Concentration Dependencies of Ethylene on Shuck Dehiscence and Fruit and Leaf Abscission of *Carya illinoensis* [Wang.] K. Koch

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Ethylene was applied to pecan branches enclosed within plastic bags as a continuous flow (1.0 l./min). Four replications of ethylene at 0, 0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5, and 10.0 μl/l. were applied continuously for 14 days to two pecan cultivars. The relative sensitivities of the three responses tested to ethylene were shuck dehiscence > leaf abscission > fruit abscission. Cultivars Desirable and Big Z had one-half maximum concentration dependencies for shuck dehiscence of 0.320 and 0.540 μl/l. of ethylene while leaf abscission required 1.195 and 1.155 μl/l. of ethylene, respectively. Kernel darkening increased with increasing ethylene treatment concentration.

Over 100,000 metric tons of pecans, *Carya illinoensis* [Wang.] K. Koch, were produced in the United States in 1973 (Galloway and Parks, 1974). While on the tree, the pecan fruit progress through a series of developmental stages from initial fertilization to shuck (involucre) dehiscence and nut drop (Dozier and Amling, 1965; Woodroof and Woodroof, 1927). The chronological time of shuck dehiscence, however, varies considerably within and between cultivars (Brison, 1974; Love and Young, 1970). Due to the high cost of multiple harvests, substantial predator losses, and loss of quality with delayed harvest (Heaton, 1974) and early freezes, considerable interest has been displayed in the possibility of chemically enhancing the normal shuck dehiscence process to facilitate a concise time of nut drop.

The response of pecan shucks to exogenous ethylene and its possible endogenous role have been known for some time (Finch, 1937). Not until recently, however, was ethylene implicated in a natural regulatory system controlling the dehiscence process (Lipe and Morgan, 1970, 1972, 1973). Endogenous ethylene at a concentration substantially higher than during the predehiscence period is produced by the seed (Lipe and Morgan, 1972). In addition to the role of ethylene in shuck dehiscence, it is also implicated in the control of both leaf (Beyer and Morgan, 1971; Jackson and Osborne, 1970) and fruit abscission (Cooper and Henry, 1971).

Much of the research on the chemical induction of shuck dehiscence has resulted in early leaf abscission, which is detrimental to yield in the following year (Hinrichs, 1962; Worley, 1971). Since the detrimental effect varies with yield (Sparks and Brack, 1972), premature defoliation is especially serious in seasons of high yields. As a consequence, the need to assess the underlying assumptions governing the use of ethylene enhancing or releasing compounds (e.g. the concentration dependencies of shuck dehiscence, leaf abscission, and fruit abscission) resulted in the present investigation.

EXPERIMENTAL SECTION

Methods. Individual shoots containing three fruits of pecan cultivars Desirable and Big Z were enclosed within 7 l. plastic bags. Treatments were applied in the field on intact shoots since the sensitivity of the dehiscence process to ethylene is altered with fruit detachment (Lipe and Morgan, 1972). Gas was applied as a continuous flow (1.0 l./min) at ethylene concentrations of 0, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5, and 10.0 μl/l. using a flow system similar to that used by Goeschl and Kays (1975). Because of the large total volume of air used, an initial dilution was made from a bottle of 1% ethylene in nitrogen. The initial dilution was to 10.0 μl/l. with subsequent concentrations derived from a secondary dilution. Background levels of ethylene were removed from humidified air with columns of vermiculite and Celite (4:1, v/v) moistened with a saturated solution of potassium permanganate. Ethylene levels were monitored both at the flow boards and from the exhaust of individual bags by gas chromatography. Three and four replications of nine concentrations were used for cultivars Desirable and Big Z, respectively.

Treatments were started 2 to 2.5 weeks prior to natural shuck dehiscence and applied continuously for 14 days. This period was selected to minimize sensitivity changes to applied ethylene. Readings were taken daily to determine the occurrence of shuck dehiscence, leaf abscission, and fruit abscission.

