Table 2. Mean style length and distance of stigma from the highest anther in flowers of 6 almond cultivars.

Cultivar	Style length (mm)	Distance of stigma from the highest anther (mm)[z]
Ardechoice	11.3 ± 0.5[y]	+2.0 ± 0.2
Desmayo Larguetta	12.7 ± 1.0	+2.6 ± 0.5
Ferragnes	13.3 ± 0.7	+ 3.1 ± 0.2
Retsu	10.9 ± 0.9	+ 0.2 ± 0.8
Texas	11.3 ± 0.8	+ 3.3 ± 0.2
Truoito	8.6 ± 0.9	− 2.3 ± 0.3

[z] + Stigma above highest anther; − Stigma below highest anther.
[y]Mean and SD (n = 50).

pollinated flowers (Table 1), but significantly lower than the hand self-pollinated.

The stigmas of 'Ferragnes', 'Ardechoice', 'D. Larguetta', and 'Texas' were positioned above the highest anther (Table 2), while the stigmas of 'Retsu' were at the same level as the highest anther. Stigmas of the 'Truoito', however, were between the lowest and highest anthers (Fig. 1). Most of the 'Truoito' flowers examined had stigmas in contact with anthers. The morphology of the 'Truoito' flower is ideal for cool climate pollination (too cool for bee flight) because the short style length maintains a stigmatic surface below and in contact with the anthers (30 per

Pollen tube growth was temperature related as has been reported for other almond cultivars (1, 6). Pollen tube growth in selfed styles of 'Truoito' was continuous without any sign of incompatibility at all temperatures tested, except 30°C, in 1982 and 1983 (Fig 2). It took 144 hr for pollen tubes to penetrate the base of all styles in both treatments at 10°C, while 72 hr were required in the selfed and 96 hr in crossed styles at 15°. At 20° and 25°, pollen tube extension to the stylar base took 54 hr for both self- and cross-pollination. Pollen tube growth in selfed styles at 30°C was inhibited, however, and only 55% had pollen tubes at their base after 54 hr.

Pollen tube growth in the field was slow in both treatments, taking 120 hr to penetrate the base of most styles. No differences in pollen tube growth rate were noticed between selfed or crossed styles, which explains the high fruit set from selfing.

The self-compatibility of 'Truoito' has been confirmed by examining the pollen tube growth and the fruit set level from self- or cross-pollination. The ratio of stamen number to pistil length in 'Truoito' flowers was 2.5 and it was higher than the range of 2.0 to 1.7 found in self-incompatible cultivars examined or reported elsewhere (7). Screening of other almond cultivars and seedlings for flowers having similar morphological characteristics would be desirable in a breeding program.

Literature Cited

1. Godini, A. 1981. Observing pollen tube growth in self-compatible almond cultivars by means of fluorescence. In G.R.E.M.P.A. colloque. 1980, Izmir Options mediterraneennes Institute Agronomique Mediterraneen, Zaragosa, 1981/I:77–82.
2. Kantartgis, N. 1961. Incompatibility and blooming period of the commercial almond and cherry cultivars grown in Greece. Ann. of the School of Ag., Univ. of Thessaloniki. 6:18–56.
3. Kester, D.E. and H.W. Griggs. 1959. Fruit setting in the almond: the effect of cross-pollinating various percentages of flowers. Proc. Amer. Soc. Hort. Sci. 74:206–213.
4. Martin, F.W. 1958. Staining and observing pollen tubes in the style by means of fluorescence. Stain. Tech. 33:125–127.
5. Muhturi-Stylianidu, E. 1979. Incompatibility of the almond *cv* Truoito. Agr. Res. III:64–73.
6. Socias i Company, D.E. Kester, and M.V. Bradley. 1976. Effects of temperature and genotype on pollen tube growth in some self-incompatible and self-compatible almond cultivars. J. Amer. Soc. Hort. Sci. 101(5):490–493.
7. Suranyi, D. 1976. Differentiation of self-fertility and self-sterility in *Prunus* by stamen number/pistil length ratio. Hort Science 11(4):406–407.

