Identification of Prunin (Naringenin-7-Glucoside) in Dormant Peach Buds as a Wheat Coleoptile Growth Inhibitor[1]

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Naringenin in peach buds was first reported by Hendershott and Walker (5) in 1959 and later by Dennis and Edgerton (2), Samish and Lavee (8), and Corgan (1). Naringenin is of interest because of its inhibition of wheat coleoptile elongation and possible effect on rest of peach buds.

In January 1965, Redhaven peach flower buds were extracted with methanol, and a portion of the crude extract was chromatographed with n-butanol: Acetic acid: water 4:1:5 (BAW). Spraying the developed chromatograph with 2% alcoholic $AlCl_3$ disclosed two distinct zones which fluoresced green under UV light, possibly indicating flavanone compounds. The zone at Rf .90 corresponded to naringenin. The Rf of .71 for the other zone was much like values reported for prunin (naringenin-7-glucoside) (3,9).

Volumes of the above crude extract corresponding to 0.1 gm fresh weight of buds were chromatographed in BAW, and the chromatographs were steamed for 30 minutes to remove the acetic acid. Portions of the chromatographs corresponding to the fluorescing zones were cultured with wheat coleoptile sections (7) in one cc of growth medium. Both zones completely inhibited growth of the coleoptiles at this concentration, representing a 1:10 dilution of the buds. The portions of the chromatographs between the two zones also were cultured with wheat coleoptiles but did not inhibit, indicating good separation of the two compounds.

When a crude methanol extract of 1.0 gm fresh weight of flower buds was evaporated and then partitioned between diethyl ether and water at pH 8, nearly all the naringenin (Rf .90 BAW) was in the ether fraction. Most of the unknown (Rf .71 BAW) was in the water fraction. Repeated extraction of the water fraction with ethyl acetate removed the unknown. The ethyl acetate was evaporated and the residue chromatographed with BAW. The fluorescing zone, which this time occurred at Rf .78, was eluted with warm methanol and further purified by chromatographing with distilled water. The Rf of the unknown in water was .40, again close to that reported for prunin (3).

A sample of known prunin was made by partial hydrolysis of naringenin with a 50:50 mixture of methanol and 2.0 N HCl as described by Seikel (9). The reaction mixture was evaporated to a small volume under vacuum and extracted with diethyl ether to remove naringenin. The aqueous layer was saturated with NaCl and extracted with n-propanol. The propanol was evaporated and the residue recrystalized from methanol. The prunin was further purified by chromatographing with distilled water. UV absorption spectra for prunin and the unknown in methanol are shown in figure 1.

The purified unknown was hydrolyzed with 10% H_2SO_4 as previously described (4). The reaction mixture was diluted with water and extracted with ether. The ether was evaporated and the residue purified by chromatographing in distilled water. UV absorption maxima for the hydrolyzed unknown and for naringenin in methanol are shown in table 1.

Reduction of flavanones with sodium borohydride followed by acidification with HCl produces blue, purple, or red colors typical for particular flavanones (6). Absorption peaks in the visible range for the reduced unknown and prunin and for the reduced hydrolyzed unknown and naringenin are shown in table 1. Reduction was with sodium borohydride in methanol, followed by addition of concentrated HCl.

The data leave little doubt that the unknown was prunin. Its inhibition of

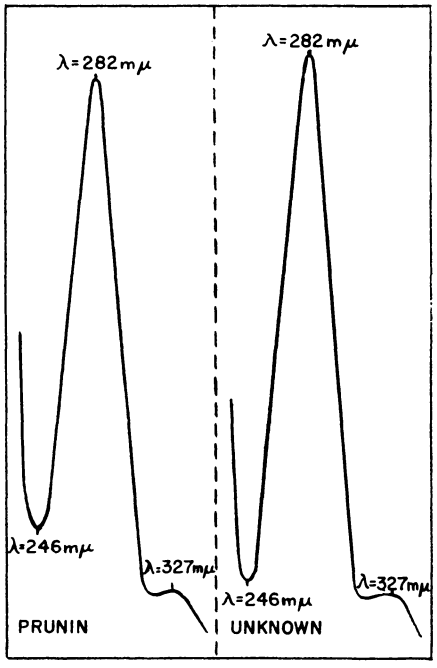

Fig. 1. UV absorption spectra of prunin (left) and the unknown (right) indicating wave lengths at maximum and minimum absorption.