Each of the ethylene responses tested will, as in nature, go to completion; however, the length of time required will vary considerably with each response. As a consequence, the time of measurement of the response is critical since early or late reading will yield an artificial dose–response curve. Daily readings were plotted as the average number of days required to reach 75% shuck dehiscence vs. ethylene concentration (Figure 1). Concentration dependencies were then plotted using the degree of completion of the response at the day of a marked rate change in the response. For shuck dehiscence of C.V. Big Z the degree of response plotted (Figure 2) was that achieved on day 7. This was also done for leaf and fruit abscission but is not presented since the rate change is extremely pronounced (approaching 90° to the x-axis) and occurred prior to that of shuck dehiscence. As a consequence, virtually no changes were incurred from that point through the termination of the treatment period for these responses.

Gas Chromatography. A Bendix Model 2500 gas chromatograph with flame ionization detector and 1.83 m × 6.4 mm i.d. glass column packed with 80–100 mesh activated alumina was utilized for ethylene detection. The operating conditions were: 50°C injection port and column temperature and 75°C detector temperature. Nitrogen carrier gas (90 ml/min) was utilized with oxygen (600 ml/min) and hydrogen (70 ml/min). Minimum detection limit by peak height was 6 nl/l. with 1-ml samples.

Nut Quality. Nuts of Big Z were gathered at the termination of the experiment, air dried at room temperature, and cracked and the kernels (seed coat, embryo, and endosperm) were graded into fancy, standard, and amber grades as described by Worley and Carter (1973).

RESULTS AND DISCUSSION

The concentration dependencies of shuck dehiscence, leaf abscission, and fruit abscission to exogenous ethylene are presented in Figure 2. Shuck dehiscence was substantially more sensitive to ethylene than either leaf abscission or fruit abscission. The cultivar Desirable, with a one-half

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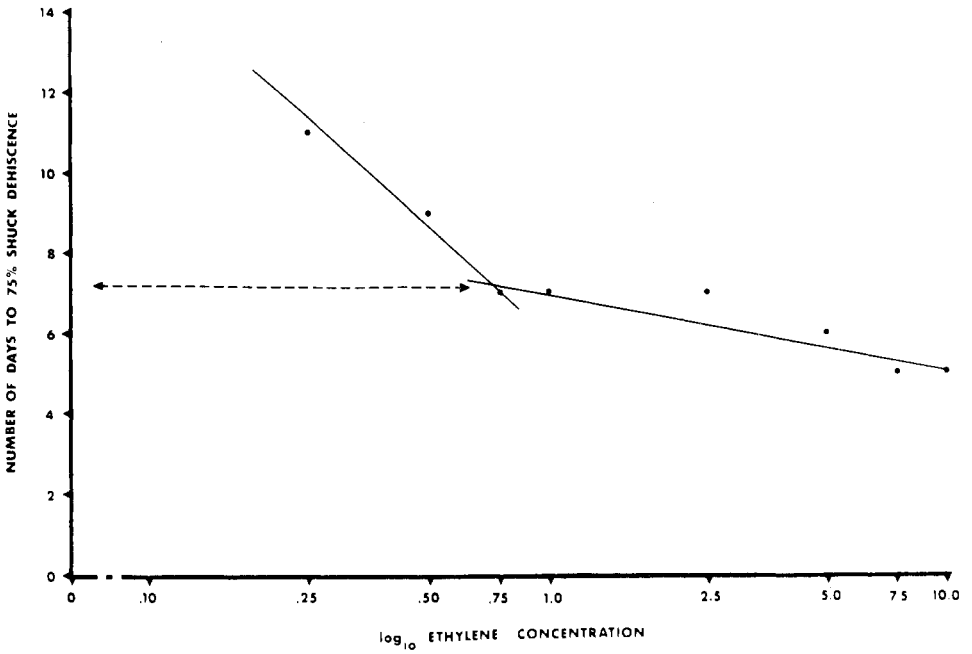

Figure 1. Relationship between exogenous ethylene concentration and the number of days required to attain 75% shuck dehiscence, cultivar Big Z.

