

AN ABSTRACT OF THE THESIS OF

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Title: Fall-Applied Ethephon and Temperature Effects on Flower Bud Growth  
and Ovule Longevity in *Prunus domestica*.

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In the Pacific Northwest some prune cultivars often exhibit inconsistent fruit set. Several factors have been associated with this characteristic. Temperature, genotype and boron content are among the most frequently mentioned.

Studies were conducted to determine the effect of fall ethephon application on flower bud growth and ovule longevity in two *Prunus domestica* L. cultivars, 'Italian' and 'Brooks'. Ethephon was applied at the 60% leaf drop stage in the years 1988 and 1989. Growth of terminal buds, as well as tissue mineral content was determined from 50 days prior to bloom until immediately prior to bloom. Ovule longevity was determined by UV fluorescence microscopy after staining ovules with aniline blue. Temperature effects on ovule longevity were determined in flowers of excised twigs held for 18 days after full bloom in growth chambers at 5, 10, 15 and 20°C.

Ethephon application reduced the growth rate of buds for the cultivars by having an effect on both fresh and dry weight. Mineral content of buds was markedly affected in the case of 'Italian'. Higher concentration of Ca and lower concentration of P, as well as higher B content were present in the ethephon treated buds of this cultivar, when compared to the untreated control buds. No difference was seen for N or K. 'Brooks' seemed not to be affected by ethephon application in terms of the mineral content of its buds.

Under field conditions 'Brooks' showed a higher ovule viability than 'Italian'. Eighty percent of the 'Brooks' flowers had at least one viable ovule at 20 days after full bloom (DAFB). On the same date, only 40% of the flowers of 'Italian' had viable ovules, and 60% showed total ovule senescence. Ethephon application altered these genotypic differences. Flowers of ethephon-treated 'Italian' trees started with less viable ovules at full bloom, but these remained viable for a longer time. At 20 DAFB, ethephon-treated trees, in 80% of the cases, had at least one viable ovule. Ethephon did not change the pattern of ovule longevity of 'Brooks'.

Increasing temperatures significantly reduced the viability of the ovules across the days, in both cultivars. At 5 °C, both cultivars showed a low rate of ovule senescence and differences between 'Brooks' and 'Italian' were explained by the initial lower viability of 'Italian' at bloom time. As temperature increased, ovule senescence was faster in 'Italian'. At 15 °C, only one ovule per flower remained viable by 8 DAFB. At 20 °C, total ovule senescence in some flowers had already occurred by 2 DAFB, four days earlier than in 'Brooks'.

Ethephon application markedly affected the rate of ovule senescence, depending on the temperature treatment and the genotype. In 'Italian', a clear effect on delayed ovule senescence was seen at 15°C and 20°C. At both temperatures, total ovule senescence was delayed at least by four days. As the temperature decreased, there appeared to be little difference between the ethephon-treated flowers and their controls. For 'Brooks' only a slight effect on ovule longevity was observed at 20°C.

Fall-Applied Ethephon and Temperature Effects on Flower  
Bud Growth and Ovule Longevity in *Prunus domestica*

by

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Fall-Applied Ethephon and Temperature Effects on Flower Bud Growth and  
Ovule longevity in *Prunus domestica*.

CHAPTER 1

Introduction

More than 2,00 varieties of plums and prunes, comprising 15 species have been grown in the United States. Some are native to North America, however all commercially grown cultivars in Oregon, belong to the European plum (*Prunus domestica* L.), the Japanese plum (*Prunus salicina* Lindl.) or the hybrids of the later. The best known and most important are the European plums and prunes of which 'Italian' is one of the most important cultivars (Mcgregor, 1976).

'Italian' prune was originated in Italy where is called 'Italian Quetsche'. It has been grown mainly in the Pacific Northwest and only on a limited basis in California. In Oregon, it has been the leading canning variety where is also dried and sold fresh (Chaney, 1981). It is a high quality fruit but the trees have the tendency to erratic fruit set and periodic crop failures.

There have been several explanations for this erratic bearing habit, including a genetic predisposition to abnormal embryo sac development (Thompson and Liu, 1973), extremely high or low temperature conditions during bloom time (Jaumien, 1968) and inadequate B levels (Chaplin et.al., 1977).

The effect of low temperatures on fruit set has been explained as influencing the effective pollination period or by causing frost damage (Webster, 1984). For these reasons there have been several attempts to delay bloom and consequently avoid these low temperature regimes. For several species 1 to 13 days in bloom delay has been obtained by means of a fall application of ethephon (2-chloroethylphosphonic acid) (Browne et al., 1978; Buban and Turi, 1979; Crisosto et al., 1987; Proebsting and Mills, 1973). Even though by delaying bloom, frost damage was avoided, in some cases the ethephon application resulted in poorer fruit set (Proebsting, 1985; Webster, 1984; Webster, 1986)) but in some others no deleterious side effects were reported (Gianfagna et al., 1986; Crisosto, 1987).

The objectives of the present study were to determine the effect of fall ethephon application on flower bud growth, mineral content of buds and ovule longevity of two different genotypes 'Italian' and 'Brooks'. In addition to this, the effect of temperature on ovule viability was studied.

## CHAPTER 2

### Literature Review

#### Introduction

The primary goal of the orchard manager is the production of maximum tonnage of high quality fruit. The ways by which this goal can be achieved are many and varied. In general, it is possible to say that the yield of a prune orchard depends on the number of flower buds that are induced, the development of these flower buds into flowers that bloom, the number of retained fruit and finally the size and weight of the individual prunes at harvest (Martin, 1981; Ryugo, 1988).

In this review, emphasis will be placed on some internal and external factors that influence the development and set of prune flowers, an important part of a complete process that will determine the final yield of the orchard.

#### Fruit set

Fruit set is one of the most important determinants of the productivity of fruit trees (Bernier et al., 1981; Marcelle, 1984). This process is a complex multi-dimensional phenomenon under both genetic and environmental control (Chaplin and Westwood, 1980). In general, fruit set can be defined as the

retention of the fruit on the tree for a certain imprecise period after bloom (Jackson, 1986). With the completion of pollination, pollen grains germinate under the stimulus of the stigmatic fluid, and the pollen tube grows down the style, enters the micropyle and penetrates the nucellar wall to the embryo sac. The two sperm nuclei then break through the tube wall. One unites with the egg (fertilization) and the other unites with the two polar nuclei giving rise to the triploid endosperm of the seed of many angiosperms (Westwood, 1978). After fertilization is completed, presumably the young embryo releases an hormonal stimulus that prevents the fruit from abscising and causes the ovary and adjacent tissues to enlarge into the developing fruit (Westwood, 1978).

For plants, in which fruit set is dependent upon fertilization and subsequent seed development, ovules must first have normal embryo sacs and coordination in timing of stigma receptivity, rate of pollen tube growth and ovule longevity (Thompson and Liu, 1973). Williams (1965) showed the significance of the time lag between pollination and fertilization. Though stigmas may be receptive for many days after anthesis, the duration of the 'effective pollination period' (EPP) is determined by the longevity of the ovule minus the period of time necessary for the pollen-tube to reach the ovule. In other words, EPP is the time interval following anthesis during which pollination will result in a commercial crop.

The EPP is directly related to several factors that might influence the difference between the rate at which the pollen tube grows and the longevity of

the egg. If growth of the pollen tube is rapid, the EPP will be longer than if growth is slow, provided that the longevity of the ovule remains constant.

Pollination can occur over several days after bloom and still result in fertilization. The sperm nucleus of a fast growing tube could reach the embryo sac while the egg is still viable, so poor pollination is not always the main reason of poor fruit set (Thompson and Liu, 1973). In a later report, Stösser and Anvari (1983), working with sweet cherries, found that the germination of pollen grains and tube growth are not critical. The limiting factor for fertilization and fruit set was ovule longevity, if pollination was delayed.

A poor crop or none in some cases has led to considerable speculation about the different causes. Several factors have been shown to affect fruit set of trees. Among these it is possible to mention genotypic differences, nutritional status of the tree and temperature conditions during and after bloom time (Thompson and Liu, 1973; Westwood et al., 1973).

### **Genotype effects**

Williams (1966), working with pears and apples, found important differences in the EPP of several cultivars. Having a different EPP, the cultivars had the ability to set different crops when exposed to the same environmental conditions. Many authors have reported differences in the behavior of several cultivars of the same species in the relation to fruit set (Surkova and Skipina,

1966). Jaumien (1968) stated that 'Comice' pear flowers had a genetic predisposition towards a high proportion of sacs with abnormal development or early degeneration. This tendency was reported as the cause of poor fruit set. Similar effects were reported by Eaton and Jamont (1965) for 'Constant' apricot and by Hartman and Howlett (1954) for 'Delicious' apples.

Keulemans (1984), working with the effect of temperature on pollen tube growth for different cultivars, found notable differences in the response for a given temperature. Some cultivars not only showed a faster rate of pollen tube growth than others, but were also characterized by a good germination of pollen grains. Kliewer (1977) tested the effects of high daytime temperatures (35 or 40°C, from 1 a.m. to 7 p.m.) from 2 to 8 days before bloom until 12 to 18 days after bloom (bloom set period) on fruit set, ovule fertility and berry growth of several *Vitis vinifera* L. cultivars. He found that the ovule fertility was greater at 25°C than at 35°C in 'Carignane' clusters, but not in cv. 'Pinot noir'. In 'Pinot noir' clusters, however, the percentage of ovule fertility and berries with seeds were significantly greater at 35°C than at 40°C. Thompson and Liu (1973), concluded that the erratic cropping of 'Italian' prune is based in a genetic predisposition for prolonged embryological processes such as: pollen tube growth, fertilization, endosperm divisions and zygotic divisions, which are accentuated by cool post-bloom temperatures. It would seem probable that similar differences in ovule longevity may be found in inter-varietal comparisons. This may be another explanation of inconsistent cropping between cultivars.

