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The Effects of Chemical and Cultural Treatments on Gibberellin Levels in Strawberry Leaves and on the Induction of Secondary Flowering

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THE EFFECTS OF CHEMICAL AND CULTURAL TREATMENTS ON GIBBERELLIN LEVELS IN STRAWBERRY LEAVES AND ON THE INDUCTION OF SECONDARY FLOWERING

by

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Richard N. Arteca

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

ABSTRACT

The Effects of Chemical and Cultural Treatments on Gibberellin Levels in Strawberry Leaves and on the Induction of Secondary Flowering

by

Richard N. Arteca, Master of Science

Utah State University, 1976

Major Professor: Dr. J. LaMar Anderson
Department: Plant Science

Gibberellins 3, 4 and 7 were isolated from "Shasta" Strawberry (Fragaria X ananassa Duch.) leaves and identified by gas and thin layer chromatography. In young expanding leaves GA_3 occurred at 5 times the concentration of either GA_4 or GA_7.

CCC (2-chloroethyl-trimethylammonium chloride), SADH (Succinamic acid-2,2-dimethyl hydrazide), ethephon (2-chloroethylphosphonic acid), and UBI-P293 (2,3-dihydro-5-6-diphenyl-1,4-oxathiin) were applied to established plantings of three June-bearing strawberry (Fragaria X ananassa Duch.) cultivars: "Shasta," "Fresno" and "Tioga." Treatments were applied on alternate days for three weeks following anthesis of the king blossom. Levels of GA_3 and GA_4 were reduced by all treatments, but GA_7 occurred at such low concentrations that treatment effects could not be measured statistically. Three weeks' exposure to short-daylengths (8 hours of light and 16 hours of darkness) resulted in no change in GA_3 or GA_7, but GA_4 concentrations

were significantly reduced. Leaf tissue was analyzed to evaluate treatment effects on chlorophyll content; no significant changes were observed. No secondary flowering as a result of photoperiod, post-harvest defoliation or growth retardant treatments was observed.

(76 pages)

INTRODUCTION

The strawberry has been classified as a short-day plant requiring short light periods and long dark periods to flower (18), but there is some variability within the species as to photoperiodic response. If exposure to light is longer than the critical period (the dark period not being long enough) plants will develop vegetatively without completing their reproductive cycle.

By shortening the length of daylight during the blossoming period, secondary flowering can be induced in the strawberry. Benoit (6, 7) has shown that an 8-hour day and a 16-hour night for 20 consecutive days has an inductive effect on the June-bearing strawberry cultivar "Redgauntlet" and that the second harvest could be obtained 98 days after the start of the short-day treatment.

Guttridge (28) showed that vegetative growth promoters are formed under long days. These vegetative growth promoters are contained within the leaf (65). Therefore, if the strawberry plant is completely defoliated or mowed down after the first crop, secondary flowering could be induced. There are unconfirmed reports that mowing has been effective in stimulating secondary flowering in tropical areas.

There is evidence for gibberellin-like substances in the strawberry (24, 55), but there is no conclusive evidence as to exactly which gibberellins are contained in strawberry leaves.

CCC (2-chloroethyl-trimethylammonium chloride) can effectively block the biosynthesis of GA_3 and other gibberellins (16, 38, 42, 52, 53). According to kinetic studies performed by Lockhart (48), CCC is

an anti-gibberellin. CCC and AMO-1618 (2-isopropyl-4-dimethylamine-5-methyphenyl-1-piperidine-carboxylate methyl chloride) inhibit vegetative growth in higher plants and gibberellin biosynthesis in Fusarium. It has been postulated by Harada and Land (38) that CCC and AMO-1618 inhibit stem growth and other responses in higher plants by inhibiting GA-biosynthesis, which is required for the growth process.

The purpose of this study was to determine the effects of growth retardants, short daylength and defoliation on endogenous gibberellin levels and secondary blossoming of "Tioga" strawberries.

REVIEW OF LITERATURE

Photoperiodic classification and comparison of June-bearing and everbearing strawberries

Everbearing varieties of strawberries are long-day plants, forming fruit-buds under long days of summer in the northern states (18). June-bearing varieties are short-day plants, rarely forming fruit-buds under natural conditions except in the fall when days become short and temperatures low. Downs and Piringer (23) compared June-bearing and everbearing varieties of strawberries at fluctuating temperatures and varying photoperiods. They reported that:

1. Runner production increased with increasing daylengths up to 15 hours in the June-bearing varieties, and with longer than 15 hour daylengths the runners were longer and in greater quantities.
2. Everbearers produced few runners under any of the photoperiods, but the trend was to produce more runners during the 13-hour days.

As a group, the everbearers produce very few, if any runners (18).

Interrelationship of temperature and photoperiod on vegetative and reproductive growth and on rest in strawberries.

Hartman (39) showed that although the June-bearing strawberry is a short-day plant, flower-buds can be initiated during long days provided the temperatures are low enough (15.5 C or less). All varieties used in his experiment formed flowers when the temperature was

maintained at 15.5 C, even under long day conditions. No flowers were formed under long days at temperatures of 21 C. The plants under short days initiated flowers at about the same rate at 15.5 and 21 C. These results in general agree with the findings of Darrow (19) and Darrow and Waldo (18) that at low temperatures flower buds may form in the strawberry under long-day light periods.

The strawberry plant in the northeastern United States becomes dormant with the onset of low temperatures in the fall (17). However, temperatures alone are not responsible for dormancy, for many plants may remain dormant under warm greenhouse conditions. When strawberry plants were left in the field until January 1 and then brought into a warm greenhouse, all varieties tested grew vigorously even though the days were short (17).

Rest in the strawberry is considered to be induced by a short-day low-temperature complex and is broken to a degree in some varieties by additional daily light exposures and in all varieties by temperature at or below freezing (18, 20). Long days and high temperatures can be very effective in breaking the rest period in strawberries (18). The strawberry differs from many other plants in that it retains its green leaves while in the resting stage unless subjected to temperatures below freezing. For this reason light and high temperatures can be very useful in breaking dormancy (20).

Piringer and Scott (54) showed that long photoperiods will stimulate runner formation without chilling, but a short period of chilling depresses runner formation, and additional chilling is then required for optimum runner formation. The longer the chilling (within the limits of their experiment (54)) the greater the number of runners.

The maximum runner production on all varieties tested occurred after long periods of winter chilling combined with long natural or artificial daylengths. Plants of all tested varieties formed runners during long days without chilling (54). Others have also shown that plants form fewer runners if they receive a short period of initial chilling, but many more runners if they receive prolonged chilling periods (3, 18, 19, 39).

Arney (2) has shown that the shortening photoperiod of autumn is the primary cause of floral initiation under natural conditions in southern England, and low night temperatures play a minor part unless they occur on consecutive nights just before the beginning of the inductive period.

Induction of flowering by mineral starvation and drought

Flowering is readily induced in some cultivars by mineral starvation before deficiency symptoms appear, by drought, or by transplanting (32). Mineral starvation induced flowering in the cultivar "Templar" in a pot plant experiment (32). Guttridge (32) showed that after five months in the summer, all 24 plants growing in a 1/3 soil-2/3 sand mixture flowered in spite of long day lengths. The limitation of fruit yields by flower inhibition in highly fertile soils or following heavy nitrogen feeding is not uncommon on commercial plantations.

A flower forming substance in the strawberry plant

Hartmann (40) hypothesized that a flower forming stimulus is manufactured in strawberry leaves under proper environmental conditions.

This stimulus is presumably translocated to the meristematic areas of the plant where it results in the differentiation of floral parts. Plants under long photoperiods become reproductive when portions of the total leaf area were subjected to short days. The number of flower clusters produced were in direct ratio to the percentage of the total leaf area placed under short day conditions. The formation of runners was inversely proportional to the percentage of the total leaf area exposed only to long days. Fully expanded leaves of the short-day plant Xanthium, exposed to long photoperiods, inhibits transmission of the stimulus through the stem to which the leaves are attached (36). Stout (63), working with sugarbeet, a long-day plant, believes that the substance conducive to reproductive development might be translocated with carbohydrates.

A vegetative growth promoter and flower inhibitor in strawberry leaves

Thompson and Guttridge (65) postulated that photoperiod control of flowering in the strawberry operated through a flower inhibitor produced in the leaves. Although strawberry leaves at any maturity can hinder flower initiation, mature leaves have a greater effect than the immature, and decreasing the photoperiod decreases the inhibitory activity, while young actively growing strawberry leaves show high gibberellin activity (24, 55). Photoperiodic response in the strawberry is controlled by a hormone produced in the leaves under long daylengths (28). This hormone was thought to induce vegetative growth and inhibit flower promotion. The removal of mature leaves removes the photoperiodic requirements for flower initiation, even with the

younger leaves present (65). When June-bearing strawberry plants were exposed (after a prolonged cold treatment) to 15.5 C and daylengths of 10, 12, 14 or 16 hours, and varying amounts of leaves were removed, the following occurred: 1) neither intact plants nor plants bearing only mature leaves initiated flowers freely when daylengths were longer than 12 hours, 2) plants with all mature leaves removed initiated flowers at 14 but not at 16 hours, and 3) totally defoliated plants initiated flowers at all daylengths. Induction was not entirely dependent upon a flower promoting substance (39), but was influenced by an inhibitor produced in the leaves (65).

Guttridge (28) presented evidence that floral induction in plants sensitive to photoperiod resulted from action at the growing apices of specific flower promoting substances or hormones. The existance of these substances is inferred by the observation that photoperiodic perception is located in the leaves, whereas response occurs at the apices, thus implying a translocatable chemical stimulus or hormone. Guttridge (28) traced P_{32} movement from the parent to the daughter plant but not in the reverse direction unless the parent plant was defoliated, or if the daughter plant was in a 17-hour and the parent in a 9-hour light period. A light-break treatment promoted vegetative growth not only to the parent plants directly exposed to it, but also in receptor plants attached by stolons. Increased promotion of vegetative growth was observed (28) when plants receiving lightbreak were exposed to a daylength three hours shorter than their attached receptor plants. The vegetative growth of plants in short photoperiods was increased either by promotive substances received via stolon from plants receiving a light break or by draining away from plants in short

photoperiods the substance which inhibits vegetative growth. Failure of the activity of a supposed flower promoting substance in this experiment and the failure to induce floral initiation in an earlier experiment (65) except under extreme conditions as mentioned earlier, is evidence for a vegetative growth promoter.

A secondary crop in June-bearing strawberries through the use of photoperiod

By controlling the photoperiod, Benoit (6, 7) obtained a secondary crop with reasonable yields from the June-bearing variety, "Redgauntlet." A shading period between the hours of 5 pm to 9 am (16-hour dark period) was the most beneficial and profitable treatment, the shorter dark periods leading to smaller yields.

Mowing and burning strawberry crops for increased yields

In such strawberry varieties as "Cambridge Favorite" that have strong photoperiodic control of inflorescence initiation, removal of the leaves fails to promote flower initiation but disturbs the normal pattern of the process (49). In varieties where photoperiodic control of flowering is weaker and subject to modification, defoliation permits the initiation of inflorescences despite the inhibitory conditions imposed by long days. Flower truss initiation in defoliated plants (cultivars "Talisman" and "Redgauntlet") exceeded that of the untreated plants. This may have resulted from a decrease in the concentration of an inhibitor of inflorescences, as old leaves are more inhibitory than young ones (28, 65). In the varieties "Cambridge Favorite" and "Royal Sovereign," which do not initiate inflorescences in the summer, autumn

initiation was reduced by defoliation. Although the rate of truss initiation subsequently increased, it frequently failed to reach the levels attained by the untreated plants before growth ceased in the winter (49).

Guttridge and Mason (31) showed that early defoliation (before mid-August) was better than late defoliation irrespective of the variety. Varietal differences in yield response to defoliation paralleled differences in truss initiation. Yields were not increased unless initiation of trusses were promoted.

