Approximately 20 μg of pure patulin were obtained by gas chromatography (Figure 1) of part of the tlc-pure material on a Hewlett-Packard F&M 810 instrument equipped with a flame detector and 9:1 stream splitter. Conditions were as follows. Column: 6-ft × 4-mm i.d. glass column packed with 5% OV 210 (Chromatographic Specialities Ltd., Brockville, Ontario) on 60–80 mesh Diatoport S (Hewlett-Packard). Injection port temperature: 250°C. Column temperature: 135°C. Detector temperature: 250°C. Helium carrier gas flow: 60 ml/min at 45 psi pressure. Patulin had a retention time of 7.6 min and was collected in a glass capillary (1 mm i.d.).

Mass spectra (Figure 2) of isolated patulin and standard patulin were obtained using the direct probe of a Hitachi Perkin-Elmer RMS-4 mass spectrometer, operating at 80 eV with an ion source temperature of 195°C and probe temperature of 120°C. The spectra were virtually identical after small corrections for machine background.

DISCUSSION

This is the first reported occurrence of patulin in a commercial food product. To evaluate patulin as a health hazard requires additional toxicological data (chronic oral feeding studies in animals are particularly lacking) and a year-round survey of apple-derived foods for this mycotoxin.

Gas chromatography of patulin has not previously been described, although the trimethylsilyl ether, acetate, and chloroacetate derivatives were used by Pohland *et al.* (1970). Assignment of structures to seven principal fragment ions in the mass spectrum of deuterated patulin was made by Scott and Yalpani (1967); an actual spectrum was not published.

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Identification of Abscisic Acid in Bartlett Pears and Its Relationship to Premature Ripening

A growth inhibitor in Bartlett pear was identified as abscisic acid (ABA) by paper chromatography, ultraviolet spectrometry, and gas–liquid chromatography. Fruit from limb enclosures kept at 16–24°C showed only a slight increase in the amount of ABA. Premature ripening and greater accumulation of ABA was found in fruit from branches kept at 7–18°C during 30-day preharvest period.

Exposure of Bartlett pears to abnormally cool temperature for short periods prior to harvest causes an early development and acceleration of the biochemical and physiological changes normally associated with maturation and ripening (Wang *et al.*, 1971). As a result, ripening is initiated and develops on the tree prior to anticipated time of normal harvest. While the nature of this physiological disorder is not fully understood, the level or ratio of certain growth substances within the fruit may be involved in stimulation of ethylene production and development of ripening capacity (Dilley, 1969).

Abscisic acid (ABA), known to stimulate fruit ripening (Addicott and Lyon, 1969), occurs in unripe Clapp's Favourite pears and increases in concentration during ripening (Rudnicki *et al.*, 1968). The present study was initiated to determine if ABA also occurs in the Bartlett pear cultivar and if significant changes in concentration develop during cold-induced premature ripening.

EXPERIMENTAL

The experiments were initiated 30 days prior to the estimated harvest date. Installation of Mylar covered limb enclosures and methods of temperature control were described previously (Wang *et al.*, 1971). Cooled cages were maintained at 18°C during the day and at 7°C during the night. Temperatures in the heated cages were maintained at 24°C daytime and 16°C at night. The first samples were collected 10 days after start of the experiment.

Extraction and purification of the sample followed the procedures described by Strausz (1970). The acidic ether fraction was found to contain the most inhibitory activity and was used exclusively in this study. The residue of the acidic ether fraction was separated by paper chromatography, using Whatman No. 1 and 2-propanol:ammonia:water (8:1:1 v/v/v). Bioassay procedures described by Nitsch and Nitsch (1956) were used. The activity of the growth inhibitor was determined by the coleoptile straight growth test using

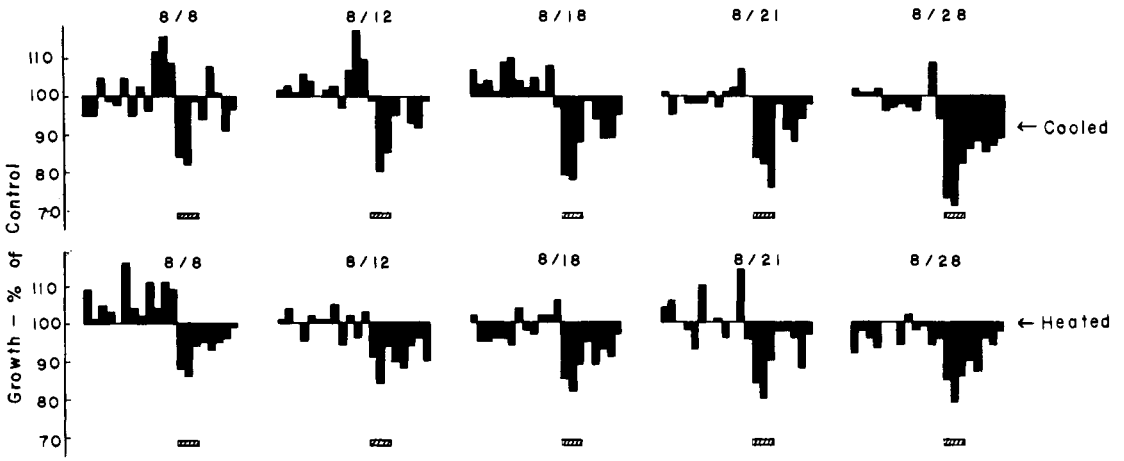

Figure 1. Comparison of oat coleoptile assays of the acidic ether fraction of extracts from cooled and heated Bartlett pear. Each histogram represents inhibitor from 25 g of fresh weight purified by paper chromatography in 2-propanol:ammonia:water (8:1:1 v/v/v). Cross-hatched inserts represent the location of ABA. The origin and the solvent front are on the far left and far right bands, respectively