## Nutritional status of the tree

Dorsey (1929), working with apples, first recorded a relationship existing between the longevity of embryo sacs and blossoms of different quality. The quality of the flower buds was understood in terms of mineral content and the element connected with fruit set was nitrogen. Murneek (1930) recognized that buds are high in nitrogen and it moves into buds from old branches during the spring season. Harley et al., (1958) clearly showed that early spring growth and fruit set in apple was based on nitrogen reserves of the tree. Evidence exists that the use of summer nitrogen applications results in flowers which are more 'vigorous'. These flowers are more likely to set fruit than those produced on trees receiving only spring applications of fertilizer. Williams (1965) explained that relationship in terms of differential ovule longevity. Flowers from trees receiving nitrogen only in the spring of the previous year contained viable ovules for about 7 - 9 days. Whereas, flowers from trees given additional nitrogen in the previous summer contained ovules capable of remaining viable for 12 - 13 days. In addition to this, trees and flowers with reduced nitrogen levels resulted in a reduction in the rate of pollen tube growth that finally reflected a less extensive EPP (Williams, 1963).

Hill-Cottingham and Williams (1967) evaluated the effect of nitrogen on the differentiation of flower buds in apple. They found that ovary and pollen tetrad development was enhanced if the trees were supplied with nitrogen during

the summer. Summer or fall nitrogen applications increased nitrogen in the trees and also extended the life of the leaves and enhanced photosynthetic activity. Trees with an appropriate nitrogen supply produced more reserve substances which increased the chances of producing 'strong', 'well developed' flower buds (Mishara, 1984).

Another mineral element that has been closely associated with fruit and seed set in a number of plants is boron. Flowers have been found to contain higher concentrations of boron than vegetative organs (Crassweller et al., 1981; Hartman and Howlett, 1954; Montgomery, 1951; Woodbridge et al., 1971). Plants marginally deficient in boron have exhibited blossom wilting and necrosis in pear (Bajter et al., 1953), poor seed set in clover (Montgomery, 1951) and reduced pollen production and viability in maize (Agarwala et al., 1981) whereas vegetative tissue was unaffected. In an attempt to overcome poor fruit set as a result of blossom blast in pear, Bajter and Thompson (1949) applied boron sprays during the bloom period which resulted in reduced blossom blast and increased fruit set. In a later report, Bajter et al. (1953) indicated that this response was due to the correction of an incipient boron deficiency and both fall and spring sprays were equally effective in reducing blossom blast.

Chaplin et al. (1977), found that the practice of applying boron sprays during the bloom period to prunes, either did not affect or slightly reduced fruit set. They postulated that this effect could either be a result of a toxic effect of the boron on the tender flower parts or simply a physical damage incurred

during the spraying process. Callan et al. (1978), found that a pre- or post-harvest foliar boron application increased fruit set of 'Italian' prune and that a pre-bloom application failed to increase set. Fall-foliar applications increased boron levels in dormant buds and spur tissues, flower buds and flowers (Callan et al., 1978). Bud break was slightly delayed and the size of flower buds and mature flowers was decreased. A reduction of style and pedicel length was also observed. The authors did not attribute the higher fruit set obtained in fall-treated trees to the reduction in style length although this may have resulted in a longer EPP.

Several authors (Dickinson, 1978; Kamali and Childers, 1970; Stanley and Loewus, 1964; Thompson and Bajter, 1959) have demonstrated the importance of an adequate level of boron for 'in vitro' pollen germination and pollen tube growth. However, for 'Italian' prune, the high boron levels of the buds, measured at budbreak, had no effect on 'in vivo' pollen tube growth rates (Callan et al., 1978) and on 'in vitro' pollen tube growth (Hanson and Breen, 1985a). Further investigations found that fall boron sprays were not effective in increasing fruit set in a warm spring when the set was high but increased fruit set by 32% in a cool spring when set was low (Hanson and Breen, 1985a). The reason why boron sprays have a variable effect on fruit set is not clear. Scott and Schrader (1974) reported that remobilization of boron from grape leaves occurs only when boron concentrations on those leaves were above certain level. Remobilization of boron reserves in prune branches, to supply flowers, may be

also limited when tissue concentrations are low. Fruit set appears to be influenced by boron concentrations in flower tissues and year to year fluctuations in these tissues may partially explain why fruit set response to fall boron sprays varies (Hanson and Breen, 1985a).

### Temperature effects

As was stated early in this review, fruit set is a complex phenomenon which can be affected by a wide range of cultural and climatic conditions. Temperature appears to be a major factor limiting fruit set in several species and particularly in areas in which low temperatures are frequent during bloom time (Keulemans, 1984). The effect of the temperature on fruit set has been explained as influencing the EPP or by causing frost damage (Webster, 1984; Webster, 1986). In relation to EPP, reduced fruit set due to adverse temperature effects on pollen tube growth rates, embryo sac development, fertilization or early embryo growth has been reported in several species: cherries (Eaton, 1959; Eaton, 1962; Postweiller et al., 1985); apple (Bradbury, 1929; Child, 1966); plums (Keulemans, 1984) and beans (Ormrod et al., 1967).

Because low temperature presumably increases ovule longevity while reducing the rate of pollen tube growth, the EPP may be reduced or lengthened by cool weather, depending upon relative effects on the two processes (Dennis, 1976). Jefferies et al. (1982), studying pollen tube growth in 'Victoria' plum

(*Prunus domestica* L.) found that there was a threshold of 2.5 °C above which the maximum growth rate was 0.34 mm per day-degree. Under those conditions, the tubes reached half of their final length at 16.6 day-degree. Fertilization of plum flowers required 16 - 20 days after pollination at constant temperature of 5 °C and only 3 - 4 days at 15 °C. Ovule development was retarded at 5 °C and after 25 days under this temperature regime, there were still some ovules in which no embryo sac had developed. At 10, 15 and 20 °C, fertilized embryo sacs with developing endosperm were present in all samples.

Thompson and Liu (1973), showed that temperatures for 3 weeks after full bloom had a pronounced effect on the rate of all embryological processes. In fact, after a comparison of the percentage of fertilized ovules in two years of study and direct observations of pollen tubes in the micropyle, nucellus or embryo sac, they demonstrated that cool post-bloom temperatures delayed pollen tube growth in 'Italian' prune. Keulemans (1984), working with plum trees, found the same relationship but clearly stated that all the varieties did not react in the same way to temperature. Some cultivars could be classified as 'slow pollen tube growers' in contrast to other examined cultivars that were classified as 'fast pollen tube growers'. At low temperatures, the differences in pollen tube growth rates between cultivars were large. At high temperatures this was not the case. The same author also reported that the cultivars with fast pollen tube growth rates at low temperatures were also characterized by a good germination of the pollen grains at those temperatures. At low temperatures,

cultivars with slow growth of the pollen tube had a low percentage of pollen grain germination. Since flowers which are pollinated in the orchard have a reduced number of pollen grains transmitted to the stigma, the influence of low temperature on pollen tube growth rates becomes very important.

A direct relationship also exists between post-bloom temperatures and ovule longevity. A shorter period of ovule receptivity in warm seasons has been reported to adversely affect fertilization in two cross pollinated crops, sweet cherry (Eaton, 1959) and apple (Dorsey, 1929; Williams, 1965). Postweiller et al. (1985) studied the effect of temperature on the senescence of ovules in two sweet cherry and three sour cherry cultivars. Their results indicated that ovule longevity was significantly influenced by temperature. At higher temperatures, the senescence of the ovules was accelerated. At 5 °C, viable ovules were found in all cultivars 5 days after anthesis (DAA). When temperature was 10 or 15 °C, the number of viable ovules decreased and at 20 °C no viable ovules were found after 2 or 3 DAA. Thompson and Liu (1973), observed high fruit set in self-pollinated 'Italian' prune trees in a season with high post-bloom temperatures. A shorter period of ovule receptivity was not detrimental in this particular case. Whereas cool temperatures prolonged ovule longevity, pollen tube growth was retarded sufficiently so that even if fertilization finally did occur, fruit set failed because ovular breakdown had already commenced (Williams, 1970).

## Bloom delay

Stone fruit species usually flower early in the spring when conditions are often unfavorable for pollination and fruit set. For this reason, there have been several trials of plant growth regulators for delaying bloom. The risk of frost damage would be reduced and the probability of good fruit set increased (Webster, 1984).

Sprays of plant growth regulators have been shown to influence the bloom-time of stone fruits. Autumn sprays of gibberellic acid ( $GA_3$ ) have delayed blossoming of peaches, apricots, sweet and sour cherries (Soni and Yousif, 1978; Walser et al., 1981). However, Dennis (1976) reported that sprays of potassium gibberellate ( $KGA_3$ ) had no effect on time of bloom of sour or sweet cherry. Moderate to severe winter injury resulted from treatment to the cambium and flower buds of the sweet cherry.

Delays in blossoming and occasionally increased floral bud hardiness, have been obtained by fall sprays of ethephon (2-chloroethylphosphonic acid) to sweet cherries, apricots, almond, prunes and peaches (Browne et al., 1978; Buban and Turi, 1979; Crisosto, 1987; Proebsting and Mills, 1973). Fall treatments of ethephon delayed bloom from 1 to 13 days, depending on the year, species and location. Proebsting (1985) reported that subsequent fruit set and yield were reduced on sweet cherries sprayed with ethephon in the previous fall. Webster (1984; Webster, 1986) also determined that ethephon delayed bloom of

plums and that fruit set was poorer on the treated trees. These findings were supported by Dennis (1976), who also stated that the beneficial effects of bloom delay in cherry were offset by deleterious side effects of gummosis, bud abscission or failure to open, and reduced fruit set. Gianfagna et al. (1986) reported up to 7 days of bloom delay in peach with no adverse effects on tree health. In a later report Crisosto (1987) and Crisosto et al. (1989) found no negative effects on tree performance when applying ethephon to 'Italian' prune.

The physiological mechanism for bloom delay is not clear yet.

Ethephon may act either directly on bud physiology or indirectly by inducing early abscission. According to Couvillon and Lloyd (1978), a significant effect in bloom delay can be obtained by post-harvest hand-defoliation in the case of peaches. Similar results in bloom delay were reported by Fuchigami (1977) and Fuchigami et al. (1977) in *Cornus stolonifera* after early hand-defoliation at the onset of rest. Reduced carbohydrate reserves from leaf abscission has been suggested as the mode of bloom delay from ethephon (Fuchigami, 1977).