In strawberry plants that were burned or cut after cropping, the following occurred: 1) crops following burning produced higher yields, except when wilted plants were burned, and 2) cutting the strawberry plants increased yields over the controls (70). It appeared that removal of leaves is advantageous to increased yields (28, 40, 65, 66).

Gibberellin biosynthesis

The isoprenoid nature of the gibberellins and their general biosynthetic relationship to diterpenes has been well defined. An overall scheme (70) is as follows:

1. Mevalonate is a precursor of the acylic diterpene geranyl-geranyl-pyrophosphate,
2. Cyclization of this substance to kaurene,
3. Modification kaurene by oxidation, and ring contraction and elimination of an angular methyl group to form C_{19}-gibberellins.

West and Fall (69) stress that this scheme pertains to studies in fungus and that sequences may differ from one organism to another even though the patterns are similar.

Studies on the biosynthesis of gibberellic acid in Fusarium moniliforme have established (-)-kaurene as a precursor (15, 25). In higher plants this pathway is still unresolved. Concentrations as low as 0.1 mg/ml CCC in the growth medium will reduce fungus gibberellin production by 50 percent, whereas 10 mg/ml or more fully suppresses GA-biosynthesis (52). Tracer studies (4) with intact fungus show that 0.13 M CCC blocks GA-biosynthesis between geranyl-geranyl-pyrophosphate and kaurene (at kaurene synthetase). Similar conclusions were reached by Cross and Meyers (16) on the effects of CCC on metabolite production in cultures of Fusarium moniliforme and by Kende (42) who has shown that Fusarium moniliforme grown in the presence of CCC does not produce gibberellins.

Kinetic studies on growth retardants

Kinetic studies by Lockhart (48) showed that CCC and Phosphon D (tributyl-2,4-dichlorobenzyl-phosphonium chloride) retard elongation by partially blocking the system that provided active gibberellin to the growth mechanism. CCC and Phosphon D acted as competitive inhibitor of growth, whereas excess gibberellin added to the system overcame their effects, thereby classifying them as anti-gibberellins. However the inhibition of stem growth by maleic hydrazide was shown (33) to be independent of the promoting effect of gibberellin. Therefore, maleic hydrazide is a noncompetitive inhibitor of growth and GA-biosynthesis since additional gibberellin will not cause a reversal of its effects. Maleic hydrazide is not classified as an antigibberellin (33).

Effects of growth retardants on gibberellin biosynthesis

Harada and Lang (38) showed that CCC and AMO-1618 (2-isopropyl-4-dimethylamino-5-methyl-phenyl-1-piperidinecarboxylate methyl chloride)

inhibit growth responses in higher plants and GA-biosynthesis in Fusarium. They postulated that CCC and AMO-1618 inhibited stem growth and other responses in higher plants due to the inhibition of GA-biosynthesis, which was required for the growth process. The following studies support this conclusion: CCC does not affect the induction of amylase synthesis in barley endosperm by exogenous gibberellin (53), but CCC lowers the content of extractable gibberellins in seedlings of peas (44) and Pharbitis nil (74), and long term treatments of CCC on balsam fir resulted in the absence of gibberellin-like substances in its sap (57).

The growth retardants CCC and AMO-1618 are more effective inhibitors of GA-biosynthesis when Fusarium cultures were maintained in continuous light (55). Large amounts of GA_3 and GA_7 were produced in the light, whereas only GA_7 was produced in the dark. Mertz and Henson (50) showed that these growth retardants caused complete disappearance of gibberellins in the dark, but this was not possible in the light. GA_3 was always detectable. Fusarium moniliforme showed variation in sensitivity to growth retardants, thereby leading to the possibility of more than one pathway of gibberellin synthesis, as has been observed in higher plants (50).

CCC shortens petioles and decreases top and root growth of strawberry plants (30). The addition of gibberellic acid overcame this depression of petiole length and increased top growth (fresh weight) but did not affect root growth. CCC caused a decrease in petiole lengths and suppressed the initiation and elongation of runners, but it did not induce the formation of flower trusses under long photo-

periods (29). CCC promoted the effects of short daylengths and had effects opposite to those of gibberellic acid and long daylengths with respect to vegetative growth, but there was no truss formation (64).

In a study by Jones and Phillips (41), CCC was found to significantly decrease the gibberellin content in excised sunflower organs. This decrease was overcome by the addition of gibberellic acid. Young and Cooper (72) showed similar results in redblush grapefruit.

Lesham and Koller (47) hypothesize that peduncle elongation may be associated with an increase in gibberellin activity. Gibberellin-like substances are lacking in the leaves of strawberry plants during flower initiation, but during peduncle elongation there was an increase in two substances, possibly GA_3 and GA_7. Exogenous GA_3 applied at the time of flower differentiation enhanced peduncle elongation, whereas CCC, an inhibitor of GA-biosynthesis, caused the opposite effect.

Tolbert (68) showed that CCC and related substances caused shorter thicker stems, broader greener leaves, earlier stronger tillering, and more uniform growth of wheat. These changes are similar to those produced by high light intensity and opposite to the effects of gibberellins.

Reid and Crozier (59) showed with the use of a rice bioassay that 1 mg/l CCC stimulated gibberellin levels 150 fold. However, plants treated with 1000 mg/l CC contained approximately the same amount of gibberellins as the untreated pea seedlings. Similar results are reported by several authors (8, 10, 35, 43, 58). In contrast, many studies found large decreases in gibberellin activity (15, 29, 39, 38, 41, 42, 44, 48, 52, 53, 57, 73). Additional research is required in this area.

CCC and AMO-1618 when added to cultures of Gibberella fujikouri at the beginning of gibberellin biosynthesis completely suppressed the biosynthesis of gibberellic acid and the diterpene(-)-kaurene,7-hydroxykarnolide and 7,18-dihydroxykaurenolide (16).

CCC in high concentrations inhibits chlorophyll and protein synthesis (8). At low concentrations CCC inhibits gibberellin biosynthesis in Jack bean leaves. Greenwald (27) showed that 0.1 mM of CCC destroyed chlorophyll and thereby dropped the carotinoid levels. The decrease of carotinoids prior to chlorophyll reduction and their rise before chlorophyll resynthesis strongly suggested that CCC played a role in bleaching of the Jack bean leaves. Greenwald (27) hypothesized that this bleaching was a result of photooxidation of chlorophyll.

Ethephon (2-chloroethylphosphonic acid) induced flowering and growth regulatory properties in pineapples (13). Ethephon degrades to form ethylene, thereby, inducing flowering. Ethephon also induces flowering in bromeliad and inhibits flowering in hydrangeas (1, 21).

In immature peach ovules the synthesis of kauren-19-01, a gibberellic acid precursor, was depressed in the presence of SADH (succinamic acid-2,2-dimethyl hydrazide) (60). SADH inhibits internode elongation in shoots (5, 12, 34, 56, 75), hastens the maturity of stone fruits (5) and enhances anthocyanin formation. Low concentrations of SADH enhanced flower initiation in the Fuschia cultivars, "Lord Byron" and "Hollydale" (60). SADH depressed the synthesis of gibberellin precursors in Cucumis satiuus (34) and in Echinocystis endosperm (22), but in two studies (38, 52) SADH has been shown to be ineffective in blocking GA-biosynthesis.

Cathey (12) stated that growth retardants are highly specific and that there is a great deal of variation with respect to plant response. Cathey and Stuart (11) showed that CCC has the ability to retard growth on a greater number of plants than many other growth retardants. There is no known interaction between growth retarding chemicals and phytochrome (12). A study by Kuraishi and Muir (46) showed that retardation of stem growth by CCC is due to the lack of IAA and not dependent upon gibberellins. Classifying CCC as an antigibberellin (48) may be misleading (46). Retardation of growth by CCC may be counteracted in intact plants with gibberellins because such treatments increase the levels of diffusable auxin (45, 46).

Moore and Hough (51) showed a major decrease in auxin levels within the strawberry during floral induction. After flowering the auxin level increases even though short-day conditions still prevail. As cited by Moore and Hough (51), Hashizume (37) found that the IAA content in Cryptomeria japonica decreases prior to floral induction and is increased immediately after under short-day conditions. Thompson (66) also supports this theory by showing that auxin levels decrease during flower initiation and later increase in the strawberry. In the past, treatments with auxin have been shown to inhibit flower initiation in petunias (26) and Xanthium (9, 67). Present research shows that auxin effects are almost certainly indirect. Salisbury (61) has shown that quantities of auxin required to inhibit flowering in Xanthium also causes epinasty indicating that these effects are not typical of untreated plants.

MATERIALS AND METHODS

Plots of established June-bearing strawberry cultivars "Fresno," "Shasta" and "Tioga" were set up in a randomized block design at the Horticultural Field Station, Farmington, Utah, on May 9, 1975. Each plot was 1.8 m in length and contained double row beds of each variety. On May 13, 1975 anthesis of the king blossom was evident, and repeated low concentration applications of growth retardants were initiated along with a 10-hour daylength treatment.

Growth retardant applications

The growth retardants CCC, SADH, ethephon and UBI-P293 were applied with a pressurized knapsack sprayer. The treatments, according to the rates outlined in Table 1, were applied 3 times a week from May 13, through June 3, 1975.

Table 1. Treatments to determine effects on gibberellin biosynthesis and secondary flowering.

Treatment	Rate
UBI-P293	1080ppm
UBI-P293	540ppm
Ethephon	560ppm
Ethephon	280ppm
10 Hr. Daylength Control	
CCC	920ppm
CCC	460ppm
SADH	500ppm
SADH	250ppm

On May 20, 1975 no application was made due to extremely wet conditions. On May 26 a heavy frost killed many king blossoms. The low temperature for the day was -1.1°C. The black plastic covered plots sustained less damage than the uncovered plots. Thermograph readings showed that temperatures under the black plastic were 1.5 to 2.0°C higher than the outside temperature.

Photoperiod

A photoperiod study was conducted following the procedure outlined by Benoit (7), shading from 5 pm to 9 am for 21 consecutive days following anthesis of the king blossom. Shading was accomplished by covering the beds with 1.5 x 2.5 M strips of 5 mil black polyethylene plastic supported by wooden slats 5 cm wide and 1 M long bent in an arc-shape and held in position by wires attached at each end. After the supports were in place, 0.3 M of plastic was buried on one side of the row. The remainder was draped over the top of the arc-shaped slats extending 0.3 M beyond the bottom of the support. This excess was stapled to a 2.5 M post expediting the daily covering and uncovering of the plants. After the 3-week short-day period, plants were subjected to normal summer daylengths.

Harvesting methods

Fruits were harvested from June 20 through July 7, 1975 when the later cultivar, "Tioga," was through bearing. The entire experimental area was harvested 5 times during this period. The sixth picking consisted of only "Tioga." Berry weights were taken per variety per

treatment. Sizes were evaluated according to commercial sizing standards, the #1 (1.9 cm diameter), #2 (1.6 cm diameter) and #3 (.9 cm diameter).

On July 15, 1975, one row of each variety was mowed, and all rows were immediately watered. This treatment removed the foliage of half the plants in each plot, allowing the evaluation of post-harvest defoliation alone and in combination with growth retardant treatments on inducing secondary flowering.

Gibberellin extraction and purification

Leaf samples were harvested July 3, 1975 and stored at -20°C to prevent breakdown or significant concentration change of naturally occurring gibberellins until analysis. Gibberellins were extracted and identified following the procedure outlined by Seeley (62), with slight modifications. First, a one gram sample size was used because with higher amounts, chlorophyll and fat globules were in larger quantities and harder to remove. Second, the centrifugation periods in the fractionating steps were 60 minutes at 8,000 g. Third, the solvent solution used in the thin layer chromatography was that of Coombe and Tate (14), $CHCl_3$;EtOH;HCOOH:85;15;1; for it provided good separation of GA_3, GA_4 and GA_7 from each other and from impurities remaining within the sample.

