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Antifungal diene in leaves of various avocado cultivars

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Abstract

Leaves (both young and mature) of seventeen commercial avocado (*Persea americana*) cultivars have been analysed monthly throughout the year to measure the concentration and seasonal variation of the antifungal diene (Z,Z)-2-hydroxy-4-oxohenicosa-12,15-dien-1-yl acetate (persin). © 1999 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Persea americana*; Lauraceae; Avocado; Persin; Seasonal variations; Hass; Fuerte

1. Introduction

The leaves of the avocado *Persea americana* Mill. are known to be toxic to a range of animals (Kingsbury, 1964). Recent work by Seawright (Oelrichs *et al*., 1995) has shown that the primary effects of avocado toxicity in goats and mice, namely damage to the heart and lactating mammary gland, are due to ingestion of the antifungal diene compound (**1**). This compound has been isolated from avocado leaves (Chang *et al*., 1975) and green fruit (Prusky, Keen, Sims & Midland, 1982) and was shown (Prusky *et al*., 1982) to inhibit germination and growth of the anthracnose fungus *Colletotrichum gloeosporioides*, the cause of the most significant post-harvest disease of commercial avocados. The growth inhibitory, insecticidal, and feeding deterrent effects of the compound have recently been explored against *Spodoptera exigna*, a generalist herbivore (Rodriguez-Saona, Millar & Trumble, 1997). We have been interested in the chemistry and anti-cancer activity (Bull & Carman, 1994; Seawright, Oelrichs, Ng, Carman & MacLeod, 1995) of the molecule and its analogues.

Avocado cultivars in commercial use are hybrids bred from three horticultural races; the Mexican race, *P. americana* var. *drymifolia* (= *P. drymifolia*), the Guatemalan race, *P. americana* var. *guatemalensis* (= *P. schiedeana*) and the West Indian or Columbian race, *P. americana* var. *americana* (= *P. americana*) (Burger, Naudé, van Rensburg, Botha & Pienaar, 1994). Although no previous quantitative comparison of antifungal diene levels between different cultivars has been made, it has been noted that leaves from some Guatemalan-derived cultivars were toxic, whereas a Mexican one was not (Appleman, 1944; Craigmill, Seawright, Mattila & Frost, 1989). Hass leaves and fruit are more toxic than Fuerte (Burger *et al*., 1994). Previously (Carman & Duffield, 1995) we have obtained supplies of compound (**1**) and its tetrahydro derivative (**2**) (which is equally active in anti-cancer and anti-lactation screens) from leaves of the Reed cultivar (Guatemalan) without knowing whether this is the optimum source. The toxicity of the avocado towards animals has lead to accidental poisonings in commercial orchards (Grant, Basson, Booker, Hofherr & Anthonissen, 1991) and a knowledge of the relative concentrations of the toxic component in a range of cultivars could be useful in preventing further loss of stock. We now report the results of a survey of the

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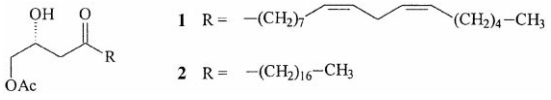
leaves of seventeen avocado cultivars, most with commercial significance.

2. Results and discussion

The study was performed over a period of about two years. Leaves of the cultivars listed in Table 1 were analysed monthly to determine the concentration of compound **1**. Both mature leaves and young leaves (when available) were surveyed. Two different individual trees of the Reed and Gwen cultivars were included to provide a comparison between individuals of the same variety. Leaves were also analysed from



two individual Hass cultivars, one from a tree reputed by the grower to consistently yield fruit particularly susceptible to anthracnose fungus, the other from a tree reputed to yield anthracnose-resistant fruit.

The averaged monthly results are collected into the Table. Our experimental error and reproducibility in analysis is estimated to be ~10%. The monthly data showed random variations outside this figure as illustrated by the standard deviations reported in the Table. These deviations must be due to uncontrolled variables such as rainfall, air temperature and the amount of recent exposure to sunlight. However, no systematic seasonal variation could be detected, and the concentration of compound **1** in the leaf of any particular cultivar appears to be independent of the season of the year. Perhaps surprisingly, mature and young leaves also showed no significant difference in the concentration of compound **1** and the concentration of this compound also appears to be independent of the age of the leaf.

Concentrations of compound **1** in leaves varied from 4.5 mg/g in the Guatemalan Hass cultivar down to undetectable amounts in the Guatemalan natural hybrids G775b and G775c. The two most significant commercial varieties, Hass and Fuerte, both contain high levels of the compound. No significant difference could be seen between the Hass anthracnose-susceptible and Hass anthracnose-resistant trees; both individuals contain significant amounts of diene **1** in their leaves.