Perhaps photosynthesis in the fall or remobilization of nitrogen to the buds is limited by early defoliation reducing both carbohydrates and nitrogen reserves required for autumn flower bud development. However, Crisosto et al., (1986) found that the effect of late fall ethephon application on bloom delay was independent of leaf abscission. In the same report, no statistical difference in reducing sugars or starch levels of flower nodal buds was detected among treatments throughout the dormant period. In fact, they proposed the ethephon

delayed bloom not by changing carbohydrate levels in buds but by affecting flower bud development during the dormant period. They proposed that elevated levels of ethylene and ABA in ethephon treated 'Redhaven' flower buds apparently delayed bloom in part by slowing the rate of floral development, possibly through effects on cell division. According to their results the time of first appearance of primordial stages was delayed two weeks in the case of pistils. Similar results were found by Gianfagna et al., (1986), reporting no difference in soluble nitrogen or carbohydrates in the buds in November after an application of ethephon in October.

Durner and Gianfagna (1989) did observe an alteration of carbohydrate and water content of different flower parts during dormancy after a fall ethephon application in peach. In this report, sucrose concentration was higher in pistils of ethephon-treated flower buds than in the untreated controls. On the other hand, sorbitol levels were lower for bud axis tissue, pistil connective tissue and scales of ethephon treated buds.

According to Bielisky (1982), sorbitol is the primary translocated carbohydrate in *Prunus spp.* The metabolism of this carbohydrate in peach and prune has only been studied to the extent of its changes during fruit ripening where sucrose is the predominant sugar (Moriguchi et al., 1990; Walsh et al., 1989). Metabolism of this sugar in the leaf has only been studied in apricot (*Prunus armeniaca*). For this species, sorbitol can be synthesized and utilized by young leaves but mature leaves are only able to synthesize but not utilize

sorbitol (Bielisky and Redgwell, 1985). According to Durner and Gianfagna (1989), the reduced levels of sorbitol may, in part, influence bud hardiness and through this delay bloom.

## CHAPTER 3

Fall-Applied Ethephon and Temperature Effects on Flower Bud Growth and  
Ovule Longevity in *Prunus domestica* L.

## Abstract

The effects of fall-applied ethephon and temperature on flower bud growth and ovule longevity in *Prunus domestica* L. were studied. Ethephon was applied at the 60% leaf drop stage at 0 and 500 mg liter<sup>-1</sup> on 9 Nov 1988 and 26 Oct 1989 to cultivars 'Italian' and 'Brooks'. In 1988, fresh and dry weights of terminal flower buds were measured from 50 days prior to bloom until the day before bloom at one week intervals. Buds were analyzed for N, P, K, Ca and B levels on each sampling date. Ovule longevity was determined for flowers of both cultivars using a fluorescence method of samples taken every two days from full bloom until 20 days after full bloom (DAFB). In 1989, twigs from untreated and ethephon-treated trees of both cultivars were excised at full bloom and held for 18 days at 5, 10, 15, and 20 °C in growth chamber with a 16 hr light/8 hr dark period. Ovule longevity was also determined for each treatment.

Buds treated with ethephon of both cultivars had different growth curves in comparison to untreated buds. The fresh and dry weights were less for buds treated with ethephon. 'Brooks' flower buds showed a higher accumulation of

fresh and dry weight than 'Italian'. There were no differences between the treatments in relation to N or K concentration in the buds. For 'Italian' a higher concentration of Ca and a lower concentration of P was seen in the ethephon-treated flower buds. Boron content was higher on the ethephon-treated buds of 'Italian' trees on some sampling dates. No effect of ethephon was observed on mineral content of flower buds for the 'Brooks' cultivar.

'Brooks' had a higher ovule longevity than 'Italian' in both years of study. Under field conditions 80% of the 'Brooks' flowers had at least one ovule viable until 20 DAFB. At anthesis, senescence of one ovule had already occurred in 20% of the 'Italian' flowers and by 20 DAFB, only 40% of the flowers had viable ovules. Ethephon treatment of 'Italian' flower buds increased the percent of flowers with one viable ovule at 20 DAFB. The pattern of ovule senescence seemed not to be affected by ethephon treatment of 'Brooks' flowers. Increasing temperatures induced a faster ovule senescence in 'Italian' and 'Brooks'. At the same temperature, 'Brooks' ovules remained viable longer when compared to 'Italian'. Ethephon enhanced the ovule longevity in 'Italian' flowers at the higher temperature, but had a lesser effect on 'Brooks' flowers.

## Introduction

Several causes have been proposed to explain the lack of sufficient fruit set that plum trees often exhibit. One explanation is the genetic predisposition towards a high proportion of embryo sacs with abnormal development or early degeneration (Thompson and Liu, 1973). Temperature conditions may be nonconducive for pollination and fertilization during and after bloom time (Jaumien, 1968). Boron levels in the flowers may be deficient, resulting in poor pollen tube growth (Hanson and Breen, 1985a).

Poor fruit set may also be due to a short effective pollination period (EPP) (Stösser and Anvari, 1982; Williams, 1966; Williams, 1970). The duration of the EPP equals the period of ovule longevity minus the time required for the pollen tube to grow and reach the egg sac. Fruit set can be decreased if the EPP is relatively short. Pollen tube growth rates might be too slow or ovule longevity too short to result in fertilization.

A delay of flowering in fruit trees by fall ethephon (2-chloroethyl phosphonic acid) applications has been reported by several researchers (Proebsting and Mills, 1973; Dennis, 1976; Coston et al., 1985; Gianfagna et al., 1986). The effect on fruit set and subsequent yield of the ethephon treatment has been erratic. The benefit of bloom delay has often been offset by side effects such as gummosis, floral abscission or failure of floral buds to open (Proebsting and Mills, 1973; Dennis, 1976; Coston et al., 1985). Crisosto et al.

(1990) reported bloom delay in peaches but yield was decreased. Bloom was delayed when ethephon was applied to 'Italian' prune but a significant increase in yield was observed in only one year of trials. Durner et al. (1990) reported that yield was enhanced by ethephon treatment in the case of 'Cresthaven' peach in a two-year trial. For 'Jerseydawn' yield was enhanced only in one year and in the other remained unaffected. Smaller pistils and heavier pre-thinning crop loads lead to smaller fruit and a later harvest date. Webster (1986), working with plums, reported that the temperature regimes at or just after blossom opening may be related to the inconsistent effectiveness of the ethephon application from season to season.

Thompson and Liu (1973) showed that in the case of 'Italian' prune, temperatures up to 3 weeks after full bloom had a pronounced effect on the rate of embryo sac development and abortion. Keulemans (1984) found the same relationship but clearly stated differences across genotypes in relation to their sensitivity to cool post-bloom temperatures. A shorter ovule longevity in warm seasons has been reported to adversely affect fertilization in sweet cherry (Eaton, 1959) and apple (Dorsey, 1928; Williams, 1965). Whereas, cool temperatures may prolong ovule longevity, pollen tube growth is retarded sufficiently so that fertilization does not occur prior to ovular breakdown (Williams, 1970).

The objectives of the present study were to determine the effect of fall ethephon application on flower bud growth, mineral content of buds and ovule longevity of 'Italian' and 'Brooks' prunes. In addition to this, the effect of temperature on ovule viability was determined.

## Materials and methods

Ethephon was applied to prune trees (*Prunus domestica* L.) cvs. 'Italian' and 'Brooks' at the 60% leaf drop stage on 9 Nov 1988 and 26 Oct 1989 at 0 and 500 mg liter<sup>-1</sup>. Regulaid (0.1% by volume) was added to assist spreading. The orchard used in this study was planted in 1975 and located at the Lewis-Brown Horticulture Research Farm, Oregon State University, Corvallis. Ten single-tree replicates were used for each treatment.

### Growth measurements.

Three replicates of 100 terminal flower buds were excised from 5 to 8 cm spurs at approximately one-week intervals, beginning on 17 Feb 1989 for the four treatments. Fresh and dry weights were obtained for each sampling date.

### Tissue mineral analysis.

Nutritional analysis of the buds was done for each sampling date. The modified Kjeldahl method (Schuman et al., 1973) was used for total N analysis and ICP spectrometry (Isaac and Johnson, 1985) for the following nutrients: P, K, Ca and B.

### Ovule longevity determination.

#### *Field experiment.*

Prior to bloom, flower buds were emasculated to prevent self-pollination and covered with cheesecloth bags to prevent bee pollination. Ten flowers, excised from the apical position of a spur (approx. 2 cm.) for each treatment, were

sampled every two days from full bloom until 20 days after full bloom (DAFB). Periodic measurements of the number of open flowers per limb were done and full bloom was considered when at least 80% of the flowers were open. The full bloom dates for the first year of study were 11, 12, 12 and 13 Apr 1989 for 'Brooks', 'Brooks' + ethephon, 'Italian' and 'Italian' + ethephon trees, respectively. In the second season, the recorded full bloom dates for the same treatments were: 30 Mar, 3, 1 and 10 Apr 1990. Samples were collected and fixed in formaldehyde : propionic acid : 95% ethanol (FAP) (5 : 5 : 90) until observations were made.

Flowers were removed from the FAP solution, washed in distilled water for 30 min, soaked in 1% sodium bicarbonate for 1 hr and rinsed three times in distilled water. Pistil and ovary samples were softened by autoclaving in 1% sodium sulfite for 2 min. Pistils were excised, ovaries were split longitudinally and the two ovules were removed with the aid of fine forceps under the light microscope. Ovules were then mounted on slides, squashed directly in 0.5% w/v aqueous aniline blue ('Baker chemical Co.' C.I. 42755) in 0.15M potassium phosphate buffer, and observed under a Carl Zeiss universal fluorescence microscope equipped for epi-illumination using a near-UV excitation (Pimienta et al., 1983; Crisosto, 1987).

Longevity of the ovules was based on the differential intensity of ovule fluorescence after staining with aniline blue. Strong fluorescence of the ovule at the chalazal end was used to indicate non-viable ovules (Polito and Pimienta,

1982; Stösser and Anvari, 1982). Each flower was rated for the number of viable (non-fluorescing) ovules per ovary. The mean number of viable ovules per flower were calculated for the 10 replicate flowers.