Chromatographic analysis

Gas chromatographic analysis of gibberellins was performed with a Hewlett-Packard 5750 gas chromatograph. Glass coiled columns 180 cm long with an inside diameter of 3 mm were used. The carrier gas was a mixture of 95 percent argon and 5 percent methane with a flow rate of 60 cc per minute. A nickle 63 electron capture detector connected to a

Tracor MT recorder was used for detection. Three percent OV-17 liquid phase on Gas-Chrom Q, 100-120 mesh was used. Three percent DC-200 liquid phase on Gas-Chrom Q, 100-120 mesh was used to confirm results attained from OV-17. Injector port, oven and detector temperatures were 250°C, 220°C and 275°C respectively. Data were analyzed quantitatively by using a standard curve, peak height in mm's vs. amount in nanograms. The amounts injected were 1/4 ng, 1/2 ng, 1 ng and 2 ng, which produced a linear relationship for the range involved.

Chlorophyll determinations

Total chlorophyll and chlorophyll A and B were determined following the method of Whitham, Blaydes and Devlin (71) with slight modifications. Chlorophyll extraction from a 1 g sample (fresh weight) of strawberry leaves in 75 ml of chilled acetone was homogenized with a Virtis-45 homogenizer for ten minutes. After the leaves were thoroughly ground, the green liquid was transferred to a Buchner funnel, containing a pad of Whatman No 1 filter paper. Following chlorophyll removal from the leaf tissue, it was transferred to a graduated cylinder where the final volumn was brought up to 100 ml with 80 percent acetone for convenience of calculation. The extract was then transferred to a 125 ml Erlenmeyer flask and refrigerated until spectrophotometric analysis. In each of the previous steps the extract was kept on ice and under low light conditions to prevent chlorophyll breakdown. For the chlorophyll determination the optical density of the chlorophyll extract in a 10 mm cell was read and recorded with a Beckman model DB-6 spectrophotometer set at 645, 663, and 652 nm. An 80 percent acetone aqueous solvent was used as a blank. The amount of chlorophyll present in the extract was

calculated on the basis of mg chlorophyll per gram of leaf tissue extracted, by the following equations (71):

$$\text{mg of chlorophyll a/g tissue} = (12.7(D663) - 2.69(D645)) \times \frac{V}{1000 \times W} \quad [1]$$

$$\text{mg of chlorophyll b/g tissue} = (22.9(D645) - 4.68(D663)) \times \frac{V}{1000 \times W} \quad [2]$$

$$\text{mg of total chlorophyll/g tissue} = (20.2(D645) + 8.02(D663)) \times \frac{V}{1000 \times W} \quad [3]$$

$$\text{mg of total chlorophyll/g tissue} = \frac{D652 \times 1000}{34.5} \times \frac{V}{1000 \times W} \quad [4]$$

Equation [4] was used as a check on equation [3].

RESULTS AND DISCUSSION

Effects of growth retardants and photoperiod on gibberellin and chlorophyll levels in strawberry leaves

The effects of the growth retardants CCC, SADH, Ethephon, UBI-P293 and photoperiod on levels of GA_3, GA_4 and GA_7 are shown graphically in Appendix Figures 1, 2 and 3 respectively. Orthoganol comparisons (Appendix Tables 2, 3 and 4) along with a Duncan's multiple range test (Appendix Tables 6, 7 and 8) were used to determine the significant differences between the various treatment means as compared to the controls. All tests were run at the 95 percent confidence level unless otherwise stated.

High levels of GA_3 were found in the untreated plots (Appendix Figure 1); 8.925 nanograms was the mean for the four one gram samples. Orthoganol comparisons (Appendix Table 2) indicated: 1) both concentrations of UBI-P293 significantly decreased GA_3 levels as compared to the controls at the 95 percent confidence level, and with the lower concentration there was significance at the 99 percent confidence level; 2) ethephon at the higher concentration showed no significant decrease in GA_3, while at the lower concentration there was a definite decrease; 3) 10-hour photoperiod produced no significant decrease in GA_3; 4) CCC produced no significant decrease at the higher concentration, but the lower concentration produced a significant decrease in GA_3 levels; and 5) SADH at the higher concentration produced a significant decrease in

GA_3 at the 95 percent confidence level and at the lower concentration there was s significant decrease at the 99 percent confidence level.

The above data show that the lower concentrations reduced GA_3 levels to a greater extent than the higher concentrations. The Duncan's multiple range tests (Appendix Table 6) verified the results from the orthoganol comparisons (Appendix Table 2).

GA_4 occurred in lower concentrations than GA_3, but treatment effects were still measurable (Appendix Figure 2). The orthoganol comparisons (Appendix Table 3) showed: 1) at the higher concentration of UBI-P293 there was no significant decrease in levels of GA_4, although at the lower concentration there was a significant decrease; 2) ethephon did not produce a significant decrease at either concentration; 3) photoperiod produced a significant decrease in GA_4; and 4) CCC and SADH each produced significant decreases at both concentrations. The Duncan's multiple range test (Appendix Table 7) verified the results attained from the orthoganol comparisons (Appendix Table 3).

Natural levels of GA_7 were lower than either GA_3 or GA_4. Orthoganol comparisons (Appendix Table 4) and the Duncan's multiple range test (Appendix Table 8) indicated no significant differences in levels of GA_7 for any of the treatments. Levels of GA_7 are shown graphically in Appendix Figure 3.

A Duncan's multiple range test was run in order to evaluate data (Appendix Figure 4) attained from the chlorophyll determination study. There was no significant increase in chlorophyll levels with any of the treatments (Appendix Table 5).

There has been evidence on the occurrence of gibberellin-like substances in actively growing strawberry leaf tissue (24, 55), but there has been no conclusive evidence as to exactly which gibberellins were contained in strawberry leaves. The preceding results constitutes present evidence or the occurrence of GA_3, GA_4 and GA_7 in young actively growing leaf tissue taken from the June-bearing cultivar, "Tioga."

Evidence has been presented that CCC effectively blocks GA-biosynthesis in Fusarium moniliforme (38, 42, 52, 53), Pharbitis nil (74), Gibberella fujikuori (16), Balsam fir (57), pea seedlings (44), redblush grapefruit (72), and excised sunflower organs (41). Thus agreeing with the results obtained in this experiment.

SADH was ineffective in blocking GA-biosynthesis in Fusarium moniliforme (52), but the above results show effective blocking of GA_3 and GA_4 in strawberry leaves.

According to the findings of this experiment, it may be concluded that: 1) multi-low rate applications of all growth retardants used produced a significant decrease in GA_3 and GA_4 but not in GA_7, 2) chlorophyll content was unaffected by growth retardants at the concentrations used, and 3) a 10-hour photoperiod decreased GA_4 but not GA_3 or GA_7.

Effects of growth retardants and photoperiod on weight and size of the June crop

The mean weight of 4 replications (three varieties combined) are shown separately for each treatment and for each day of harvesting (Appendix Figures 5 through 14). There was a progressive increase in yields for all treatments from the first day of harvest until the last, when the weights began to level off. There was a large deviation around

the mean. This error was due in part to: 1) differences in picking procedures of the people who aided in harvesting and 2) inconsistency in the number of plants contained within the plots prior to treatment; in some areas plants were densely matted, while other areas contained a sparse amount of plants.

Orthoganol comparisons were made from the overall weight difference between each individual-growth retardant-treated plot and the controls showed no significant difference (Appendix Table 10). Lack of significance was due to a large sampling error. This is shown graphically by comparison of Appendix Figures 5, 6, 7, 8, 11, 12, 13, 14 with Appendix Figure 10.

Orthoganol comparisons of the mean weights per day were made between photoperiod plots and controls (Appendix Table 9). The comparisons showed that on the second day of harvesting, the yield from plots covered with black plastic were significantly higher than the controls. The rest of the comparisons showed no significant differences (Appendix Figures 9 and 10). The overall difference in weight was not significant (Appendix Table 10, comparison 5).

Overall yields were reduced due to a heavy frost on May 26, 1975, which killed many of the king blossoms. Plants under the black plastic canopy sustained less damage than the uncovered plants.

Size comparisons were made between the treated plots and the controls (Appendix Figures 15 through 24). The mean number of each size grade for four replications (three varieties combined) are shown separately for each treatment, for each day of harvesting.

Orthoganol comparisons were made between the number of size grade ones in each treatment and the control. The overall amount of size one

berries in the plots with a 10 hour photoperiod were significantly higher than the controls (Appendix Table 11 comparison 5 and Appendix Figures 19 and 20). There was no significant difference between the growth retardant-treated plots and the controls (graphically shown by comparison of Appendix Figures 15, 16, 17, 18, 21, 22, 23, 24 with Appendix Figure 20). The significantly higher number of size one fruit may have been due to a warmer temperature under the black plastic canopy, which protected many of the king blossoms. The only blossoms that were killed under the black plastic were those that were in contact with the black plastic during the frost.

Orthanoganol comparisons were also made between the number of size two fruit in the treated and untreated plots. These comparisons indicated no significant differences (Appendix Table 12 and graphically by comparison of Appendix Figures 15, 16, 17, 18, 19, 21, 22, 23, 24 with Appendix Figure 20).

The number of size three fruit for all treatments was very low (Appendix Figures 15 through 24), insignificant, and not analyzed.

According to these findings, the following may be concluded: 1) growth retardants at the concentrations used did not affect the yield and size of the June crop, and (2) plastic covering of the photoperiod treated plots protected king blossoms against late frosts, thereby increasing yields in the earlier stages of harvesting. This treatment had no effect on the overall yield, which is in agreement with the results attained by Benoit (6, 7).

Effects of growth retardants, photoperiod and post-harvest defoliation on secondary flowering

The growth retardant-treated plots did not produce a secondary crop, possibly due to the timing of application.

A 10 hour daylength was ineffective in producing a secondary crop. This may have been due to timing; it is quite possible that the varieties, "Shasta," "Fresno" and "Tioga," require different photoperiod manipulation than Redgauntlet, the variety used by Benoit (6, 7). "Redgauntlet" naturally produced a secondary crop, but the yields are extremely low, and manipulation of photoperiod increases these yields; whereas, the varieties used in this experiment do not naturally produce a secondary crop. More research is required in this area in order to determine the exact photoperiodic requirements for these varieties.

Post-harvest defoliation was also ineffective in producing a secondary crop. With varieties having strong photoperiodic control of inflorescence, such as "Cambridge Favorite," removal of the leaves fails to promote initiation and disturbs the normal pattern of this process (49). "Shasta," "Fresno" and "Tioga" may also have strong Photoperiodic control over flowering, therefore not initiating flower buds under noninductive cycles.

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APPENDIX

Table 2. Analysis of variance table for orthoganol comparisons between mean levels of GA_3 per gram of strawberry leaf tissue ((c.v. Tioga) for each of the treatments listed below and the mean.

ANOV	d.f.	S.S.	M.S.	Fcal.	Ftable
Treatments	9	88.65	9.85	1.70	2.21
UBI-P293 (1080 ppm)	1	30.42	30.42	5.241	*4.17
UBI-P293 (720 ppm)	1	54.08	54.08	9.32	**4.17
Ethephon (564 ppm)	1	13.26	13.26	2.284	4.17
Ethephon (282 ppm)	1	33.21	33.21	5.72	*4.17
Photoperiod (10 hr. daylength)	1	15.68	15.68	2.70	4.17
CCC (920 ppm)	1	15.125	15.125	2.61	4.17
CCC (460 ppm)	1	42.78	42.78	7.37	*4.17
SADH (502 ppm)	1	34.03	34.03	5.863	*4.17
SADH (251 ppm)	1	54.60	54.60	9.41	**4.17
Residual	30	174.14	5.8045		
Corrected Total		262.79			

*Significant at the 95 percent confidence level.
**Significant at the 99 percent confidence level.

Table 3. Analysis of variance table for orthoganol comparisons between mean levels of GA_4 per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments listed below and the mean.

