EARLY DEVELOPMENT OF THE PEACH (PRUNUS PERSICA (L.) Batsch.) FRUIT AND THE TIME OF ENDOSPERM CYTOKINESIS

by

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TABLE OF CONTENTS

LIST OF TABLES AND PLATES

Page

LIST OF FIGURES

Page

INTRODUCTION

Peach trees set more fruit than they are capable of maturing to the desired marketable size. Chemical thinning of the fruit is an advantageous method for the orchardist, provided application is made at the correct developmental period of the fruit. A criterion for accurate chemical application timing must be established for successful fruit thinning in commercial peach orchards.

Fruit from the same cultivar will differ in development rate from year to year at the same location, depending upon climatic conditions and will differ during the same year at different geographical locations. The length of development in 'Elberta' was reported by Blake (2) to vary from 123 to 144 days upon the same trees in different years due to differences in climatic conditions. Lott (23) indicated that stage I of fruit development varied in Illinois from 44 to 63 days depending upon temperature. Tukey (32), working with several early and late maturing cultivars, found stage II was almost absent and embryos were aborted in the earlier cultivars, while in the late maturing cultivars stage II was pronounced and embryos were fully developed. Weinberger (33) stated that the period between full bloom and fruit maturity is specific for each variety with some modifications.

The process of cell wall formation is known as cytokinesis. The period of cytokinesis in the endosperm tissue is most favorable for chemical fruit thinning. The danger of frost damage is reduced, fruit set is accurately evaluated and the fruit is at the proper stage for maximum chemical thinning activity (14,15,19,20).

The purpose of this research was to determine the growth curve of five cultivars of peach, Prunus persica (L.) Batsch. ('Glohaven', 'Cresthaven', 'Redhaven', 'Redskin', and 'Babygold 5') and to determine the time of cytokinesis as related to morphological indices.

LITERATURE REVIEW

Fruit growth stages.

Conners (7) divided peach fruit growth into three stages based upon suture diameter increases of the cultivars 'Greensboro', 'Belle', and 'Elberta'. Stage I was a period of rapid development of the fruit due mainly to the increase in size of the ovule lasting up to 44 days from the beginning of measurement. Stage II was termed a rest period; during this time the ovule was formed and the endocarp became hard. Stage II began 4 to 5 weeks prior to ripening and was a period of rapid growth of the mesocarp to maturity. Stage II was subject to the greatest modification with 1 to 2 weeks for the early maturing cultivars and 4 to 7 weeks for the later maturing cultivars.

The cyclic growth pattern has been confirmed by Blake (1), Dorsey and McMunn (9), Lilleland (17), Lott (21), and Tukey (29). The pattern can be illustrated by a double sigmoidal curve.

Blake (1) studied the size increase in 'Elberta' from blossom bud to maturity by measuring polar, suture, and cheek diameters. The polar diameter increased more rapidly during the first 51 days, then the suture diameter increased more rapidly than the other two, and during stage III, both the suture and cheek diameters increased more rapidly than the polar diameter.

Dorsey and McMunn (9) studied the development of component structures of the fruit using the cultivar 'Elberta'. During stage I the endocarp and ovule grew to approximately maximum size. The initiation of stage II was marked by the hardening of the endocarp, the attainment of approximately maximum size by the ovule, and the beginning of rapid growth in the endosperm.

Lott (17) measured the development of 'Hiley' peach from the beginning of endocarp hardening until flesh maturity. Measurements of diameter, volume,

fresh weight and dry weight were made. No definite second period of growth was found when measuring dry weight as the endocarp and ovule increased rapidly during this time. A second growth period or stage II is a correct concept when considering only pericarp measurement of diameters, volume, and fresh weight.

Tukey (29) measured polar diameter increases of the pericarp, endocarp, nucellus and integuments, and embryo for the cultivars 'Greensboro', 'Triumph', 'Carman', 'Elberta', and 'Chili'. In addition to determining three growth periods of the pericarp, he also found: 1) the duration of stage I was similar in all cultivars; 2) the duration of stage II was directly correlated with the date of ripening, that is, a short period for early maturing cultivars (5 days in 'Greensboro') and longer period in later maturing cultivars (40 days in 'Chili'); 3) the initiation of endocarp hardening began at stage II in all five cultivars; 4) the nucellus and integuments in all cultivars reached maximum size near the end of stage I; and 5) embryo development in all cultivars was similar, with rapid enlargement beginning about the termination of stage I and reaching maximum size within 29 to 31 days.

Development of the vascular system.

Ragland (25) studying 'Phillips Cling' reported that two ventral bundles and one dorsal bundle was initiated in late December or early January from the pedicel vascular system which terminated in the carpel base. The ventral or dorsal bundles were not embedded in the endocarp. For two-thirds the distance to the apex the dorsal bundle was contained in a shallow groove on the dorsal side of the endocarp. It then continued directly to the fruit apex. The two ventral bundles were in deep grooves, one on either side of the ventral suture and extended the entire length of the endocarp. The ventral and dorsal bundles converged at the fruit apex and passed into the style. Ventral bundles branched freely in the apical half of the fruit while

the dorsal bundles branched more in the basal half.

Ten to twelve bundles entered the endocarp from the pedicel and branched through depressions in the endocarp and into the mesocarp. Ragland reported the bundles were initiated about 6 weeks prior to full bloom, and those extending into the mesocarp were initiated about 2 weeks before full bloom. The funicular bundles were soon initiated and located between the ventral bundles, completely within the endocarp, with one leading to each ovule. A few small bundles entered the mesocarp directly from the receptacle, but most of the vascular system in the mesocarp was supplied by bundles from the endocarp.

Tukey (29) noted that differentiation of the endocarp tissue from the mesocarp was evident microscopically at full bloom and could be observed macroscopically 2 weeks after bloom. Lignification began at the apex and proceeded down the ventral suture and from the inside of the endocarp tissue outward.

Ovule development.

Two anatropous ovules are differentiated within a single carpel. Each ovule is attached to a funiculus which is differentiated from the placenta, the inner surface of the ovary. Ragland (25), working with 'Phillips Cling', found at full bloom that one ovule was usually smaller than the other. This condition continued for 10 to 12 days, at which the smaller ovule usually aborted. Harrold (12) reported that the ovules in 'Carman' develop equally until about 3 days prior to bloom, then one aborted. Lott (22) found the two ovules in 'Hiley' peach were the same size at full bloom and after about 7 days one was thicker and longer than the other. This condition persisted about 31 days after bloom then the smaller one aborted. Occasionally, both ovules developed to maturity.

Harrold (12) observed that the integuments attained their maximum size by the end of stage I.

The nucellar tissue consisted of the central portion of the ovule, surrounded by two integuments with the micropylar opening opposite the apex of the nucellus. A subepidermal cell of the nucellus opposite the micropyle differentiated as a megasporocyte, and by two divisions gave rise to four megaspores. The inner most megaspore sometimes called the chalaza megaspore developed into the female gametophyte and the remaining megaspores disintegrated (4).

Harrold (12) indicated that three divisions of the functional megaspore followed so that one or two days prior to full bloom the megagametophyte was in the 8-nucleate stage. Maturation and migration of the two polar nuclei to the middle of the megagametophyte coincided with full bloom while the antipodals were disintegrating. Prior to fertilization the megagamete and the fused polar nuclei positioned themselves near each other.

Brunk and Cooper (3,4) describe the process of double fertilization as the formation of the embryo from the union of one sperm nucleus, from the pollen tube, with the egg cell in the embryo sac and the central cell of the female gametophyte, which resulted from the union of an antipodal polar nuclei and a micropylar polar nuclei, being fertilized by the other sperm nucleus, thereby developing into the endosperm.

Evidence of fertilization was indicated by the style beginning to fade (12). The synergids persisted for about a week after fertilization and then disintegrated.

Harrold (12) found that the embryo sac began to elongate following the union of the polar nuclei and had doubled in length by the time of fertilization. The embryo and endosperm, both 1-nucleate, remained close together, then there was a gradual movement of the endosperm nucleus down the embryo sac toward the chalaza. The first divisions of the endosperm nucleus began

to occur at a point one-half the distance to the chalaza, 9 to 10 days after bloom. Then rapid divisions of the endosperm nuclei occurred so that the embryo sac was lined with a peripheral layer of free-nuclear endosperm. The free-nucleate state persisted until about the end of the sixth week after bloom, which corresponded to the end of stage I.

