that the flowers were emasculated in this study (Wellington, 1913; Hardy, 1931b).

The "method of pollination" studies showed that autogamy was as effective as allogamy in 'Willamette', 'Meeker' and 'Nootka' (Table 15). This means that each flower contains sufficient viable pollen to pollinate itself. This is important because it means that insects or wind have only to spread pollen among the pistils within a flower, and do not have to carry it between flowers, to be effective. Although not all of the varieties were examined for this, the fact that stamen numbers were all very similar (means of 76.7 - 92.6) suggests that this is the case in at least all of the summer-fruiting cultivars studied (Table 14).

In 'Willamette' and 'Meeker', flowers pollinated by autogamy or allogamy for 3 consecutive days produced as many drupelets as open-pollinated flowers visited by bees. Similarly, Daubeney (1971) found that drupelet set of 'Willamette' and 'Meeker' flowers pollinated only with their own pollen or that from adjacent flowers was nearly as good (66 - 73 %) as that from open pollination (91 - 97 %). Since the planting used in this study consisted of several cultivars and bee activity was high, open-pollinated flowers may have received pollen from other cultivars. The fact that 'Nootka' set a significantly lower number of drupelets by hand pollination than by open pollination may indicate that this cultivar benefited from cross-pollination.

Screen-covered flowers produced a considerable set (mean of 30.4 - 51.3 drupelets), showing that self-pollination and/or the effects of wind will set drupelets in the absence of bees. This factor amounted to 35.4 - 68.6 % of the set obtained in open pollination, depending on the variety. Self-pollination seems to be due to an overlap between the outermost pistils and the innermost stamens, which may contact one another as a raspberry flower opens (McGregor, 1976; Free, 1970). Observations of flowers in the laboratory showed that pistils immediately adjacent to stamens were heavily covered with pollen. Other workers have also obtained considerable set in isolated flowers. Couston (1963) obtained 38 % of the number of seeds produced from open pollination in screen-covered plots of 'Malling Jewel', and McCutcheon (1976) and Shanks (1969) obtained 57 - 59 % of the number of drupelets produced from open pollination in caged plots of 'Woolama' and 'Sumner'. An effect of wind on pollination could be from either transport of pollen by the wind (anemophily) from flower to flower or within a flower, or by moving the flower parts so that the anthers and stigmas contact one another. The fact that autogamy was as effective as allogamy in this study suggests that movement of pollen within a flower may be sufficient. Daubeney (1969) showed that a jarring effect on the flowers would not increase set in 'Newburgh' or 'Sumner'. Shanks (1969) found that a simulated wind did not increase drupelet number in 'Sumner', but McCutcheon (1976) obtained a higher number of drupelets from flowers of 'Woolama' in cheesecloth "bags" than from flowers in plastic bags. Clearly the

subject of wind pollination in red raspberry needs further investigation.

Flowers exposed to bees (open pollination) produced from 1.5 - 2.8 times more drupelets than those in the screen "bags". This difference can most likely be attributed to visitation by bees, as the bee density was very high in the study area and few other insects were seen. It can be seen that bees were least important in 'Nootka' and most important in 'Willamette', with an intermediate importance in 'Meeker'.

The importance of bees to the pollination of raspberries is readily apparent from these studies, as is the need to have them available in sufficient numbers during the 2 - 3 days in which the flowers are most receptive. Strong fluctuations in the weather, especially during the spring bloom period, further emphasize the need for bees as cool temperatures may inhibit bee flight (Free, 1970). A one to two-day delay in pollination due to poor weather for bee flight should not cause a problem, however, as most cultivars seem to be receptive for two to three days and one day of pollination seems to be sufficient for a good set of drupelets. Pollination by wind or self-pollination in the absence of bees is likely to be irregular and unpredictable, and may reduce drupelet number by as much as 35 to 68 %. Although bee flight may be less restricted during the flowering period of primocane varieties due to higher air temperatures, the development of the flowers is also accelerated and their receptivity period may be shorter. Thus the importation of bees could also be justified for these varieties.

Table 12. Study periods and associated weather data for effective pollination period (EPP) studies, 1981-83.

Variety ¹	Type ²	Year	Study period	Air temp. (°C)			Total precip. (cm)
				Max.	Min.	Mean	
W	SF	1981	5/26 - 6/2	22.3	8.1	15.2	0.05
W,M,N,S,C	SF	1982	5/29 - 6/5	19.6	8.2	13.9	0.10
W,M,N	SF	1983	5/25 - 6/1	25.3	10.9	18.1	0.81
F	PF	1982	8/5 - 8/12	27.0	11.8	19.4	0.28
H,SC	PF	1982	8/28 - 9/4	25.5	11.6	18.5	0.28

¹ W=Willamette, M=Meecker, N=Nootka, S=Skeena, C=Chilcotin, F=Fallred, H=Heritage, SC=Scepter.