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Pecan Kernel Proteins and Their Changes with Kernel Development

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Abstract. **Pecan [*Carya illinoensis* (Wangenh.) Koch] kernel development is characterized by rapid accumulation of dilute acid and dilute alkali soluble proteins and decline of buffer and alcohol soluble proteins during embryo and cotyledon expansion. Mature kernels contained 7.8% protein, consisting of 51% acidic glutelins, 27% alkali glutelins, 9% concentration alkali, 7% prolamine, 4% albumin, and 1% globulin. Each fraction was composed of at least 2 proteins throughout kernel development. Proteins in each fraction were comprised primarily of neutral amino acids, but individual amino acid levels were highest for basic amino acids, with relatively high levels of lysine and sulfur containing amino acids. Electrophoresis of acid soluble glutelins revealed at least 7 subunits with molecular weights of 102, 58, 37, 30, 26, 19, and 16 (× 10^3). The data are considered in relation to alternate bearing and manipulations of fruit maturity.**

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Annual pecan production in the United States approximates 154 million kg of which essentially all comes from trees exhibiting some degree of irregular bearing. Irregular bearing creates uncertainly in income, marketing, and kernel quality. It seems largely dependent upon energy reserves, such that low reserves correlates with reduced nut yield (12, 13, 15, 16). Factors, such as late-season kernel filling, that prevent accumulation of a high level of energy reserves in storage tissues by the conclusion of the growing season contribute to irregular bearing (12). Early kernel development and maturation in relation to autumn leaf abscission would likely contribute to consistent nut production and quality. The developmental processes of kernel growth (filling of cotyledons) are intimately associated with both quantitative and qualitative changes in endogenous kernel proteins (8). These proteins accumulate rapidly after shell hardening (7) to about 10% of kernel weight at nut maturity (5, 7). The relationship of protein types and composition to kernel development is unknown, especially the relationship with initiation of rapid kernel filling. This report presents baseline information concerning protein classes and their association with kernel development.

Source of plant material. Pecan fruit were collected from ten 65-yr-old 'Moneymaker' pecan trees maintained free of disease and insect pests. Samples were from trees with moderate crops; thus, kernel development was normal and had well-filled cotyledons. Fruit were collected 10 Aug. to 27 Oct. at intervals of 1–3 weeks, packed in dry ice, bulked, transported to the laboratory, and lyophilized. The kernel (embryo, endosperm, and testa) was separated from other fruit tissues and analyzed for lipid and protein characteristics.

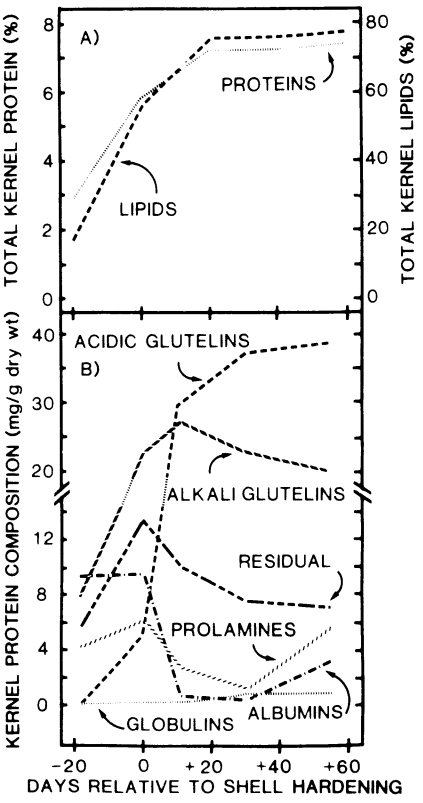

Fig. 1. Total lipids and proteins in developing pecan kernels (**A**), and accumulation of different classes of protein in developing kernels (**B**). Protein classes are designated according to their soluability in alcohol (prolamines), aqueous buffer (albumins), aqueous salt (globulins), dilute acid (acidic glutelins), dilute alkali (alkali glutelins), and concentrated alkali (residual). Kernel development is indexed to shell hardening (defined as complete lignification of the shell), which occurred on 3 Sept.

Lipid extraction and quantitation. Crude lipids were measured by extracting kernel tissue with diethyl ether, weighing the kernel residue and determining lipid content by subtraction.

