2. Cobb, W. Y. and B. R. Johnson. 1973. Physiochemical properties of peanuts. p. 209–263. *In* Peanuts: culture and uses, a symposium. Stone Printing, Roanoke, Va.
3. Davis, J. T. and D. Sparks. 1974. Assimilation and translocation patterns of carbon-14 in the shoot of fruiting pecan trees, *Carya illinoensis* Koch. J. Amer. Hort. Sci. 99:468–480.
4. Evans, H. J. and G. J. Sorger. 1966. Role of mineral elements with emphasis on the univalent cations. Annu. Rev. Plant Physiol. 17:47–77.
5. Fore, S. P., N. J. Morris, C. H. Mack, A. F. Freeman, and W. G. Bickford. 1953. Factors affecting the stability of crude oils of 16 varieties of peanuts. J. Amer. Chem. Soc. 30:298–301.
6. Heaton, E. K., J. E. Marlon, and J. G. Woodroof. 1966. Pecan oil is highly unsaturated. Peanut J. Nut World 45:36–38.
7. Heaton, E. K., A. L. Shewfelt, A. E Badenhop, and L. R. Beuchat. 1977. Pecans: handling, storage, processing and utilization. Univ. Georgia Res. Bul. 197.
8. Holley, K. T. and R. O. Hammons. 1968. Strain and seasonal effects on peanut characteristics. Ga. Agr. Expt. Sta. Res. Bul. 32.
9. Metcalf, L. D. 1975. The gas chromatographic analysis of derivatives of fatty acids. J. Chromat. Sci. 13:516.
10. Nowakowski, T. Z. 1971. Effects of potassium and sodium on the contents of soluble carbohydrates and nitrogenous compounds in grass. p. 45–49. In: Potassium in Biochemistry and Physiology. 8th Coloq. Intern. Potash Institute, Berne, Switzerland.
11. Sparks, D. 1975. Alternate fruit bearing — a review. Pecan South 2:44.
12. Worthington, R. E., T. S. Bogges, Jr. and E. K. Heaton. 1972. Fatty acids of channel catfish (*Ictalurus punctatus*). J. Fish. Res. Bd. Canada 29:113–115.

HortScience 17(1):70–71. 1982.

Gibberellin-like Substances in Developing Fruits of Pecan[1]

B. W. Wood[2]

U. S. Department of Agriculture, ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA 31008.

Additional index words. phytohormones, irregular bearing, biennial bearing, *Carya illinoensis*

Abstract. **Several gibberellin-like substances were detected by cucumber, dwarf pea, and lettuce bioassays after liquid chromatographic (HPLC) fractionation of liquid endosperm from the developing seed of pecan [*Carya illinoensis* (Wang.) K. Koch cv. Moneymaker]. The response of bioassays to authentic gibberellin standards and fractions eluted from the HPLC column at the same times as gibberellin A_3, A_4, and A_7 suggests that these 3 gibberellins may be present in the liquid endosperm.**

Most economically important pecan cultivars bear crops in alternate years, yield being positively correlated with carbohydrate reserves (17, 18, 19, 20, 21). Factors that deplete carbohydrate reserves reduce or prevent yield the following year (19, 20, 21). The more severe the carbohydrate stress, the greater the effect on pistillate flowers (18). Although carbohydrates play a major role in irregular bearing, they have not been shown to be the sole regulator and do not preclude a role for endogenous phytohormones, which have not been investigated.

In apple, the developing seed inhibits flowering in some cultivars (3). The seeds are a rich source of gibberellins (5, 11) which can inhibit floral initiation (10, 12). The spur tissues of certain biennial bearing cultivars receive more gibberellin than those cultivars which initiate flowers with more regularity (8). Gibberellins also have been implicated as inhibitors of flowering in pear (7, 13) and citrus (15, 16) and may exercise a similar role in pecan. However, gibberellins are not likely to exercise the major role as with some apple cultivars, because the production of pistillate flowers is not always reduced by the production of seed (9). In immature apple (12) and *Echinocystis macrocarpa* (4) seeds the quantity of gibberellin-like substances was greatest when the endosperm reached maximum volume. Immature pecan endosperm reaches maximum volume at the onset of shell hardening (14); the purpose of this study was to determine if gibberellin-like substances occur in this tissue.

