

Jonathan Spot Induced by Ultraviolet Light

By

E. BALÁZS¹ and Á. TÓTH²

¹ Research Institute for Plant Protection, Department of Pathophysiology, Budapest, H-1525.

² University of Horticulture, Department of Chemistry, Budapest, H-1502

Experiments were conducted to demonstrate the spot-inducing effect of ultraviolet light in Jonathan spot of mature apples (*Malus sylvestris* Miller). Ultraviolet light irradiation either from the sun under orchard conditions or artificially induced by UV germicidal lamp causes Jonathan spot. In the spotted apple peel tissues anthocyanins (mainly cyanidine-3-galactoside) were oxidized or destroyed. Simultaneously with UV light irradiation, there were increases in the activities of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase.

In the last decade considerable quality reduction in stored Jonathan apples is due to formation of brown spots in the peel tissue. Both the commercial value and export potential are reduced in apples showing these symptoms. The causes of spot formation are not clear and opinions are conflicting in the literature.

PENTZER (1925) observed that the pH of spotted tissue was higher than that of adjacent normal tissue and suggested that the spot symptoms originated from the anthocyanin color conversion from red to blue. The spotted tissue was characterized by a marked accumulation of cations, mainly K^+ , Ca^{++} and Mg^{++} . Perhaps there is a relationship between pH changes, binding of the organic acids by cations and peel tissue neutralization.

The relationship between cation accumulation and organic synthesis and the development of Jonathan spot on apple fruit was demonstrated by RICHMOND *et al.* (1964). They found that the epidermal tissues of Jonathan apples affected with Jonathan spot contained more than twice as much K^+ and four times as much Mg^{++} and Ca^{++} as adjacent normal tissues. The total acidity of spotted tissue was twice that of normal tissue while the pH was 0.7 unit higher. They also found that the citric acid content of spotted tissue was considerably higher than that found in normal tissue.

In spite of these data, several investigators have traced the Jonathan spot back to calcium deficiency and have offered calcium-fertilizer spray application as a control measure for Jonathan spot (BEYERS, 1963., SCHACHRESTANY, 1964., SCHUMACHER and FANKHAUSER, 1966., PAIS and PETHŐ, 1971). Apparently these recommendations were connected with the decreased calcium fertility levels of orchards. On the other hand, JERMY (1962) measured the temperatures of apple trees on different sides of the crown and the sun-tanned side and the shaded side

of the apple fruit. He found a 12°C difference between the two sides in the sunny heat of September. According to observations, by orchardists if the weather is sunny and hot during the ripening period, apples have increased spotting under storage.

HADWIGER and SCHWOCHAU (1971) demonstrated that pea pods irradiated with ultraviolet light showed increased phenylalanine ammonia lyase (PAL) activity. In our preliminary experiments we established that the PAL activity was higher in spotted peel tissue than in the normal tissue (BALÁZS and TÓTH, 1973). However we did not observe any biochemical differences between the Jonathan spot and Lenticel spot as described by TRECCANI (1960), in spite of the fact that the two types of peel spots are different from a histological point of view.

On the basis of these former observations, we believed that the ultraviolet light of the sun causes increased enzyme activity in the irradiated peel tissue and destroys the anthocyanins. This study demonstrated that the Jonathan spot was induced by ultraviolet light and that UV light promoted through increased enzyme activity, the oxidation of anthocyanin and caused the Jonathan spot.

Materials and Methods

Plant material

Mature Jonathan apples (*Malus sylvestris*, Miller) were harvested and maintained in storage at approximately +4°C for six months to obtain fruit with varying degrees of spot development.

Artificial Jonathan spot induction

Jonathan apples were placed under a germicidal lamp (Germicid F type, Tungfram) with an energy output between 185–578 nm. The major energy output is at 253.7 nm.

The apples were irradiated 40 cm from the lamp surface. The controls were the unirradiated sides of the apples. The time of UV irradiation was dependent upon the colour of the peel, and the amount of light received by the plant during its growing period. After UV treatments (for 1, 2, 3, 4 and 5 h) the apples were stored at 4°C for six months. The apples were observed at weekly intervals during the storage period.