Protein extraction and quantitation. Kernel proteins were extracted and classified according to the solubility method of Osborn (11). Kernel meal was extracted consecutively with 0.1 M sodium phosphate buffer (pH 7), 10% NaCl, 70% ethanol, 0.05 N HCl and 0.05 N NaOH to solubalize albumins, globulins, prolamines, and glutelins, respectively. Fifteen grams of freeze-dried kernel (in triplicate) from each of several sampling dates were ground in a mortar with a pestle with acetone to remove lipids prior to protein extraction. Protein quantitation was by the Bradford method (2).

Electrophoresis. Protein fractions that exhibited marked changes during kernel development were precipitated by adjusting sample solution pH in preparation for polyacrylamide gel electrophoresis (PAGE). The acid soluble glutelin precipitate then was centrifuged and the pellet subjected to sodium dodecyl sulfate (SDS) PAGE with and without β-mercaptoethanol. [SDS sample buffer was 391 mg $NaH_2PO_4 \cdot H_2O$; 1.02 g Na_2HPO_4; 1 g SDS; 1 ml β-mercaptoethanol (or absent); and 15 mg Bromphenol Blue diluted to 100 ml with deionized water.] After tubes containing sample or marker were heated in boiling water for 10 min, they were ready for electrophoresis.

Ten percent SDS polyacrylamide gels were prepared by combining 15 ml of gel buffer (7.8 g $NaH_2PO_4 \cdot H_2O$; 20.45 g Na_2HPO_4; 2.0 g SDS; water to one liter, pH 7), 13.5 ml of 10% acrylamide solution (22.2 g acrylamide; 600 mg N, N-methylene-bis-acrylamide, 100 ml water), 1.5 ml ammounium persulfate solution (100 mg ammonium persulfate in 15 ml water, and 50 µl TMEDA (N,N,N,N-tetramethylethylenediamine). This mixture was dispersed in glass gel tubes (5 mm × 102 mm) and layered with water.

Gel tubes were placed in a Pharmacia (Model GE-2/4) electrophoresis unit filled with 3.3 liters of gel buffer diluted 1:2 with water. Samples of 10 µg of treated protein were layered on each gel. Molecular weight markers, (phosphosylase b, bovine serium albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and gamma-lactalbumin) were treated in the same manner as the sample proteins and subjected to electrophoresis concurrently with the samples. Gels were subjected initially to 4 mA per gel tube and increased to 8 mA per tube after the tracking dye penetrated all gels. Upon completion of the run, gels were fixed (400 ml methanol; 70 ml glacial acetic acid; 530 ml water) for 2 hr, stained [454 ml 50% (v/v) methanol; 46 ml glacial acetic acid; 1.25 g Brilliant Blue R (Comassie Brilliant Blue R)] for 2–5 hrs and then destained with frequent changes in destaining solution (50 ml methanol; 75 ml glacial acetic acid; 875 ml water).

Gel profiles were determined with a gel scanner (Instrumentation Specialties Co., Model 1310) and an absorbance/fluorescence monitor (Instrumentation Specialties Co., Model UA-5).

Amino acid analysis. Fifty milligrams of protein from each protein fraction was acid hydrolyzed, then purified in preparation for derivatization by the procedure described by product data bulletin 9702–2 (Alltech Associated, 2051 Waukegan Road, Deerfield, IL 60015) which is a slight modification of procedures developed by Adams (1). The N-acetyl amino acid n-propyl esters then were subjected to flame ionization GLC analysis using an amino acid column (Alltech Associates). Chromotography conditions were helium (12 ml/min), hydrogen (30 ml/min), air (300 ml/min), injector at 250°C, FID detector at 300°, oven temperature program from 110° to 182° at 8°/min and then 182° to 275° at 31°/min held at 275° for 5 min. Norleucine was used as an internal standard.

Crude lipids and proteins comprise about 80%–85% of mature kernel dry weight from well-filled 'Moneymaker' nuts (Fig. 1-A). They began rapid accumulation at the time of rapid cotyledon growth when there is a cessation of cotyledon cell division and initiation of rapid cell expansion (6, 9), which also corresponds to the beginning of lignification of the shell (4). This period of rapid dry weight accumulation is marked by completion of total protein accumulation (dry weight basis) about 40 days before shuck dehiscence. Thus, protein accumulation primarily occurs early in the period of cotyledon expansion and is essentially complete 3 weeks after complete lignification of the shell. Total protein of the mature kernel was determined to be 7.8%, which compares to 10% reported by Meredith (10) and 9.5% by Hammar and Hunter (7).