*Growth chamber experiment.*

To determine the temperature effect on ovule longevity across the genotypes, 3 replicates of 20 twigs up to 150 mm long each were cut from flowering trees and placed in shallow trays, in moist florist's foam ('Oasis', Oasis floral products, Smithers-oasis, France F-67000) (Jefferies et al., 1982). Three flowers per twig were emasculated to prevent self-pollination. The pots were then transferred to controlled environment chambers maintained at 5, 10, 15 and 20°C, with a 16 hr light/ 8 hr dark period. Initially, a Murashigue and Skoog basal salt mixture ('Sigma' M5524) solution was added to the trays to provide nutritional requirements. Distilled water was then periodically added to keep sufficient moisture in the foam. Samples of 10 flowers were taken every two days for at least 18 DAFB depending on the temperature. The flowers were fixed in FAP solution and prepared the same way as the samples taken in the field experiment.

Statistical analysis.

Both experiments were conducted as a complete randomized design. Data for the tissue mineral analysis and growth measurements was analyzed using two different models. The responses fresh weight (FW), dry weight (DW), N and P were fitted to a nonlinear analysis of covariance, exponential growth model

$$Y_{ij} = \alpha_i \exp(\beta_i \cdot \text{day}_j) + \tau_i + \epsilon_{ij}$$

$$i = 1, \dots, 4 \quad j = 1, \dots, 8.$$

The  $ij^{\text{th}}$  response is denoted  $Y_{ij}$ . The  $j^{\text{th}}$  regressor is denoted  $\text{day}_j$ , and  $\text{day } 1 = 0$  corresponds to 17 Feb. The index  $i = 1, 2, 3, 4$  corresponds to the treatments 'Italian' (I), 'Italian' + ethephon (I + E), 'Brooks' (B) and 'Brooks' + ethephon (B + E), respectively. The random error terms are assumed to be independent and identically distributed with a mean of zero. The 12 parameters  $\alpha_i$ ,  $\beta_i$  and  $\tau_i$  were estimated by nonlinear least squares. The parameters  $\alpha$ ,  $\beta$  and  $\tau$  do not have a simple geometrical and biological interpretation.

Consequently, instead of testing whether each individual parameter differs among treatments, it was decided to test jointly whether all three parameters differed among treatments. This corresponds to testing whether the regression functions coincide. The hypotheses of coincident responses among treatments were tested with likelihood ratio, approximate F-tests (Seber and Wild, 1989).

The responses P and Ca were fitted to a linear analysis of covariance model

$$Y_{ij} = \alpha_i + \beta_i \text{day}_j + \epsilon_{ij}$$

$$i = 1, \dots, 4 \quad j = 1, \dots, 8.$$

The notation is the same as in the exponential growth model. The 8 parameters  $\alpha_i$  and  $\beta_i$  were estimated by least squares. To keep the analysis

consistent, it was tested only whether the lines coincide (whether both the slope and intercept differ among the treatments). The hypotheses of coincident responses among treatment were tested with an F-test.

For the response variable B, instead of analyzing the data as B concentration on buds, actual B content for a sample of 100 buds was obtained. Ovule longevity data for the field experiment as well as B content of buds were analyzed by using a general linear model procedure in SAS (SAS Institute Inc., 1987). Differences among treatments were tested by using orthogonal contrasts. Data from the growth chamber experiments was combined for a response surface regression analysis. Separate regressions were performed for each cultivar/ethephon treatment/temperature combination for a total of four analyses. The regression model used was a complete quadratic response surface model of the form:

$$Y = \beta_0 + \beta_1 X_1 + \beta_{11} X_1^2 + \beta_2 X_2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \beta_{1122} X_1^2 X_2^2$$

where Y=number of viable ovules,  $X_1$ =temperature and  $X_2$ =days after full bloom. Components were deleted from the model if their parameter estimate ( $\beta$ ) was not significant ( $\alpha=0.05$ ). If a component increased the mean square error ( $\delta^2$ ) or if its deletion did not decrease the  $R^2$  of the model, it was also deleted. Results are presented as graphs of the predicted values from the models.

## Results

### Growth rate and mineral content of buds.

Ethephon treated flower buds for both cultivars showed a different pattern of growth when compared to their controls. Tests of hypothesis and probability levels for the equations fitted to each particular treatment (Table 3.1) show the significance of these differences. The model for the responses fresh weight and dry weight has an  $R^2 = .989$  and  $.986$ , respectively. In both cases the ethephon treatment resulted in a lower rate of bud growth (Figure 3.1). 'Brooks' flower buds showed a higher accumulation of fresh and dry weight when compared to 'Italian' buds.

The model for the response N has an  $R^2 = .982$  (Table 3.2). However there were no differences in N concentration between the treatments (Figure 3.2). In relation to the response variable K, the model has an  $R^2 = .988$  (Table 3.1). For both genotypes, there were no significant differences between the ethephon treatment and the controls (Figure 3.2). However, when looking at the comparison between ethephon-treated 'Italian' and ethephon-treated 'Brooks' flower buds, there was a significant increase in K concentration.

The tests of hypothesis and probability levels for the responses P and Ca appear in Table 3.2. There was no effect of ethephon application for P responses ( $R^2 = .884$ ) in 'Brooks' flower buds. In the case of 'Italian' flower buds, there was a decrease in P concentration in the ethephon treated buds when compared to the control (Figure 3.3).

For the response Ca ( $R^2 = .965$ ), a similar situation occurred. The ethephon treatment showed no effect in the case of the 'Brooks' cultivar. A significantly higher Ca concentration was observed in the case of 'Italian' buds treated with ethephon in comparison to untreated buds (Figure 3.3).

There was no difference in B content per bud between the ethephon treatment and the controls during the first 3 dates of sampling (Table 3.3). From 10 April, 'Italian' + ethephon had significantly higher B content than the control treatment. This situation was different in the case of 'Brooks' in which there were differences only at two sampling dates, 10 March and 7 April. When comparing the overall B content per cultivar only the last two sampling dates resulted in significant differences. Boron content was significantly higher in 'Brooks' for these two dates.

**Ovule longevity.**

*Field experiment:*

A clearly different ageing process between the two cultivars was observed in the field experiment. During the first 8 days of study, untreated and ethephon treated 'Italian' flowers showed less number of viable ovules when compared to flowers of 'Brooks'. After this date, this difference became non significant and both genotypes show differences again only at the last two sampling dates (Table 3.4). The rate of ovule death was the same for the ethephon treatment and its control in the case of 'Brooks'. There was no difference in any of the sampling dates except for one (Table 3.4). In 'Italian',

flowers started with less number of viable ovules and this situation continued until 8 DAFB when there was no difference between the ethephon treated and control flowers. From that date on, ethephon treated flowers showed a significantly higher number of viable ovules than untreated ones.

The frequency distribution of the number of viable ovules per flower is presented in Figure 3.4. In the case of 'Brooks', at 0 DAFB, 100% of the flowers had two viable ovules. By 8 DAFB, 70% of the flowers had two viable ovules and the rest had at least one viable ovule. Only 30 % of the flowers had two viable ovules at 11 DAFB, but in the remaining samples at least one ovule remained viable. At the last sampling date (20 DAFB) only 10 % of the flowers had no viable ovules, 10% had two viable ovules and the remaining 80% had at least one non-senescent ovule per ovary.

For 'Italian', some ovule death had already occurred by anthesis when only 80% of the flowers had two viable ovules and the remaining had only one. After 6 days, 20% of the flowers had two dead ovules and another 20 % had only one viable ovule. By 11 DAFB, only 60% of the flowers had viable ovules. Ovule senescence continued until 20 DAFB when 40% of the flowers had only one viable ovule and the remaining were all senescing.

'Italian' flowers excised from ethephon-treated trees displayed a clearly different senescence pattern in their ovules when compared to untreated 'Italian' buds. At 0 DAFB, only 20% of the flowers had two viable ovules but the rest of the flowers had at least one non-senescent ovule. By 11 DAFB, ovule

senescence did not increase considerably and only 10% of the flowers had no viable ovules. By 20 DAFB, in 80% of the flowers at least one ovule remained viable.

Ethephon sprays did not seem to affect the pattern of ovule longevity in the 'Brooks' cultivar. In the case of ethephon-treated flowers, only 80% of them had two viable ovules by 0 DAFB. The number of viable ovules remained the same until 6 DAFB. By 11 DAFB, 80% of the flowers had at least one viable ovule, this being the case for the next 9 days of study.

*Growth chamber experiment:*

Regression equations and the corresponding surface responses are presented in Table 3.5 and Figures 3.5 and 3.6, respectively. The predicted number of viable ovules in 'Italian' flower buds at 0 DAFB was less than two, indicating that in this cultivar ovular senescence had already occurred before bloom. This was not the case for 'Brooks' and in all flowers two viable ovules were present at full bloom.

Across all temperature regimes, 'Italian' had less number of viable ovules when compared to 'Brooks'. At 5°C, the rate of ovule death was similar for both cultivars and the difference on the predicted average number of ovules across days are explained by the initial lower ovule viability of 'Italian'. As temperature increased, ovule senescence was faster in 'Italian'. At 15°C, only one ovule per flower remained viable by 8 DAFB. For 'Brooks', higher temperature also resulted in a more rapid decrease in ovule longevity over time

but at a lower rate of senescence in comparison to 'Italian' ovules. Total ovule senescence for some 'Italian' flowers had already occurred by 2 DAFB for the 20°C treatment. At the same temperature, for 'Brooks' flowers, senescence of both ovules began 4 days later (6 DAFB).

Fall ethephon application had an effect on ovule longevity. The rate of ovule death was reduced in both cultivars (Figures 3.5 and 3.6). For 'Italian', ethephon application translated into a less number of viable ovules at anthesis. However, those ovules remained viable for a longer period of time. Ovule longevity was enhanced but this effect was also dependant on temperature. This interaction was particularly important at 20°C. Without ethephon treatment, at 6 DAFB, some flowers were already lacking of viable ovules. However, when treated with ethephon at least one viable ovule was present at 10 DAFB. Also at 15°C, more ovules per flower remained viable in the ethephon treated flowers. As temperature decreased, this situation changed and at 5°C there appeared to be little difference between the ethephon treatment and the control.