² SF=Summer-fruiting, PF=Primocane-fruiting.

Table 13. Effective pollination periods (EPP) and drupelet set of 8 cultivars of red raspberry, 1981-83.¹

Variety (Type) ²	Year(s)	EPP	Poss. set ³	Mean drupelet number				Check
				Days from Emasculation				
				1	2	3	4	
Willamette (SF)	1981-82	1-3	76.5a	63.9b	54.9b	55.0b	39.3c	1.9
Meeker (SF)	1982-83	1-2	77.4a	67.9a	70.0a	54.1b	50.0b	5.6
Nootka (SF)	1983	1-2	72.7a	55.2b	62.8a	42.5d	49.4c	17.8
Skeena (SF)	1982	1-3	85.6a	78.1a	68.8ab	63.8ab	50.1b	15.3
Chilcotin (SF)	1982	1-4	79.1a	39.6c	52.9bc	82.3a	64.0ab	6.8
Fallred (PF)	1982	1-2	58.2a	23.4bc	58.1a	32.3b	12.5c	1.2
Heritage (PF)	1982	1-2	82.8a	76.6a	80.9a	45.1b	8.3c	0.3
Scepter (PF)	1982	1-2	48.1a	37.8b	29.2bc	23.9cd	16.6d	0.4

¹Mean of 3 replicates, 4 flowers sampled per replicate. Mean separation within rows by Duncan's Multiple Range Test, 5% level.

²SF=Summer-fruiting; PF=Primocane-fruiting; EPP= Effective Pollination Period, considered to be the time from flower opening until set dropped significantly below its peak value.

³Number of drupelets set by hand pollination for 3 consecutive days.

Table 14. Pistil and stamen numbers in 7 cultivars of red raspberry, 1982-83.

Variety	Type ²	Year(s)	Pistil no ¹ (S.E.)	Stamen no ¹ (S.E.)
Willamette	SF	1982-83	90.8 (3.3)	92.6 (2.2)
Meeker	SF	1982-83	90.0 (2.0)	82.9 (1.2)
Nootka	SF	1982-83	73.2 (2.1)	88.4 (3.6)
Skeena	SF	1982-83	87.7 (1.5)	83.8 (1.1)
Chilcotin	SF	1982-83	88.1 (1.3)	76.7 (0.9)
Fallred	PF	1983	98.1 (2.4)	----
Heritage	PF	1983	90.1 (2.4)	----

¹ Mean of 50 flowers sampled from each variety.

² SF=Summer-fruited, PF=Primocane-fruited.

Table 15. Effects of various methods of pollination on drupelet number in 3 cultivars of red raspberry, 1983.

Variety	Number of drupelets ¹			
	Open	Hand Selfed ²	Hand Crossed ³	Self + Wind (screen "bag")
Willamette	85.8a	78.8a	75.6a	30.4b
Meeker	78.8a	80.8a	77.6a	41.6b
Nootka	74.8a	63.0b	61.7b	51.3c

¹ Mean of 3 replicates, 4 flowers per replicate. Mean separation within rows by Duncan's Multiple Range Test, 5% level.

² Selfed=Pollination by movement of pollen within a flower on 3 consecutive days (autogamy).

³ Crossed=Pollination by transferring pollen from 4 other flowers of the same cultivar on 3 consecutive days (allogamy).

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III. EFFECTS OF TEMPERATURE ON FLOWER DEVELOPMENT, DRUPELET SET AND POLLEN-TUBE GROWTH IN RED RASPBERRY

ABSTRACT

Development of 'Willamette' red raspberry flowers from flower opening to petal fall took place in 3 days at 22° C, but required 18 days at 6° C. Petals were retained for only 2 days at 18 and 22° C, but for as long as 10 days at 6° C. The lower threshold for flower development appears to be close to 6° C.

Anther dehiscence began almost immediately after the flowers opened in 'Willamette' 22° C, but not all flowers had dehiscing anthers until the second or third day they were open at 6 - 14° C. Pollen of 'Willamette' remained viable (>15 %) for at least 4 days at temperatures from 6 - 22° C.

Nectar secretion followed several different patterns, depending on the temperature. The general pattern in 'Willamette' was for low production over 2 - 3 days at high temperatures, high production for 3 - 4 days at intermediate temperatures, and intermediate production for at least 4 days at low temperatures. Nectar production peaked on the first day at 22° C, but not until the second and third days at 14° C and 6° C, respectively.

The first sign of decline in the pistils of 'Willamette', seen as minute black specks on the tips of the outermost stigmas, occurred as soon as 3 days after flower opening at 22° C, but was delayed for 16 days at 6° C. The anthers remained healthy for the same length of time as the stigmas at higher temperatures, but at least a week longer than the stigmas at lower temperatures.