Long strands of cytoplasm appeared throughout the free-nuclear endosperm about a week prior to cell wall formation in 'Halehaven' and 'Redhaven' (18). Cell wall formation is a process known as cytokinesis (11). Lombard (18) found that the greatest concentration of cytoplasmic strands surrounded the vacuolated areas near the micropylar end of the endosperm. The process of cytokinesis began at the micropylar end and along the periphery of the embryo sac, and continued until the vacuole in the center became occupied with cells. This process is completed during stage II. The endosperm grew rapidly after cytokinesis reaching one-third the volume of the ovule within 18 days.

Ragland (25) reported that the embryo sac development began at the time the bud began to burst, and was ready for fertilization 10 to 16 days later. The growth rate of the embryo sac was accelerated and within 3 to 5 days it had reached the chalaza. The embryo sac extended in the form of a thread-like tube and expanded at the chalazal end to almost the sizes as it was at the embryo end. The embryo sac was lined with a thin layer of protoplasm and was filled with a clear watery fluid. Cytokinesis occurred 40 to 50 days after fertilization in the cultivar 'Phillips Cling'. The endosperm continued to grow until it had reached its maximum size, 30 to 35 days after cytokinesis.

The zygote nucleus did not divide until about 12 days after fertilization (Harrold, 12). Embryo development was slow until about a week prior to stage II. Acceleration of embryonic growth coincided with cellular endosperm development of 1/3 to 1/2 the length of the nucellus. Cotyledons were

differentiated and the embryo extended almost to the chalaza. Cellular endosperm and nucellular tissue were digested as the embryo advanced. Elongation was completed approximately twelve weeks after bloom.

Ragland's (25) work with 'Phillips Cling' agrees very closely with Harrold's. Ragland indicated that approximately 60 days were required for the cotyledons to reach their mature size. The embryo developed slowly for 20 to 35 days after fertilization and prior to initiating cotyledons.

Lilien-Kipnis and Lavee (16) described the initial embryo structure as flat, then changed to spherical at approximately the same time as when the endosperm was changing from free-nuclear to the cellular stage. This occurred 35 days after bloom in 'Ventura' peach. Lombard and Mitchell (20) described the initial embryonic stage as filamentous in which the embryo grew linearly for 28 days after fertilization, beginning 14 days after full bloom in 'Redhaven'. The change to the spherical stage occurred 43 days after full bloom. The embryo was designated as spherical since the distal tier of cells had increased in size three dimensionally. The embryo remained in this stage 7 to 10 days. The period of cytokinesis of the endosperm occurred during the spherical stage, 42 to 46 days after full bloom, after which was completely cellular. The spherical stage began 3 to 4 days prior to endosperm cellular formation. Lombard and Mitchell (20) indicated that cytokinesis was completed 3 to 4 days prior to the transitional stage. The initiation of the transitional stage, 50 to 52 days after full bloom, marked the beginning of 'June drop', a natural fruit thinning phenomenon. Cotyledons were developing 7 days after cytokinesis.

The next stage described by Lombard and Mitchell (20) was the transitional in which the embryo assumed a definite elliptical appearance at the distal end, about 52 days after bloom. It was during the termination of this stage and the beginning of the cotyledonary phase that the greatest growth in all planes of the embryo occurred. Similar developmental periods were reported

for 'Halehaven'.

Tukey's (29) study on the duration of embryo development for five cultivars indicated an almost identical period of approximately 30 days whereas Harrold's (12) study on 'Carman', included in Tukey's study, indicated a growth period of 42 to 49 days. The work of Lilien-Kipnis and Lavee (16) with 'Ventura' coincided with Harrold's.

Fruit abscission.

Harrold (12) reported three periods of fruit drop. The first drop occurred during a three-week period which began 1 to 3 days prior to full bloom and consisted of fruits with ovules which had degenerated between the megaspore mother cell stage and the early megagametophyte stage. Developing fruits had shed the calyx cup about the middle of the first drop. The second drop lasted one week and occurred five weeks after bloom or during the middle of stage I. The second drop occurred during the time of free-nuclear endosperm. The third drop also lasted one week and occurred about the seventh week after bloom. The only structural difference between the aborting ovules of the second and third drops was in the degeneration of the nucellus in the chalazal region of third drop fruits.

Detjen (8) found that the majority of fruits abscising in the later stages showed evidence of ovule fertilization, therefore lack of fertilization was not the major factor in immature fruit abscission. Harrold (12) indicated that a disorder in the chalazal region would disarrange the vascular system and possibly caused a failure of nutrient conduction, thereby resulting in abscission.

Lilien-Kipnis and Lavee (16) found that the endosperm of abscissing fruit did not have uniform structure, but possessed one or more of the following: embryo sac disintegration; endosperm nuclei which had moved from

the embryo sac wall to the center of the sac; or limited endosperm cellular formation with larger than usual cells that appeared to be empty. The embryo was always found to be small and appeared to cease development early.

Tukey (31) artificially destroyed cherry and peach embryos of various periods of growth. When the embryo was destroyed early in the development of the fruit, during stage II or earlier, there was an abrupt check in fruit growth, and the fruit eventually abscissed. Destruction of the embryo in the transition period between stages II and III resulted in continued growth of the fruit, but with earlier ripening and failure to achieve full size. Destruction of the embryo in stage III resulted in increased growth rate and earlier ripening, and occasionally larger fruit size. Lott (23) reported that freezing temperatures during the first three weeks after bloom may kill the ovules without killing the mesocarp. This usually causes heavy drop soon after injury although some may drop later in the season. Fruits with dead ovules may develop to maturity.

Lott and Simons (24) described morphological characteristics of ten developmental stages in the peach flower and fruit from full bloom to style abscission based upon work with 'Redhaven' and 'Redskin'. Stage IV and X were selected as morphological markers because the interval for both was only a few days. Stage IV was described as having the "abscission zone evident as a pale greenish-yellow line around the outside of the floral cup about 25% of the distance from the bottom of the cup to its rim". There was no visible separation at this stage, 7 to 10 days after full bloom, which was prior to fertilization. Stage X was described as having the style abscised or if not abscised it would fall when touched. Stage X occurred 20 to 24 days after full bloom, and occurred within the first growth phase of the pericarp corresponding to the period of free-nuclear endosperm. Lott and Simons indicated that stage X probably coincided with the initiation of embryo development but remained to be investigated. Variations are likely to exist

among cultivars.

The time of endosperm cytokinesis as an indice for fruit thinning.

Dorsey and McMunn (9,10) reported that thinning is effective throughout stage II. Lombard (19) indicated that the period of cytokinesis is a favorable indice for chemical fruit thinning because fruit set can be evaluated since it occurs after fertilization and after likely frost danger. Fruit of optimum size can be produced when thinning takes place during this period. A 7 to 10 mm ovule length for long cycle peaches coincides with the period of cytokinesis in the endosperm. Random sampling of varying fruit sizes should be conducted to determine ovule length. One-third of the fruit should have an ovule length of 11 to 13 mm, completed cytokinesis, and unaffected by the chemical.

Lombard and Mitchell (20) determined that cytokinesis in 'Redhaven' occurred 42 to 46 days after full bloom. They applied NAA (30 ppm) at 35, 42, and 49 days after full bloom. Significant thinning occurred with treatments 35 and 42 days, but none was achieved at 49 days when the endosperm was completely cellular. The natural ovule hormone level was decreasing rapidly at the 49 day treatment which coincided with the beginning of 'June drop'.

Leuty (14) applied NAA (30 ppm) to 'Redhaven' and 'Halehaven' peaches 6 days prior to, during, and 6 days after cytokinesis. Prior to cytokinesis in 'Redhaven', NAA failed to thin while after cytokinesis, NAA gave almost as much thinning as the period during cytokinesis. No significant abscission was obtained in 'Halehaven' and histological studies showed that the endosperm was either totally free-nuclear to completely cellular.