ANOV	d.f.	S.S.	M.S.	Fcal.	Ftable
Treatment	9	71.42	7.935	1.62	2.21
UBI-P293 (1080 ppm)	1	9.57	9.57	1.95	4.17
UBI-P293 (720 ppm)	1	29.26	29.26	5.97	*4.17
Ethephon (564 ppm)	1	9.90	9.90	2.02	4.17
Ethephon (282 ppm)	1	20.16	20.16	4.12	4.17
Photoperiod (10 hr. daylength)	1	40.04	40.05	8.17	*4.17
CCC (920 ppm)	1	40.05	40.05	8.17	*4.17
CCC (460 ppm)	1	26.28	26.28	5.36	*4.17
SADH (502 ppm)	1	26.48	26.48	5.4	*4.17
SADH (251 ppm)	1	40.05	40.05	8.17	*4.17
Residual	30	147.0	4.9		
Corrected Total		218.426			

*Significant at the 95 percent confidence level.

Table 4. Analysis of variance table for orthoganol comparisons between mean levels of GA_7 per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments listed below and the mean.

ANOV	d.f.	S.S.	M.S.	Fcal.	Ftable
Treatment	9	21.8	2.42	.609	2.21
UBI-P293 (1080 ppm)	1	.396	.396	.1	4.17
UBI-P293 (720 ppm)	1	1.78	1.78	.45	4.17
Ethephon (564 ppm)	1	.5832	.5832	.15	4.17
Ethephon (282 ppm)	1	3.73	3.73	.94	4.17
Photoperiod (10 hr. daylength)	1	5.37	5.37	1.35	4.17
CCC (920 ppm)	1	2.27	2.27	.57	4.17
CCC (460 ppm)	1	1.67	1.67	.42	4.17
SADH (502 ppm)	1	8.632	8.632	2.17	4.17
SADH (251 ppm)	1	13.16	13.16	3.29	4.17
Residual	30	119.69	3.98		
Corrected Total		141.48			

Table 5. Duncan's Multiple Range Test for mean levels of chlorophyll per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments listed below.

Treatments	Yi	P_{10} D=.467	P_9 D=.464	P_8 D=.460	P_7 D=.456	P_6 D=.450	P_5 D=.443	P_4 D=.433	P_3 D=.420	P_2 D=.389
CCC (920 ppm)	2.06	*.70	*.63	*.47	.34	.34	.31	.29	.23	.18
SADH (502 ppm)	1.88		*.52	.45	.29	.16	.16	.13	.11	.05
CCC (460 ppm)	1.83			*.47	.40	.24	.11	.11	.08	.06
Photoperiod (10 hr. daylength)	1.77				.41	.34	.18	.05	.05	.02
UBI-P293 (1080 ppm)	1.75					.39	.32	.16	.03	.03
Ethephon (564 ppm)	1.72						.36	.29	.13	0
Control	1.72							.36	.29	.13
Ethephon (282 ppm)	1.59								.23	.16
SADH (502 ppm)	1.43									.07
UBI-P293 (720 ppm)	1.36									

*Significant at the 95 percent confidence level.

Table 6. Duncan's Multiple Range Test for mean levels of GA_3 per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments listed below.

Treatments	Yi	P_{10} D=4.08	P_9 D=4.05	P_8 D=4.02	P_7 D=3.98	P_6 D=3.93	P_5 D=3.87	P_4 D=3.79	P_3 D=3.67	P_2 D=3.4
Control	8.92	*5.225	*5.2	*4.625	*4.125	*4.075	*3.9	2.8	2.75	2.57
Ethephon (564 ppm)	6.35		2.65	2.62	2.05	1.55	1.50	1.32	.225	.175
CCC (920 ppm)	6.175			2.47	2.45	1.875	1.37	1.32	1.15	.05
Photoperiod (10 hr. daylength)	6.125				2.42	2.4	1.82	1.32	1.27	1.1
UBI-P293 (1080 ppm)	5.025					1.32	1.3	.72	.225	.175
Ethephon (282 ppm)	4.85						1.15	1.125	.55	.05
SADH (502 ppm)	4.8							1.1	1.07	.5
CCC (460 ppm)	4.3								.6	.57
UBI-P293 (720 ppm)	3.725									.025
SADH (251 ppm)	3.7									

*Significant at the 95 percent confidence level.

Table 7. Duncan's Multiple Range Test for mean levels of GA_4 per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments listed below.

Treatments	Yi	P_{10} D=3.71	P_9 D=3.68	P_8 D=3.65	P_7 D=3.62	P_6 D=3.57	P_5 D=3.52	P_4 D=3.44	P_3 D=3.44	P_2 D=3.17
Control	4.47	*4.475	*4.475	*4.475	*3.825	*3.637	*3.625	3.17	2.22	2.18
UBI-P293 (1080 ppm)	2.28		2.28	2.28	2.28	1.63	1.44	1.43	.98	.03
Ethephon (564 ppm)	2.25			2.25	2.25	2.25	1.60	1.41	1.4	.95
Ethephon (282 ppm)	1.3				1.3	1.3	1.3	.65	.46	.45
CCC (460 ppm)	.85					.85	.85	.85	.20	.01
SADH (502 ppm)	.837						.837	.837	.837	.18
UBI-P293 (720 ppm)	.65							.65	.65	.65
Photoperiod (10 hr. daylength)	0								0	0
CCC (920 ppm)	0									0
SADH (251 ppm)	0									

*Significant at the 95 percent confidence level.

Table 8. Duncan's Multiple Range Test for mean levels of GA_7 per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments listed below.

Treatments	Yi	P_{10} D=3.36	P_9 D=3.34	P_8 D=3.31	P_7 D=3.28	P_6 D=3.24	P_5 D=3.19	P_4 D=3.12	P_3 D=3.03	P_2 D=2.87
Control	2.56	2.56	2.07	1.64	1.36	1.06	.94	.91	.54	.35
UBI-P293 (1080 ppm)	2.21		2.21	1.72	1.28	1.01	.71	.59	.56	.18
Ethephon (564 ppm)	2.02			2.02	1.53	1.1	.82	.52	.40	.37
CCC (460 ppm)	1.65				1.65	1.16	.72	.45	.15	.03
UBI-P293 (720 ppm)	1.62					1.62	1.13	.69	.42	.12
CCC (920 ppm)	1.5						1.5	1.01	.57	.3
Ethephon (282 ppm)	1.2							1.2	.71	.27
Photoperiod (10 hr. daylength)	.925								.925	.43
SADH (502 ppm)	.487									.487
SADH (251 ppm)	0									

Table 9. Analysis of variance table for mean weight comparisons between 10 hour daylength treatment and the control for each day of harvesting.

ANOV	d.f.	S.S.	M.S.	Fcal.	Ftable	
Treatments	9	397.1	44.12	8.57	2.21	Sign.
1 - June 20, 1975	1	.8625	.8625	.167	4.17	N.S.
2 - June 23, 1975	1	21.7	21.7	4.22	*4.17	Sign.
3 - June 25, 1975	1	12.65	12.65	2.45	4.17	N.S.
4 - June 30, 1975	1	3.125	3.125	.61	4.17	N.S.
5 - July 3, 1975	1	.3825	.3825	.07	4.17	N.S.
Residual	30	154.71	5.15			
Corrected Total		551.81				

*Significant at the 95 percent confidence level.

Table 10. Analysis of variance table for the overall mean weight comparison for each of the treatments listed below and the control.

ANOV	d.f.	S.S.	M.S.	Fcal.	Ftable	
Treatments	10	45.3188	4.53	.298	1.88	N.S.
UBI-P293 (1080 ppm)	1	7.456	7.456	.49	3.84	N.S.
UBI-P293 (720 ppm)	1	.4645	.4645	.03	3.84	N.S.
Ethephon (564 ppm)	1	1.55	1.55	.1	3.84	N.S.
Ethephon (282 ppm)	1	2.25	2.25	.14	3.84	N.S.
Photoperiod (10 hr. daylength)	1	21.25	21.25	1.39	3.84	N.S.
CCC (920 ppm)	1	8.677	8.677	.57	3.84	N.S.
CCC (460 ppm)	1	.9765	.9765	.06	3.84	N.S.
SADH (502 ppm)	1	2.71	2.71	.17	3.84	N.S.
SADH (251 ppm)	1	.0122	.0122	.0008	3.84	N.S.
Residual	190	2,898.43	15.2			
Corrected Total		2,943.8				

Table 11. Analysis of variance table for orthoganol comparisons between the number of ones in each of the treatments listed below and the control.

ANOV	d.f.	S.S.	M.S.	Fcal.	Ftable
Treatment	9	59,548.4	6,616.5	1.08	1.88
UBI-P293 (1080 ppm)	1	931.225	931.225	.15	3.84
UBI-P293 (720 ppm)	1	144.4	144.4	.023	3.84
Ethephon (564 ppm)	1	756.9	756.9	.12	3.84
Ethephon (282 ppm)	1	2,480.62	2,489.62	.4	3.84
Photoperiod (10 hr. daylength)	1	30,747.025	30,747.025	5.00082	*3.84
CCC (920 ppm)	1	2,958.4	2,958.4	.48	3.84
CCC (460 ppm)	1	396.9	396.9	.965	3.84
SADH (502 ppm)	1	1,612.9	1,612.9	.26	3.84
SADH (251 ppm)	1	600.625	600.625	.097	3.84
Residual	190	1,168,206.2	6,148.4		
Corrected Total		1,227,754.6			

*Significant at the 95 percent confidence level.

Table 12. Analysis of variance table for orthoganol comparisons between the number of twos in each of the treatments listed below and the control.

ANOV	d.f.	S.S.	M.S.	Fcal.	Ftable
Treatment	9	6,072.72	674.74	.52	1.88
UBI-P293 (1080 ppm)	1	3,686.4	3,686.4	2.87	3.84
UBI-P293 (720 ppm)	1	115.6	115.6	.09	3.84
Ethephon (564 ppm)	1	483.025	483.025	.37	3.84
Ethephon (282 ppm)	1	245.025	245.025	.19	3.84
Photoperiod (10 hr. daylength)	1	70.625	70.625	.055	3.84
CCC (920 ppm)	1	3.025	3.025	.0023	3.84
CCC (460 ppm)	1	648.025	648.025	.505	3.84
SADH (502 ppm)	1	8.1	8.1	.006	3.84
SADH (251 ppm)	1	403.225	403.225	.315	3.84
Residual	190	243,379.8	1,280.9		
Corrected Total		249,452.52			

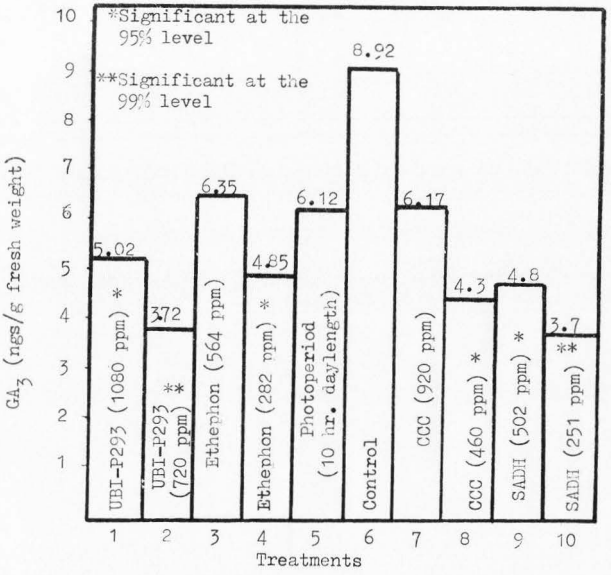

Figure 1. Mean levels of GA_3 per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments.