Drupelet set was significantly higher in 'Willamette' flowers pollinated at 14, 18 and 22° C than at 10° C, and significantly higher at 10° C than at 6° C. Pollen tubes of 'Scepter' required only 24 hours to reach the base of the style at 18 - 22° C, but 3 - 4 days were necessary at 6 - 10° C. The low set in the 'Willamette' flowers was probably related to a reduction in pollen tube growth rate so that the ovules degenerated before the pollen tubes reached them.

The results of this study indicate the importance of providing sufficient numbers of bees to adequately pollinate the flowers in the event of undesirable weather conditions.

INTRODUCTION

Several studies exist relating to temperature effects on floral initiation, bud development, and time of flowering in red raspberry (Rubus idaeus L.) (e.g. Lockshin et al., 1981; Vasilakakis et al., 1979, 1980). Hill (1958) followed the weight, diameter and chemical changes of raspberry fruits at two day intervals from full bloom to maturity. Effects of temperature on development of the flowers and fruit, however, have not been documented.

Otterbacher et al. (1983) showed that high temperatures will result in rapid loss of viability of raspberry pollen. High temperatures have also been shown to shorten the period of stigma receptivity in pear (Lombard et al., 1972), and to hasten the rate of ovule degeneration in cherry (Eaton, 1959), though they may enhance the rate of pollen-tube growth in apple (Child, 1966), pear (Lombard et al., 1972) and cherry (Lombard et al., 1983).

Temperatures below freezing, which can occur during flowering and fruit set of fruit crops in the Northwest, may result in freezing injury to flower tissues or young fruits of many species including cherry, apple and pear (Westwood, 1978). Less severe cold may also affect fruit set by reducing activity of honey bees (Apis mellifera L.) (Free, 1970) or decreasing pollen germination and the rate of pollen-tube growth so that the ovules degenerate before fertilization can occur (Lombard et al., 1983; Thompson and Liu, 1973). The optimum temperature for pollen-tube growth, fertilization and fruit set in tree fruits seems to be between 18 and 27° C (Free, 1970).

In addition to direct effects on fruit set and bee flight, temperature may affect the attractiveness of flowers to bees by influencing anther dehiscence, nectar secretion and petal retention (Percival, 1979). Nectar volume and concentration and the presence of petals are particularly important in bee attraction (Free, 1970).

The periods of anther dehiscence, nectar secretion and petal retention in red raspberry have been studied, but this has not been related to temperature. Wellington (1913) and Hardy (1931) found that dehiscence began almost immediately after the flowers had opened. McGregor (1976) and Free (1970) stated that anther dehiscence in red raspberry occurred for 2 - 9 days, but gave no reference to temperature effects. Nectar secretion in raspberry ranges from 3.8 to 14 mg per day (Sazykin, 1952; Simidchiev, 1976) and a total production of 13 - 27 mg during the life of a flower (Petkov, 1963; Szklanowska, 1972). Again, these figures have not been related to temperature and no mention was given as to over how many days the nectar was secreted. McGregor (1976) stated that raspberry petals are shed "a day or so after the flower opens", but no mention was made as to temperature.

Rates of pollen-tube growth have been more extensively investigated in tree fruits than in caneberries. The time of growth of pollen tubes from the stigma to the ovary has been reported as 3 - 6 days for apple at 11 - 20° C (Child, 1966), 3 - 9 days for cherry at 7.5 - 15° C (Lombard et al., 1983) and 3 - 12 days for pear at 6 - 15° C (Lombard et al., 1972). Engelhardt and Stosser (1979) found that pollen tubes reached the base of the style of

blackberry flowers in about 18 hours and the ovule in about 30 hours at field temperatures of 11 - 24° C. No data could be located on pollen tube growth rates in raspberry.

The purpose of this paper was to study the process of flower development in relation to temperature, including petal retention, pistil and stamen maturation, pollen-tube growth and drupelet set. These relationships may be used to make predictions regarding pollination and fertilization in the field.

MATERIALS AND METHODS

In June 1982, laterals containing flower buds of 'Willamette' red raspberry were collected from mature plants in the field at the Oregon State University Lewis-Brown Horticulture Farm. Sections 15 cm in length, each containing a large flower bud one day from anthesis and two leaves, were cut from the laterals. Three of these bud-sections were placed in each of 3 flasks of distilled water, then the flasks were placed in temperature cabinets at 6, 12 and 24°C. At intervals of 24, 48, 72 and 96 hours, a random sample of pollen was removed from each 'Willamette' flower and checked for percent germination in an 18 % sugar solution using the hanging-drop method (Lewis, 1942). In addition, the nectar which had accumulated in each 'Willamette' flower was removed with a 10 microliter micro-pipet and the volume recorded. The entire experiment was replicated three times at one-day intervals.