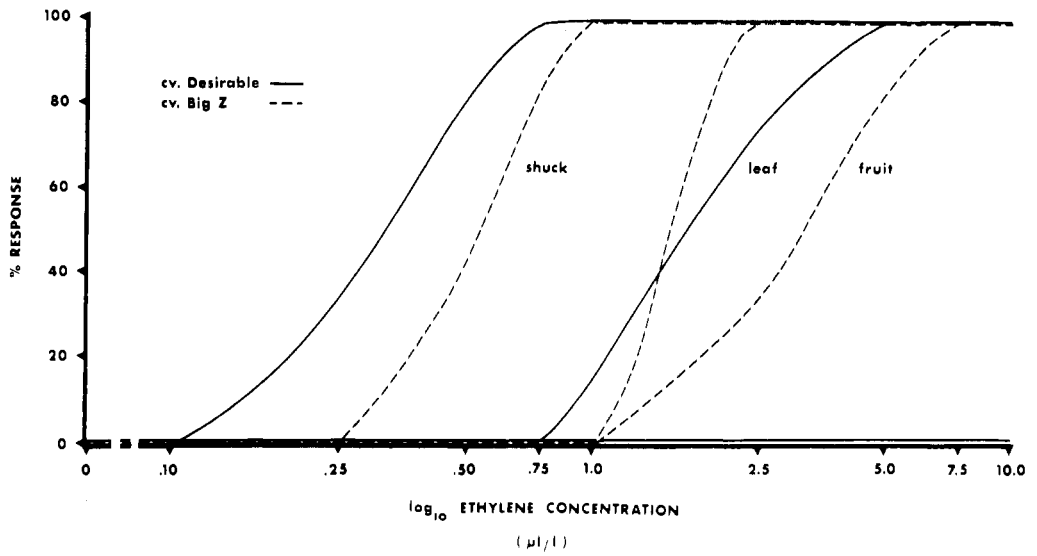

Figure 2. Relationship between ethylene concentration and the percent shuck dehiscence, leaf abscission, and fruit abscission of pecan cultivars Desirable and Big Z.

maximum concentration dependency for shuck dehiscence of 0.320 μl/l. of ethylene, required 1.330 μl/l. less ethylene to attain one-half maximum response for shuck dehiscence than for leaf abscission. Big Z responded in similar fashion, with a one-half maximum concentration dependency for shuck dehiscence of 0.540 μl/l. and a spread of 1.010 μl/l. between shuck dehiscence and leaf abscission.

Abscission of pecan fruits of both cultivars required higher levels of exogenous ethylene than either leaf abscission or shuck dehiscence. The magnitude of differences in concentration required between cultivars for this response as well as shuck dehiscence suggests a high potential for variation between the many commercially grown pecan cultivars. The one-half maximum dose requirement for abscission of fruits of Big Z was 3.20 μl/l. of ethylene while Desirable was totally unaffected at even the highest ethylene concentration tested (10.0 μl/l.).

The concentration requirement of shuck dehiscence is within the same general concentration range as a wide variety of growth responses in the Fabaceae family (syn. Leguminosae) (Goeschl and Kays, 1975) as well as the climacteric response in some fruits (Burg and Burg, 1965). The shift in the response curves of leaf and fruit abscission to higher concentration requirements, while suggesting differences in bind site requirements, may also be explained by the possibility of varying levels of endogenous ethylene and/or carbon dioxide or a differential state of sensitivity between the particular organs during the time period tested. From the standpoint of potential harvest facilitation, however, the gross ethylene requirement is the primary factor of concern. Hence, the data indicate a substantial spread in the dose requirements between shuck dehiscence and leaf abscission and a strong potential for the manipulation of shuck dehiscence without the undesirable side effect of leaf abscission.