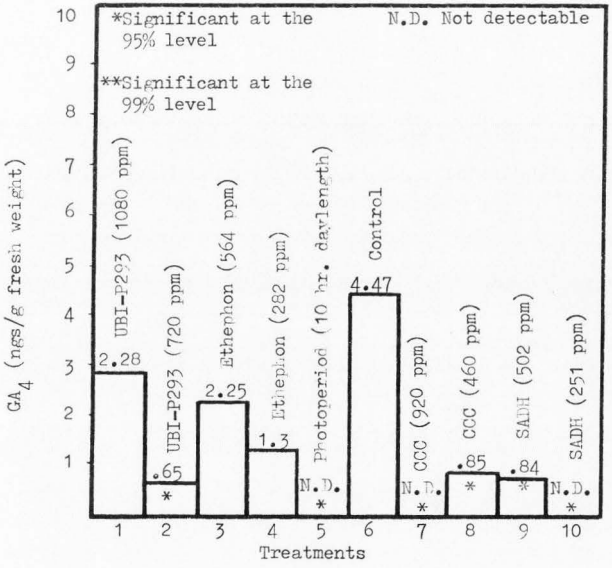

Figure 2. Mean levels of GA_4 per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments.

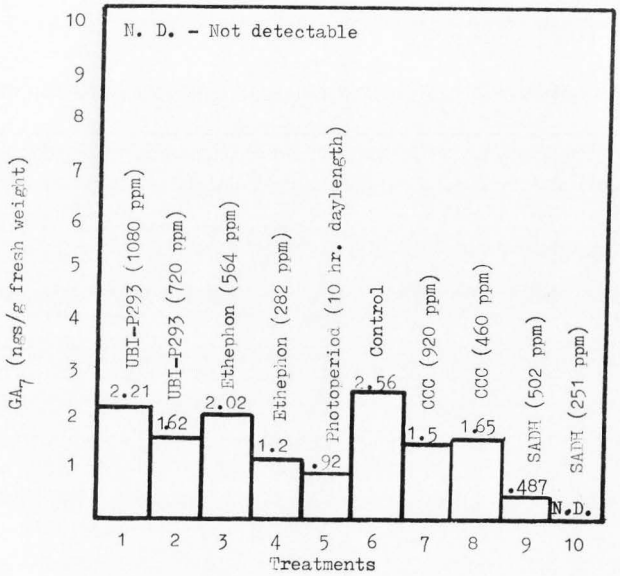

Figure 3. Mean levels of GA_7 per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments.

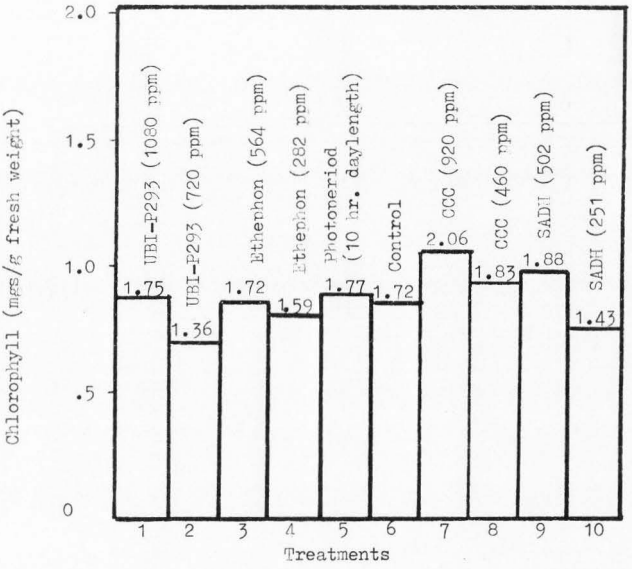

Figure 4. Mean levels of total chlorophyll per gram of strawberry leaf tissue (c.v. Tioga) for each of the treatments.

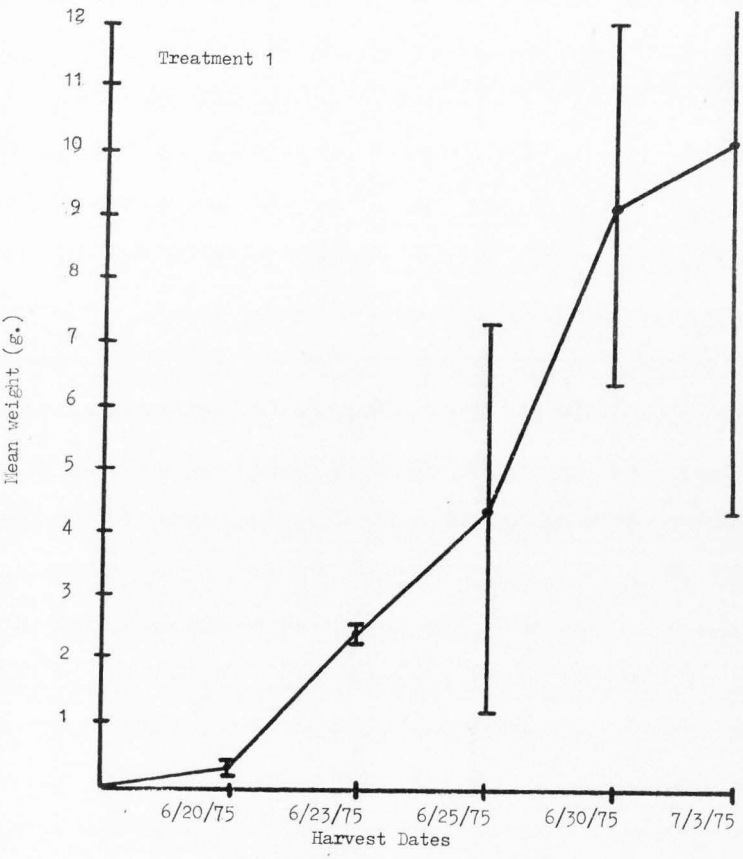

Figure 5. The mean weights of 4 replications (c.v. Shasta, Fresno and Tioga combined) of UBI-P293 (1080 ppm) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

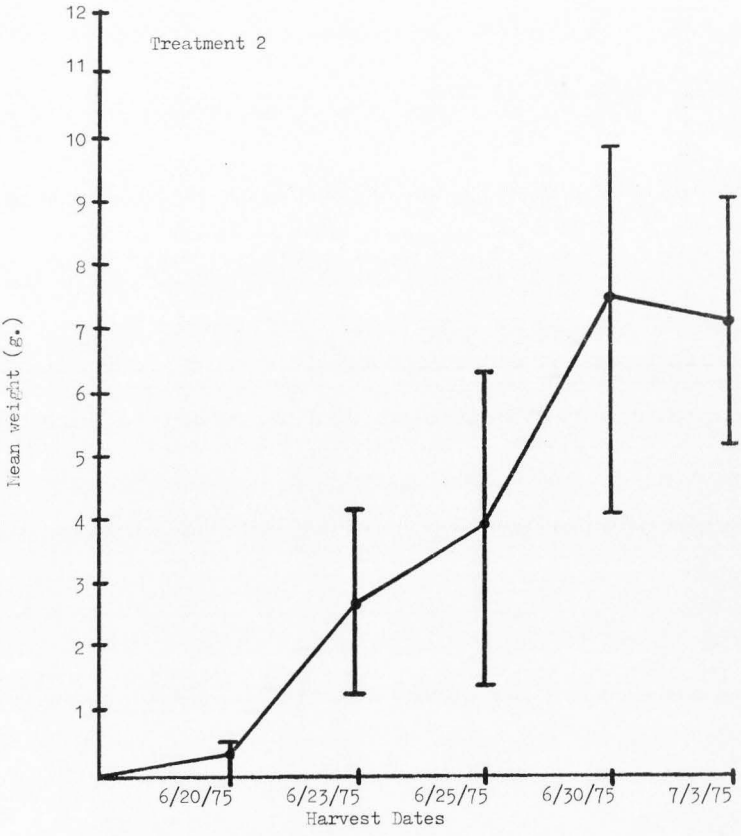

Figure 6. The mean weights of 4 replications (c.v. Shasta, Fresno and Tioga combined) of UBI-P293 (720 ppm) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

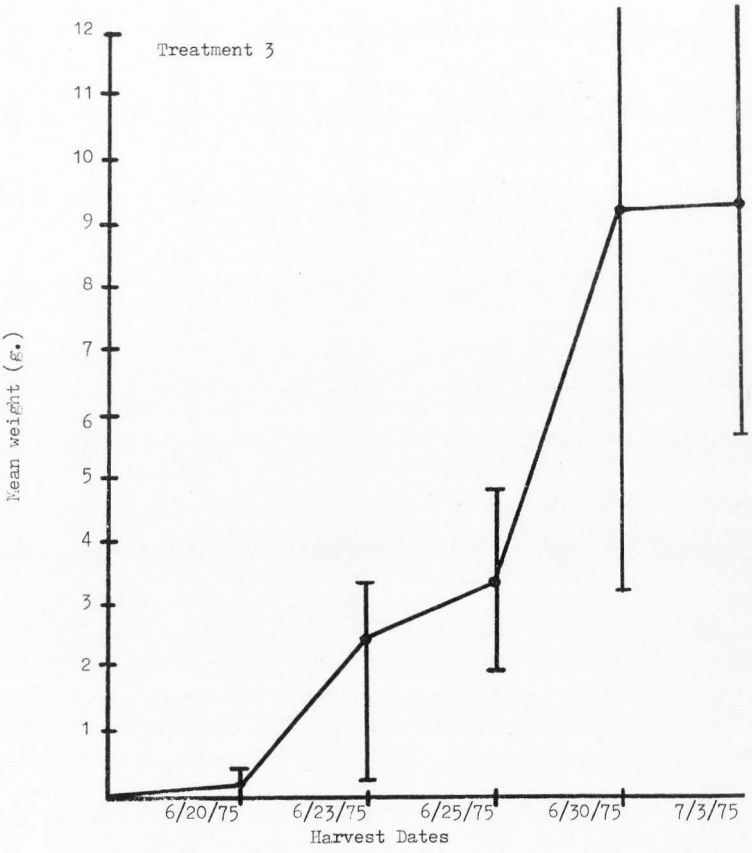

Figure 7. The mean weights of 4 replications (c.v. Shasta, Fresno and Tioga combined) of Ethephon (564 ppm) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

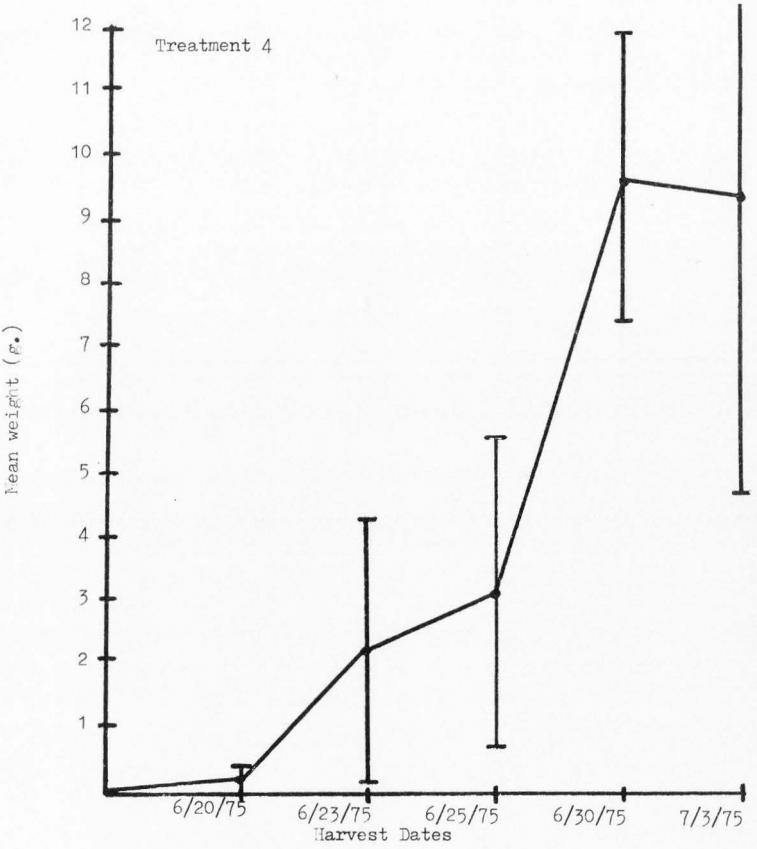

Figure 8. The mean weights of 4 replications (c.v. Shasta, Fresno and Tioga combined) of Ethephon (282 ppm) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

