Scientia Horticulturae, 21 (1983) 19—28
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

ENGLISH WALNUT FRUIT GROWTH AND DEVELOPMENT

KATHERINE PINNEY and VITO S. POLITO
Department of Pomology, University of California, Davis, CA, 95616 (U.S.A.)
(Accepted for publication 4 December 1982)

ABSTRACT

Pinney, K. and Polito, V.S., 1983. English walnut fruit growth and development. *Scientia Hortic.*, 21: 19—28.

Samples of the 'Ashley', 'Hartley' and 'Franquette' cultivars of English walnut (*Juglans regia* L.) were collected at weekly intervals from bloom to harvest, and development of the fruit, embryo and surrounding tissue was followed. The first division of the zygote occurred approximately one week after pollination, by which time the endosperm, nucellus and integument had shown considerable growth. The endosperm first formed cell walls at the 8-celled embryo stage and was completely cellular by the time the embryo contained approximately 64 cells. Rapid growth of the cotyledons started as the shell began to harden at Week 7. Measurement of fruit growth indicated that fresh weight gain in 'Ashley' and 'Hartley' fruits followed a double sigmoid pattern, characterized by a 3-week period of slowed growth that began approximately 7 weeks after bloom. Rate of increase in total length and diameter also slowed greatly at Week 7. Fresh weight gain in 'Franquette' appeared to follow a single sigmoid curve, with no prolonged period of slowed growth corresponding to that in the other cultivars. Kernel growth also described a double sigmoid curve, with its initial, rapid phase nearly coincident with the beginning of Stage III of the whole-fruit, fresh-weight curve at Week 10.

Keywords: fruit growth; *Juglans regia* L.; walnut.

INTRODUCTION

The English, or Persian, walnut (*Juglans regia* L.) is the second most widely planted tree-crop species in California (over 80 000 ha), yet there has been little research on the growth and development of the fruit of any of the important commercial cultivars during the California growing-season. Details on such basic parameters of fruit growth as the timing, sequence and anatomy of embryo, endosperm, shell and husk development are lacking in the literature.

Early researchers (Lubbock, 1891; Benson and Welsford, 1909; Holm, 1921) concerned themselves with gross morphology. Subsequent workers (Langdon, 1934, 1939; Nast, 1935; Manning, 1938, 1940) concentrated on flower histology and early embryology, but little attention was given to fruit development over the growing-season. More recently, Lin et al. (1977)

0304-4238/83/$03.00

examined floral organogenesis, while Roth (1977) and Westwood (1978) have briefly reviewed the subject of walnut fruit structure.

The objective of the present work was to determine the timing, pattern and sequence of structural changes which occur during fruit development from the pistillate flower stage to harvest in 3 commercially important (early 'Ashley', mid-season 'Hartley' and late 'Franquette') cultivars in the California walnut industry, and to generate a detailed description of fruit growth and development in these cultivars.

MATERIALS AND METHODS

The trees were growing in a well-managed commercial orchard on the Sacramento River, 16 km north of Davis. Walnut pistillate bloom extends over a period of several days or weeks, depending on the cultivar and climatic conditions. An effort was made to minimize variation due to differences in age by tagging flowers at the stage of maximum stigma receptivity. Fifteen to 20 trees of each cultivar were randomly selected and 250—400 flower pairs of each cultivar were tagged at full bloom. To further minimize variation, only those flowers which were present in pairs (no singles or triples) were tagged. Twenty flowers of each cultivar were collected at full bloom and 20 fruits of each cultivar were collected at weekly intervals until harvest.

Freshly collected samples were weighed and measurements taken of their length, from the calyx to the base of the ovary, and the radial circumference at the widest point. As the fruit developed, additional measurements were taken of shell length and width, and embryo fresh and dry (after 48 h at 65°C in a vacuum oven) weights.

The early samples were fixed in CRAF III (Sass, 1958) for 1 week, washed, dehydrated in 2,2-dimethoxypropane and acetone (modified from Lin et al., 1977), and embedded in paraffin. As an aid to fixation and infiltration, 0.5—1-mm thick pieces were sliced off the sides through the ovary wall in the plane parallel to the suture. Thicker slices were cut from the larger fruits, but the ovule was always left intact and supported by surrounding tissue. Once the shell began to harden, the developing seed was removed prior to fixation and the pericarp was fixed separately.