Leuty and Bukovac (15) found that abscission was induced by NAA (30 ppm) applied at 42 to 48 days after full bloom in 'Redhaven', but not at 33 days. The endosperm was free-nuclear at the 33-day treatment and had a mean pericarp

length of 24 mm. The 42-day treatment was during the period of cytokinesis with a mean pericarp length of 30.8 mm. The endosperm was cellular and the mean pericarp length was 33 mm at the 48-day treatment. The mean pericarp length was used since it was found to be the most consistent index. Thinning did not occur in 'Halehaven' as the endosperm was either free-nuclear or cellular at the application dates. The mean pericarp length of 'Halehaven' did not vary significantly from 'Redhaven'.

A second experiment was conducted at the Vineland Station, Ontario, by Leuty and Bukovac (15) using 'Redhaven' and 'Loring' cultivars. The NAA application was 34, 38, and 43 days after full bloom. 'Redhaven' was under going cytokinesis at 38 days after full bloom and 'Loring' was in this stage at the 34- and 38-day treatment. Treatments during cytokinesis resulted in significant thinning for both cultivars.

Sherman and Buchanan (28) found the period of cytokinesis in the short cycle 'Early Amber' peach grown in Florida to occur at an ovule length of 12.1 mm and a pericarp diameter of 25.3 mm. Buchanan, et al. (5) using the cultivar 'Early Amber' obtained adequate thinning during the 4-day period the endosperm was under going cytokinesis. Sherman and Arnold (27) investigated seven short cycle peach cultivars and found that they have a longer ovule, 11.4 to 13.6 mm, at the time of cytokinesis than long cycle peach cultivars.

MATERIALS AND METHODS

The rate of pericarp and ovule growth, and the period of endosperm cytokinesis was studied using 5-year old peach trees: 'Glohaven', 'Crest-haven', 'Redhaven', 'Babygold 5', and 'Redskin' growing at the Horticulture Experimental Field, Wichita, Kansas.

Field procedure.

The experimental orchard was planted into six replicated randomized complete block (RCB) design utilizing the five cultivars studied. All five cultivars were sampled every other day beginning two days after full bloom for a total of 25 sampling days covering 50 days. Ten fruits were sampled from one tree of each cultivar in two replicates per sampling day. A randomized sampling order was used. A limb was randomly selected on each sampling day and fruits were removed consecutively beginning with the basal most fruit and until ten had been collected. Fruits along with floral structures were placed in collection bottles, submerged in FAA killing fluid, and evacuated over-night.

Immediately after picking, the pericarp measurements of samples 19 through 25 were taken, ovules removed and placed in collection bottles containing FAA and evacuated since fruits were too large for sample bottle. A sample was collected and pericarp measurements were taken prior to killing and four weeks after killing to determine if pericarp size changed as a result of killing treatment.

Measurements.

Pericarp measurements taken were polar diameter, suture diameter, and volume. Polar diameter was the distance from the point of attachment of the peduncle to the base of the style, or the point at which the style and ovary were fused. Suture diameter was the widest point measuring across the ovary

and perpendicular to the suture. Volume was measured by water displacement.

Ovule measurements were length and width. Ovule length was measured from the micropylar end to the chalazal end of the integuments. Ovule width was the greatest transverse diameter.

An E.R. Bogusch Measuring Slide was used for measuring small ovules and fruit. A vernier caliper was used for measuring the larger samples.

Laboratory procedure.

Standard histological procedures were followed as described by Sass (26) and Johansen (13). Ovules were soaked in distilled water two hours then dehydrated in tertiary butyl alcohol and infiltrated in Paraplast as described by Sass (26). As recommended by Lenty and Bukovac (15) a small portion of the integuments were shaved from the sides to prevent ovule shriveling, embryo sac collapse, and to facilitate infiltration. Hot Paraplast was poured into molds, an inpregnated ovule was added and positioned, and molds were placed on ice to solidify the Paraplast.

Six to 10 μ longitudinal sections of the ovules were prepared and stained in Safranin-O, Fast-Green, and Orange-G. Slides were prepared by floating sections on 4% Formalin and mounted using Haupt's adhesive. Permount was used to mount cover slips.

Slides were examined using a light microscope at 60X magnification to determine endosperm cellular structure.

Climatological data.

Thermograph recordings were made for the duration of the experiment. Degree days were calculated using the formula:

$$\left[\frac{\text{Daily maximum temperature } ^{o}C + \text{Daily minimum temperature } ^{o}C}{2}\right] - 10^{o}C$$

RESULTS

Fruit pericarp and ovule measurements of the five cultivars are illustrated in Figure 1 a, b, c, d and e. Each datum point is the mean of a twenty fruit sample. The results of histological investigation of the endosperm tissue for each cultivar are represented symbolically as free-nuclear. undergoing cytokinesis, or cellular. Photomicrographs (Plate I a, b and c) of 'Redskin' peach ovules show endosperm development.

Measurement data for cultivars are similar, 'Glohaven' grew slightly faster and 'Babygold-5' grew somewhat slower than 'Cresthaven', 'Redhaven', and 'Redskin'. The growth rate accelerated rapidly for all measurements from full bloom through the cytokinetic period, after which the rate of increase was less rapid.

'Glohaven' underwent cytokinesis 32 days after full bloom (AFB). Two days prior it was free-nuclear and completely cellular two days after the cytokinetic period.

'Cresthaven' was undergoing cytokinesis at 34 and 36 days AFB. Ovules sampled 32 days and before were in the free-nuclear state. Endosperm was as completely cellular 36 days AFB.

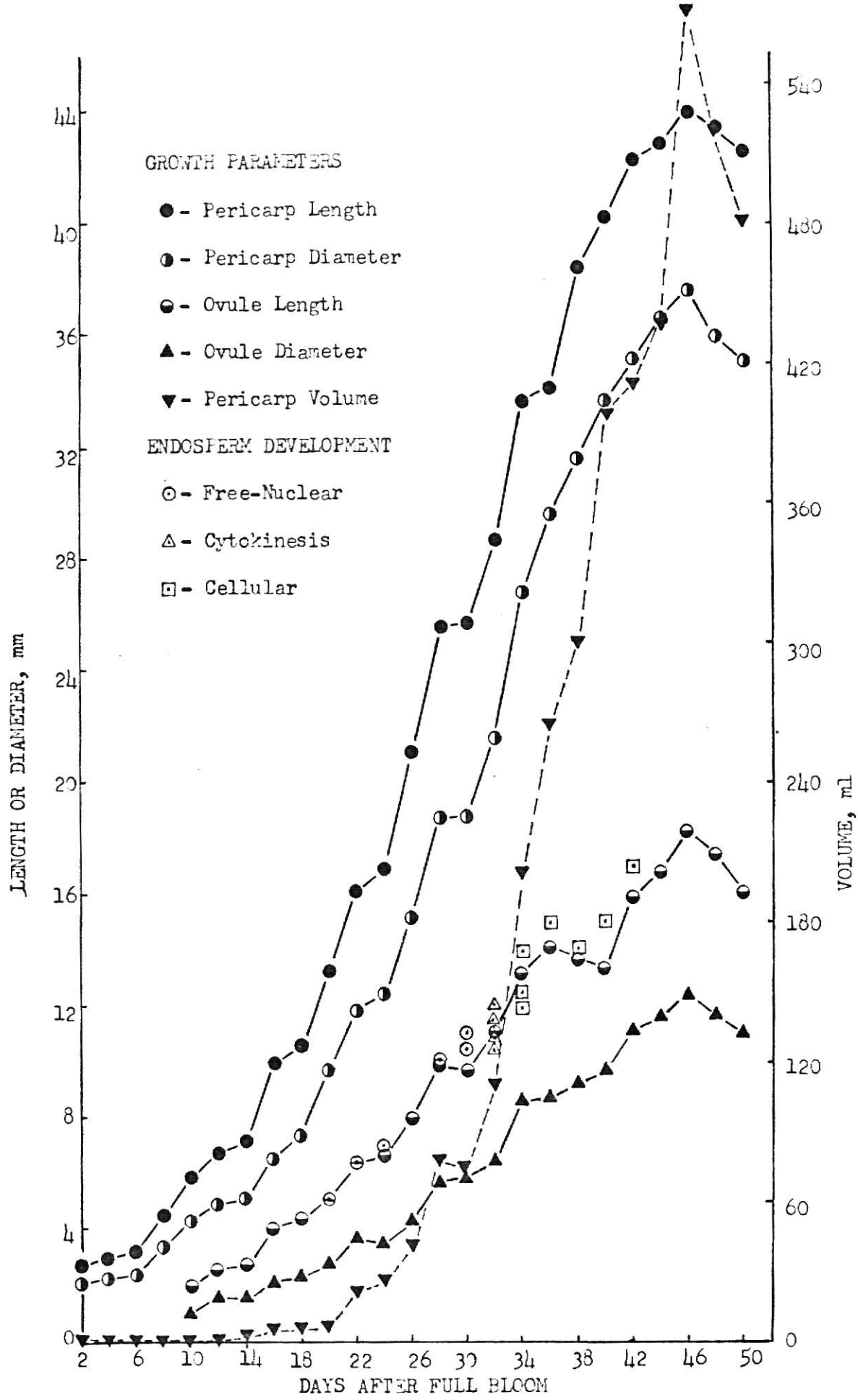
'Redhaven' was undergoing cytokinesis at 34 to 38 days AFB and also during this time endosperm was identified as being completely cellular. The free-nuclear stage was 34 days and prior.