For 'Brooks', the same number of viable ovules were present at anthesis when comparing the two treatments (Figure 3.6). The surface response for the ethephon treated buds was different than that of the untreated control buds (Figure 3.6). At low temperatures, the rate of ovule senescence was similar in both cases and a clear difference only appeared between 15°C and 20°C. At 20°C, ovule longevity was slightly enhanced by ethephon treatment. By 18 DAFB, the ethephon treated flowers had more viable ovules than the control.

## Discussion

Flower buds treated with ethephon grew more slowly during the winter. This difference was maintained during the period of flower bud swell in the spring. There was a considerable difference in terms of fresh weight and dry matter content. According to Gianfagna (1989), in the case of 'Cresthaven' peach, both overall flower bud length and the length of the pistil were reduced within one month of the ethephon application. In that report, pistil length was only 80% and the length of the flower buds 90% of the untreated buds. In our case, buds from the ethephon treated trees were considerably smaller than buds not treated with ethephon, approximately 80% of the controls. This might be explained as a result of reduced cell division for the ethephon treated buds (Gianfagna, 1989). Apelbaum and Burg (1972) have shown that ethylene inhibits cell division in the plumular hook of pea. Inhibition of the growth of dormant buds suggests that ethylene released in the tissues may inhibit cell division during the later phases of flower differentiation in the autumn. Delayed differentiation of flower primordia could result in a delay in flower development the following spring.

Being the process of fruit setting a complex phenomenon under both genetic and environmental control, it can be directly and indirectly related to a number of nutritional factors both organic and inorganic. Unfortunately, the literature does not provide much evidence for categorizing the essential elements

in this regard. In addition to this, there is no report in the literature regarding the effect of ethephon application on mineral element content of flower buds. Our results show that nitrogen and potassium concentrations in flower bud tissue were not changed by the ethephon application. Phosphorus and calcium concentrations were changed. Several reports have shown that increased fruit set can be obtained by various N treatments. Westwood et al., (1964) in a long term nitrogen experiment with pears, reported that fruit set was higher in trees receiving higher N levels. Williams (1965) reported that late summer N applications to apple trees resulted in much better fruit set the following spring than did regular spring applications. In this study, even without having a higher N concentration in the tissue, the ethephon treatment resulted in enhanced ovule longevity of 'Italian' flowers. Perhaps, there are some other factors that play a more important role and N is not limiting in ovule longevity of prune.

Few reports are available on the effects of K and P on fruit set in deciduous tree fruits species. According to Taylor and Goubran (1975), 'Jonathan' apple trees deficient in P showed delayed bud burst and retarded development of a reduced number of vegetative and floral meristems. This translated into an indirect effect on fruit set since delayed emergence reduced the opportunity for cross pollination and subsequent fruit set. Our results agree with this report to the extent that the greater effect on bloom delay was observed in 'Italian' and that in this cultivar P concentration was lower with the ethephon application. But instead of reducing fruit set, ovule longevity was

enhanced. In the case of Ca, the higher concentration of this element as a result of the ethephon application could be associated to a delayed rate of growth rather than to an increased movement of  $\text{Ca}^{++}$  into the buds. According to Sun et al., (1990) in the case of prunes, the vascular bundles of the ethephon treated buds developed more slowly than those of untreated buds.  $\text{Ca}^{++}$  shows relatively low mobility in the plant and it is translocated mainly via xylem sap in an upward direction with the transpiration stream (Mengel and Kirby, 1982). As was the case for ethephon treated buds, where growth was delayed and transpiration rates were most likely low, it is improbable that new  $\text{Ca}^{++}$  was going into the buds. What we probably observed was rather a dilution effect in the case of the untreated control as growth started early and no new  $\text{Ca}^{++}$  was translocated into the buds.

As in the case of Ca, B levels were higher in the ethephon treated buds of 'Italian'. A dilution effect can not account for the lower levels of B in the case of the untreated controls as B was expressed as total content per 100 buds. Hanson (1984) studying B accumulation in 'Italian' prune, stated that remobilization of this element in branches was more limited than for other elements. In intact trees, B accumulated in buds more slowly before bud swelling, but rapidly as buds accumulated dry matter from swelling to bloom. Hanson and Breen (1985b) explained these results saying that xylem in the axis of flower buds seems to become functional only when buds begin to swell. However, they were able to account for only 26% of the B entering buds as

being supplied via the xylem. In the same report, they stated that limited dry matter accumulation in buds indicated that these are weak carbon sinks at this time and therefore suggested that symplastic flow to buds would also be limited. Perhaps ethephon changed this situation by altering the intrinsic physiology of the buds and in this way allowed for an enhanced B movement to buds in the spring.

The longer ovule viability of 'Brooks' flowers was clearly observed under both field and growth chamber conditions. The ethephon application did not change this pattern markedly. 'Brooks' flowers seem to fit the description of a "strong" flower as stated by Williams (1965). These flowers were larger and bloomed earlier. 'Brooks' flowers were strong enough to have two viable ovules in 10% of the cases until 20 DAFB. At this time senescence of both ovules was only 10% of the total number of flowers. The remaining 80% still had one viable ovule. Ethephon application seemed to induce an early senescence of one ovule prior to anthesis. This effect was less obvious in 'Brooks' flowers and was not seen in the growth chamber experiments during the second season of study.

'Italian' flowers can be classified as "weak" blossoms according to the criteria outlined by Williams (1965). Some ovule senescence is already apparent at the time of bloom. The ovules also senesce at a faster rate than in 'Brooks' flowers. Ethephon treated flowers started with a less number of viable ovules but these showed a delayed senescence that in practical terms might translate into better chances of fruit setting.

Efforts to prolong ovule longevity have been successful in apple by improving cultural conditions (Dorsey, 1929; Williams, 1963; Williams, 1965). Williams (1965) found that summer N application provided optimal nutritional conditions at the time of flower initiation which greatly increased the proportion of so called "strong" flowers. Kliewer (1977) stated that grape ovule fertility may be markedly affected by a supply of organic nutrients to the ovules. A situation that is critical as temperature increases. Perhaps the natural tendency of the 'Italian' genotype to a lower fruit set as reported by Thompson and Liu (1973) can be explained by a limited supply of nutrients to the flowers buds. If the development of one of the ovules is incomplete, the other one may be able to compete better for a 'limited' supply of nutrients to the flower buds. In addition to this the increase amount of B and Ca in the ethephon treated buds may be playing an important role in enhancing the longevity of the ovules.

The results clearly illustrated that ovule senescence was influenced by the temperature in which flowers were maintained. At higher temperatures, ovule senescence was accelerated. However, genotype and ethephon treatment interacted with temperature. Kliewer (1977) and Ewart and Kliewer (1977) reported that grape fruit set was much better at 25 °C than at 35 or 40 °C. They indicated that ovules degenerated earlier as temperature increased, relating this to an insufficient supply of organic nutrients to the ovules. El-Ahmadi and Stevens (1979), working with tomatoes, found that ovules were affected by high temperatures more drastically than was pollen. Ovule viability was reduced in

all cases by high temperatures but cultivars differed significantly in this respect. Levy et al., (1978) found that in this species male gamete viability was affected more drastically than female gametes at high temperature. Whether the male or female gamete was affected more seriously was also found to depend on the genotype. In our experiments, 'Brooks' which consistently sets a crop seemed to be less dependant on temperature effects than 'Italian'. The effect of ethephon in preventing ovule senescence in 'Brooks' flowers was less marked and was only seen at high temperatures. Perhaps as a result of a natural tendency of 'Brooks' to produce strong ovules, these ovules can resist a higher temperature stress threshold than 'Italian' does. On the contrary, for the 'Italian' cultivar, the pattern of ovule senescence was greatly changed by ethephon. Ovule longevity was enhanced in contrast to the untreated controls. It seems that the effect of ethephon on ovule longevity is indirect rather than a direct effect on the ovules themselves.

Table 3.1. Tests of hypothesis and probability levels for the exponential growth model fitted to the responses fresh weight (FW), dry weight (DW), nitrogen (N) and potassium (K), for untreated and ethephon-treated 'Italian' and 'Brooks' flower buds<sup>z</sup>.

Null hypothesis	Response variable							
	FW		DW		N		K	
	F	P-value	F	P-value	F	P-value	F	P-value
B, B+E, I & I+E <sup>x</sup> all coincide	21.60	.000	15.79	.000	1.21	.341	4.64	.002
B and B+E coincide	10.42	.000	7.77	.001	--	--	2.90	.060
B and I coincide	25.42	.000	22.49	.000	--	--	2.13	.129
B and I+E coincide	60.33	.000	41.04	.000	--	--	4.14	.020
B+E and I coincide	3.60	.031	4.65	.013	--	--	7.69	.001
B+E and I+E coincide	20.89	.000	14.85	.000	--	--	9.92	.000
I and I+E coincide	9.09	.001	4.01	.022	--	--	1.16	.348

<sup>x</sup> B=Brooks; B+E= Brooks + ethephon; I=Italian; I+E= Italian + ethephon.

<sup>z</sup> Based on data obtained from 3 replicates of 100 terminal buds each.

Table 3.2. Tests of hypothesis and probability levels for the simple linear model fitted to the responses phosphorus (P) and calcium (Ca) for untreated and ethephon-treated 'Italian' and 'Brooks' flower buds<sup>z</sup>.