Young fruits and ovules were sectioned at 8 μm and stained with safranin-fast green (Sass, 1958). Sections of pericarp used to follow the sequence of shell hardening were cut at 10 μm and stained in Toluidine Blue O (Sakai, 1973).

Because 'Hartley' is currently the most widely grown walnut cultivar in California, investigation of the early stages of embryo development concentrated on it and, except where noted, descriptions of the early embryology refer to this cultivar.

RESULTS

The walnut pistillate flower contains a single orthotropic, unitegmic ovule. Analysis of the first collection of 20 flowers at pollination revealed that, in the majority of these, the ovule was in an early stage of embryo sac development.

One week later, the zygote was clearly evident at the micropylar end of the embryo sac (Fig. 1, AB) and the endosperm was large and still in the free nuclear division stage of development. Thus, although syngamy was not observed, it appeared that by this time fertilization had occurred.

Fruit development

Timing and sequence of the key stages in fruit development for the 3 cultivars are summarized in Table I. Details of embryogeny in 'Hartley' are described in the sections below.

Embryo. — The zygote remained as a single cell through the first week after fertilization and the nucleus contained 2 prominent nucleoli (Fig. 1, B). Two or 3 of the nucellar cells in the region of the zygote were slightly enlarged and vacuolate at Week 1. As the embryo grew, as many as 5 cells in the nucellus above the embryo enlarged and appeared to become internally disorganized. By the 64+ cell embryo stage, this condition had disappeared and only the crushed cell walls remained.

The first division of the zygote was always nearly transverse to slightly oblique (Fig. 1, C), and the second was always perpendicular to the plane of the first division. Subsequent divisions (Fig. 1, DEFG) occurred in all planes, forming a large globular embryo (Fig. 1, H) with a short blocky supensor about 6 weeks after pollination. The basal cell of the walnut embryo continued to divide, forming the suspensor and contributing significantly to the body of the embryo itself. Zygotes and early embryos had a dense, dark-staining mass adhering to them (Fig. 1, AB). This material was probably derived from degenerate synergids which had collapsed against the zygote and persisted there for an extended period.

About 6 weeks after pollination, the apex of the embryo began to flatten, a definite protodermal layer of cells became evident, and cotyledon primordia began to form (Fig. 2, ABC). Two weeks later, bifurcation of the 2 cotyledons was evident and the embryo assumed its 4-lobed form. The cotyledons continued to elongate, but gained little in thickness, remaining thin, transparent, bifurcate lobes until fully expanded at about 10 weeks after pollination (Fig. 3).

Endosperm. — During the interval between fertilization and the first division of the zygote, the endosperm had undergone numerous free nuclear divisions, which were not observed but are described by Nast (1941) as being

TABLE I

Stages of walnut fruit development (1980). "Date" as day/month

Date	Week	'Hartley'	Corresponding date and time after bloom in other cultivars			
			'Ashley'		'Franquette'	
			Date	Week	Date	Week
29/4	0	Full bloom, early embryo sac development				
6/5	1	Zygote present at the micropylar end of embryo sac				
14/5	2	2—8 cells in embryo, endosperm becoming cellular at the micropylar end				
21/5	3	8—32-celled embryo				
28/5	4	64+ celled embryo, completely cellular endosperm	21/5	4	4/6	5
4/6	5	Multicellular globular embryo to flattening of embryo apex, initiation of shell-hardening noted in stained sections, nucellar cap at maximum size	28/5	5	11/6	6
11/6	6	Cotyledon primordia evident, embryo approximately 1.0 mm	28/5	5	18/6	7
25/6	8	Macroscopic embryo (0.2—1.0 cm long), total growth slows, shell suture line sclerification from tip to base	11/6	7	25/6	8
1/7	9	Rapid growth of kernel begins	18/6	8	1/7	9
9/7	10	Final length and diameter of shell attained	1/7	10	23/7	12
16/7	11	Overall growth (fresh weight) resumes, kernel growth slows	25/6	10	9/7	10
23/7	12	Sclerification of the shell complete	16/7	12	20/8	16
13/8	15	Kernel growth resumes and remains rapid until harvest	7/8	15	20/8	16
3/9	18	Maximum total fresh weight	7/8	15	27/8	17
16/9	20	Ethephon application approximately 12 days prior to harvest	29/8	18	none	
25/9	21	Last collection — approximate harvest date	3/9	19	30/9	22

simultaneous. By the time of the first zygotic division, the endosperm occupied a parietal position surrounding a central vacuole. The first cell walls formed at the micropylar end about 2 weeks after pollination (see Fig. 1, D), when the embryo was 8-celled and the endosperm contained from 100 to 200 nuclei. Subsequent formation of walls proceeded basipetally and, by 2 weeks later, the endosperm was completely cellular. The endosperm be-

came much thicker at the micropylar end than at the chalazal end, where it remained as a few-layered parietal band surrounding the central vacuole, which persisted until the embryo reached its full length.