In May 1983, 'Willamette' bud-sections prepared as in 1982 were placed in temperature cabinets at 6, 10, 14, 18 and 22° C. Each flower was hand-pollinated daily by brushing it with the anthers of four other flowers of the same cultivar from the time the pistils first became visible until the stigmas no longer appeared receptive. At 24-hour intervals from anthesis, the stage of development of each flower, presence/absence of dehiscing anthers, and appearance of the pistils and stamens was recorded. Observations were continued for 19 days (May 27 - June 14). On the 20th day the beakers containing the partially-developed fruits were transferred to a laboratory at 22° C for one week, and finally left outdoors to complete berry

development. Once development appeared to have ceased, the enlarged, green berries were dissected and a count of berry and drupelet set was made. This experiment was replicated three times at one-day intervals.

In August 1983, a study was conducted to determine the effect of temperature on pollen tube growth rate. Flowers of the primocane-fruiting variety 'Scepter' were removed from the field at the swollen bud stage, emasculated, and placed in moist florist's foam using the method of Jefferies et al. (1982). Four of these flowers were inserted in each of five pieces of foam, then the foam was placed in temperature cabinets at 6, 10, 14, 18 and 22° C. Each flower was hand-pollinated daily in the same manner as in the May studies, beginning at the time of emasculation. A random sample of 10 pistils was removed from each flower 24, 48, 72 and 96 hours after placing them in the temperature cabinets. The pistils were then fixed in FAA and cleared and softened with concentrated sodium hydroxide using the method of Martin (1958). The cleared pistils were placed on microscope slides, stained with aniline blue dye, and viewed through a microscope equipped with ultraviolet light in order to see the pollen tubes. The percentage of penetration of the pollen tubes in the style was then determined for each flower. This experiment was also replicated three times at intervals of two days.

RESULTS AND DISCUSSION

In order to quantify the stage of bloom, a numerical scale was developed from 0 to 5, with 0 representing a swollen, unopened bud and 5 representing a flower with one or no petals left. These stages are shown diagrammatically for 'Meeker' in Fig. 7.

In stage 1, the calyx has split, but still remains tightly pressed to the petals. The tips of the stigmas are exposed at this stage, but the styles and the stamens are not yet visible. In stage 2, the sepals have spread out to a horizontal position, exposing the petals which are tightly pressed against the stamens. The styles and stamens remain hidden at this stage. In stage 3, the sepals are in a horizontal position, the petals are in a vertical position, and both the pistils and stamens are clearly visible. In stage 4, the sepals are reflexed beyond the horizontal, and the pistils are separated so that the outer ones intersect the innermost stamens. This is when self-pollination seems most likely to occur. In 'Meeker', the petals are usually folded back against the sepals at this stage. In 'Willamette', the petals usually remain in a vertical position and begin to decay. Petal drop also begins to occur at this stage. At stage 5, one or no petals remain attached, and the sepals are reflexed to the point where they may touch the petiole. The stamens lean outward, away from the pistils at this stage and the nectariferous area is clearly visible. This description generally agrees with that of McGregor (1976), though his description is somewhat simplified. Both McGregor (1976) and Free (1970) pointed out correctly that both anther dehiscence and

stigma maturation occur from the outside of the flower towards the center as a raspberry flower matures.

The data in Tables 16 - 18 show that flower development, nectar secretion and pollen germination were all affected by temperature. The rates of flower development are summarized in Table 16. As would be expected, the flowers developed more rapidly as the temperature increased. 'Willamette' flowers reached stage 5 in 3 days at 22° C, but required as long as 18 days to reach stage 5 at 6° C. Development at 6° C began very slowly, not reaching stage 2 until 4 - 5 days after anthesis. This temperature appears to be close to the threshold for flower development. Other workers have reported a threshold of 6° C for flower development in peach (Richardson et al., 1975) and pear (Anonymous, 1960).

Anther dehiscence occurred almost immediately upon flower opening (stage 1) at 20° C, but 33 % of the flowers did not release pollen until the second day, and 20 % not until the third day the flowers were open at 9° C. This agrees favorably with the results of other workers. Wellington (1913) and Hardy (1931) found that raspberry anthers dehisced almost immediately after flower opening at field temperatures. Snyder (1942) found that dehiscence was delayed at least one day at low temperatures in apple flowers. Dehiscence of raspberry flowers at temperatures as low as 12° C has also been reported (Percival, 1955).

Pollen could easily be collected from 'Willamette' during stages 1-4 at each temperature, but the viability of this pollen rapidly decreased over time (Table 18). At 6 and 14° C, viability

increased on the second day, suggesting that some of the pollen may not have been mature on the first day. At 22° C, however, viability dropped rapidly after the first day. Some pollen remained viable (>15 %) at all temperatures through the fourth day after anthesis. Similarly, Otterbacher et al. (1983) found that pollen of 'Fallred' retained a viability greater than 1 % for at least one week at 23°C.