Null hypothesis <sup>x</sup>	Response variable			
	P		Ca	
	F	P-value	F	P-value
B, B+E, I & I+E all coincide	2.65	.041	3.34	.015
B and B+E coincide	0.50	.612	1.27	.300
B and I coincide	1.07	.360	1.09	.353
B and I+E coincide	2.42	.110	9.01	.001
B+E and I coincide	0.17	.847	0.49	.616
B+E and I coincide	5.02	.015	3.61	.043
I and I+E coincide	6.70	.005	4.58	.021

<sup>x</sup> I= Italian; I+E= Italian + ethephon; B= Brooks; B+E= Brooks + ethephon

<sup>z</sup> Based on data obtained from 3 replicates of 100 terminal buds each

Table 3.3. The effect of fall-applied ethephon (E) on boron content (mg) of 'Brooks' and 'Italian' prune flower buds sampled from 49 days prior to bloom until just prior to full bloom.<sup>z</sup>

Treatment	February				March			April
	17	24	03	10	18	25	31	07
Italian	11.13	12.17	14.23	12.60	15.20	14.87	20.10	45.66
Italian + E	12.43	13.83	15.13	17.53	20.40	21.60	27.07	48.60
Brooks	10.20	10.37	12.00	16.47	15.40	15.10	31.13	54.43
Brooks + E	8.76	9.87	11.03	13.13	16.50	16.10	30.77	64.00
Significance	NS	NS	NS	**	*	**	**	**
Contrasts								
Italian vs Italian + E	NS	NS	NS	**	*	***	*	*
Brooks vs Brooks + E	NS	NS	NS	*	NS	NS	NS	*
Italian + (Italian + E) vs Brooks + (Brooks + E)	NS	NS	NS	NS	NS	NS	**	**

<sup>z</sup> Average B content of 3 samples (100 buds each) from 10 trees.

Table 3.4. The number of viable ovules in 'Brooks' and 'Italian' prune flowers treated with or without ethephon, from full bloom until 20 days after full bloom, under field conditions.<sup>z</sup>

Treatment	Days after full bloom								
	0	4	6	8	11	13	15	17	20
Italian	1.8	1.5	1.4	1.0	0.8	0.8	1.0	0.6	0.4
Italian + E	1.2	1.2	1.1	1.1	1.1	1.0	0.9	1.0	0.8
Brooks	2.0	1.8	1.8	1.7	1.2	1.1	1.2	1.1	1.0
Brooks + E	1.8	1.8	1.8	1.5	1.2	1.2	1.1	1.1	1.2
Significance	***	*	*	*	NS	NS	NS	*	**
Contrasts									
Italian vs Italian + E	***	*	*	NS	*	*	NS	**	**
Brooks vs Brooks + E	NS	NS	NS	*	NS	NS	NS	NS	NS
Italian + (Italian+E) vs Brooks + (Brooks+E)	**	*	**	**	NS	NS	NS	*	**

<sup>z</sup> Average of 10 replicate flowers from 10 trees

Table 3.5. Regression equations, coefficients of determination ( $R^2$ ) and model significance probabilities ( $P>F$ ) of the number of viable ovules for untreated and ethephon-treated 'Italian' and 'Brooks' prune flower buds held at 5, 10, 15 and 20°C.

Treatment	Equation	$R^2$	$P>F$
Italian	$Y = 1.2872 + 0.1233*X_1 + 0.0831*X_2 - 0.0053*X_1^2$ $- 0.0029*X_2^2 - 0.0143*X_1X_2 + 0.00002315*X_1^2X_2^2$	0.51	0.0001
Italian + E	$Y = 1.3450 + 0.0471*X_1 - 0.0024*X_1^2 - 0.0034*X_1X_2$	0.31	0.0001
Brooks	$Y = 1.5679 + 0.0924*X_1 + 0.0635*X_2 - 0.0039*X_1^2$ $- 0.0015*X_2^2 - 0.0105*X_1X_2 + 0.00001546*X_1^2X_2^2$	0.41	0.0001
Brooks + E	$Y = 1.9345 - 0.0038*X_1 - 0.0011*X_2 - 0.0034*X_1X_2$	0.25	0.0001

$X_1$  = Temperature (°C)  
 $X_2$  = Days after full bloom

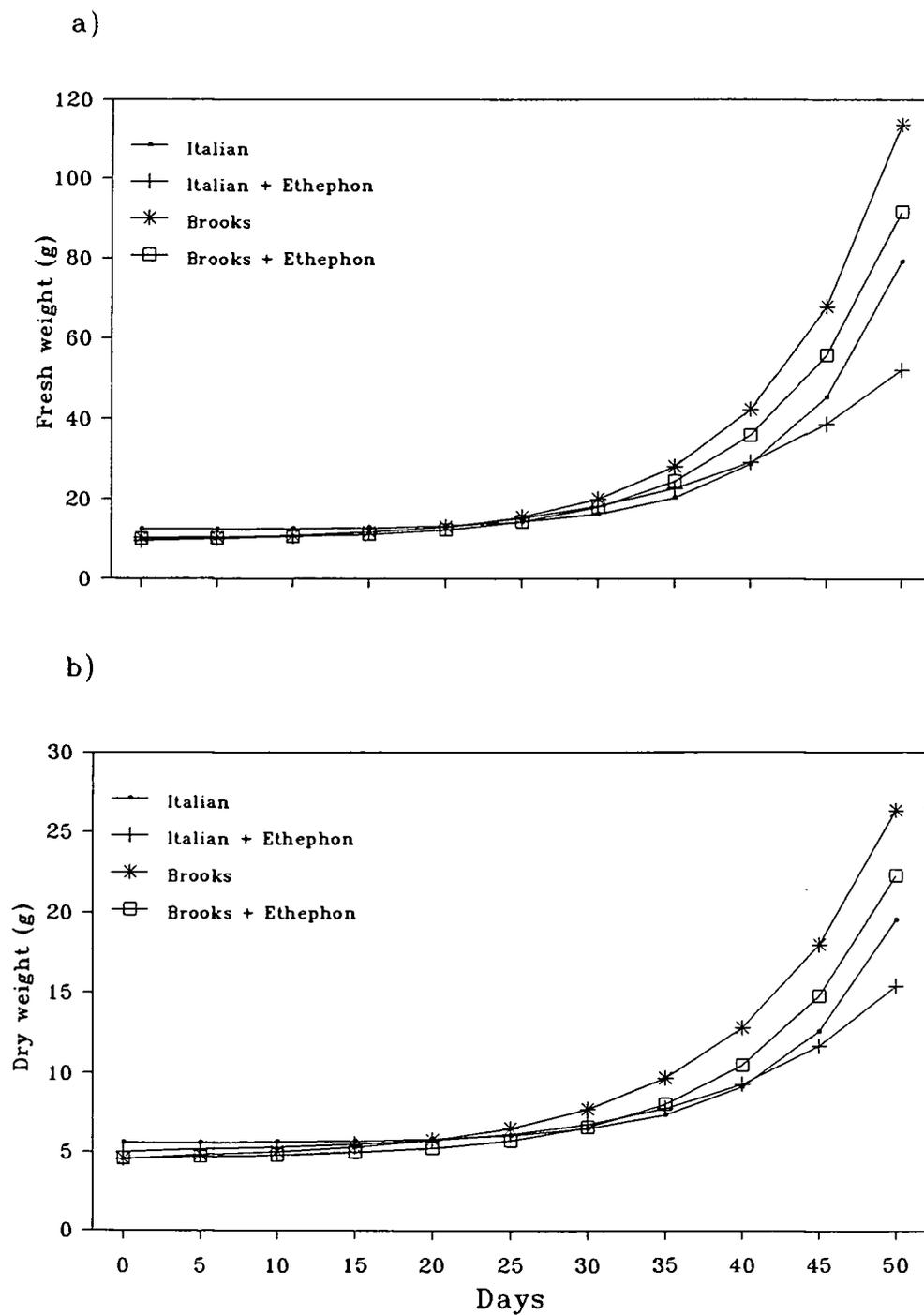


Figure 3.1. Predicted (a) fresh weight and (b) dry weight for untreated and ethephon-treated flower buds, excised from 'Italian' and 'Brooks' prune trees. Day 50 = full bloom.