Nucellus. — The nucellus reached its maximum size about 4 weeks later, and persisted as a conical cap above the embryo and as a thin band of cells surrounding the endosperm until the embryo reached full size, crushing the nucellar cells against the integument.

Integument. — The single integument changed little in thickness as the fruit matured. At anthesis, the micropyle was relatively large, but prior to fertilization the integument had lengthened to cover the expanding nucellus completely and close the micropyle. As the ovule enlarged, the integument, as a differentiating seed coat, developed as a uniform layer of cells surrounding the nucellus, endosperm, and embryo.

Shell. — The pattern of shell hardening was basipetal in the longitudinal plane and centripetal in the transverse plane. The first cells to become sclerified were those at the micropylar end. Sclerification proceeded basipetally from this point, with the cells along the suture lines becoming sclerified very rapidly. Sclerification of the cells along the sutures was complete before those perpendicular to the suture had progressed more than 1 mm. Completion of sclerification from this point required less than a week. By the fourth week after pollination, the tissue which would become the shell had differentiated as small isodiametric cells located between the hull and the ovule. Only the outermost one-half to two-thirds of this tissue actually contributed to the shell; the remainder contributed to the packing tissue.

Growth

Whole fruit. — In 'Ashley' (Fig. 4, A) and 'Hartley' (Fig. 4, B), the increase in whole fresh weight followed a double sigmoid pattern of growth. The initial accelerated phase (Stage I) began soon after fertilization and continued until the 6th or 7th week after bloom. This was followed by a slowed period of growth (Stage II), which lasted between 3 and 4 weeks. A second accelerated phase (Stage III) began about the 9th week in 'Ashley' and the 11th week in 'Hartley', and was followed by a gradual loss in weight as the fruits began to dry prior to dehiscence, 4—6 weeks later.

'Franquette' showed a similar pattern of growth (Fig. 4, C), but no extended period of slowed growth was clearly evident. The use of whole fresh weight as a measure of growth led to some difficulty in interpretation of the data due to irrigation-induced variation, which became evident in later stages. Collections were made every week on Tuesdays or Wednesdays, and the trees were watered on alternate Mondays or Tuesdays. 'Franquette'