Pollen viability was considerably lower in the pollen-tube growth study on 'Scepter' the following year (Table 20). Little or no germination of pollen occurred after 2 days at 18 and 22° C, 3 days at 18° C, and 4 days at 10 and 14° C. Good germination occurred through the fifth day, however, at 6° C. It is unknown if short pollen life is a characteristic of the 'Scepter' cultivar, or if the poor germination was a result of the deterioration of the detached flowers, which did not hold up well in the humid environment of the temperature cabinets. Other workers have reported similar difficulties with maintaining the condition of detached flowers in temperature cabinets (Lombard et al., 1972; Jefferies et al., 1982).

Nectar secretion followed several different patterns, depending on the temperature (Table 17). The general pattern in 'Willamette' was for low production over 2 - 3 days at low temperatures, high production for 3 - 4 days at intermediate temperatures, and intermediate production for at least 4 days at low temperatures. Nectar production at 22° C peaked on the first day the flowers were open. The low volume recovered at this temperature may reflect a high evaporation rate rather than lower production, as the open

nectaries of raspberry are strongly affected by the relative humidity of the surrounding atmosphere (Free, 1970). It has been pointed out that visitation of flowers by insect pollinators may not be reduced simply because the nectar volume drops if the concentration of the nectar also increases (Percival, 1979).

Production at 6 and 14°C increased on the second day the flowers were open. Nectar secretion dropped rapidly after its peak at 14 and 22° C, but continued at an intermediate level through the fourth day at 6° C. Simidchiev (1976) also noted a decrease in nectar secretion with increasing age of a raspberry flower. The total volume of nectar produced also varied with temperature, ranging from 30.3 - 62.3 µl. This volume was highest at 14° C, followed by 6° C and 22° C, respectively.

No directly comparable data on nectar secretion could be found in the literature, as other workers have measured nectar secretion of raspberry in mg rather than µl. Sazykin (1952) found differences in nectar secretion by variety, soil and climatic conditions, with an average of 4 - 6 mg produced per day. Petkov (1963) and Szklanowska (1972) obtained mean total weights of 13 mg and 27 mg per day, respectively. Simidchiev (1976) found a mean secretion of 3.8 - 14 mg per day. Increased production of nectar at moderately high temperatures, as observed in this study, is common in many plants (Percival, 1979). Lower and upper thresholds for nectar secretion have also been reported. Prunus avium does not secrete nectar below 8° C, and white clover ceases to produce nectar at temperatures above 25° C (Percival, 1979).

Petal retention, which is represented by the number of days through stage 4, was prolonged to 10 days at 6° C. Clearly the observation by McGregor (1976) that raspberry petals are shed "a day or so after the flower opens" is only true at high temperatures. Petal retention seems to be related to fertilization in many plants (Percival, 1979). Thus the delay in petal loss at lower temperatures may have been related to slow pollen-tube growth, resulting in delayed fertilization of the ovules.

The first sign of decline in the pistils was the appearance of minute black specks on the tips of the outermost stigmas. This occurred as soon as 3 days after anthesis at 18 and 22° C, but was delayed until 15 - 16 days at 10 and 6° C (Table 19). These black marks gradually appeared on more and more pistils toward the center, and the stigmas lost their glistening, sticky appearance. The disappearance of the sticky secretion on the stigmas is associated with a loss of receptivity in many plants (Percival, 1979). A dieback of the styles then began to occur from the stigmas toward the ovaries. This proceeded about 90 % of the way down the styles. Soon after pistil dieback began, the ovaries began to expand. The bases of the styles remained green throughout this drupelet expansion phase.

The anthers remained healthy about the same length of time as the stigmas at higher temperatures (3 days), but at least a week longer than the stigmas at cooler temperatures (14 - 19 days). In comparison, McGregor (1976) and Free (1970) stated that anther dehiscence in raspberry occurred over 2 - 9 days. Decline in the

stamens was seen first as a darkening in color of the anthers, followed by a shriveling of the attachment between the anther and the filament and finally a dieback of the entire filament. Very little pollen remained on the anthers by the time the first signs of decline were seen.

Drupelet set was significantly higher in 'Willamette' flowers pollinated at 14, 18 and 22° C than at 10° C, and significantly higher at 10° C than at 6° C (Fig. 8). All flowers set fruit at 14° C and above, but two flowers did not set at all at both 6 and 10° C. The drupelets produced at 6° C did not hold together sufficiently to produce an acceptable berry. Berries produced at 10° C held together, but had an irregular appearance. Those produced at 14, 18 and 22° C, in contrast, were large and well-proportioned and showed more than 80 % drupelet set. These results suggest that pollen tube growth was inhibited to the point where ovule degeneration occurred at the lower temperatures. The period of pollen-tube growth is greatly extended in apple, pear and cherry at temperatures of 6 - 11° C (Child, 1966; Lombard et al., 1972; Lombard et al., 1983).