Kernel quality, as measured by color, decreased progressively with increasing ethylene concentration from 0.10 to 2.5 μl/l. (Table I). Color changes occurred at ethylene con-

Table I. Kernel Quality (C.V. Big Z)

Ethylene μl/l.	% by weight		
	Fancy	Standard	Amber
0	91	9	0
0.10	61	39	0
0.25	57	53	0
0.50	34	53	13
0.75	14	77	9
1.00	18	78	4
2.50	9	91	0
5.00	0	73	27
7.50	0	100	0
10.00	0	83	17

centrations substantially below the one-half maximum concentration requirement for shuck dehiscence. The change in color of pecan kernels to exogenous ethylene could be due to a direct effect of ethylene on kernel color and/or an indirect effect, e.g. enhancing the rate of physiological processes which in turn affect pigment synthesis.

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Cycocel Plant Growth Regulant. Fate of Carbon-14 Chlorocholine Chloride in Sugarcane Grown in Hawaii

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Studies of the biochemical behavior of ^{14}C-labeled chlorocholine chloride in sugarcane using thin-layer chromatography showed evidence of no other radioactive material in the foliage than the labeled chlorocholine chloride. Only very low concentrations of radioactivity were found in the sugarcane stalks. Radioisotope analysis and chemical analysis for chlorocholine chloride in the stalk and in foliage were in good agreement.

Cycocel Plant Growth Regulant (registered trademark of American Cyanamid Company), 2-chloroethyl trimethylammonium chloride (also referred to as CCC and chlorocholine chloride), has shown promise as a ripener for sugarcane. Indications from field studies are that foliage sprays at late stages of maturity bring about reductions in the vegetative growth and an increase in the sugar content in the cane.

The purpose of the present study was to determine the metabolic fate of this compound in the sugarcane plant to provide the basis for residue analytical methodology responding to all of the toxicologically significant components of the residue. The metabolism of chlorocholine chloride labeled with carbon-14 had been studied in wheat plants (Blinn, 1967) where the compound was not significantly converted by metabolic processes. Faust and Bier (1967), working with nitrogen-15 labeled chlorocholine chloride in cereal plants, were also unable to find any labeled conversion products of CCC. Willemot and Belzile (1970) in their study with alfalfa leaflets showed slow conversion of Cycocel-^{14}C to phosphatidylcholine (0.5% in 5 hr). By contrast, Jung and EL-Fouly (1966) and EL-Fouly and Ismail (1969) showed that chlorocholine chloride from aqueous extracts of wheat and cotton plants was quickly converted into choline. Also, Stephan and Schütte (1970), working with 5- to 10-day-old barley embryos, separated from their roots and placed in beakers containing ^{14}C-labeled chlorocholine chloride, showed that 10 to 20% of the radioactivity isolated was present as choline. Belzile et al. (1972) found chlorocholine chloride to be weakly metabolized by winter barley to choline and unidentified compounds.

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MATERIALS AND METHODS

Radiolabeled Chlorocholine Chloride. Chlorocholine-*1,2-*^{14}C chloride was obtained from New England Nuclear Corporation, Boston, Mass. It had a specific activity of 6 mCi/mmol. The radiochemical purity was checked by two-dimensional thin-layer chromatography with a solvent system consisting of acetonitrile–water–acetic acid (60:40:2) and the radiopurity of the radiotracer was found to be 98%.

Application to Sugarcane. Carbon-14 labeled chlorocholine chloride was applied to mature sugarcane plants (approximately 18 months of age) in Hawaii. Applications were made by representatives of the Hawaiian Sugar Planters Association. Two experiments were conducted, one in May 1972 and the other in October 1972. For the May experiment, the plants were sprayed with undiluted radioactive chlorocholine chloride and each plant received 0.67 mg of the plant growth regulant. For the October experiment, the ^{14}C-labeled chlorocholine chloride was mixed with 33 parts of unlabeled chlorocholine chloride and this mixture was applied at a rate of 4 lb of active ingredient per acre to ten isolated and tagged stalks representing one stool (5 ft × 5 ft microplot). Both applications were made with a chromatograph sprayer. No cultural practices were conducted