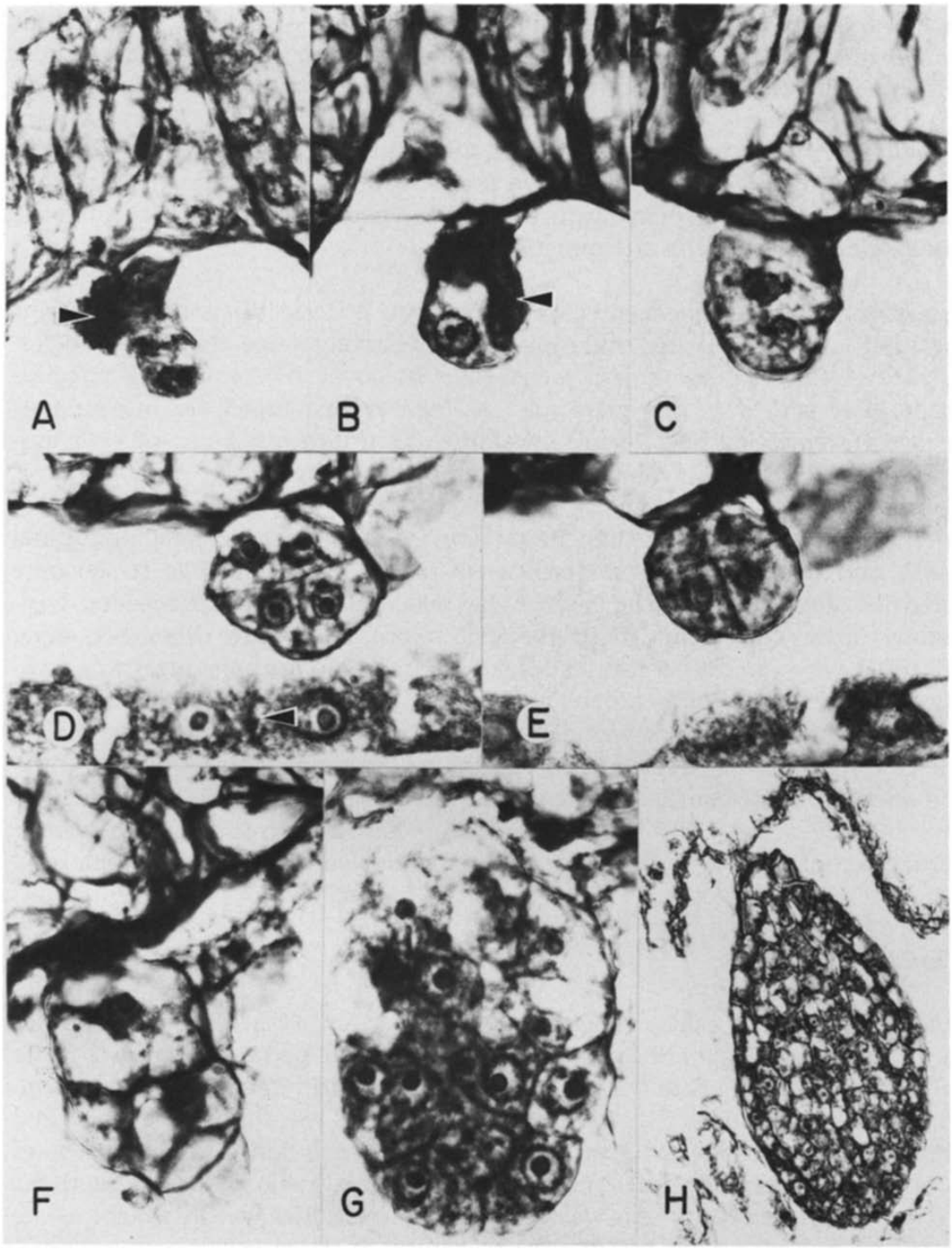

Fig. 1. Early embryo development in 'Hartley' walnut. A, B, zygote. Note the 2 nucleoli, the densely stained material adhering to the zygote (arrow) and enlarged nucellar cells above the zygote. C, 2-celled embryo. D, E, adjacent serial sections of an embryo at the 8-cell stage. Note the cell walls formed in the endosperm (arrow). F, 16-celled embryo. G, 64+ cell stage. H, globular embryo. Figures are oriented such that the micropylar end of the orthotropous ovule is toward the top of the page.

was especially affected by the irrigation schedule. Its fruits lost 50% of the weight gained the first week following irrigation. This variation was found to a lesser extent in 'Ashley' and 'Hartley', and here too it corresponded exactly to the irrigation schedule. A smaller and corresponding fluctuation could also be seen in whole fruit length and width, and in kernel fresh weight.

When the whole fruit length and width were plotted, the data for all 3 cultivars (Fig. 4) showed very similar, single sigmoid curves. The initial accelerated phase began, as with the weight curves, soon after fertilization and lasted about 4 weeks. This was followed by a period of slowed growth, which leveled off 3—4 weeks later as the fruits approached final size.

Kernel. — Kernel (embryo, endosperm, nucellus and integument) fresh and dry weight also showed a marked double-sigmoid pattern. Stage I of kernel fresh weight growth began 8—9 weeks after pollination and lasted for 1—2 weeks. Growth slowed during Stage II, which lasted until Week 15, after which rapid growth continued until harvest. The kernel dry weight curves were similar to those obtained for fresh weight. As the endosperm was absorbed and the embryo began to accumulate solids and dry matter near harvest, water was lost from the kernel and the curves moved much closer together. At the last collection date, 1 week after the actual harvest date, 'Franquette' kernels contained less than 5% water.