'Redskin' was undergoing cytokinesis at 32 to 36 days AFB. The free-nuclear stage was 34 days AFB and before. Completely cellular endosperm was at 36 days and beyond.

'Babygold-5' was undergoing cytokinesis at 30 to 34 days AFB. One endosperm was identified as being completely cellular at 32 days but all other endosperm that had completed cytokinesis occurred at 36 days or beyond. Free-

Figure 1. Growth parameters of peach pericarps and ovules sampled at two-day intervals from 2 to 50 days after full bloom (AFB), April 15, 1975. Ovule measurements began 10 days AFB. Endosperm development is designated as free-nuclear, cytokinetic, or cellular based on microscopic observations and plotted according to sampling date and ovule length.

a 'Glohaven'


GROWTH PARAMETERS
● - Pericarp Length
◑ - Pericarp Diameter
◒ - Ovule Length
▲ - Ovule Diameter
▼ - Pericarp Volume
ENDOSPERM DEVELOPMENT
⊙ - Free-Nuclear
△ - Cytokinesis
⊡ - Cellular
LENGTH OR DIAMETER, mm
VOLUME, ml
DAYS AFTER FULL BLOOM
0
4
8
12
16
20
24
28
32
36
40
44
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120
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240
300
360
420
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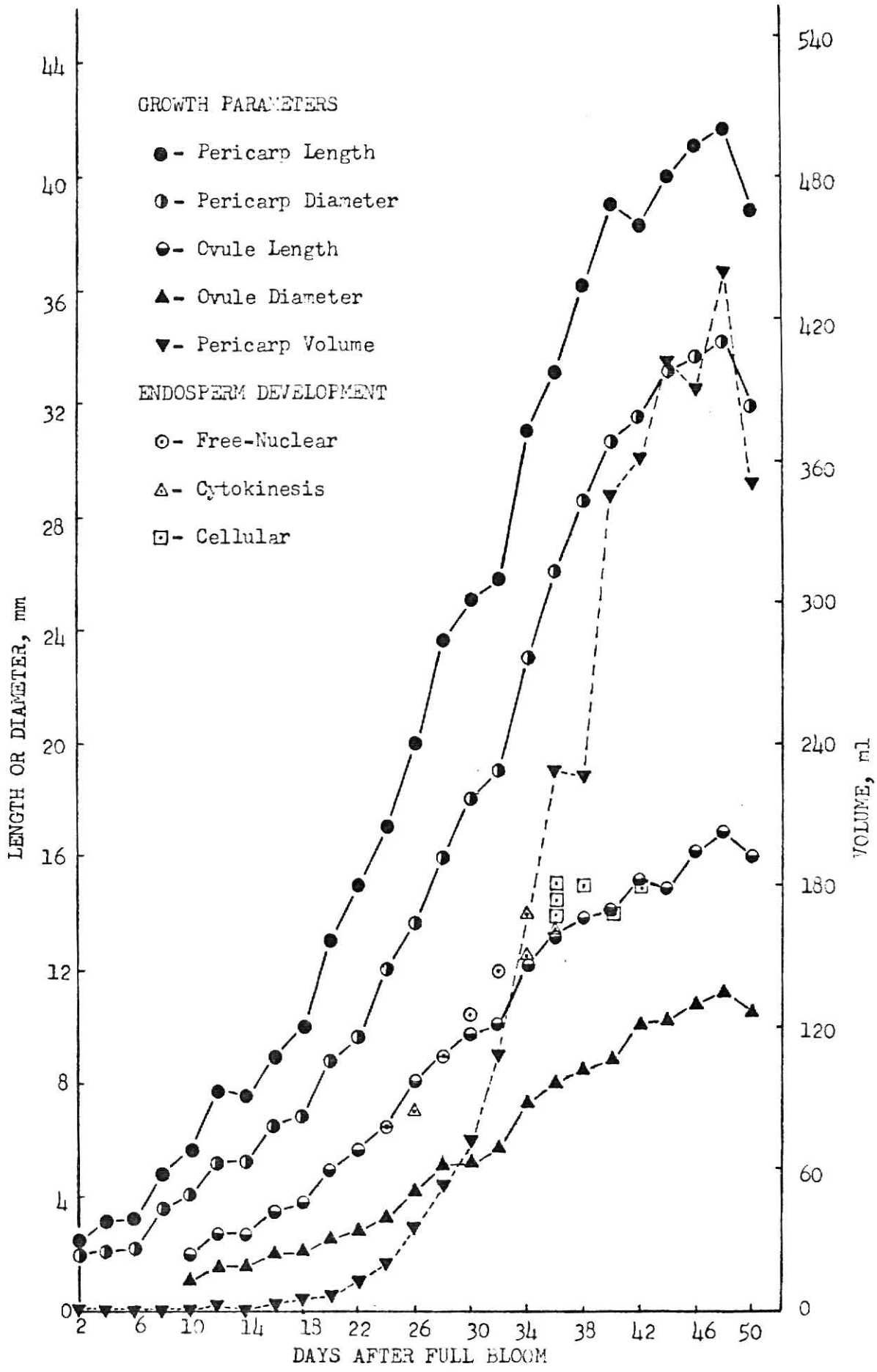
46
50

Figure 1. (continued)

b 'Cresthaven'


GROWTH PARAMETERS
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ENDOSPERM DEVELOPMENT
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LENGTH OR DIAMETER, mm
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DAYS AFTER FULL BLOOM
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Figure 1. (continued)

c 'Redhaven'