The values in the Table can be compared with various literature values; 2 mg/g isolated yield from fresh Reed leaf (Carman & Duffield, 1995), 9-10 mg/g isolated yield from freeze-dried Reed leaf (Oelrichs, Seawright, Ward, Schäffeler & MacLeod, 1995),

Table 1
Concentration of diene (**1**) in avocado leaf (mg/g of fresh leaf)

Cultivar	Race	Average (SD)
Hass	Guatemalan	4.5 (0.7)
Sharwil	Guatemalan (Australian-bred)	3.3 (0.7)
Reed (2)	Guatemalan	3.1 (0.8)
Pinkerton	Mexican/Guatemalan	2.9 (0.8)
Plowman	Guatemalan	2.6 (0.5)
Reed (1)	Guatemalan	2.5 (0.4)
Fuerte	Mexican/Guatemalan	2.5 (0.4)
Wurtz	Guatemalan	2.3 (0.6)
Gwen (2)	Guatemalan	2.2 (0.5)
Gwen (1)	Guatemalan	1.8 (0.2)
Rincon	Mexican	1.4 (0.3)
Shepard	West Indian/Mexican	1.4 (0.2)
Hazzard	Guatemalan	1.1 (0.2)
Duke-7	Mexican	0.9 (0.2)
Zutano	West Indian/Mexican	0.9 (0.2)
Velvick	West Indian	0.7 (0.1)
Edranol	Mexican/Guatemalan	0.4 (0.1)
G775b (Martin Grande)	Guatemalan hybrid	< 0.01
G775c (Martin Grande)	Guatemalan hybrid	< 0.01
Hass (Sus)*	Guatemalan	4.1 (0.7)
Hass (Res)†	Guatemalan	3.9 (0.6)

*Anthracnose-susceptible fruit-bearing tree †Anthracnose-resistant fruit-bearing tree.

0.6 mg/g isolated yield from fresh leaves of an unspecified variety (Chang *et al.*, 1975), 1.4 mg/g (Fuerte) and 0.6 mg/g (Hass) from freshly harvested fruit peel (Prusky, Kobiler & Jacoby, 1988).

3. Experimental

3.0.1. Leaf analyses

Avocado leaves were collected monthly from labelled mature trees growing at the Birdwood Nursery, Nambour, Queensland. Leaves were normally collected from similar heights on the same side of the tree. Reed (1) and Reed (2), and Gwen (1) and Gwen (2), were duplicate trees from the same area. Hass (Sus) and Hass (Res) were trees considered to consistently provide fruit respectively susceptible to, and resistant to, anthracnose. Both mature and young (when available) leaves were stored in airtight bags at -20°. The leaf (0.9-1.1 g) was weighed and chopped finely with scissors. The leaf material was ground in a mortar with EtOAc (10 ml). The green liquid was decanted and filtered. Two further extractions were usually necessary to ensure that all the green colour was extracted, leaving brown fibrous residue which was shown to contain no remaining diene (**1**). The combined organic filtrate was treated with an aliquot of internal standard [octadecanol (1.00 mg) in EtOAc]. The mixture was then taken to dryness ($< 40°$ under vacuum). Trimethylsilylimidazole (0.3 ml) was added with swirling to ensure complete dissolution, and the stoppered flask was allowed to stand (> 15 min). Excess reagent was quenched with MeOH (6 drops; violent reaction) followed by distilled water (5 ml) and NaCl (3-4 mg). The resultant mixture was shaken with hexane (distilled, 1-2 ml) and the organic layer was pipetted off and filtered through a plug of cotton wool into a GC vial. Samples were stored (-20°) until analysis using a Varian 3300 gas chromatograph [30 m nonpolar (5%-phenyl)-methylpolysiloxane column (BP-5 or DB-5); He carrier gas; 100° for 2 min rising to 250° at 16°/min, then 250° for 17 min; flame ionisation detection]. Peak areas were measured relative to the derivatised octadecanol peak. Detector response factors were determined frequently by separate injection of a derivatised soln containing octadecanol (1.00 mg) and compound **2** (1.00 mg). Frequent checks of the derivatised leaf sample by GC-MS analysis (Hewlett Packard MSD 5970 spectrometer using a GC inlet with a column identical or similar to those reported above) confirmed the identity of the peaks for both compound **1** and the octadecanol standard. For most leaf samples the peak for compound **2** was the major GC peak.

Results for both young and mature leaves are collected into the Table as an average value. Young leaves, when available, showed only marginally higher values than did mature leaves. Results over a two year period show no apparent seasonal variation.

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