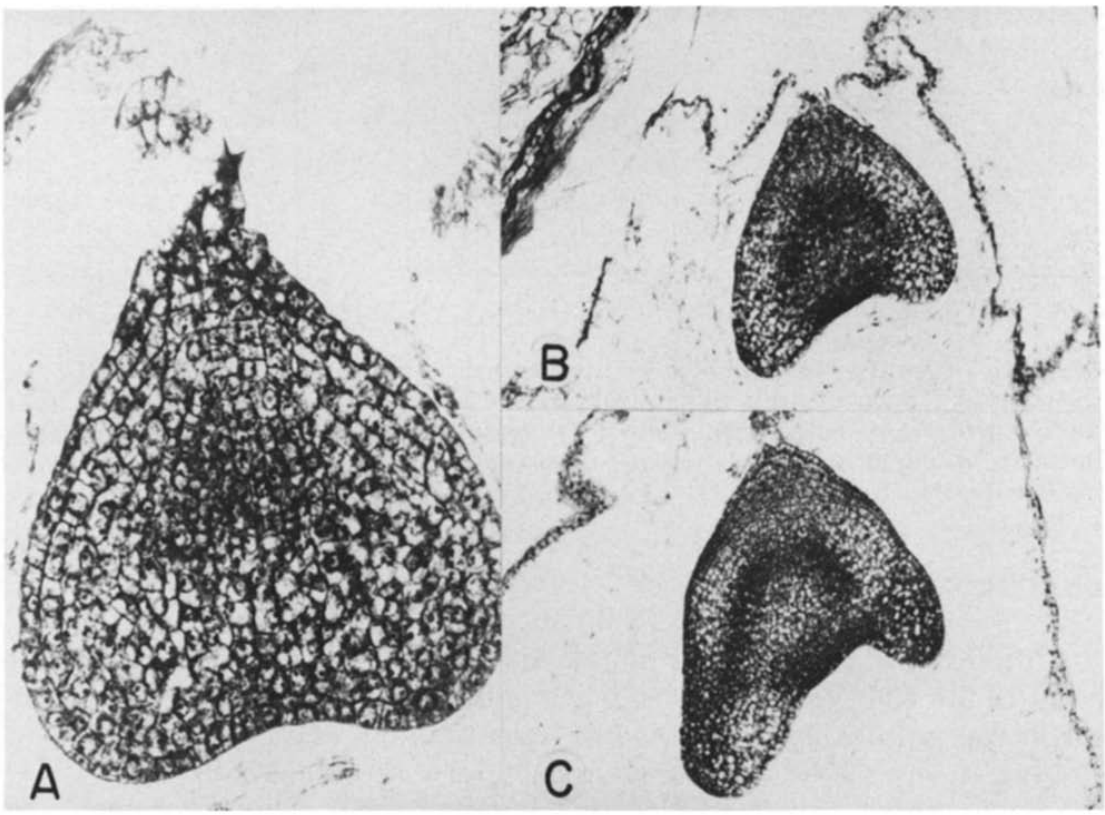

Fig. 2. Embryo development in 'Hartley' walnut. Initiation (A) and development (B, C) of the cotyledon primordia.

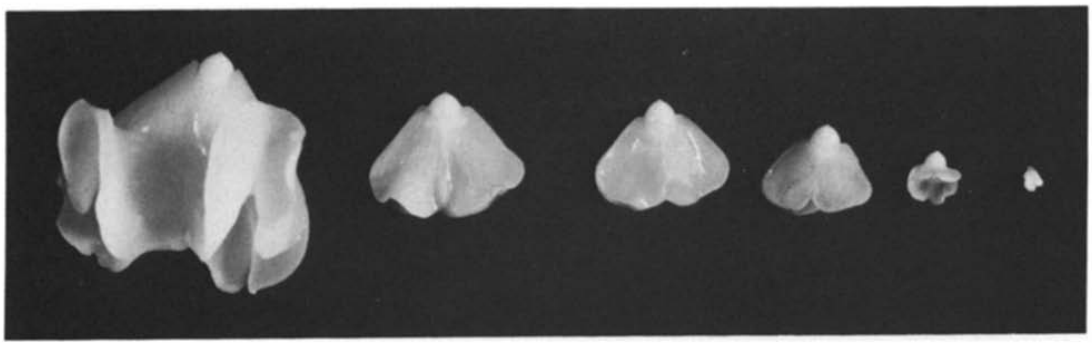

Fig. 3. Early stages in the development of embryos of 'Ashley' walnuts. Note bifurcation and infolding of the cotyledons as they expand to full size.