Pecan kernel proteins are composed of several major classes, including globulins, albumins, prolamines, and both acidic and alkali soluable glutelins, most of which vary greatly in concentration during kernel development and especially around the time of complete shell hardening (Fig. 1-B). These flucuations occur at a time of great flucua-

Table 1. Amino acid composition of pecan kernel protein classes and total kernel protein at kernel maturity.

Amino acid	mg amino acid per gram protein						Proportion of total protein (%)
	Prolamines	Albumins	Globulins	Acidic[z] glutelins	Basic[y] glutelins	Residual	
Ala	t[x]	12	70	31	17	53	2.6
Arg	105	124	177	287	366	112	24.9
Asp	12	6	13	18	8	14	1.4
Cys[w]	t	42	t	59	101	45	6.3
Glu	51	71	95	113	59	45	8.5
Gly	t	3	31	24	16	31	2.0
His	38	293	224	56	47	89	6.6
Ile[w]	118	16	36	37	20	30	3.7
Leu[w]	t	6	66	108	68	62	1.6
Lys[w]	58	123	74	37	100	49	6.0
Met[w]	20	6	10	7	16	14	1.1
Phe[w]	401	91	70	112	95	271	14.6
Pro[w]	40	7	32	18	9	38	1.9
Ser	39	6	10	16	14	52	2.0
Thr[w]	29	1	11	5	2	24	2.6
Tyr[w]	43	180	26	42	48	22	1.3
Val[w]	46	10	65	28	14	49	12.9

[z]Proteins soluble in 0.05 N HCl.
[y]Proteins soluble in 0.05 N NaOH.
[x]Detectable at trace levels.
[w]Essential amino acid.

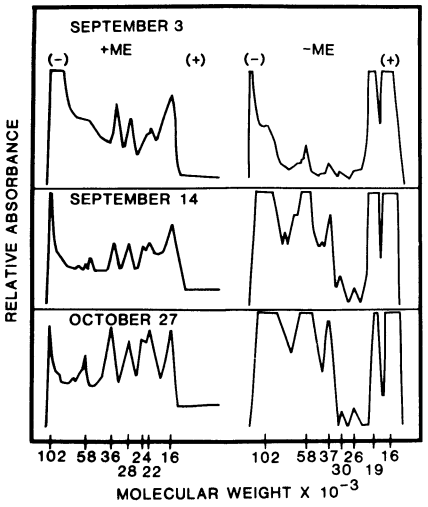

Fig. 2. Relative absorption of acidic glutelins of developing pecan kernels after SDS-PAGE. Acidic glutelins were digested in sodium dodecyl sulfate in both the presence (+ME) and absence (−ME) of β-mercaptoethanol; gels were then scanned with a gel scanner to determine relative absorption.

tions in abscisic acid and gibberellin levels in the same kernel tissues (14). Thus, there may be an important relationship between these growth regulators and protein classes. Globulins are at trace levels throughout all measured phases of kernel development and are thus a minor class of pecan protein in terms of kernel composition. Prolamines and albumins begin at relatively high levels 20 days prior to shell hardening, but decline with further kernel development. Acid soluble glutelins originally are present at only trace levels during the early stages of kernel development but increase repidly and become the predominant protein during cotyledon growth. Alkali soluble glutelins also are present at high levels in the mature kernel and comprise the most common protein during early kernel development. Thus, the major storage proteins in pecan seed are 2 classes of glutelins. In contrast, the major storage proteins in most cereals are prolamines (8).

Class composition of pecan proteins is more like that of monocots than dicots, such as peanut, soybean, and hemp (3, 8). Albumins and globulins are major proteins in most dicots, but are present at low levels in pecan.