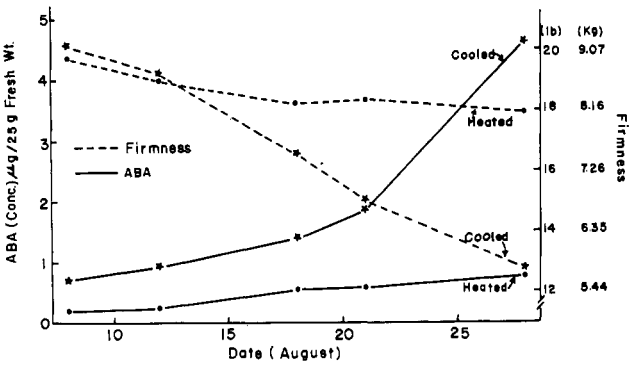

Figure 2. Effect of preharvest temperatures on firmness and concentration of ABA in attached Bartlett pears during maturation

Forkedeer oat variety (Tennessee Seed Producers, Inc., Nashville).

Paper Cochromatography of the Inhibitor and Synthetic ABA. Samples of acidic ether fraction were cochromatographed with synthetic ABA (Shell Development Co., Modesto, Calif.). The solutions of ABA standards (0.078–2.5 μg) and of the fruit extract were separately streaked on Whatman No. 1 paper and chromatographed with five different solvents (2-propanol:ammonia:water 8:1:1 v/v/v, benzene:ethyl acetate:formic acid 80:20:5 v/v/v, chloroform:benzene:acetic acid 100:100:1 v/v/v, ethanol:ammonia:water 80:5:15 v/v/v, 2-propanol:water 10:1 v/v). Each chromatogram was assayed with the oat coleoptile test.

Ultraviolet Light Absorption Spectrum. Samples of acidic ether fraction and synthetic ABA were separately purified by paper chromatography, twice in 2-propanol:ammonia:water (8:1:1 v/v/v) and once in benzene:ethyl acetate:formic acid (80:20:5 v/v/v). The R_f region which contained the most inhibitory activity was cut and eluted with 95% ethanol. The eluate was scanned spectrophotometrically.

Gas–Liquid Chromatography. Samples purified by paper chromatography were analyzed by glc (Varian Aerograph Model 1200). The method of Davis *et al.* (1968) was used, but with a linear temperature program at 9.8°C/min and a nitrogen flow rate of 20 ml/min. This modification was found to provide an improved separation of the peak for the trimethylsilyl derivatives.

RESULTS

Identification of ABA in Bartlett Pears. The R_f value which showed the most inhibitory activity from the extract separated and purified by paper chromatography coincided closely with that of synthetic ABA in the five different solvent systems used. Also, the ultraviolet absorption spectra of the ethanol eluates were identical. The trimethylsilyl derivative of the inhibitor had the same retention time as that of synthetic ABA in gas–liquid chromatography.

Relationship of ABA and Premature Ripening. The amount of ABA in heated fruit was low and showed only a slight increase during the experiment (Figure 1). Fruits exposed to cool temperatures consistently had a higher ABA level. In samples harvested after 30 days of modified temperature treatment, the cooled fruit contained five times more ABA than the heated fruit and was 5 lb softer (Figure 2).

DISCUSSION

Since Okhuma *et al.* (1963) first isolated ABA from cotton fruits, it has been identified in extractions of numerous species (Addicott and Lyon, 1969). Fruits are found to be the richest source (Milborrow, 1967). Although its role in fruit ripening is not fully understood, an increased amount of ABA was found to accompany the ripening process of Clapp's Favourite pears (Rudnicki *et al.*, 1968).

It has been reported that ABA stimulates ethylene production in bean explants (Abeles, 1967) and citrus leaves (Cooper *et al.*, 1968). The high concentration of ABA which accumulated in cooled Bartlett fruits may similarly have stimulated ethylene production. While pears in the heated cages responded normally, fruits in the cooled cages showed an early acceleration in ethylene production and developed typical symptoms of premature ripening and many had abscissed (Wang *et al.*, 1971). Cooper *et al.* (1969) reported an accumulation of ethylene in citrus fruits as a result of chilling. Cool temperature probably caused upsetting of the balance of endogenous growth regulators (Phinney and West, 1960) which in turn changed the normal pace of the maturation and ripening. An alteration in the ratio of ABA to ripening inhibitors may also be involved (Dilley, 1969). Nevertheless, the extensive drop of the premature ripened Bartlett pears was undoubtedly caused by excessive amount of this abscission agent in the fruit.

Whether the increase in ABA in cooled Bartlett pears

resulted from synthesis in the fruit or was translocated from the leaves cannot be determined from the data. In detached pears, Rudnicki *et al.* (1968) found that an increase in ABA concentration occurred during storage. Therefore, cool temperature exposure may actually stimulate ABA synthesis within the fruit. It was reported, however, that ABA can be synthesized in leaves and is readily transported to other organs (Eagles and Wareing, 1964; Evans, 1966). Thus, the source of ABA in premature ripened Bartlett pears is not clear and warrants further study.

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