Isolation and purification of anthocyanins

The spotted tissue (both from naturally induced Jonathan spot and artificially induced Jonathan spot) and the adjacent normal red peel tissue were carefully scraped from the pulp. Samples of spotted tissue and normal peel tissue were dried in a desiccator above P₂O₅ for 3 days in the dark under vacuum. 1–1 g

dried peel samples were extracted with 25 ml $\text{HCl}-\text{CH}_3\text{OH}$ (1 : 99 v/v) for 24 h at 4 °C. The peel samples were washed two times with 5 ml $\text{HCl}-\text{CH}_3\text{OH}$ (1 : 99 v/v) and the extracts were collected and filtered through G 2 glass filters. The filtered extracts were concentrated in a vacuum desiccator above anhydrous NaOH for 12 h. The final volume of the extracts was approximately 5 ml. The extracts were filtered again through Whatman No. 1 paper and were washed with 20 ml distilled water. These aqueous extracts were passed through a column containing 7 ml of DOWEX 50 W-X 4 (H^+ 400 mesh) ion exchange resin (RODNEY and LUH, 1965).

Table 1
Enzyme activities in apple peel tissue

Tissue	Activity of		
	PO	PPO	PAL
Jonathan spot (natural)	151±8	128±6	135±5
UV germicidal lamp-induced Jonathan spot	158±6	127±5	136±5

Enzyme activity was expressed in percentage of adjacent red peel tissue control (100%). The enzyme measurement was repeated five times. The enzyme activity was calculated both for fresh weight and 10 µg protein. The time of UV irradiation was 4 hours as optimum time. Measurements were made three months after UV treatment

The column was washed with 10 ml distilled water and 5 ml acetone three times. The anthocyanins were eluted from the resin with 50 ml $\text{HCl}-\text{CH}_3\text{OH}$ (5 : 95 v/v) mixture. The eluted anthocyanins were concentrated to 10 ml under vacuum at 35 °C, and the samples were concentrated further in a vacuum desiccator above anhydrous NaOH to the desired degree. This purified anthocyanin (mainly, cyanidine-3-galactoside) was measured spectrophotometrically (UNICAM SP 800) between 350–550 nm and compared to spectral data of known quantities of pure cyanidine-3-galactoside (HARBORNE, 1967).

Enzyme assays

Peroxidase (PO) and polyphenol oxidase (PPO) extractions and assays.

For examining PO and PPO activities, the samples were taken from the normal red and spotted peel tissues that were carefully scraped from pulp. Samples were homogenized (5–5 g fresh weight) in 50 ml of 0.1 M phosphate buffer pH 6.5, with quartz sand in a pestle and mortar at 4 °C. They were filtered through four layers of cheesecloth and centrifuged at 0 °C with a Janetzky K 23 type centrifuge at 5000 *g* for 30 min. The supernatant was used for the determination of the activity of PO and PPO, using the modified method of FEHRMANN and DIMOND (1967). Catechol (0.2 M) was used as a substrate for PPO and pyro-

gallol (0.2 M) for PO. Increases in absorbance at 400 nm and at 430 nm were recorded in a UNICAM SP 800 spectrophotometer, respectively.

Phenylalanine ammonia lyase (PAL) extraction and assay: 5–5 g fresh weight spotted and normal red peel tissues were carefully scraped from pulp and homogenized at 4 °C in a pestle and mortar, with 0.1 M borate buffer (25 ml) pH 8.8 and quartz sand. After centrifugation at 5000 g at 0 °C for 20 min in a Janetzky K 23 type centrifuge, the supernatant was centrifuged further at 20,000 g for 30 min (MOM G 120 Ultracentrifuge), and this supernatant was used for measuring the PAL activity. An appropriate amount (0.1 ml) of the enzyme solution, 200 mmoles of borate buffer pH 8.8 plus 60 mmoles of L-phenylalanine and distilled water for a total volume of 3 ml were mixed in a 1 cm path cuvette. After a 1-h incubation period increase in absorbance at 290 nm against controls without phenylalanine was recorded with a UNICAM SP 800 spectrophotometer (BELLINI and HILLMAN, 1971). The protein content was determined by the method of LOWRY *et al.* (1951).