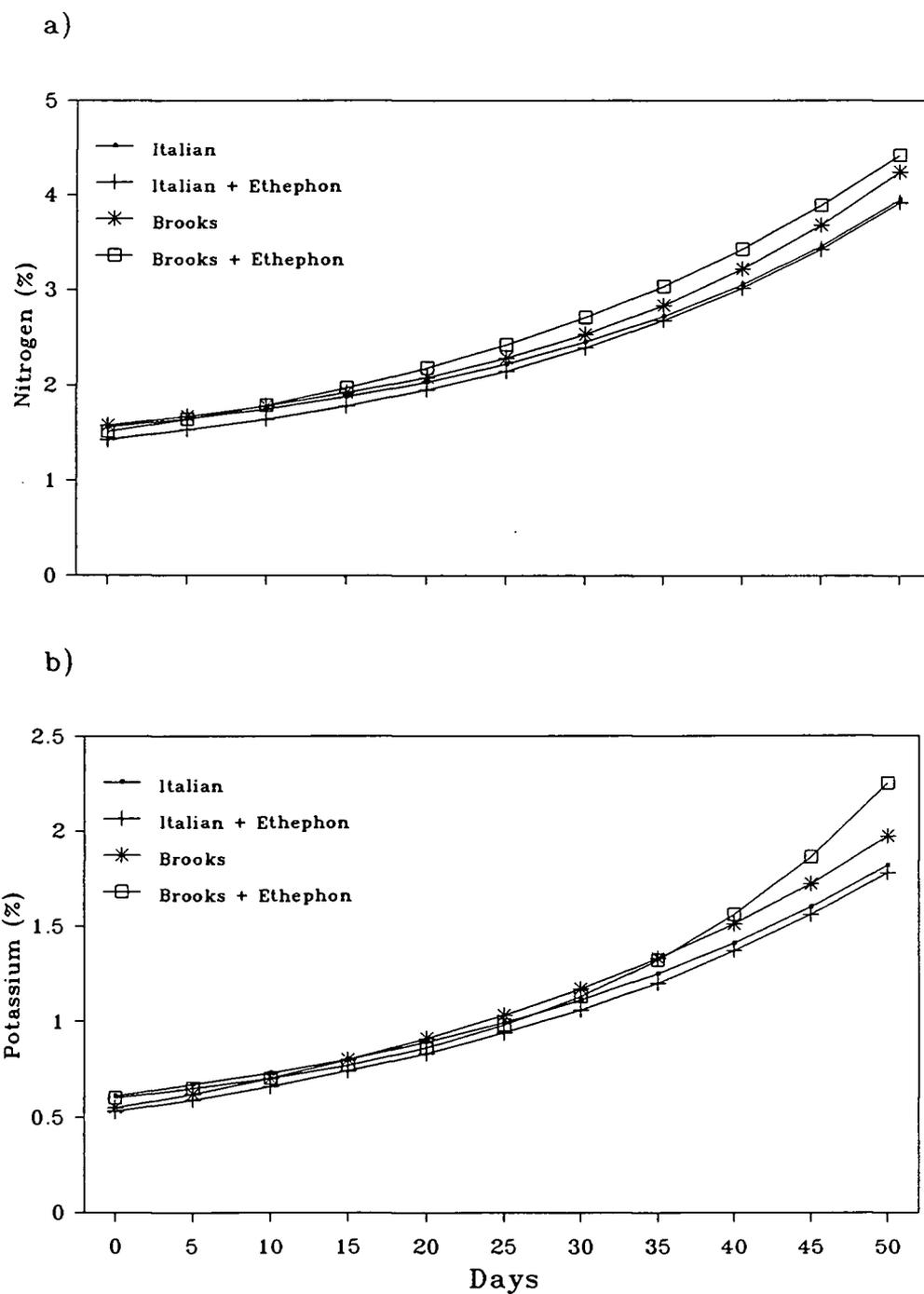


Figure 3.2. Predicted (a) nitrogen and (b) potassium concentration for untreated and ethephon-treated flower buds excised from 'Italian' and 'Brooks' prune trees. Day 50 = full bloom.

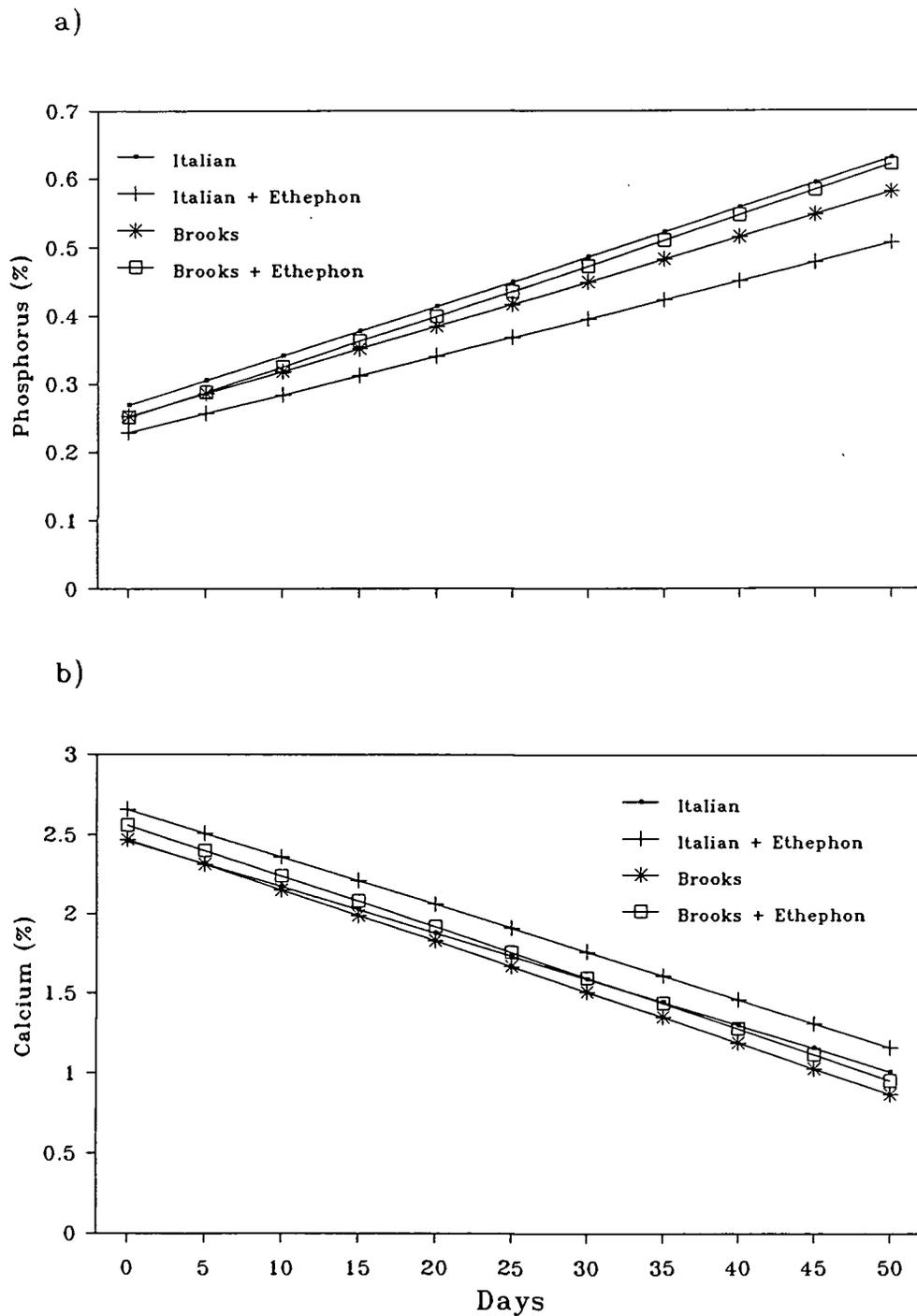


Figure 3.3. Predicted (a) phosphorus and (b) calcium concentration for untreated and ethephon-treated flower buds, excised from 'Italian' and 'Brooks' prune trees. Day 50 = full bloom.

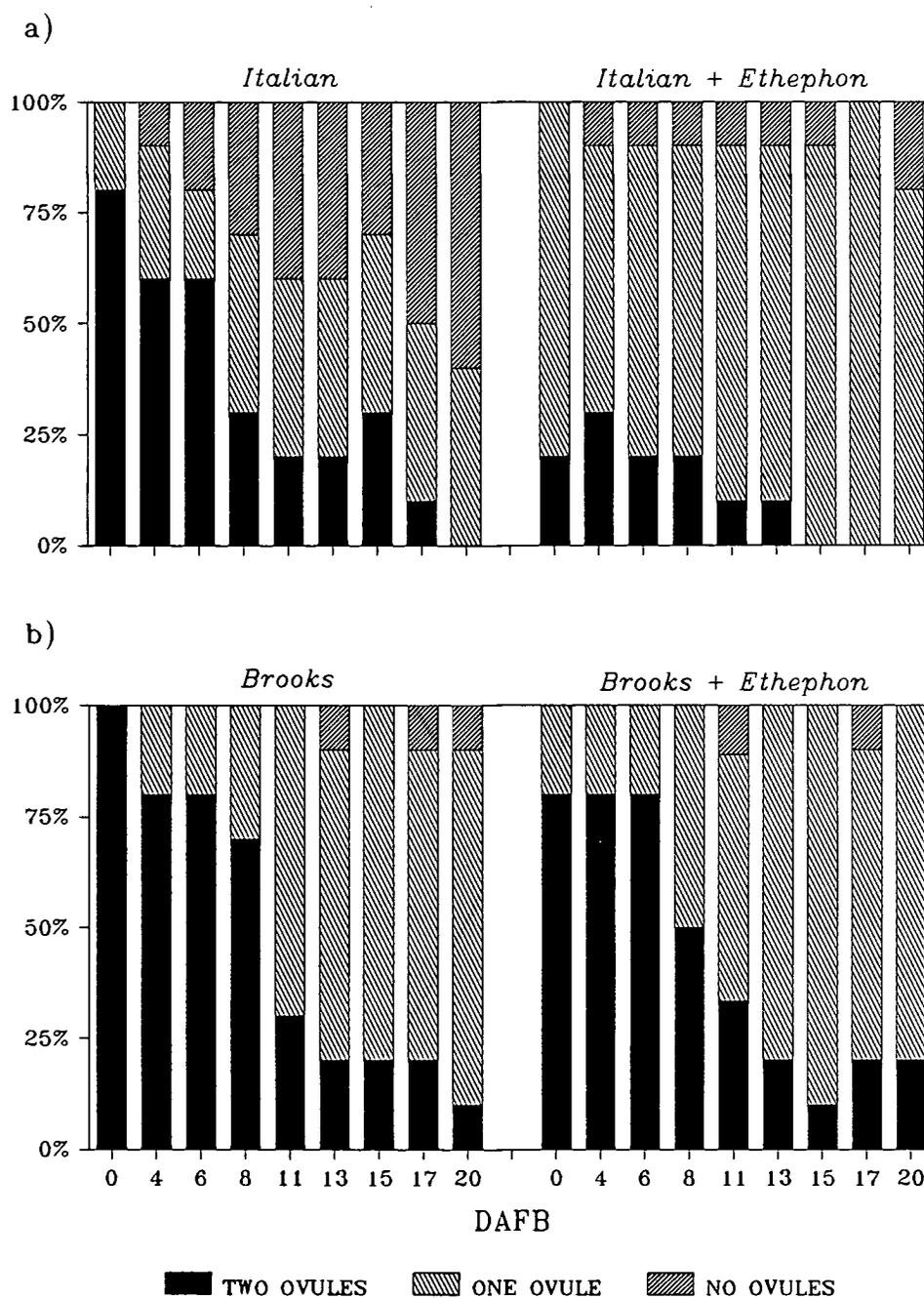


Figure 3.4. Frequency distribution of the number of flowers with two, one or no viable ovules from full bloom until 20 days after full bloom. a) 'Italian' vs 'Italian' + ethephon. b) 'Brooks' vs 'Brooks' + ethephon.