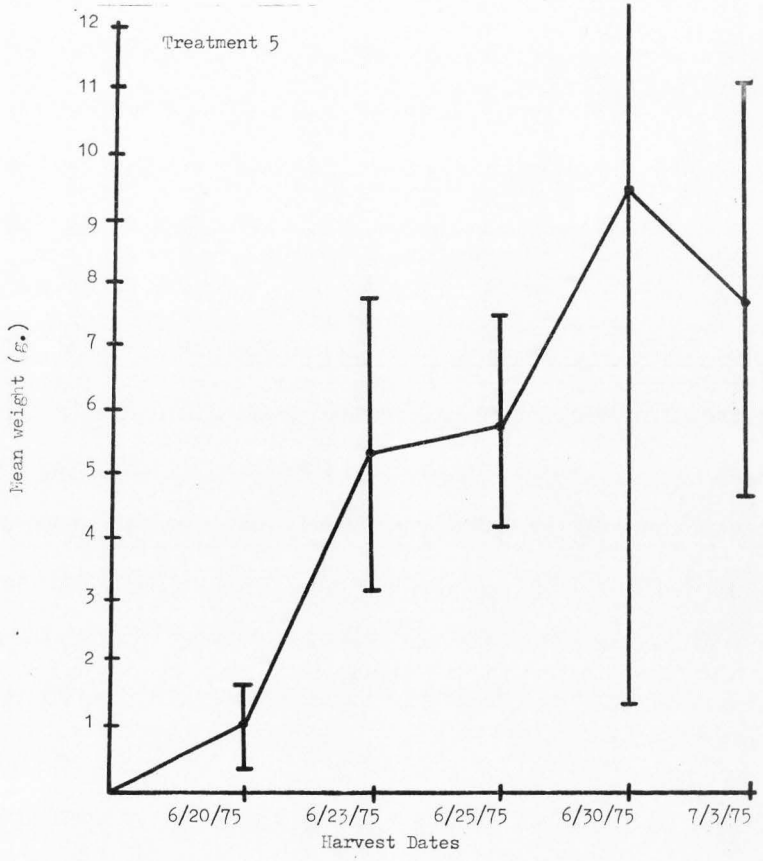

Figure 9. The mean weights of 4 replications (c.v. Shasta, Fresno and Tioga combined) of Photoperiod (10 hour daylength) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

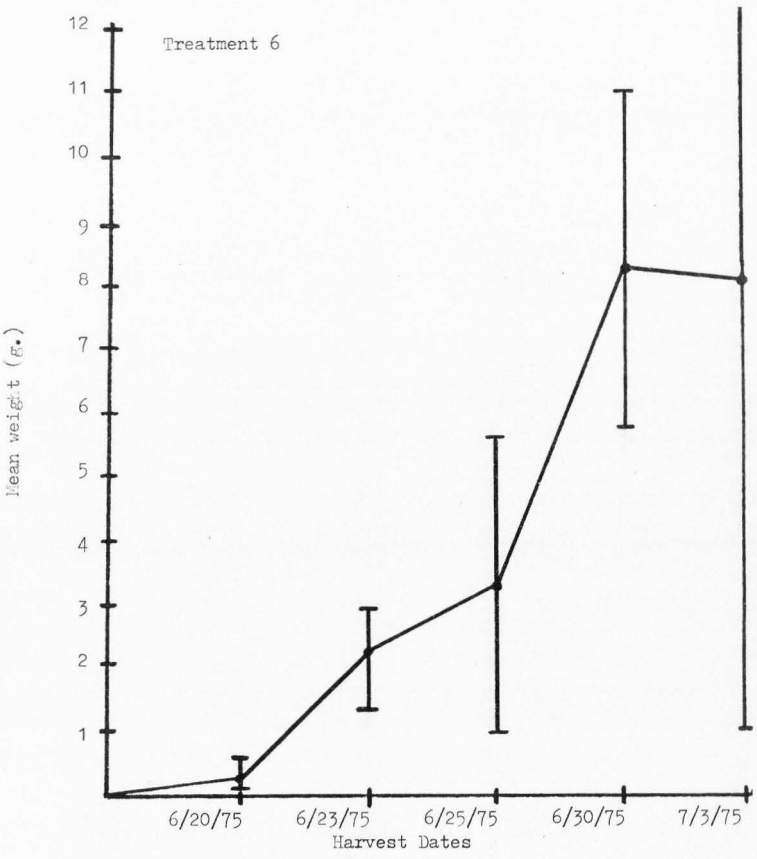

Figure 10. The mean weights of 4 replications (c.v. Shasta, Fresno and Tioga combined) of Control for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

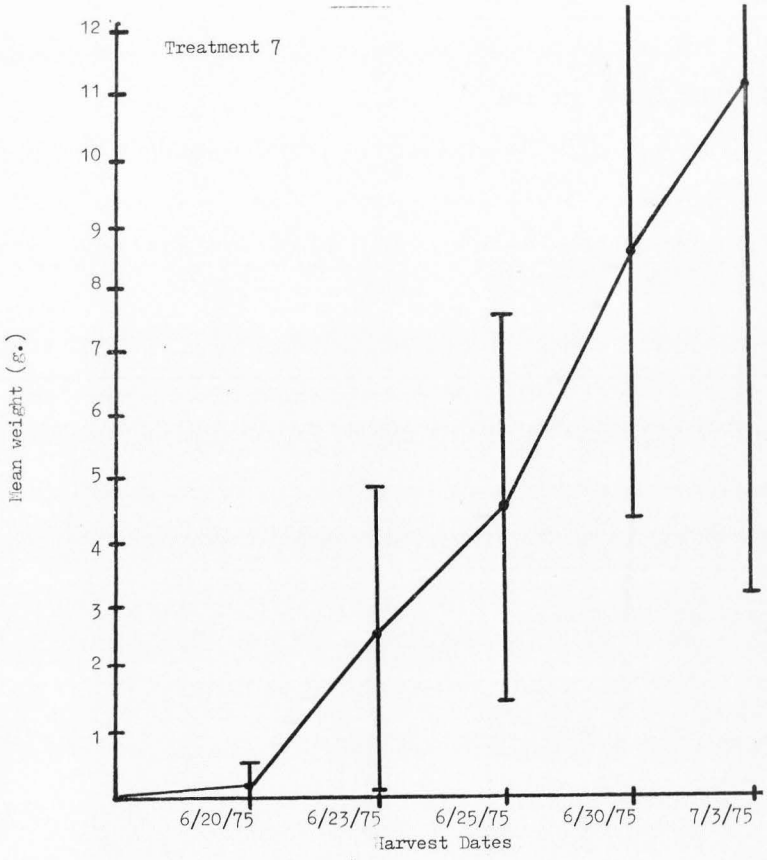

Figure 11. The mean weight of 4 replicitions (c.v. Shasta, Fresno and Tioga combined) of CCC (920 ppm) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

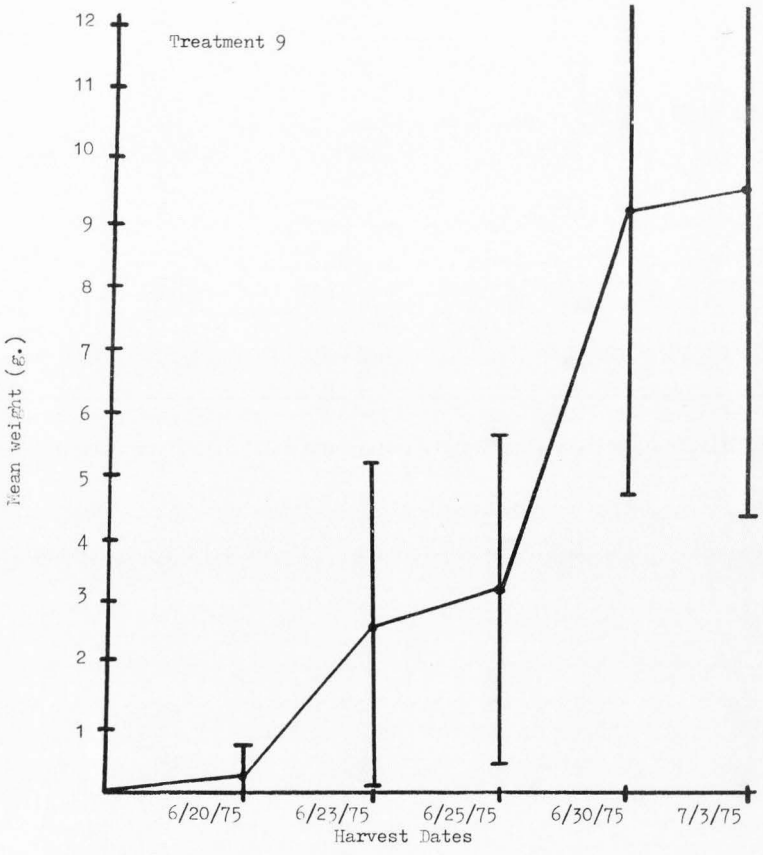

Figure 13. The mean weight of 4 replications (c.v. Shasta, Fresno and Tioga combined) of SADH (502 ppm) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

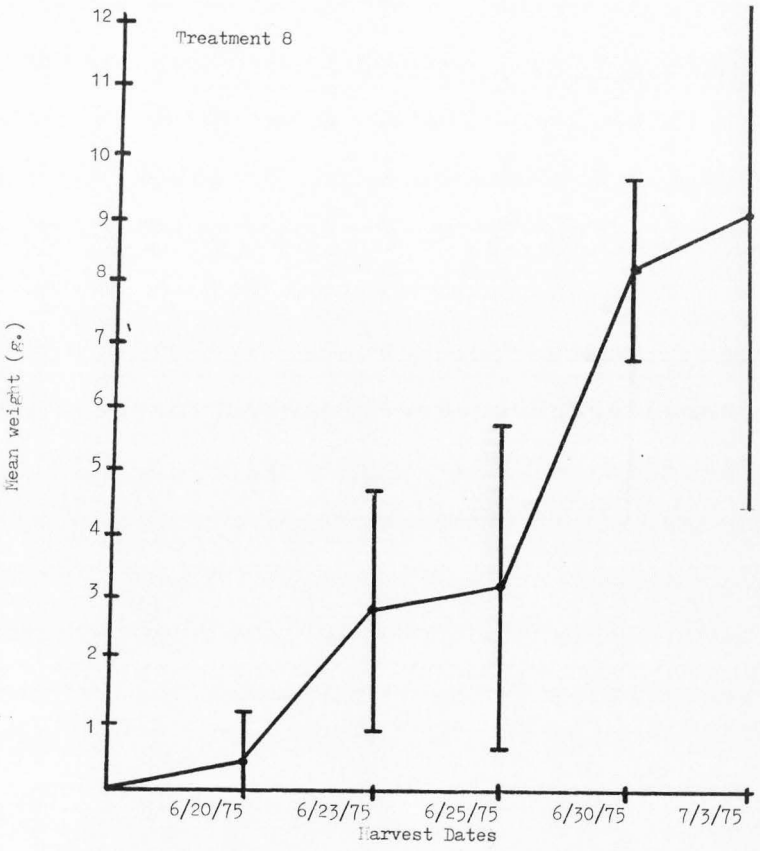

Figure 12. The mean weight of 4 replications (c.v. Shasta, Fresno and Tioga combined) of CCC (460 ppm) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