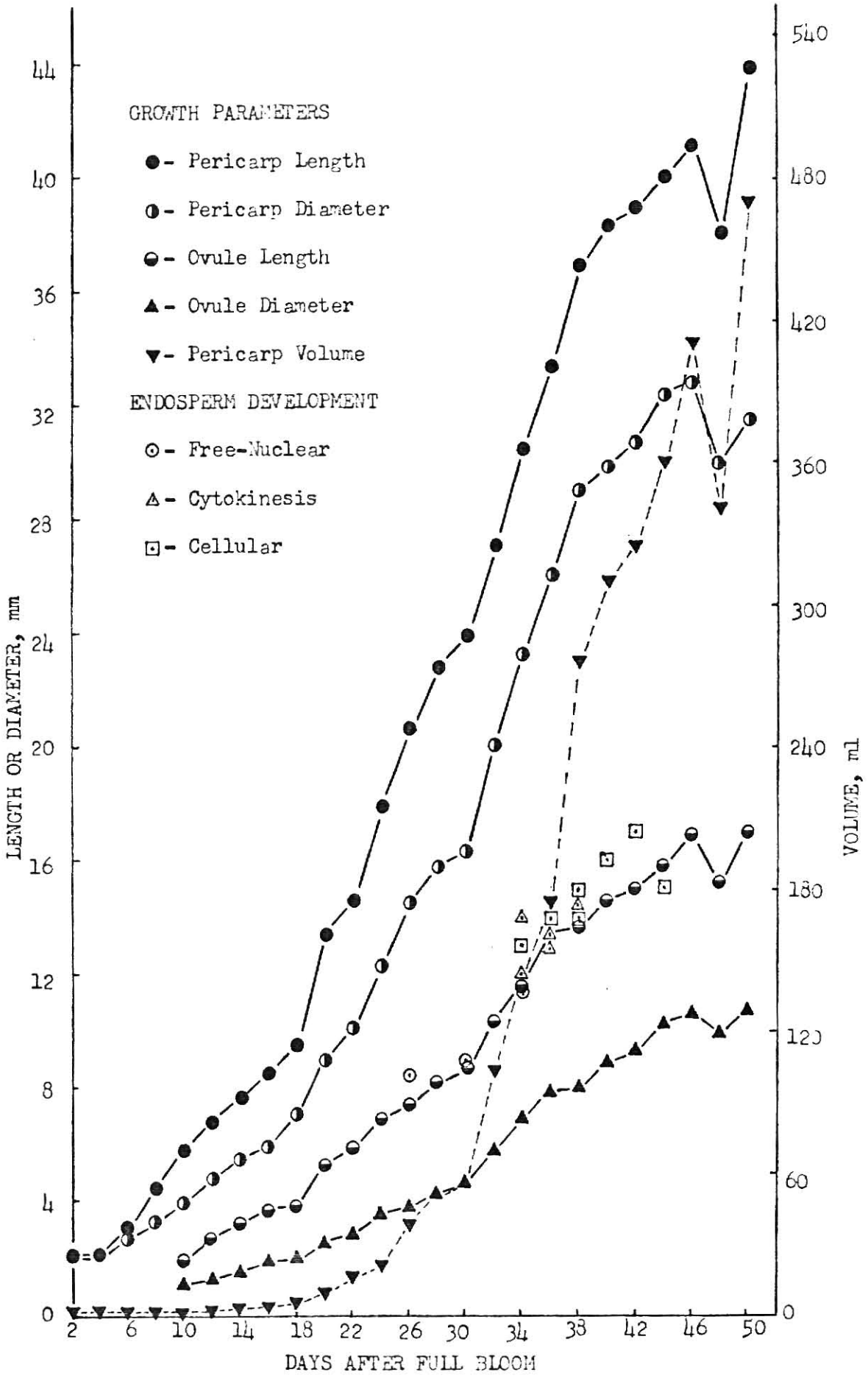
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Figure 1. (continued)

d 'Redskin'

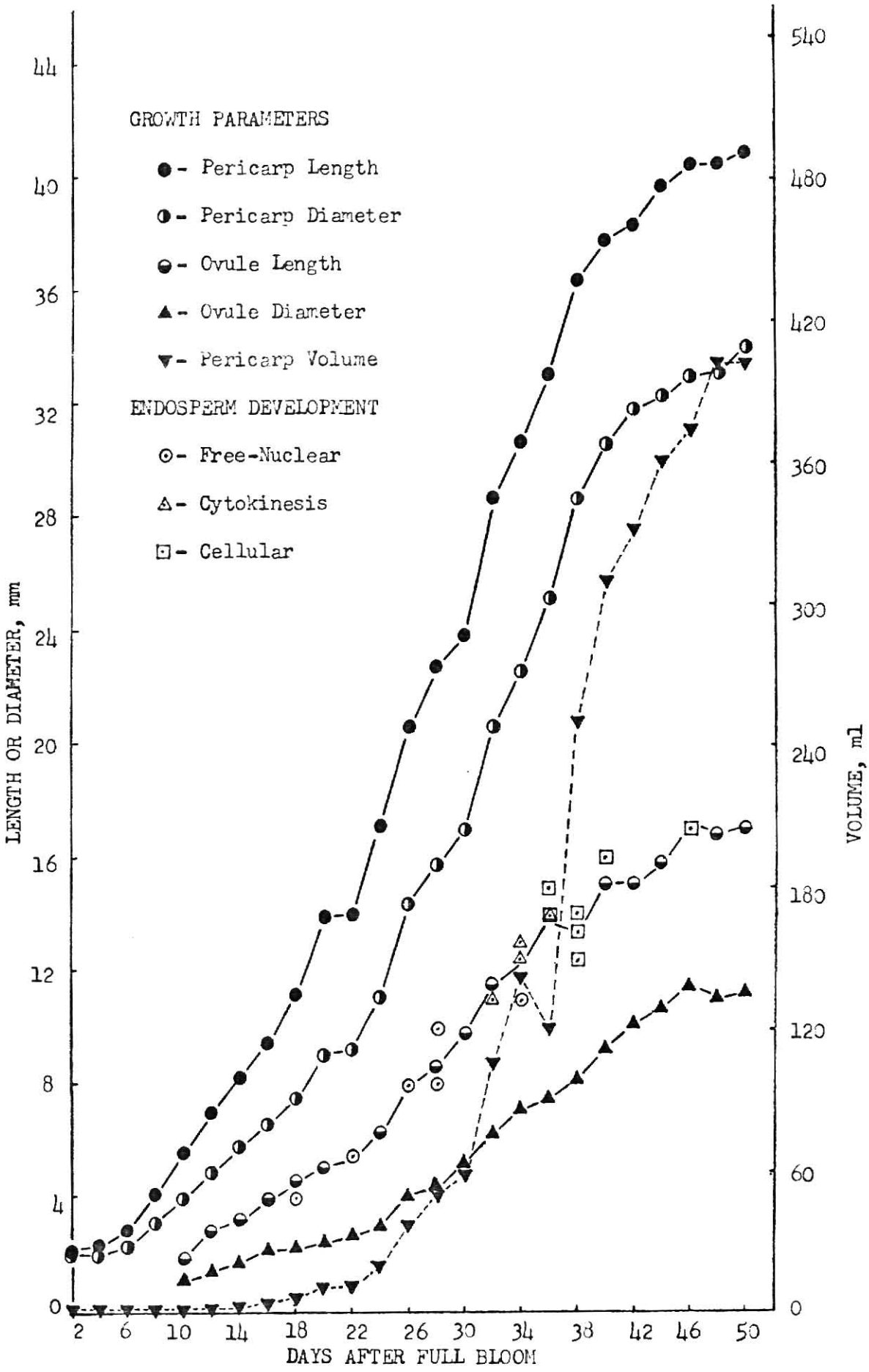
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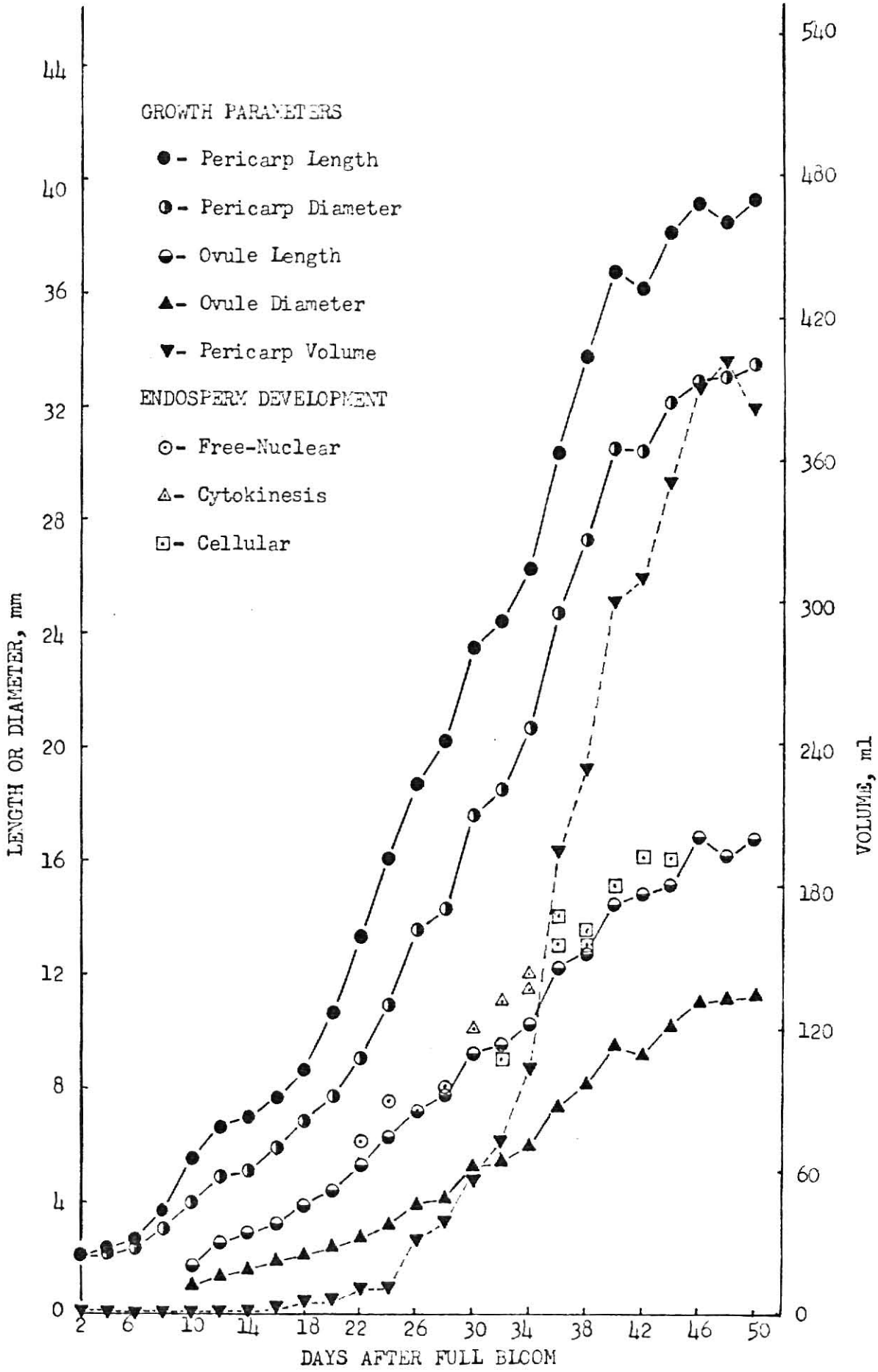
Figure 1. (continued)