Results and Discussion

The irradiation of Jonathan apples with UV light promoted the Jonathan spot development under storage. We found that the irradiated sides of Jonathan apples were affected with Jonathan spot to a large extent, while the non-irradiated sides were not spotted. The number and degree of spot development were dependent upon the time of UV irradiation and the red colour intensity of peel tissue. We also found that the artificially induced Jonathan spot appeared at the same time as the natural Jonathan spot. We demonstrated that in the Jonathan spot anthocyanin was oxidized or destroyed (Fig. 1). This caused the visible symptoms

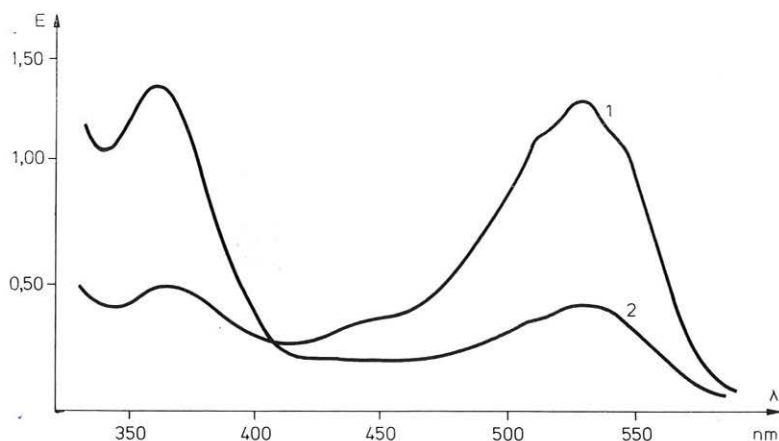


Fig. 1. Ultraviolet spectra of cyanidin-3-galactoside purified from apple peel tissue. 1. UV spectra of anthocyanin purified from normal red Jonathan apple peel; 2. UV spectra of anthocyanin purified from Jonathan spot tissue. Solvents: HCl—CH₃OH (1 : 99 v/v)

on the apple peel tissue. It is noteworthy that if we did not use ion exchange resin in the purification we were unable to demonstrate any differences between the anthocyanin content of spotted and adjacent normal tissue. Mainly in the spotted tissue we found large quantities of phenolic compounds that altered our control. No differences were observed between the anthocyanins of artificially induced and natural spots.

Our experiments proved that the peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activities were higher in spotted tissue than in normal tissue. In respect to these enzymes, we could not detect any differences between the natural and artificially induced Jonathan spot (Table I). The observed UV-induced increase in PAL activity relates well to other literature (HADWIGER and SCHWOCHAU, 1971).

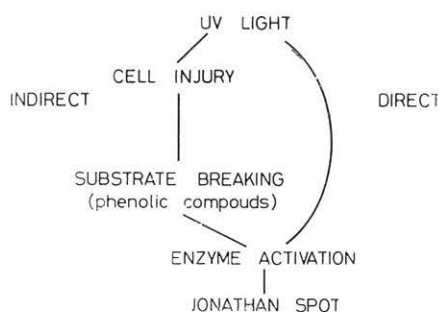


Fig. 2. Two theoretical pathways of the UV light-induced Jonathan spot formation

We proposed two theoretical pathways for the UV light-induced Jonathan spot formation: through direct enzyme activation and indirect enzyme activation (Fig. 2). Our experiment demonstrated that UV light from the sun is involved with Jonathan spot on the apple peel tissue. We were also able to induce Jonathan spot with a germicidal lamp. In the Jonathan spot anthocyanin was destroyed or oxidized. The peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activities were higher in spotted than in normal tissue. These observations correlated well with the ecological effects of high temperatures and concentrated sunshine during the ripening period in September. Enzyme activity is inhibited under low storage temperatures (0–4 °C); therefore the spots appear only in the second or third months of storage.

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