Developing fruits from 'Moneymaker' pecan were harvested during the liquid endosperm stage shortly before shell hardening, packed in ice, transported to the laboratory, stored at 4°C, and the liquid endosperm of the developing seed removed within one day. The tip of the fruit was removed to expose the seed coat, a hypodermic needle was inserted into the liquid endosperm, and the watery contents were removed and immediately stored in small flasks cooled by a solution of dry ice and acetone. The crude sample (600 ml) was purified by adjusting to pH 8 with phosphate buffer and partitioned twice with 1/2 volumes of redistilled petroleum ether. The aqueous phase was then slurried with prewashed polyvinyl pyrrolidone (PVP) and filtered through Watman no. 2 filter paper before adjusting to pH 3 with HCl and partitioning three times with equal volumes of redistilled ethyl acetate. The organic fraction was dried *in vacuo* at 35° and stored at −20°.

The extract was further purified and fractionated by preparative HPLC using a 30 × 0.4 cm i.d. stainless steel column packed with LiChroprep RP-18 (25–40 μm). The sample was dissolved in 20% methanol, filtered through a LCWP 10 μm Millipore filter, and injected. HPLC solvents were water (solvent A) and acetonitrile (solvent B). A concave gradient (0–100%) of acetonitrile was delivered by two Waters 6000A pumps controlled by a Waters 660 solvent programmer set on curve program No. 8. Solvent flow rate was 2 ml min^{-1}, and the gradient started 30 sec after injection. Fractions were collected every min for 40 min with a fraction collector. Samples were then concentrated by lyophilization. Retention times of authentic gibberellin A_3, A_4, and A_7 standards were determined by absorbence at 200 nm using a Waters 450 variable wavelength detector.

Gibberellin-like activity was located and compared by lettuce (*Lactuca sativa* L. cv. Grand Rapids) (6), dwarf pea (*Pisum sativum* L. cv. Little Marvel) (1), and cucumber (*Cucumis sativus* cv. Marketer) (2) assays of each sample fraction. Samples used for bioassay were first dissolved in ethanol and then made into an aqueous solution of 20% ethanol and 0.1% Tween 20 surfactant. Aliquots from each fraction were bioassayed in each assay and their activity compared with that of gibberellins A_3, A_4, and A_7 standards. Each assay was replicated twice.

HPLC fractionation and subsequent bioassay of 'Moneymaker' pecan liquid endosperm revealed several groups of fractions that exhibited gibberellin-like activity in all three bioassays (Fig. 1). GA_3 was eluted in fractions 11- to 13-, GA_4 and GA_7 in fractions 24- to 28-; the latter two compounds were not separated. Two of the zones of activity in the extract coincided with the GA_3 and $GA_{4/7}$ zones, although 4- to 5- other active zones were evident (Fig. 1). Several peaks of activity occurred at the same retention volumes in all three assays, suggesting that the smaller active zones represented more than background responses and may consequently represent other gibberellins. Fractions 21–23 were particular noteworthy in the dwarf pea assay, because of the large amount of activity in relation to the other 2 assays. These data

[1]Received for publication September 21, 1981.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[2]Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the USDA and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

[3] Research Horticulturist.

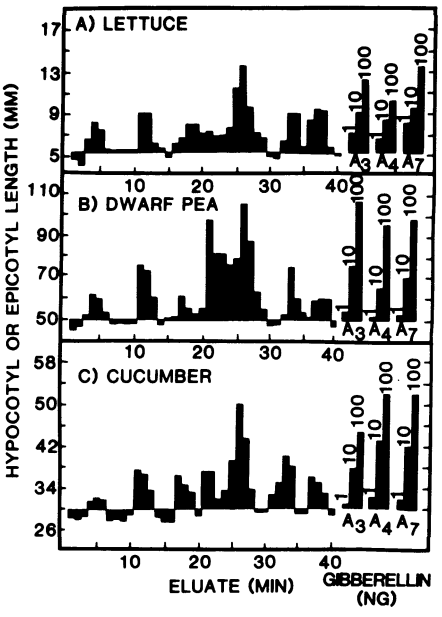

Fig. 1. Activity of methanol extracts of pecan liquid endosperm following a 40 min fractionation by HPLC on a LiChroprep RP-18 column using a water and acetonitrile solvent system. Bioassays were by A) lettuce, B) dwarf pea, and C) cucumber. Cucumber and dwarf pea plants were treated with the equivalent of 9 ml of pecan liquid endosperm; lettuce plants with 18 ml. Values are means for two replicates of each bioassay. Eluteion times for gibberellin standards were 11- to 13-min for GA_3 and 24- and 28-min for GA_4 and GA_7.

suggest that pecan liquid endosperm may contain gibberellin A_3 as well as A_4 and/or A_7 or other gibberellins having similar retention volumes.