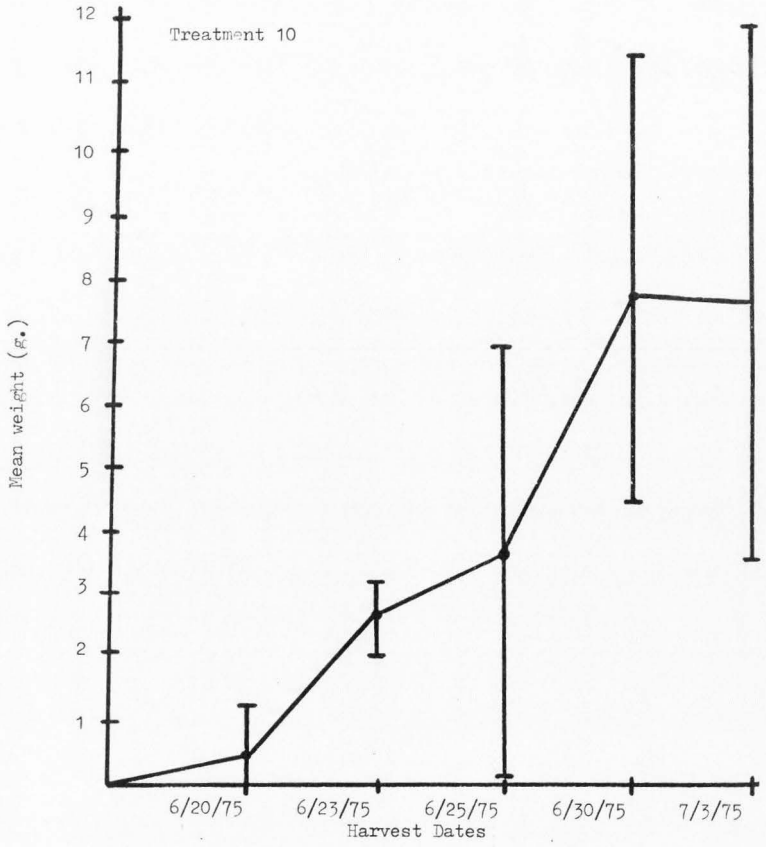

Figure 14. The mean weight of 4 replications (c.v. Shasta, Fresno and Tioga combined) of SADH (251 ppm) for each day of harvesting, plus and minus two standard deviations are represented by vertical bars running through the mean.

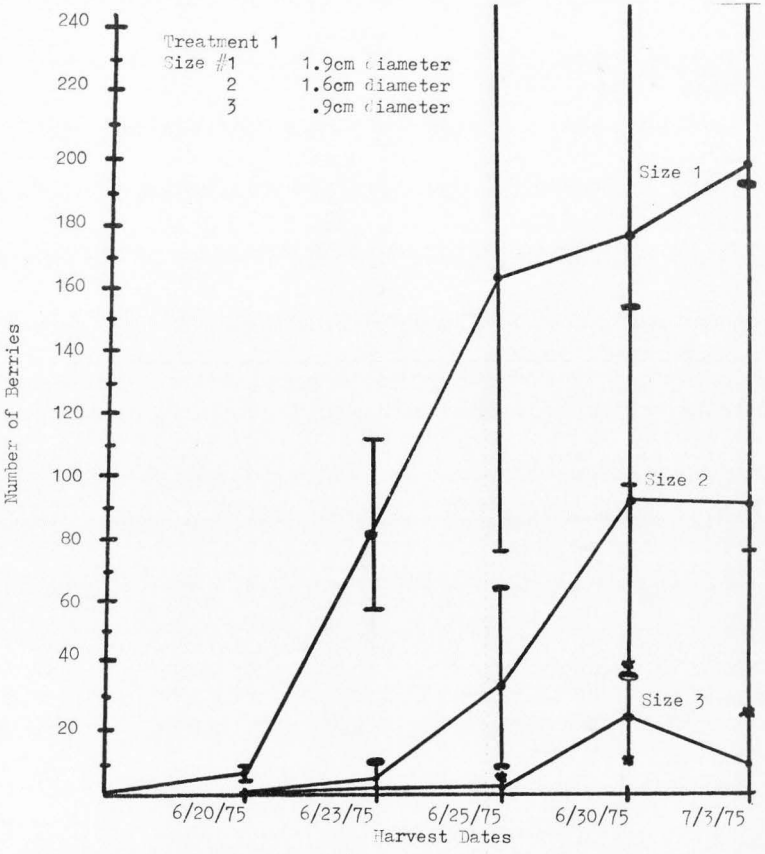

Figure 15. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with UBI-P293 (1080 ppm) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

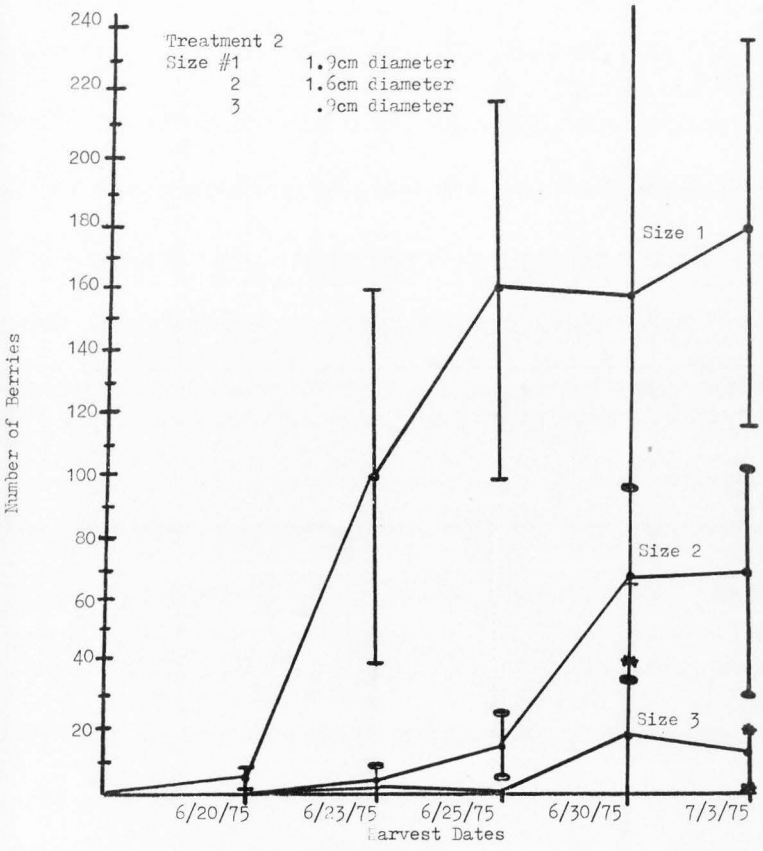

Figure 16. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with UBI-P293 (720 ppm) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

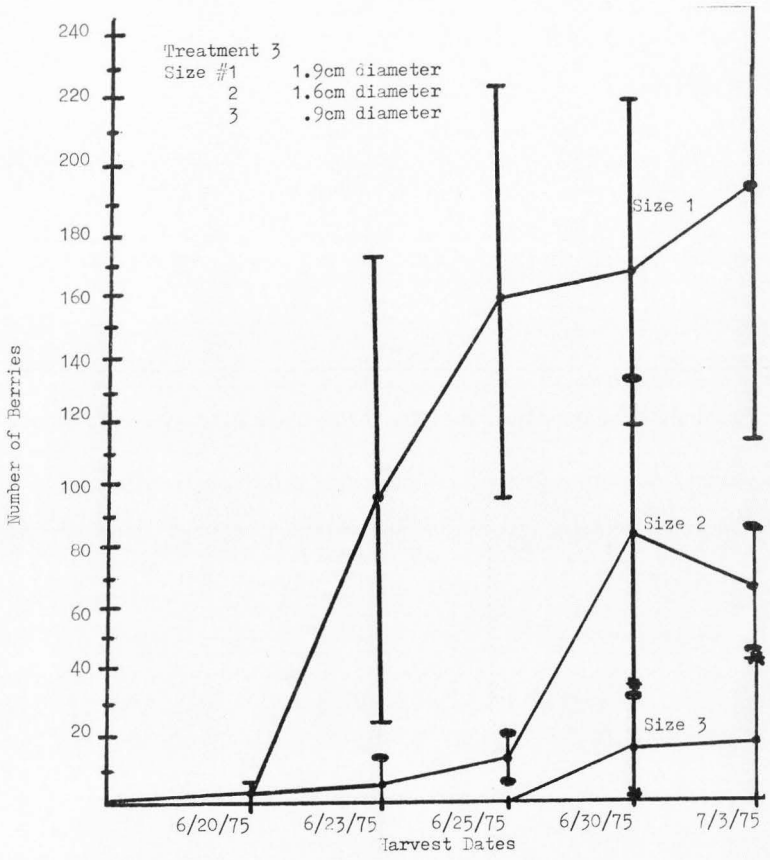

Figure 17. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with Ethephon (564 ppm) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

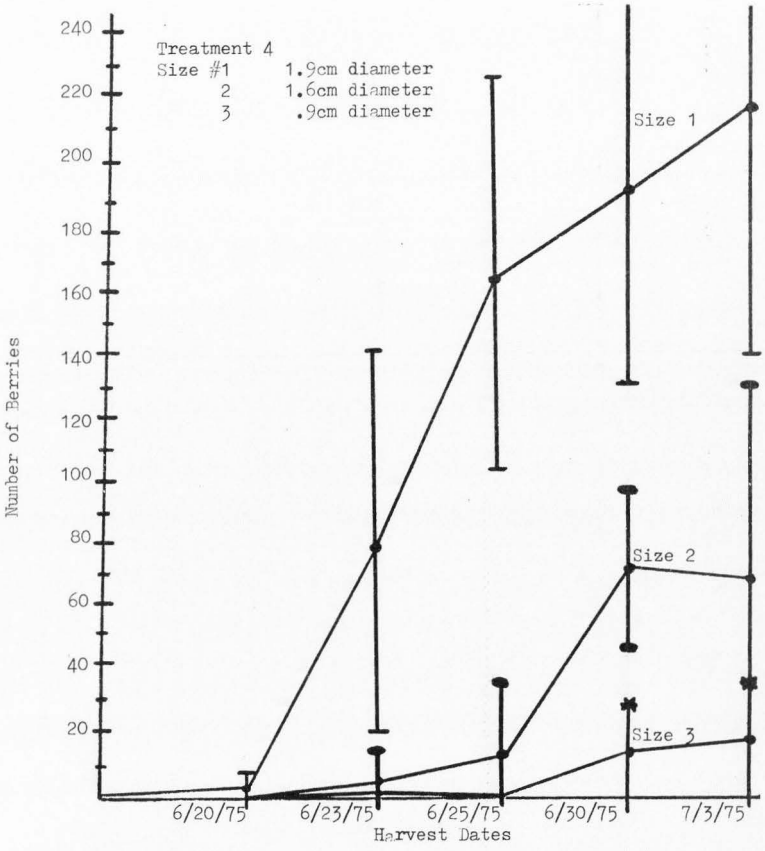

Figure 18. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with Ethephon (282 ppm) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

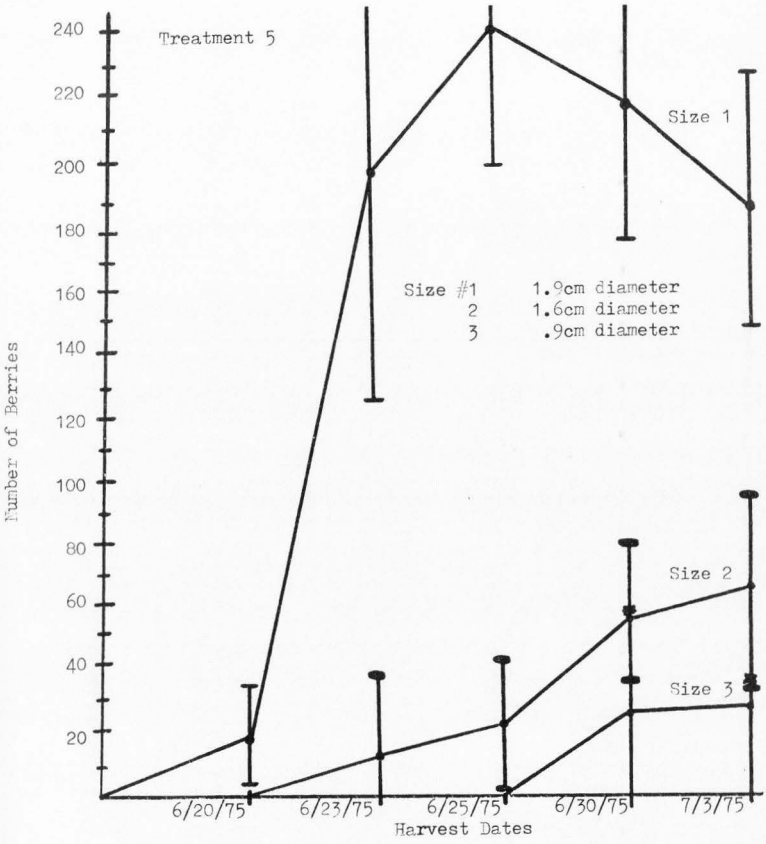

Figure 19. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with Photoperiod (10 hr. daylength) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

