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REDUCTION OF POLYPHENOLOXIDASE ACTIVITY IN PEACHES SPRAYED WITH ALAR, ETHREL OR GIBBERELLIC ACID*

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Abstract—The failure of Early Amber peaches which had been field sprayed with Alar, Ethrel or gibberellic acid to darken as expected when cut is due to a 90 per cent reduction in the activity of polyphenoloxidase (EC 1.10.3.1) in the mature fruit. The possibilities that the reduced darkening might be due to the formation of a spray-induced reversible inhibitor or inactivator, to reduced substrate levels, or to elevated ascorbic acid levels were shown to be untrue.

INTRODUCTION

BUCHANAN *et al.*[1] have observed that field spraying of immature Early Amber peaches with Alar, Ethrel or gibberellic acid (GA-3) leads to a marked reduction in browning of the fruit when harvested at commercial maturity. The fact that unsprayed mature peaches browned normally 24, 48 and 72 hr after being dipped in Alar or Ethrel indicated that direct polyphenoloxidase (PPO) inhibition was not involved. Direct inhibition of enzyme activity also seems unlikely in view of the low levels of GA and Ethrel used (30 and 50 ppm, respectively) and the time lapse between application and harvest.

Possible causes of this spray-induced behavior might be (*a*) differences in redox potential as reflected in ascorbic acid levels, (*b*) the presence of an inhibitor of PPO, (*c*) differences in substrate concentration or (*d*) differences in the level of PPO activity. The purpose of this investigation was to determine which of these factors was responsible for the failure of the treated peaches to brown normally.

RESULTS AND DISCUSSION

Abnormally high levels of ascorbic acid in the sprayed fruit might bring about inactivation of the PPO reaction before the onset of visible browning. Assay of control and treated fruit for reduced ascorbic acid by conventional *m*-phosphoric acid extraction and indophenol titration showed that, within experimental error, the levels of the compound were equal in all the fruits. The finding that all the fruits were strongly buffered at pH $3{\cdot}80 \pm 0{\cdot}04$ (s.d.) ruled out the possibility of enzyme inactivation by low pH.

Inasmuch as *in vitro* experiments had shown that ferulic acid would prevent browning if added to slurries of control fruits before the onset of darkening, a search was made for such a spray-induced enzyme inhibitor. Two-dimensional paper chromatograms of acetone

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[1] D. W. BUCHANAN, C. B. HALL, R. H. BIGGS and F. W. KNAPP, *Hort Science* **4**, 302 (1969).

extracts of peaches from treated trees were sprayed with an alcoholic solution of chlorogenic acid and catechin (5 and 2·5 mM respectively) before being sprayed with an active peach PPO preparation. Since no unbrowned area appeared on the paper the absence of an inhibitor of PPO may be inferred.

If an inhibitor was present and responsible for the low PPO activity of the enzyme from the treated fruits, adding these preparations to the enzyme from control fruit should result in decreased total activity, unless there was only a stoichiometric amount of inhibitor attached to the low-activity enzyme. The observed effect of mixing the enzymes was that neither

TABLE 1. LEVELS OF SUBSTRATES FOR ENZYMATIC BROWNING IN MATURE EARLY AMBER PEACHES FIELD-SPRAYED WHEN IMMATURE WITH ALAR, GIBBERELLIC ACID (GA) AND ETHREL

Spray treatment	*o*-Diphenols* (mg%)	Degree of browning† (units/100 g)	
		Actual	Enzyme added‡
Control	63·5	17·4§	—
Alar (2000 ppm)	60·0	5·0	72·8
GA (50 ppm)	56·3	0·0	33·0
Ethrel (50 ppm)	51·1	0·2	45·2

* Calculated as catechin, on wet weight of fruit basis.
† Degree of browning, measured and defined as by Walker.[2]
‡ Enzyme extracted from acetone powder of control fruit, added at about 2/3 of amount found in source.
§ For some unknown reason control supernatant was quite light; most of the color was in the sediment. This was true of GA + enzyme, to a lesser degree.

TABLE 2. EFFECT OF SPRAYING IMMATURE EARLY AMBER PEACHES WITH ALAR, GIBBERELLIC ACID (GA) AND ETHREL ON PPO ACTIVITY ON MATURE FRUITS AND ON ACTUAL AND POTENTIAL BROWNING

Spray treatment	PPO activity* (units/g fruit)		Degree of browning‡ (units/100 g)	
	Normal assay	PVP treated†	Actual	Potential
Control	3·64	3·45	31·2	71·4
Alar (2000 ppm)	0·34	0·33	—	—
GA (50 ppm)	0·17	0·23	0·8	1·2
Ethrel (50 ppm)	0·21	0·20	0·0	5·2

* Enzyme activity as defined by I.U.B.,[5] calculated on fresh weight of fruit.
† Enzyme extracted from acetone powder in presence of Polyclar AT (PVP).
‡ Degree of browning measured and defined as by Walker.[2]

depression nor enhancement of activity occurred. The resulting value was the sum of the separate activities, indicating the lack of any free inhibitory substance.

Polyvinylpyrrolidone (PVP) has been shown to effectively remove tannins and other phenolic substances which inactivate enzymes.[3,4,5] Acetone powders from spray-treated fruit were soaked overnight in buffer, accompanied by an equal weight of Polyclar AT (insoluble PVP, Dyes and Chemicals Division, GAF Corp.). However, levels of PPO activity in the buffer extract were hardly above those found when no PVP was used (Table 2).