e 'Babygold-5'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VOLUME, ml
DAYS AFTER FULL BLOOM
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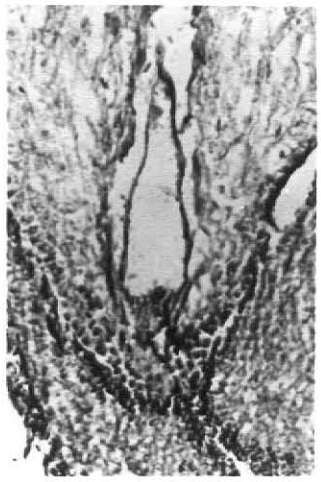
Plate I. Photomicrographs of 'Redskin' peach ovules showing endosperm development. Zeins Photomicroscope with standard tungsten illuminator and bright field illumination without condensor was used with 135 Kodak Plus-X Panchromatic film ASA 125.

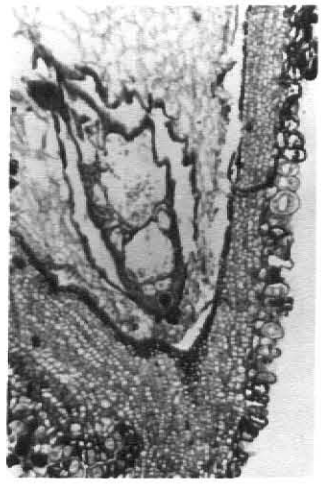
a Free-nuclear endosperm from ovule 4 mm in length and 18 days after full bloom (40X).

b Cytokinesis endosperm from ovule 14 mm in length and 36 days after full bloom (16X).

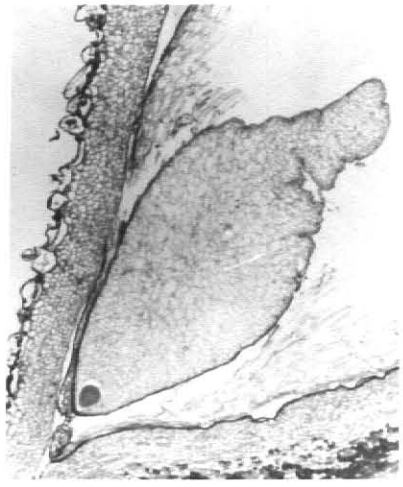
c Cellular endosperm from ovule 16 mm in length and 40 days after full bloom (10X).



a



b



c

nuclear endosperm was observed up through 28 days AFB.

The mean pericarp lengths and mean ovule lengths for all cultivars undergoing cytokinesis are presented in Tables 1 and 2, respectively. During endosperm cytokinesis, 'Babygold-5' mean ovule length was 9.0 to 10.0 mm and mean pericarp length was 23.5 to 26.0 mm, 'Glohaven' 11.0 mm and 29.0 mm, 'Redskin' 11.5 to 13.5 mm and 28.5 to 33.0 mm, 'Redhaven' 11.5 to 13.5 mm and 30.5 to 36.0 mm, and 'Cresthaven' 12.0 to 13.5 mm and 31.0 to 33.0 mm.

Pericarp volume during cytokinesis period (Table 3) Babygold-5 was 58 to 103 ml, 'Glohaven' 110.5 ml, 'Redskin' 116 to 141 ml, 'Redhaven' 141 to 277 ml, and 'Cresthaven' 168 to 228 ml.

'Babygold-5' entered the period of cytokinesis with the least number of accumulated degree days, 311.0, and an additional 60.0 degree days accumulated by the completion of cytokinesis (Table 4). 'Glohaven' was found to be undergoing cytokinesis on one sampling day and had accumulated 339.0 degree days. 'Cresthaven' and 'Redhaven' both began cytokinesis with 371 degree days accumulated, but 'Redhaven' had almost doubled the accumulated degree days by the completion of cytokinesis with 63.5 versus 35.0 for 'Cresthaven'. 'Redskin' entered the period of cytokinesis with the same number of accumulated degree days as Glohaven, 339.0 and also accumulated the longest range with 67.0 additional degree days.

Shuck split began approximately two weeks AFB and was completed approximately three and one-half weeks AFB (Appendix Table A).

'Redhaven' had the greatest number of twin ovules, 19 out of a possible 500 measured. 'Redskin' and 'Babygold-5' each had three. 'Glohaven' and 'Cresthaven' each had five.

Table 1. Peach pericarp length during endosperm cytokinesis period. Evidence of cytokinesis was obtained from samples underlined.

Cultivar	Mean Pericarp Length, mm[a] Days After Full Bloom				
	30	32	34	36	38
'Glohaven'	25.7	28.7	33.7[b]	34.1	38.4
'Cresthaven'	25.1	25.8	31.1	33.1	36.2
'Redhaven'	22.9	27.1	30.5	33.4	36.9
'Redskin'	23.9	28.7	30.7	33.1	36.5
'Babygold-5'	23.5	24.3	26.2	30.3	33.7

a Mean of 20 pericarps
b Mean of 19 pericarps

Table 2. Peach ovule length during endosperm cytokinesis period. Evidence of cytokinesis was obtained from samples underlined.

Cultivar	Mean Ovule Length, mm[a] Days After Full Bloom				
	30	32	34	36	38
'Glohaven'	9.7	11.1	13.2[b]	14.1	13.8
'Cresthaven'	9.8	10.1	12.3	13.3	13.8
'Redhaven'	8.6	10.3	11.7	13.0	13.6
'Redskin'	9.8	11.5	12.3	13.7	13.4
'Babygold-5'	9.2	9.4	10.2	12.2	12.7

a Mean of 20 ovules
b Mean of 19 ovules

Table 3. Peach pericarp volume during endosperm cytokinesis period. Evidence of cytokinesis was obtained from samples underlined.