Acidic glutelins are the major kernel protein class. These proteins are present from the beginning of kernel filling (3 Sept.) to its cessation (27 Oct.); however, their concentrations increase with filling (Fig. 1-B). SDS-poly acrylamide gel electrophoresis without β-mercaptoethanol revealed at least 7 subunits with molecular weights of 102, 58, 37, 30, 26, 19, and 16 ($\times 10^3$) (Fig. 2). The same subunits were detected in gels of each date of the acidic glutelins fraction analyzed. Their electrophoresis, using β-mercaptoethanol, revealed 5 peptides. Peptide molecular weights are 36, 28, 24, 23, and 16 ($\times 10^3$). These data suggest that at least 2 major proteins are present in the fraction and that the composition of the fraction remains the same during development.

Protein fractions differ markedly in amino acid composition (Table 1). Protein classes are mainly composed of neutral amino acids, but individual amino acid levels are highest for basic acids (lysine, arginine, and histidine), typical of many seed storage proteins. The percentage of amino acid composition of mature kernels was determined by GLC and individual percentages of amino acids differed from those determined by an amino acid analyzer, as reported by Elmore and Polles (5) and Meredith (10). Levels of lysine cystein and methionine were detected at levels higher than previously reported for pecan (5, 10), and are present at levels necessary to meet human nutrutional needs. The vast majority of essential amino acids in the mature kernel are found in glutelins.

Regulation of nut maturity by manipulating kernel development must, in part, involve either early lipid and protein accumulation in cotyledons or an increased rate of cotyledon filling or cell enlargement (9). Since filling already is rapid, the initiation of protein (primarily glutelins) and lipid accumulation may prove to be the most fruitful avenue of manipulation. Thus, the major limiting factor is more closely related to cell division and, perhaps, tissue differentiation. The advancement of cotyledon filling would likely allow the tree to accumulate increased energy reserves that would contribute to tree productivity the following year.

Literature Cited

1. Adams, R.F. 1974. Determination of amino acid profiles in biological samples by gas chromatography. J. Chromat. 95:189–212.
2. Bradford, M.M. 1967. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ann. Biochem. 72:248–254.
3. Derbyshire, E., D. Wright, and D. Boulter. 1976. Legumin and vicilin, storage proteins of legume seeds. Phytochem. 15:3–24.
4. Dozier, W. and H. Amling. 1974. Fruit growth and embryological development of the Stuart pecan, *Carya illinoensis*. Ala. Agr. Expt. Sta. Bul. 463.
5. Elmore, C. and S. Polles. 1980. Nitrogen fertilization effects on amino acid composition of pecan (*Carya illinoensis*) nutmeats. J. Agr. and Food Chem. 28:902–904.
6. Esau, K. 1977. Anatomy of Seed Plants. Wiley, N.Y.
7. Hammer, H. and J. Hunter. 1946. Some physical and chemical changes in the composition of pecan nuts during kernel filling. Plant Physiol. 21:476–491.
8. Larkins, B. 1981. Seed storage proteins: Characterization and biosynthesis, pp. 449–489. In: A. Marcus, (ed.). The biochemistry of plants: proteins and nucleic acids. Academic Press, N.Y.
9. McKay, J. 1947. Embryology of pecan. J. Agr. Res. 74:263–283.
10. Meredith, F. 1974. Amino acid composition and quality in selected varieties of pecans *Carya illinoensis*. Fla. State Hort. Soc. Proc. 87:362.
11. Osborn, T.B. 1895. The proteins of barley. J. Amer. Chem. Soc. 17:539–567.
12. Sparks, D. 1979. Physiology-site, growth, flowering, fruiting, and nutrition, pp. 211–239. In: R.A. Jaynes, (ed.) Nut tree culture in North American. Northern Nut Grow. Assn.
13. Sparks, D. and C. Brack. 1972. Return bloom and fruit set of pecan from leaf and fruit removal. Hort Science 7(2):131–132.
14. Wood, B. 1984. Free and bound abscisic acid and free gibberellin-like substances in pecan kernel tissue during seed development. J. Amer. Soc. Hort. Sci. 109(5):626–628.
15. Worley, R. 1979. Pecan yield, quality, nutlet-set and spring growth as a response to time of fall defoliation. J. Amer. Soc. Hort. Sci. 104(2):192–194.
16. Worley, R. 1979. Fall defoliation date and seasonal carbohydrate concentration of pecan wood tissue. J. Amer. Soc. Hort. Sci. 104(2):195–199.