Pollen-tube growth in 'Scepter' flowers is summarized in Table 20. Pollen tubes reached the base of the style within 1 - 2 days at 18 and 22° C, but 4 - 5 days were required at 6 and 10° C. This rate of growth is comparable to that measured by Engelhardt and Stosser (1979), who found that blackberry pollen tubes reached the base of the style in about 18 hours and the ovule in about 30 hours at field temperatures of 11 - 24° C. The rate of growth at 6 - 10° C however, seems high compared to that reported for other fruits.

Pollen tubes of pear require as long as 12 days to reach the base of the style at 6° C (Lombard et al., 1972) and cherry flowers require from 5 - 9 days at 7.5° C (Lombard et al., 1983).

In addition to their effects on flower development, extremes in temperature may have a strong influence on bee activity. Cool temperatures reduce bee flight and the distance that bees will forage from their hives. Foraging flights may occur at 12 - 14° C in early spring, but higher temperatures are required later in the year and on cloudy days (Free, 1970). McCutcheon (1976) showed that the foraging range of bees in raspberry fields is reduced considerably in cold, windy or overcast weather conditions. These observations suggest that more time may be required for adequate pollinating activity to occur under cold or overcast weather conditions than at warmer temperatures. This study has shown that cool temperatures also prolong flower development, so both bee attraction and successful pollination can occur over a longer period than at higher temperatures. Extremely low temperatures of 10° C or below, however, are likely to result in decreased set regardless of the amount of pollinating activity due to slow pollen-tube growth.

High temperatures, though increasing bee flight, may also inhibit pollination by speeding up the rate of flower development. Stigma decay began to occur as early as the second day the flowers were open at temperatures of 18 - 22° C (Table 16). Anther dehiscence, nectar secretion and pollen viability also decreased rapidly after the first day at 18 - 22° C (Tables 16 - 17, Fig. 8). Thus a high density of bees must be present soon after anthesis to

insure good pollination at high temperatures.

Although a wide temperature range was used in this study, it is not out of line with weather experienced in the field at the times of raspberry bloom in the Northwest. During May and June, the months of bloom of the summer-fruited raspberry varieties, the mean daily air temperature in Corvallis, Oregon is 14.9°C , with a mean daily minimum temperature of 8.5°C and a mean daily maximum temperature of 21.3°C . Thus we would expect a rate of development comparable to that between 12 and 16°C in this study in most years. Daily highs of only 14.4°C occur one year in four on the average, however. In those years when the mean daily temperature may be as low as $11 - 12^{\circ}\text{C}$, and both pollen-tube growth and bee activity should be restricted considerably. In contrast, daily highs exceed 27°C one year in four. These temperatures, though ideal for bee flight, would result in rapid development and decline of the flowers.

Mean temperatures during the August-September bloom period of primocane-fruited varieties such as 'Scepter' average 17.9°C , with a mean low of 9.9°C and a mean high of 25.8°C . Thus we would expect a rate of development comparable to that between 16 and 20°C in this study. Highs of 15°C or below occur only 1 year in 20 during these months, so low temperatures are not likely to be as much of a problem as in the spring bloom period. Highs exceed 30°C in one year out of four, however, so flower development and decline will often be very rapid. All of these factors emphasize the importance of providing sufficient numbers of bees so that adequate

pollination can be insured even under undesirable weather conditions.

Table 16. Rate of development of 'Willamette' red raspberry flowers at various temperatures, 1983.

Days from anthesis (S.E.) ¹					
Temp (°C)	Stage of flower development				
	1	2	3	4	5
6	2.3 (0.3)	4.7 (0.3)	8.0 (0.0)	10.0 (0.0)	18.0 (0.0)
10	2.3 (0.7)	4.5 (0.5)	7.0 (0.0)	8.7 (0.3)	15.0 (2.0)
14	1.3 (0.3)	3.0 (0.0)	4.0 (0.0)	5.0 (0.0)	9.0 (1.5)
18	1.0 (0.0)	1.0 (0.0)	1.7 (0.3)	2.0 (0.0)	3.3 (0.3)
22	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	2.0 (0.0)	3.0 (0.0)

¹Mean of 3 replicates, 4 flowers per replicate.

Table 17. Nectar secretion in 'Willamette' red raspberry at various temperatures, 1982.