Cultivar	Mean Pericarp Volume, ml[a]				
	Days After Full Bloom				
	30	32	34	36	38
'Glohaven'	73.8	110.5	200.0[b]	263.0	300.0
'Cresthaven'	73.0	107.5	168.0	228.0	225.0
'Redhaven'	55.0	103.0	141.0	175.0	277.0
'Redskin'	57.0	116.0	141.0	121.0	250.0
'Babygold-5'	58.0	73.0	103.0	194.0	230.0

a Mean of 20 pericarps
b Mean of 19 pericarps

Table 4. Accumulated heat units until peach endosperms had undergone cytokinesis.

Cultivar	Degree Days[a]		
	Beginning	Completion	Range
'Glohaven'	339.0	339.0	0.0
'Cresthaven'	371.0	406.0	35.0
'Redhaven'	371.0	434.5	63.5
'Redskin'	339.0	406.0	67.0
'Babygold-5'	311.0	371.0	60.0

a Formula for degree days:

$$\left[\frac{\text{Daily maximum temperature } ^{o}C + \text{Daily minimum temperature } ^{o}C}{2}\right] - 10^{o}C$$

DISCUSSION

Endosperms observed were classified according to one of the three developmental phases; free-nuclear, cytokinetic, or cellular. The period of cytokinesis for each cultivar was estimated from Figure 1, a - e. The mean ovule and pericarp lengths underlined in Tables 1 and 2 correspond to the days after full bloom in which cytokinesis occurred.

Ovules investigated varied as to endosperm developmental stage within a sampling date. 'Redhaven' at 34 days AFB had ovules in all three stages of development. The trend for all cultivars showed the free-nuclear stage at the smaller ovule length, the cellular stage at the larger ovule length, and the cytokinetic ovule stage of intermediate length within a sampling date. Exceptions were observed.

The object of chemical thinning application is to make application of the thinner at or during the cytokinetic stage, since significant thinning does not result when application is made during the free-nuclear or cellular stages for most thinning compounds. One-third of the fruit should be in the cellular stage to avoid abscission when the chemical thinner is applied so that the desired crop size is achieved.

Thinning of the cultivars studied should be able to be accomplished by chemicals. 'Glohaven' may be hard to thin chemically because all ovules observed were undergoing cytokinesis at the same sampling date. If this is true of all 'Glohaven' fruit, then chemical thinning must be precisely timed since there was only a short period that the compound would be effective.

Growth measurements of the pericarp and histological investigations (Figure 1, a - e) indicated that stage I was near completion at the time cellular endosperm could be identified. The growth curves for all five

cultivars coincided and were similar in duration. Tukey (29) found the length of stage I to be the same for the cultivars he tested.

The embryo and endosperm began to enlarge rapidly near the termination of stage I as observed by Tukey (29). Long strands of cytoplasm began to form at the micropylar end and along the embryo sac periphery. The central portion of the sac became cellular last.

Shuck split was complete about the middle of stage I which agrees with Harrold's observations (12).

The generally accepted ovule length indice for endosperm cytokinesis in long cycle peaches (19,27) is 7 to 10 mm. This was found to be the case only in 'Babygold-5' (Table 2). 'Glohaven', 'Cresthaven', 'Redhaven', and 'Redskin' all fell within 11.0 to 13.7 mm mean ovule length which was the range reported by Sherman and Arnold (27) for short cycle peaches.

Leuty and Bukovac (15) reported that 'Redhaven' was undergoing cytokinesis at 42 days AFB and with a mean pericarp length of 30.8 mm. The endosperm was cellular at a mean pericarp length of 33 mm and 48 days AFB in Leuty and Bukovac's experiment at East Lansing, Michigan. In a second experiment at Vineland, Ontario also using 'Redhaven', they found cytokinesis occurred 38 days AFB with a mean pericarp length of 29.5 mm.

In contrast to the results of the Michigan and Ontario experiments, the results from the collections made at Wichita, Kansas indicated cytokinesis in 'Redhaven' began 34 days AFB and was completed 38 days AFB with a mean pericarp length of 30.5 and 46.9 mm, respectively (Table 1). In Kansas the pericarp was longer, ovules were longer, and cytokinesis occurred earlier; therefore, if thinning is based on cytokinesis then the standards for Kansas should be based on larger pericarp-ovule size.

Ovule length appears to be more useful index of cytokinesis in all five cultivars as opposed to pericarp diameter, length or volume. Ovule

length grew continually but had a gradual increase which allows one to anticipate future daily growth; thereby, allowing the grower to predetermine the period that the endosperm would be undergoing cytokinesis. The pericarp parameters could be used however they grew more rapidly; thereby, not being as easy to anticipate amount of growth. Most researchers have used ovule length as one of their parameters; therefore, comparisons to past work can be made. Diameter measurements of the ovule and pericarp generally have not been used.

Accumulated degree days is more reliable parameter for estimating the time of cytokinesis than using the number of calendar days (Leuty and Bukovac, 15). Degree day calculations were based upon the standard formula; however, this formula is not adjusted for the peach in relation to minimum or maximum growth responses. Degree hours accumulated formula now being developed would take this into account.

'Babygold-5' began cytokinesis with the least accumulated degree days of 311. 'Redskin' and 'Glohaven' each had accumulated 339 degree days, and 'Cresthaven' and 'Redhaven' both had 371. This information is useful for determining how near the endosperm is to the cytokinetic stage. Frequent checks are easy to accomplish, and a tabulated record of accumulated degree days should be kept. Checking for ovule length can be delayed until accumulated degree days is nearing the time of cytokinesis for the cultivar in question.

It should be kept in mind that the accumulated heat unit range during cytokinesis was different for each cultivar. If chemical thinners are to be applied to 'Glohaven' it would be necessary to make application precisely as no range was found. A very short range was found for 'Cresthaven'. 'Redhaven', 'Redskin', and 'Babygold-5' would allow for more leaway in application as the range in this particular growing season spanned a four day period.

The importance of considering local climatic conditions in relation to growth and development and the geographic location is necessary in determining appropriate timing for cultural practices. The results from this experiment indicate a particular time period of cytokinesis at Wichita which differs from other peach growing areas.

CONCLUSIONS

Fruit growth during stage I for peach cultivars 'Glohaven', 'Cresthaven', 'Redhaven', 'Redskin', and 'Babygold-5' during the 1975 season at the Horticulture Experiment Field, Wichita, Kansas did not vary appreciably as to growth rate or duration.

The period of endosperm cytokinesis occurred toward the end of stage I. This period was approximately one day for 'Glohaven', two days for 'Cresthaven', and four days each for 'Redhaven', 'Redskin' and 'Babygold-5'. All observed ovules with endosperm undergoing cytokinesis ranged from a mean ovule length of 11.0 to 13.7 mm. Only 'Babygold-5' had a mean ovule length within the 7 to 10 mm length which is considered typical of long cycle peaches. 'Glohaven', 'Cresthaven', 'Redhaven', and 'Redskin' had a mean ovule range during cytokinesis of 11.0 to 13.7 mm which is considered typical of short cycle peaches.

Proper timing of chemical thinning sprays to correspond to cytokinesis stage of ovule development in Kansas appears to be at a greater ovule and pericarp size and at fewer days after full bloom than previously reported from other peach growing areas. Local environmental conditions along with geographic location was apparently a major contributing factor for the time, duration and pericarp-ovule lengths in determining cytokinesis.

APPENDIX

Table A. Shuck split.

Cultivar	Days After Full Bloom[a]	
	Beginning	Completion
'Glohaven'	14	18
'Cresthaven'	12	24
'Redhaven'	14	24
'Redskin'	12	24
'Babygold-5'	14	24

a Full bloom was 4-14-75.

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LITERATURE CITED

1. Blake, M.A. 1925.
The growth of the fruit of the Elberta peach from blossom bud to maturity. Proc. Amer. Soc. Hort. Sci. 22:29-39.

2. Blake, M.A. 1930.
Length of fruit development period of Elberta and some other varieties of peaches. N.J. Agr. Exp. Sta. Bull. 511. 24 pp.

3. Brink, R.A. and D.C. Cooper. 1940.
Double fertilization and development of the seed in angiosperms. Bot. Gaz. 102:1-25.

4. Brink, R.A. and D.C. Cooper. 1947.
The endosperm in seed development. Bot. Rev. 13:423-477.

5. Buchanan, D.W., R.H. Biggs, J.A. Blake, and W.B. Sherman. 1970.
Peach thinning with 3-CPA and ethrel during cytokinesis. J. Amer. Soc. Hort. Sci. 95:781-784.