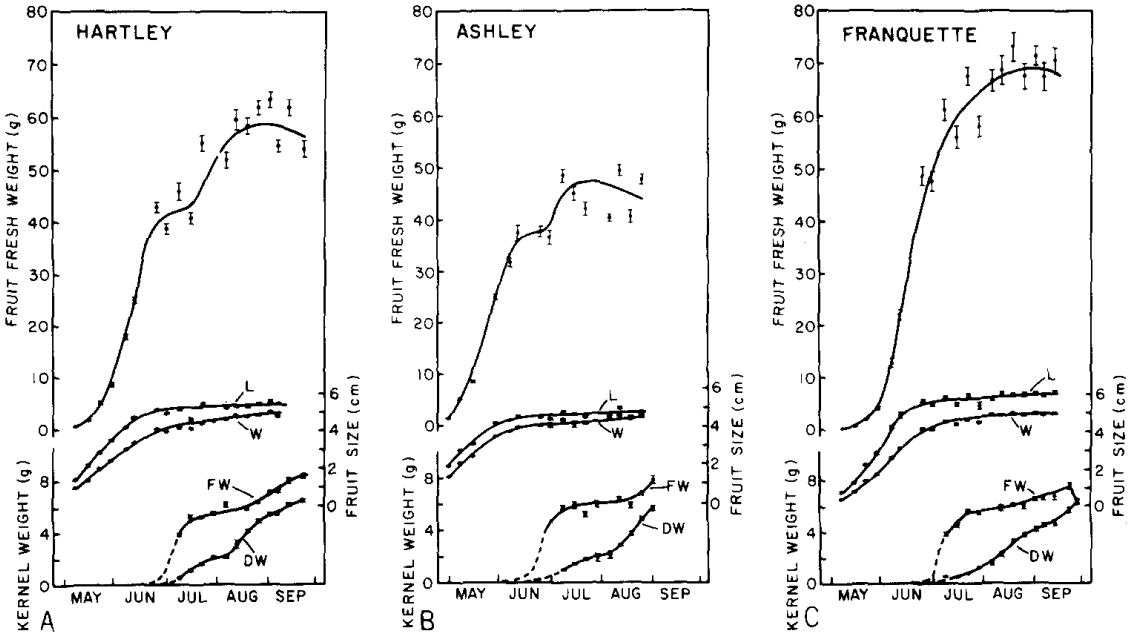

Fig. 4. Growth curves of the whole fruit and kernel of the 'Ashley' (A), 'Hartley' (B) and 'Franquette' (C) walnut from bloom to harvest. Curves represent changes in whole fruit fresh weight (top), length (L), width (W), kernel fresh (FW) and dry (DW) weights. Dotted portions of the kernel growth curves were inferred from observation of embryo growth. Bars represent 2 × S.E.M.

DISCUSSION

Although the terminal cell of the first division usually gave rise to the body of the embryo and the basal cell contributed primarily to the suspensor, it was not possible to fit the subsequent pattern of growth in the walnut embryo to any of the classical embryo types (Johansen, 1950). Nast (1941) followed embryo growth in 'Concord' in detail and she, too, was unable to

discover any consistent pattern of growth prior to what she described as the globular embryo. While the planes of the first 2 or 3 divisions were consistent from embryo to embryo, subsequent divisions were completely irregular. Cells derived from the original basal cell of the first division appeared to divide less frequently than those from the original apical cell, resulting in a globular, somewhat pear-shaped embryo about 5 weeks after pollination. One week later, the cotyledons appeared, a protodermal layer and procambium became evident, and the beginning of the hypocotyl axis was distinguishable.

Nast (1941) described growth of the cotyledons and of root and shoot apices in detail. She found that the cotyledons expanded and became shaped as a result of the activity of the meristems present. The cotyledons did not appear to touch the walls of the locule until they reached their final length, about 11 weeks after full bloom.

From the time of fertilization until the 64+ cell stage, the embryo is partially covered with a dark-staining mass of tissue, which both Nast (1941) and Langdon (1934) considered to originate from the pollen tube. Although this material may include the pollen tube or tubes, it is more likely that it consists mainly of the remains of degenerate synergid material. Nast (1941) also noted the presence of a dark-staining layer along the surface of the embryo sac adjacent to the nucellus, suggesting pollen tubes as its origin also. This material was found to cover an area several sections thick as a dense, amorphous layer in which individual pollen tubes could not be discerned. This tissue had the appearance of crushed cells, perhaps created by the pressure exerted by the expanding embryo sac.