Nectar volume (μ l) (S.E.) ¹					
Temp. (°C)	Days from anthesis				Total (1-4)
	1	2	3	4	
6	1.5 (1.5)	7.2 (3.5)	17.7 (1.9)	17.0 (4.2)	43.4
14	13.8 (3.9)	27.8 (8.7)	19.9 (3.2)	0.8 (1.1)	62.3
22	22.7 (4.8)	7.4 (4.1)	0.2 (0.2)	0.0 (0.0)	30.3

¹Mean of 3 replicates, 3 flowers per replicate.

Table 18. Pollen germination of 'Willamette' red raspberry at various temperatures, 1982.

Pollen germination (%) (S.E.) ¹				
Temp. (°C)	Days from anthesis			
	1	2	3	4
6	58.3 (15.9)	71.1 (15.8)	57.8 (7.1)	34.2 (11.2)
14	49.6 (13.5)	73.6 (0.7)	48.7 (8.8)	32.5 (5.8)
22	50.6 (9.2)	38.9 (5.3)	25.0 (5.1)	15.6 (9.1)

¹Mean of 3 replicates, 3 flowers per replicate.

Table 19. Effect of temperature on the length of four phases of flower maturation in 'Willamette' red raspberry, 1983.

Temp. (°C)	Days from anthesis (S.E.) ¹			
	Dehis. ² began	Stigma ³ life	Anther ³ life	Drupelet ⁴ expansion began
6	2 (0.6)	16.0 (1.5)	19.0 (0.6)	19.0 (0.6)
10	2 (0.0)	15.5 (1.2)	----	14.0 (0.6)
14	2 (0.0)	7.0 (0.6)	9.5 (0.5)	12.7 (0.3)
18	1 (0.0)	3.0 (0.0)	3.0 (0.0)	7.0 (0.6)
22	1 (0.0)	3.0 (0.6)	3.0 (0.6)	6.0 (0.6)

¹ Mean of 3 replicates, 4 flowers/replicate.

² Day when all flowers were dehiscing.

³ Days until first sign of decay was seen under the microscope.

⁴ Days until expansion was visible with the unaided eye.

Table 20. Pollen tube growth in 'Scepter' red raspberry, 1983.

% penetration of style (S.E.) ¹						
Days after anthesis						
Temp. (°C)	1	2	3	4	6	
6	6.3 (1.3)	8.9 (2.5)	9.9 (2.4)	39.3 (11.7)	95.7	
10	6.8 (3.9)	12.9 (0.6)	12.5 (4.2)	50.9 (15.6)	P	
14	26.3 (8.2)	67.1 (17.0)	69.0 (6.0)	97.2 ²	P	
18	62.7 (17.2)	94.0 (1.4)	P	P	P	
22	71.2 (13.6)	95.6 (4.4)	P	P	P	

¹ Mean of 3 replicates, 4 flowers per replicate. Flowers were emasculated one day before anthesis. P=poor germination of pollen.

² One replication only; poor germination in other 2.