[2] J. R. L. WALKER, *Phytochem.* **8**, 561 (1969).
[3] J. D. JONES, A. C. HULME and L. S. C. WOOLTORTON, *Phytochem.* **4**, 659 (1965).
[4] W. D. LOOMIS and J. BATTAILE, *Phytochem.* **5**, 429 (1966).
[5] A. M. FIRENZUOLI, P. VANNI and E. MASTRONUZZI, *Phytochem.* **8**, 61 (1969).

Addition of $CuSO_4$ to enzyme preparations before assay did not change the rates appreciably, showing that no compound was formed which inactivated PPO by chelation of the prosthetic Cu in the same way that diethyldithiocarbamate does.

Assay for total *ortho*-diphenols showed that there were small differences associated with the GA and Ethrel spray treatments (Table 1). The greatest decrease was only about 20 per cent of the control value. This could account for only a fraction of the observed reduction in browning. Addition of active PPO from control fruit to the slurries of sprayed fruit resulted in browning comparable to that of slurried control fruit (Table 1). This confirmed the presence of ample substrate in the non-browning peaches, as well as the absence of inhibitor.

Furthermore, two-dimensional paper chromatograms of the peach phenols showed only slight qualitative differences between the substrates from the control-, GA- and Ethrel-treated fruit. A large part of the extract from Alar-treated peaches was lost during concentration and the remainder gave very poorly defined chromatograms. The only important browning substrates were four fluorescent compounds, tentatively identified as isomeric chlorogenic acids. These appeared to be in approximately equal concentration and turned grey when sprayed with PPO. Catechin was also present, but as this compound yields a peach-flesh-yellow color when oxidized by PPO it can hardly be classified as a direct browning precursor. The ratio of catechin to chlorogenic acids did not appear to vary greatly between treatments.

The fourth possibility, that the differences in browning were due to reduced levels of PPO activity, was confirmed by assay of buffer extracts of acetone powders from the various fruits. The values given in Table 2 are averages of two extractions from duplicate acetone powders from two harvests. Agreement between duplicates, harvests and extractions was good. Whereas the control peaches had a fairly high PPO content of 3·6 enzyme units[6] per gram of fruit, the activities of treated fruit were less than 10 per cent of that value—0·34 for Alar, 0·17 for GA and 0·21 for Ethrel.

Comparison of the data for actual and potential browning, also presented in Table 2, shows that whereas there was ample PPO in the control fruit to oxidize additional substrate (pyrocatechol), supplying more of this compound to the slurries of treated fruit had essentially no effect, because enzymic activity was lacking.

Presently available data do not permit determination of the factor(s) responsible for low PPO activity in the spray-treated fruit. One possibility is that the sprays were responsible for partial failure in PPO synthesis, causing either a reduction in the amount of enzyme protein formed or a depression in its catalytic power. Enzyme induction can be the result of decreased proteolysis as well as increased synthesis of enzyme.[7] Conversely, some of the sprays, at least, may have stimulated proteolysis rather than inhibiting PPO synthesis. Although ethylene, which is a degradation product of Ethrel, has been shown to induce increased PPO activity in potato tubers, both white and sweet,[8] it seems more likely that under the conditions of this experiment PPO degradation was stimulated.

The potential of these treatments for control of browning in peaches and other susceptible fruits and vegetables, as well as concurrent effects on organoleptic and/or other qualities will be subjects for further investigation.

[6] ANONYMOUS, *Enzyme Nomenclature, Recommendations* (1964) *of the International Union of Biochemistry on the Classification of Enzymes Together with their Units and the Symbols of Enzyme Kinetics*, Elsevier, New York (1965).

[7] P. FILNER, J. L. WRAY and J. E. VARNER, *Science* **165**, 358 (1969).

[8] M. A. STAHMANN, B. G. CLARE and W. W. WOODBURY, *Plant Physiol.* **41**, 1510 (1966).

EXPERIMENTAL

Spraying and harvesting of the peaches have been previously described.[1] Duplicate acetone powders were prepared from fruit of each harvest. Samples of the powders were soaked overnight in 0·1 M phosphate + 0·1 M pyrophosphate, pH 7·5 (0·5 g in 30 ml), at 5°. After centrifugation the supernatants were assayed for PPO activity by a spectrophotometric method.[9] All PPO assays were run at least three times and adequate controls were maintained.

Total *o*-diphenols in the filtrate from acetone powder preparation were determined by a modification of the method described by Arnow[10] using catechin as standard.

The acetone-extractable phenolic materials were transferred to a small volume of ethyl acetate and chromatographed on Whatman No. 1 paper. Solvents were 2% HOAc, descending, followed by *n*-amyl alcohol–HOAc–H_2O (2:1:1, v/v) ascending in the second direction. The dried chromatograms were viewed under u.v. illumination, then sprayed with PPO prepared from control fruit.

The degrees of actual and potential browning were measured by slight modification of the method described by Walker.[2] Water was used for blending the tissue, rather than buffer. The data shown in Table 1 were collected 1 hr after blending, rather than 30 min.

[9] F. W. KNAPP, *J. Food Sci.* **30**, 930 (1965).
[10] L. E. ARNOW, *J. Biol. Chem.* **118**, 531 (1937).