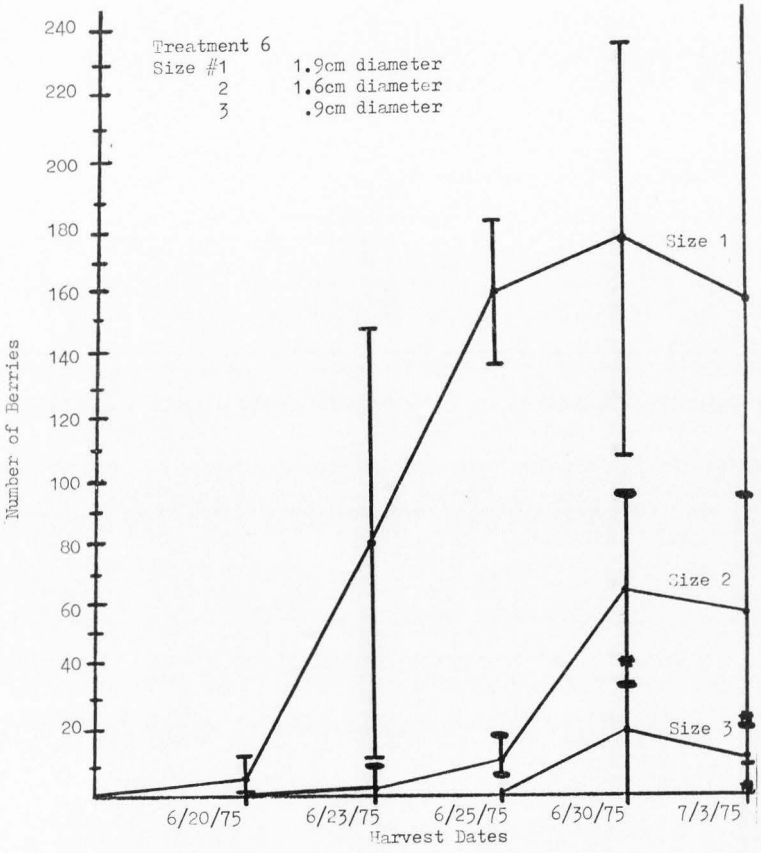

Figure 20. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with Control for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

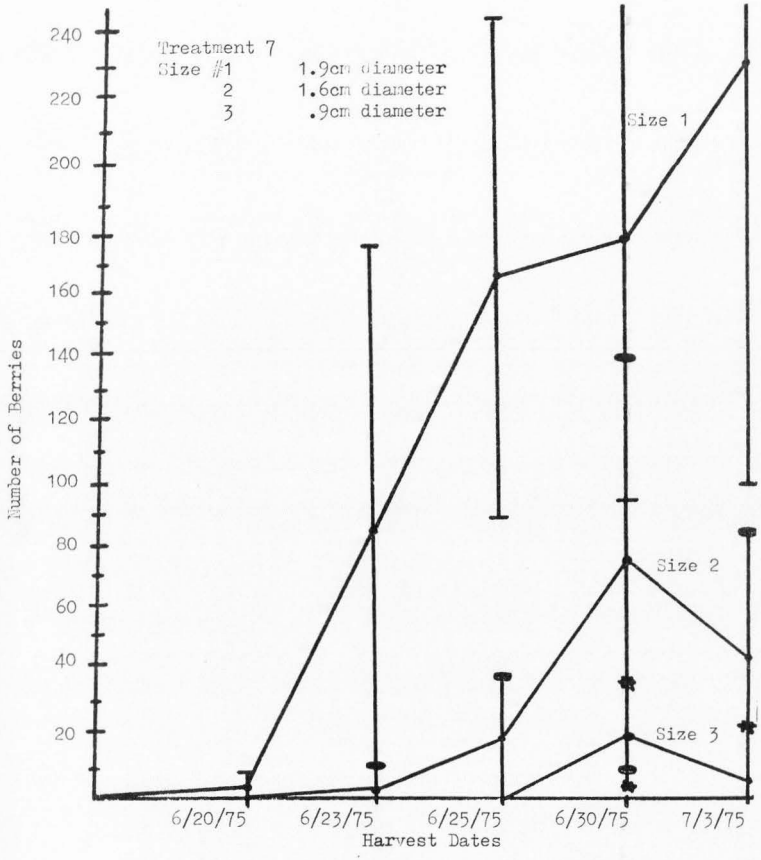

Figure 21. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with CCC (920 ppm) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

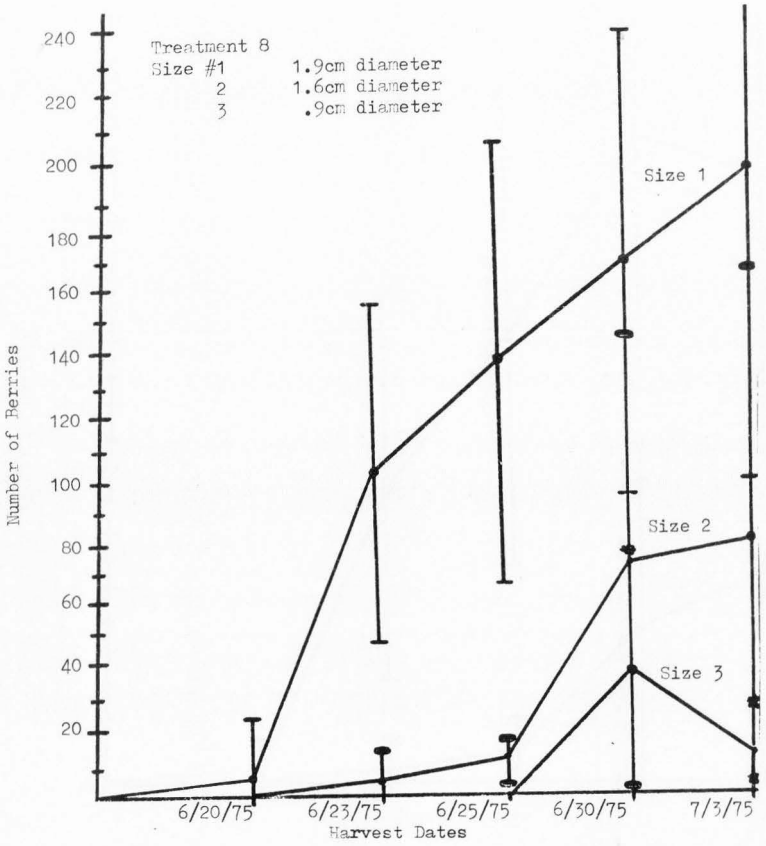

Figure 22. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with CCC (460 ppm) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

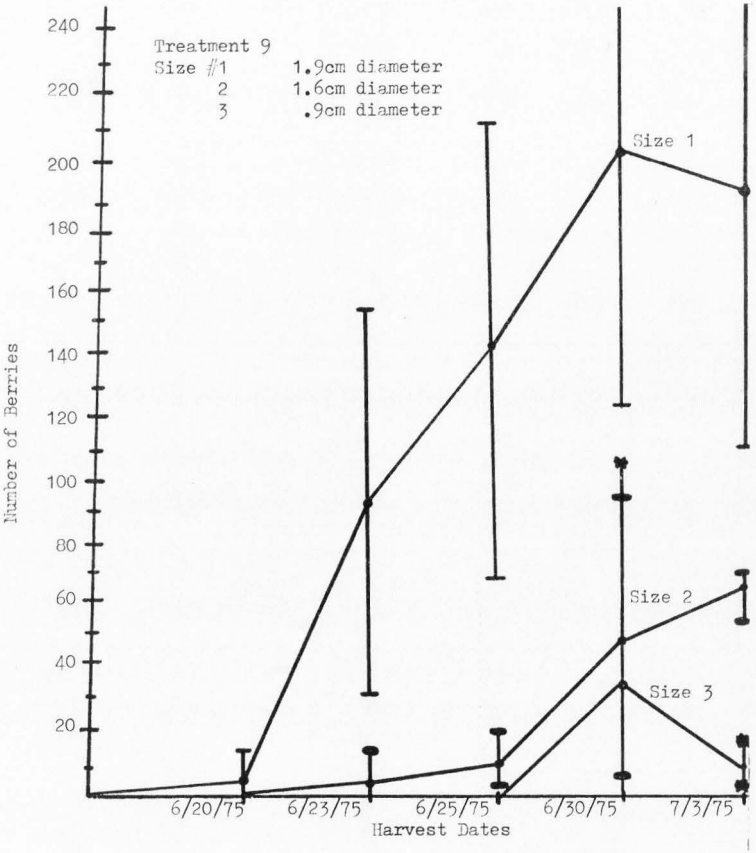

Figure 23. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with SADH (502 ppm) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.

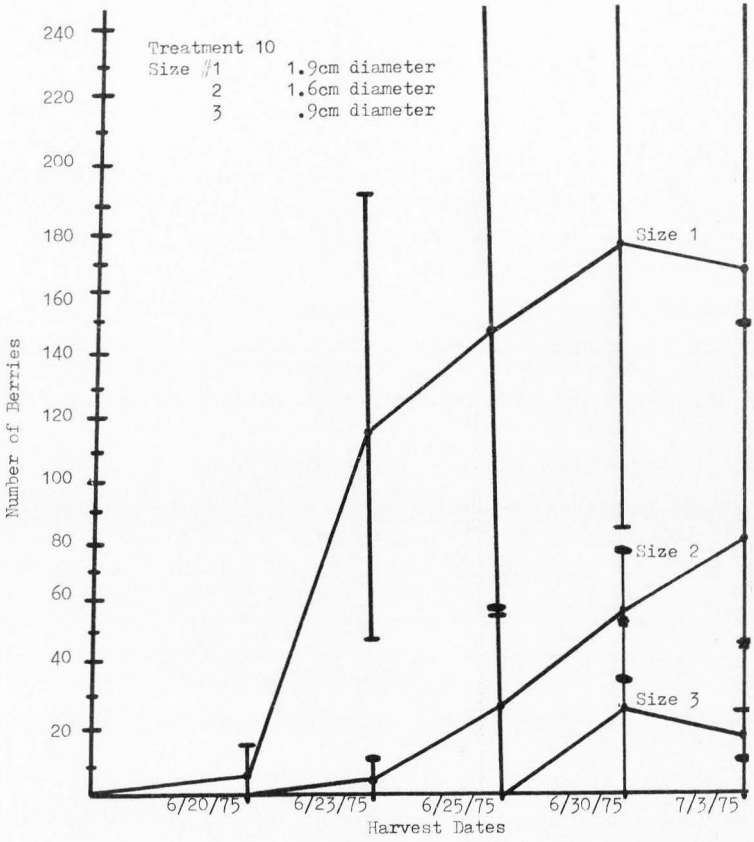

Figure 24. The mean number of size grade berries for 4 replications (c.v. Shasta, Fresno and Tioga combined) treated with SADH (251 ppm) for each harvest date plus and minus two standard deviations which are represented by vertical bars running through the mean.