Sclerification of the shell in the Juglandaceae has been variously described. In pecan (*Carya illinoensis*), McKay (1947) described it as beginning at the blossom end, while Nast (1941) described shell hardening in *J. regia* as being centripetal. Roth (1977) investigated these events in great detail and described it as being both centripetal and basipetal. This is confirmed here.

The pattern of fruit growth in relation to the time after bloom was similar in the 3 cultivars studied (see Table I). In each case, the onset of major developmental stages occurred at comparable time after bloom. For example, initiation of shell-hardening occurred 5—6 weeks after bloom, increase in fruit size slowed 7—8 weeks after bloom, and rapid kernel-growth began at 8—9 weeks.

The fresh weight growth curves for 'Ashley' and 'Hartley' were found to be double sigmoid, although there was some fluctuation in response to the irrigation schedule. In addition, there was collaborative evidence to suggest that the weight curves for all 3 cultivars are double sigmoid, although Stage II may be very brief in 'Franquette'. A pause or slowed phase of growth occurred approximately 7 weeks after pollination in all cultivars. At this time, also, growth in diameter began to decrease, tip-hardening occurred, and rapid kernel-growth began soon afterwards. Further, the basic struc-

ture of the walnut mimics that of the fleshy drupes, all of which exhibit a double-sigmoid pattern of growth, where the stage of slowed growth typically corresponds to the period of embryo growth and endocarp hardening.

ACKNOWLEDGEMENTS

We acknowledge with thanks the advice and cooperation of Dr. George Martin throughout this work, Mr. Reid Beckstrom, Manager of Desseret Farms, for allowing us access to his orchards, Ms. Valarie Stallman for her capable technical assistance, and Mr. Don Edwards for his assistance in the preparation of the figures.

REFERENCES

Benson, M. and Welsford, E.J., 1909. The morphology of the ovule and female flower of *Juglans regia* and of a few allied genera. Ann. Bot., 23: 625—632.

Holm, T., 1921. Morphological study of *Carya alba* and *Juglans nigra*. Bot. Gaz., 72: 375—389.

Johansen, D.A., 1950. Plant Embryology. Chronica Botanica, Waltham, MA, 305 pp.

Langdon, L.M., 1934. Embryogeny of *Carya* and *Juglans*, a comparative study. Bot. Gaz., 96: 93—117.

Langdon, L.M., 1939. Ontogenetic and anatomical studies of the flower and fruit of the Fagaceae and Juglandaceae. Bot. Gaz., 101: 301—327.

Lin, C.H., Falk, R.H. and Stocking, C.K., 1977. Rapid chemical dehydration of plant material for light and electron microscopy with 2,2-dimethoxypropane and 2,2-diethoxypropane. Am. J. Bot., 64: 602—605.

Lin, J., Shabany, B. and Ramos, D., 1977. Pistillate flower development and fruit growth in some English walnut cultivars. J. Am. Soc. Hortic. Sci., 102: 702—705.

Lubbock, J., 1891. On the fruit and seed of the Juglandaceae. Bot. J. Linn. Soc., London, 28: 247—254.

Manning, W.E., 1938. The morphology of the flowers of the Juglandaceae. I. The inflorescence. Am. J. Bot., 25: 407—419.

Manning, W.E., 1940. Morphology of the flowers of the Juglandaceae. II. The pistillate flowers and fruit. Am. J. Bot., 27: 839—852.

McKay, J.W., 1947. Embryogeny of the pecan. J. Agric. Res., 74: 263—283.

Nast, C.G., 1935. Morphological development of the fruit of *Juglans regia*. Hilgardia, 9: 345—381.

Nast, C.G., 1941. The embryogeny and seedling morphology of *Juglans regia*. I. DeLilloa. Rev. Bot. Inst., Buenos Aires, 6: 163—205.

Roth, I., 1977. Fruits of Angiosperms. Encyclopedia of Plant Anatomy, Gebruder Borntraeger, Berlin-Stuttgart, 614 pp.

Sakai, W.S., 1973. Simple method of differential staining of paraffin-embedded plant material using toluidine blue O. Stain Technol., 48: 247—249.

Sass, J.E., 1958. Botanical Microtechnique. Iowa State College Press, Ames, IA, 228 pp.

Westwood, M.N., 1978. Temperate-zone Pomology. Freeman, San Francisco, CA, 428 pp.