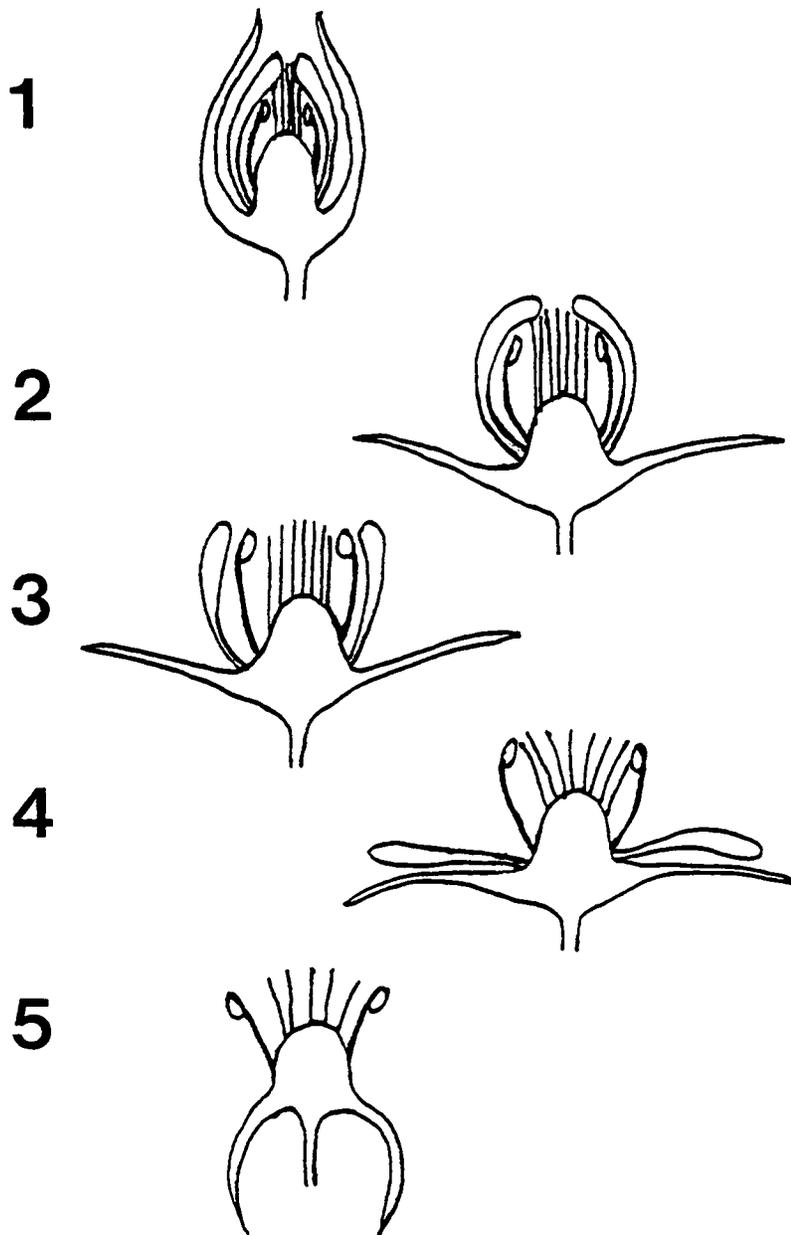


Figure 7. Stages of flower development in 'Meeker' red raspberry.

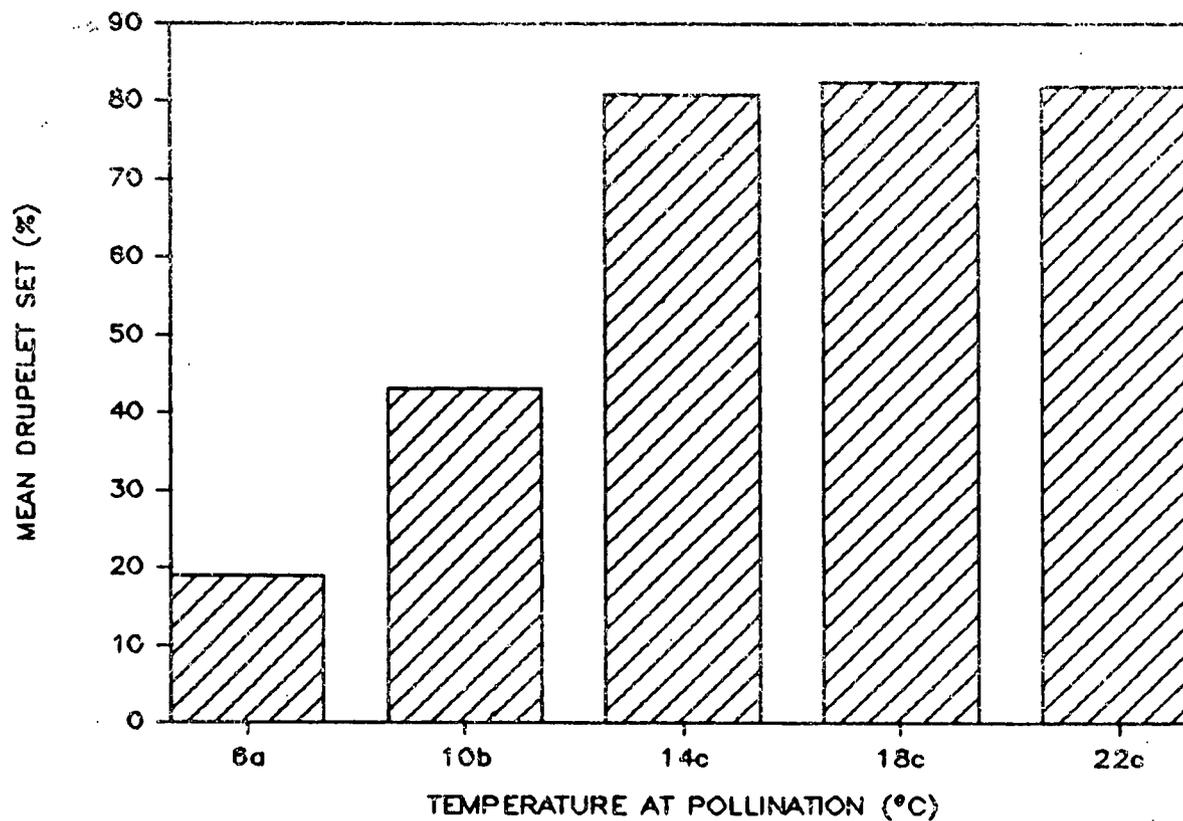


Figure 8. Effect of temperature on drupelet set of 'Willamette' red raspberry. Means followed by the same letter are not significantly different by Duncan's Multiple Range Test, 1% level.

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