Table 1. Comparison of absorption maxima for prunin and the unknown and for naringenin and the hydrolyzed unknown in methanol. Compounds were reduced with sodium borohydride.

Compounds + Treatment	λ Max. (mμ)
	UV
Prunin	282
Unknown	282
Naringenin	288
Hydrolyzed unknown	287
	Visible
Prunin reduced + HCl	548
Unknown reduced + HCl	548
Naringenin reduced + HCl	533
Hydrolyzed unknown reduced + HCl	533

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growth of wheat coleoptile sections and the fact that it was present with its aglycone, naringenin, in dormant peach buds is of physiological interest. Neither the exact concentrations nor the concentration changes of prunin in peach flower buds during dormancy were determined.

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Drop of Maturing Apples Associated with Bird Feeding[1]

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Further studies of bird damage on apples indicate that fruit dropping as a result of bird activities may be a greater economic loss than feeding damage. Since feeding wounds are caused during the fruit maturation period, the amount of dropped fruit resulting is directly related to two independent factors; first, the inherent ability of the variety to retain its fruit and second, the stage of fruit maturation when damage occurs.

A protective cage was constructed over trees of the apple varieties Cortland, McIntosh and Richared Delicious, to determine the extent of dropped fruit independent of bird activity (Figure 1). Similar type trees, even to the extent of fruit production per tree, were used to obtain control data, e.g., fruit feeding and drop damage. The fruit feeding damage that occurred in the control plots was comparable with that which had occurred in previous years (1). Drop damage between the protected and unprotected plots was compared for the 1965 season.

Fig. 1. Protective cage constructed to exclude birds and prevent their feeding activities so that apple drop damage data could be obtained.

It was assumed that the samples of dropped fruits obtained followed a binomial distribution and that the proportion of the total number of drops in a protected and similar unprotected area could be tested for equality by the X^2 (Chi-square) method of analysis (2). Differences in wind velocities were measured with standard meteorological wind equipment and were found to be negligible from place to place within and without the protective cage. High winds (gusts to 50 mph) occurred during the fruit maturation period but they did not result in a significantly greater fruit drop in the unprotected plots when compared with the protected plots.

More apples dropped sooner in the unprotected plots than in the protected plots during the 37 day maturation period from August 17 to September 23 (Figure 2). The X^2 value was highly significant and since the only ascertainable difference was accessibility of the fruit in the unprotected plots to bird feeding, it is logical to attribute the increased proportion of drops to such activities. Further analysis revealed that significantly greater numbers of fruit dropped from trees of the Cortland and McIntosh varieties than with the Red Delicious variety, however, more feeding damage occurred to the former varieties.

In this study more than twice as many Cortland and McIntosh apples dropped as a result of bird feeding. While dropped fruit does have salvage value the economic loss resulting from it is obvious. The loss is accentuated in instances when the eventual distribution of the fruit is intended for fresh market channels. Although both the Cortland and McIntosh varieties can be processed, the major share of production in this area is directed to the fresh market.

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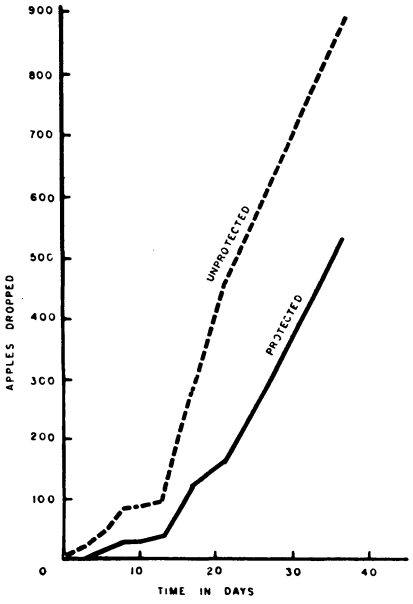

Fig. 2. Cumulative distribution of the number of apples dropped from trees in the protected and unprotected plots during the maturation period of the 1965 season.

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