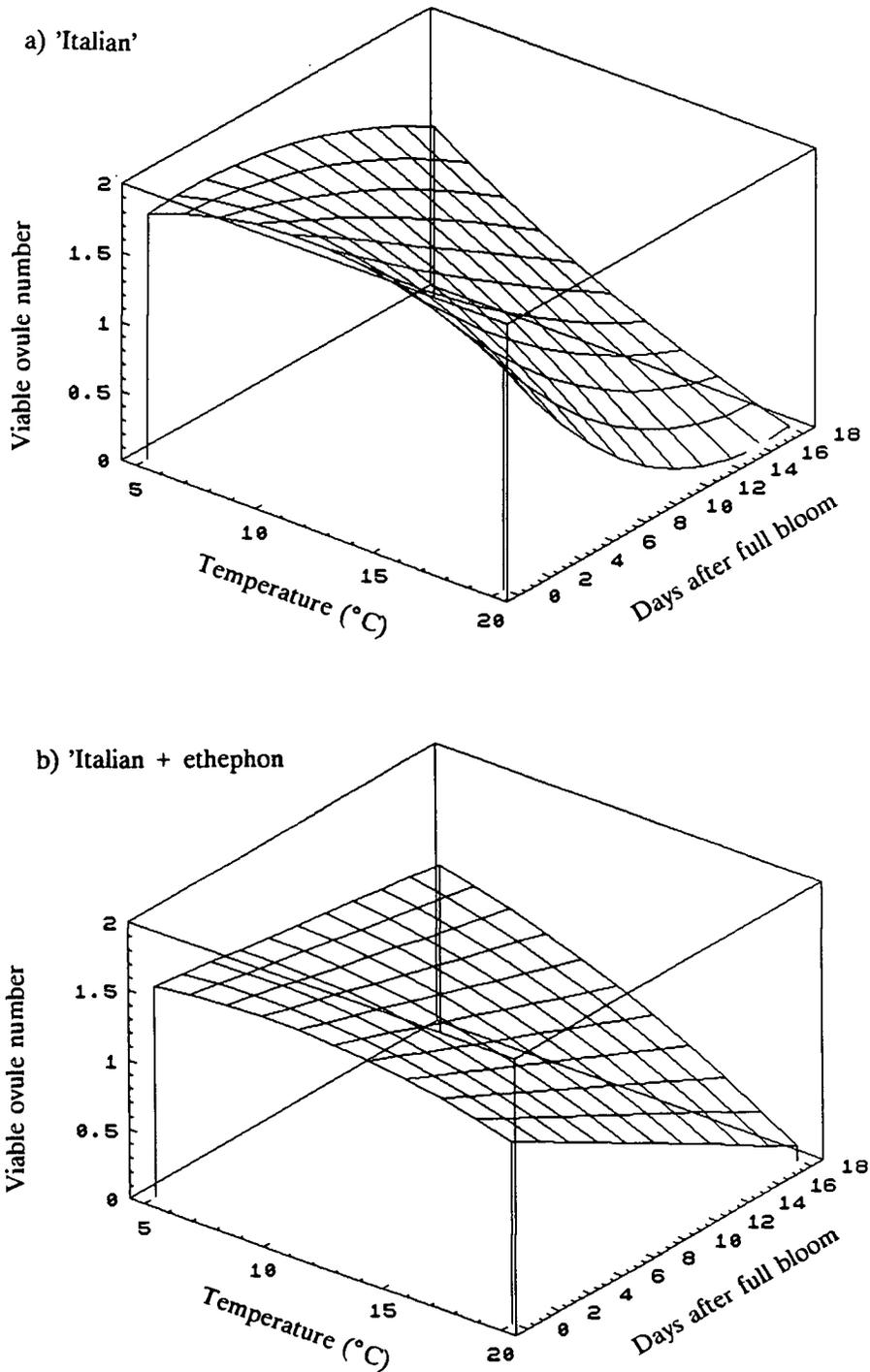


Figure 3.5. Predicted number of viable ovules derived from equations in Table 3.5 for (a) untreated and (b) fall ethephon treated ( $500 \text{ mg liter}^{-1}$ ) flowers excised from 'Italian' prune trees and held for 18 days after full bloom at 5, 10, 15, and  $20^\circ\text{C}$ .

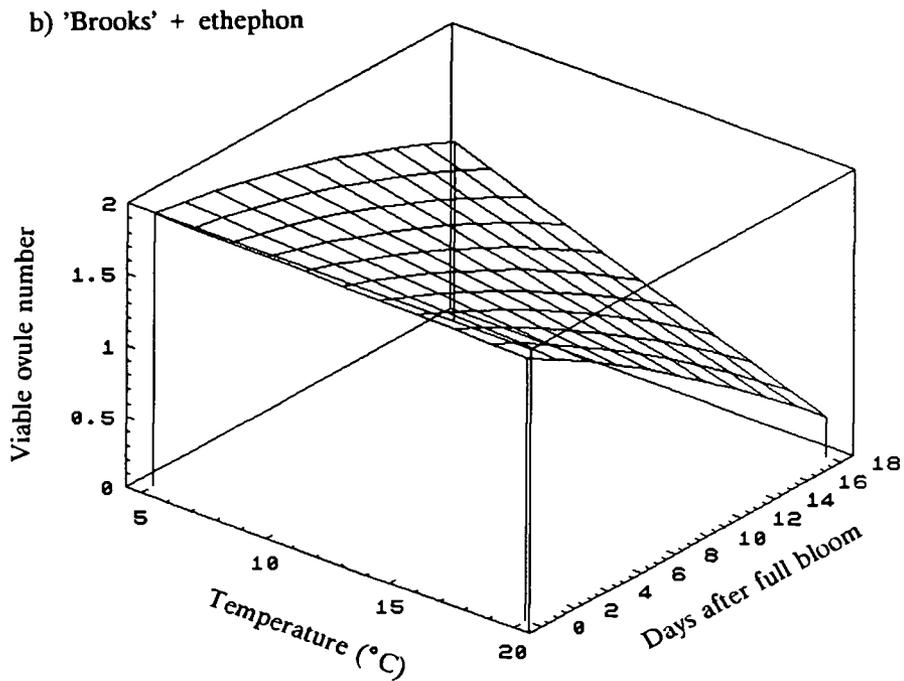
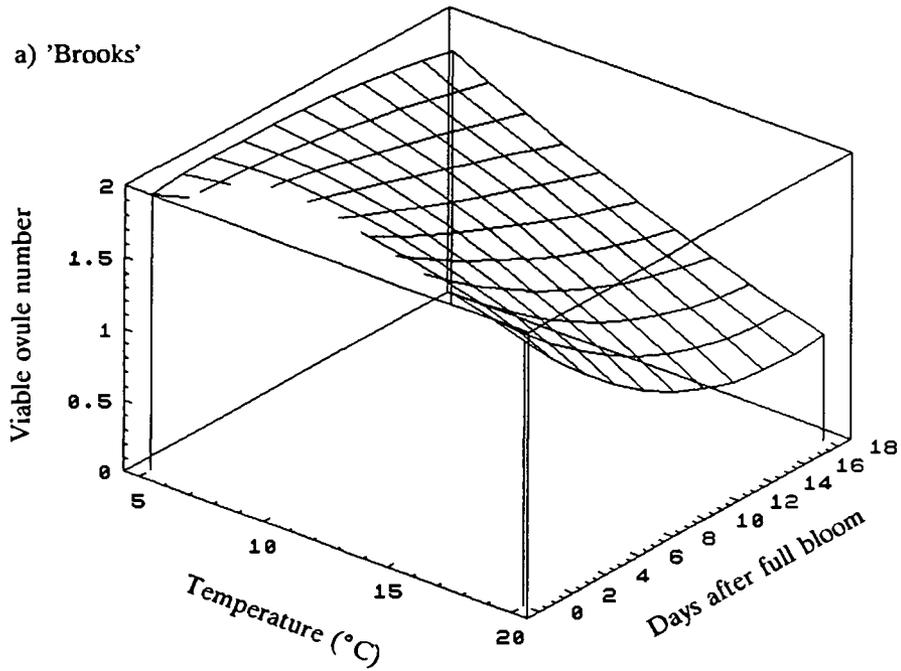


Figure 3.6. Predicted number of viable ovules derived from equations in Table 3.5 for (a) untreated and (b) fall ethephon treated ( $500 \text{ mg liter}^{-1}$ ) flowers excised from 'Brooks' prune trees and held for 18 days after full bloom at 5, 10, 15, and  $20^\circ \text{C}$ .

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## APPENDIX

Appendix 1. Parameter estimates of the exponential growth model fitted to the responses fresh weight (FW), dry weight (DW), N and K for untreated and ethephon-treated 'Italian' and 'Brooks' prune flower buds.<sup>z</sup>

Response variable	Treatment <sup>y</sup>	Parameter		
		$\alpha$	$\beta$	$\tau$
FW	I	0.058	0.141	12.30
	I + E	1.032	0.075	8.46
	B	0.314	0.116	9.87
	B + E	0.274	0.114	9.66
DW	I	0.014	0.138	5.58
	I + E	0.115	0.090	5.03
	B	0.170	0.097	4.56
	B + E	0.072	0.110	4.59
N	I	0.415	0.0382	1.143
	I + E	0.503	0.0357	0.925
	B	0.391	0.0411	1.191
	B + E	0.760	0.0315	0.750
K	I	0.320	0.0313	0.291
	I + E	0.381	0.0291	0.148
	B	0.495	0.0270	0.056
	B + E	0.160	0.0485	0.422

<sup>y</sup>I=Italian; I+E=Italian + ethephon; B=Brooks; B+E=Brooks + ethephon.

<sup>z</sup>Based on data obtained from 3 replicates of 100 buds each.

Appendix 2. Parameter estimates of the simple linear model fitted to the responses P and Ca for untreated and ethephon-treated 'Italian' and 'Brooks' prune flower buds.<sup>z</sup>

Response variable	Treatment <sup>y</sup>	Parameter	
		$\alpha$	$\beta$
P	I	0.269	0.00721
	I + E	0.229	0.00553
	B	0.253	0.00653
	B + E	0.251	0.00737
Ca	I	2.460	-0.02900
	I + E	2.660	-0.03000
	B	2.470	-0.03200
	B + E	2.560	-0.03210

<sup>y</sup> I= Italian; I+E= Italian + ethephon; B= Brooks; B+E= Brooks + ethephon.

<sup>z</sup> Based on data obtained from 3 replicates of 100 buds each.