6. Christ, E.G. and M. Bausher. 1968.
Chemical fruit thinning studies on peaches grown in New Jersey, pp. 26 to 33. In: S.R. Siemer (ed.) Symposium on Stone Fruit Thinning. Admal Co., North Chicago, Ill. 60604

7. Connors, C.H. 1920.
Measurements of peaches, pp. 82-88. In N.J. Agr. Exp. Sta. Annu. Rep. for 1919.

8. Detjen, L.R. 1926.
Physiological dropping of fruits. Del. Agr. Exp. Sta. Bull. 143. 47 pp.

9. Dorsey, M.J. and R.L. McMunn. 1926.
The development of the peach seed in relation to thinning. Proc. Amer. Soc. Hort. Sci. 23:402-414.

10. Dorsey, M.J. and R.L. McMunn. 1928.
The third report on the Illinois thinning investigations. Proc. Amer. Soc. Hort. Sci. 25:269-276.

11. Esau, K. 1965.
Plant Anatomy. 2nd ed. pp. 56-59. John Wiley and Sons, N.Y.

12. Harrold, T.J. 1935.
Comparative study of the developing and aborting fruits of Prunus persica. Bot. Gaz. 96:505-520.

13. Johansen, D.A. 1940
Plant Microtechnique. McGraw-Hill, Inc., N.Y. 523 pp.

14. Leuty, S.J. 1964.
Embryo and endosperm development in the peach (Prunus persica (L.) Batsch.) in relation to the induction of fruit abscission with naphthaleneacetic acid. Ph.D. Thesis. Mich. St. Univ. (Abstr.)

15. Leuty, S.J. and M.J. Bukovac. 1968.
The effect of naphthaleneacetic acid on abscission of peach fruits in relation to endosperm development. Proc. Amer. Soc. Hort. Sci. 92:124-134.

16. Lilien-Kipnis, H. and S. Lavee. 1971.
Anatomical changes during the development of Ventura peach fruits. J. Hort. Sci. 46:103-110.

17. Lilleland, O. 1932.
Growth study of the peach fruit. Proc. Amer. Soc. Hort. Sci. 29:8-12.

18. Lombard, P.B. 1959.
Endosperm and pericarp of the peaches as related to fruit thinning with plant regulators. Ph.D. Thesis. Mich. St. Univ. (Abstr.)

19. Lombard, P.B. 1968.
Revolution in chemical thinning in the West. Proc. Nat. Peach Council. 29:43-45.

20. Lombard, P.B. and A.E. Mitchell. 1962.
Anatomical and hormonal development in Redhaven peach seeds as related to the timing of naphthaleneacetic acid for fruit thinning. Proc. Amer. Soc. Hort. Sci. 80:163-171.

21. Lott, R.V. 1932.
The growth rate and chemical composition of the Hiley peach from stone formation to flesh maturity. Proc. Amer. Soc. Hort. Sci. 29:1-8.

22. Lott, R.V. 1939.
A study of the seasonal development of the peach fruit. Ph.D. Thesis. Univ. of Ill. (Abstr.)

23. Lott, R.V. 1952.
Some effects of temperature upon the development, maturation, and ripening of apples and peaches. Proc. Amer. Soc. Hort. Sci. 86:209-218.

24. Lott, R.V. and R.K. Simons. 1964.
Floral tube and style abscission in the peach and their use as physiological reference points. Proc. Amer. Soc. Hort. Sci. 85:141-153.

25. Ragland, C.H. 1934.
The development of the peach fruit with special reference to split-pit and gumming. Proc. Amer. Soc. Hort. Sci. 31:1-21.

26. Sass, J.E. 1971.
Botanical Microtechnique, 3rd ed. Iowa St. Univ. Press. Ames, Iowa. 288 pp.

27. Sherman, W.B. and C.E. Arnold. 1973.
Proper timing is vital to peach thinning. Fruit Var. and Hort. Dig. 26:37-38.

28. Sherman, W.B. and D.W. Buchanan. 1969.
Endosperm cytokinesis in Early Amber peach. Fla. St. Hort. Soc. 82:255-257. In Hort. Abstr. 41:77.

29. Tukey, H.B. 1933.
Growth of the peach embryo in relation to growth of fruit and season of ripening. Proc. Amer. Soc. Hort. Sci. 30:209-218.

30. Tukey, H.B. 1934.
Growth of the embryo, seed and pericarp of the sour cherry (Prunus cerasus) in relation to season of fruit ripening. Proc. Amer. Soc. Hort. Sci. 31:125-144.

31. Tukey, H.B. 1936.
Development of cherry and peach fruits as affected by destruction of the embryo. Bot. Gaz. 98:1-24.

32. Tukey, L.D. 1963.
A comparison in fruit sizing among early and late peach varieties. Fruit Var. and Hort. Dig. 18:11-13.

33. Weingerger, J.H. 1941.
Studies on time of peach thinning from blossoming to maturity. Proc. Amer. Soc. Hort. Sci. 38:137-140.

EARLY DEVELOPMENT OF THE PEACH (PRUNUS PERSICA (L.) Batsch.) FRUIT AND THE TIME OF ENDOSPERM CYTOKINESIS

of

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AN ABSTRACT OF A MASTER'S THESIS

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The early development for the peach (Prunus persica (L.) Batsch.) fruit was studied to determine the growth curve and the time of endosperm cytokinesis in the cultivars: 'Glohaven', 'Cresthaven', 'Redhaven', 'Redskin', and Babygold-5' growing at the Horticulture Experiment Field, Wichita, Kansas during the 1975 season, in order to determine a criterion for accurate chemical application timing for successful fruit thinning.

The experimental orchard had been planted into six replicated randomized complete block design utilizing the five cultivars studied. All five cultivars were sampled on every second day beginning two days after full bloom (AFB) (April 15, 1975) for a total of 25 sampling days covering 50 days. Ten fruits were sampled from one tree of each cultivar in two replicates per sampling day. A randomized sampling order was used. Fruits along with floral structures were placed in collection bottles containing sufficient FAA killing fluid and evacuated over-night.

Pericarp measurements taken were polar diameter, suture diameter, and volume. Ovule measurements were length and width. Six to ten μ longitudinal sections of the ovules were prepared, mounted and stained for microscopic examination.

The growth rate was similar for all five varieties with 'Glohaven' slightly more rapid and 'Babygold-5' slightly slower than the other three cultivars. 'Glohaven' was found to be undergoing cytokinesis 32 days AFB with a mean pericarp length of 28.7 mm; 'Cresthaven' 34 to 36 days AFB with mean ovule length of 12.3 to 13.3 mm and a mean pericarp length of 31.1 to 33.1 mm; 'Redhaven' 34 to 38 days AFB and had a mean ovule length of 11.7 to 13.6 mm and a mean pericarp length of 30.5 to 36.9 mm; 'Redskin' 32 to 36 days AFB with a mean ovule length of 11.5 to 13.7 mm and a mean pericarp length of 28.7 to 33.1 mm; and 'Babygold-5' 30 to 34 days AFB with a mean ovule length of 9.2 to 10.2 mm and a mean pericarp length of 23.5 to 26.2 mm.

Mean volume measurements during cytokinesis were: 'Babygold-5', ranged from 58 to 103 ml displaced; 'Glohaven', 110.5 ml; 'Redskin', 116 to 141 ml; 'Redhaven', 141 to 277 ml; and 'Cresthaven', 168 to 228 ml.

The period of endosperm cytokinesis occurred toward the end of stage I. This period was approximately one day for 'Glohaven', two days for 'Cresthaven', and four days each for 'Redhaven', 'Redskin', and 'Babygold-5. When endosperms were undergoing cytokinesis, ovules from all cultivars except 'Babyhold-5' ranged in length from 11.0 to 13.7 mm, which is the typical range reported for short cycle peaches but not for the long cycle cultivars studied in this experiment. 'Babygold-5' ovules ranged from 9.2 to 10.2 mm.

Local environmental conditions along with geographic location was apparently major contributing factors determining the time, duration, and pericarp-ovule lengths in determining cytokinesis.