The presence of gibberellin-like compounds in the liquid endosperm of developing pecan seeds raises questions concerning their role. Since liquid endosperm is present throughout most of the period associated with endosperm growth (14), qualitative and quantitative differences in GAs during this time could possibly affect flower initiation and fruit development. Further research is needed to provide an understanding of the role of GAs in seed, fruit, and flower development.

Literature Cited

1. Brian, P. W. and H. G. Hemming. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. Physiol. Plant 8:669–681.
2. Brian, P. W. and H. G. Hemming. 1961. Promotion of cucumber hypocotyl growth by two new gibberellins. Nature 189:74.
3. Chan, B. G. and J. C. Cain. 1967. The effect of seed formation on subsequent flowering in apple. Proc. Amer. Soc. Hort. Sci. 91:63–68.
4. Corcoran, M. R. and B. O. Phinney. 1962. Changes in amounts of gibberellin-like substances in developing seed of *Echinocystis, Lupinus*, and *Phaseolus*. Physiol. Plant. 15:252–262.
5. Dennis, F. G., Jr. 1976. Gibberellin-like substances in apple seeds and fruit flesh. J. Amer. Soc. Hort. Sci. 101:629–633.
6. Frankland, B. and P. F. Wareing. 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature 185:255–256.
7. Gil, G. F., G. C. Martin, and W. H. Griggs. 1973. Fruit-set and development in the pear: Diffusible growth substances from seeded and seedless fruits. J. Amer. Soc. Hort. Sci. 98:51–54.
8. Hoad, G. V. 1978. The role of seed-derived hormones in the control of flowering in apple. Acta Hort. 80:93–103.
9. Isbell, C. L. 1928. Growth studies on the pecan. Ala. Agr. Expt. Sta. Bull. 226:1–63.
10. Luckwill, L. C. 1964. New ways to initiate fruit bud formation. The Grower, p. 791–793.
11. Luckwill, L. C. 1970. The control of growth and fruitfulness of apple trees. p. 237–254. In: L. C. Luckwill and C. Cutting (eds.) Physiology of tree crops, Academic Press, New York.
12. Luckwill, L. C., P. Weaver, and J. MacMillan. 1969. Gibberellins and other growth hormones in apple seeds. J. Hort. Sci. 44:413–424.
13. Martin, G. C., F. G. Dennis, Jr., J. MacMillan, and P. Gaskin. 1977. Hormones in pear seeds I. Levels of gibberellins, abscisic acid, phaseic acid, dihydrophasic acid, and two metabolites of dihydrophaseic acid in immature seeds of *Pyrus communis* L. J. Amer. Soc. Hort. Sci. 102:16–19.
14. McKay, J. W. 1947. Embryology of pecan. J. Agr. Res. 74:263–283.
15. Monselise, S. P. and A. H. Halevy. 1964. Chemical inhibition and promotion of citrus flower bud induction. Proc. Amer. Soc. Hort. Sci. 84:141–146.
16. Moss, G. I. 1970. Chemical control of flower development in sweet orange (*Citrus sinensis*). Austral. J. Agr. Res. 21:233–242.
17. Sitton, B. G. 1933. Some of the influences of foliage on set and filling of the pecan. Nat. Pecan Assoc. Bul. 32:89–90.
18. Sparks, D. and C. E. Brack. 1972. Return bloom and fruit set of pecan from leaf and fruit removal. HortScience 7:131–132.
19. Worley, R. E. 1971. Effects of defoliation date on yield, quality, nutlet set, and foliage regrowth for pecan. HortScience 6:446–447.
20. Worley, R. E. 1979. Pecan yield, quality, nutlet set, and spring growth as a response to time of fall defoliation. J. Amer. Soc. Hort Sci. 104:192–194.
21. Worley, R. E. 1979. Fall defoliation date and seasonal carbohydrate concentration of pecan wood tissue. J. Amer. Soc. Hort. Sci. 104:195–199.