Laboratory Studies on the Effects of Chemicals on the Coloration of Apples[1]

R. M. Smock,
Cornell University, Ithaca, New York

Abstract. Post harvest screening of compounds that might influence coloration of apples was done in the laboratory. A number of carbonates such as glycol carbonate and carbonate buffers increased anthocyanin development. Certain compounds that were phytotoxic increased coloration. Other compounds such as chloro-IPC, quercetin, and sym-dimethyl diphenylurea decreased coloration. Diuron decreased coloration at relatively high concentrations and increased it at 10 ppm. The possible side effects of this compound are not yet known. Monuron at 10 ppm also increased coloration.

Introduction

TWO previous studies (7 and 9) reported methods of studying the effects of chemicals on apple coloration in the laboratory. Laboratory screening is desirable because only a limited number of tree spray experiments (8) can be conducted in a year. This study summarizes the results of 145 experiments in further exploration of chemicals that might induce anthocyanin development in apples.

Materials and Methods

Since dipping whole fruits seemed more comparable to what would happen in orchard trials, only this method was used (9). Greater responses have been obtained in "feeding" experiments (9), but in the orchard the chemical must either be absorbed by the leaves or the skin of the fruit or both.

The details of the method have been described (9). Treatments were replicated 5 times. Briefly, the apples were dipped in the test solutions and held in a moist chamber for 2 hrs. They were then placed under fluorescent lamps with the apple stems immersed in water for 36–72 hrs. The anthocyanins were extracted in 1% HCl in methanol at 32°F overnight. The optical density was determined at 540 mμ.

[1]Received for publication September 18, 1968. The advice of Dr. L. L. Creasy of this department during the course of this study was greatly appreciated.

Results

Compounds that had no effect. Table 1 lists the compounds that had no effect on coloration at the concentrations employed. Wide concentration gradients were not always used and it is possible that the most effective concentration was missed.

Compounds that reduced coloration. Table 2 presents a list of compounds that reduced coloration of 'McIntosh' apples at the concentrations used. The per cent of reduction was based on the color produced by the water controls. In a few cases, the compounds listed did not significantly reduce color, but the reduction was so close to significance that they were listed. With those compounds that reduced coloration there was usually a good concentration gradient. That is, the higher the concentration, the more was the color reduction.

Phytotoxic compounds that increased coloration. Phytotoxic compounds were taken out of this screening program because of the requisites of good pomological practice. A 55% liquid formulation of Malathion (3 and 5) was used at 1600 ppm on 'McIntosh'. This significantly increased coloration but there was leaf injury. When the 25% dry wettable formulation was used at 450 to 1800 ppm there was no effect on coloration of 'McIntosh' and there was no leaf injury. Cobalt chloride (.0005 to .001 *M*) increased color significantly but it was phytotoxic. The same was true of copper sulfate (9).

Compounds that increased coloration. One of the problems of this type of experimentation has been lack of reproducibility from experiment to experiment. A compound that gave a significant increase in 3 experiments might not give such a difference in the fourth. This problem of variability of results will be discussed in a later section. Compounds are presented that gave significant color increases in at least 3 experiments.

In a previous report (9) it was shown that various carbonates gave color increases. Ethylene carbonate is 50% soluble in water whereas calcium carbonate is soluble only at 12 ppm. Table 3 presents the results of one experiment with this compound with 'McIntosh' apples. In this experiment this material gave a good response in increased coloration. In direct comparisons with .001 M Ca CO_3 in other experiments, ethylene carbonate gave about an equal response. One such experiment is presented in Table 4 with 'Milton' apples.

Table 3. The influence of ethylene carbonate on the coloration of 'McIntosh' apples.

Treatment	Conc.	Anthocyanins[x]	Increase
	M	μg/cm²	%
H_2O	—	3.16 a	—
Ethylene carbonate	.0008	3.30 a	4
Ethylene carbonate	.0016	4.56 b	44

[x]Means identified by the same letter are not significantly different at the .05 level.

Table 1. Compounds that had no effect on the coloration of 'McIntosh' apples.

Compound	Concentration range		Compound	Concentration range	
	M	ppm		*M*	ppm
Aspartic acid	.0002 –.002		Methyl phloroglucinol	.0001 –.001	
Benzimidazole (1)[x]	.0001 –.0005		L-phenylalanine	.000001–.001	
Bis (methoxy-phenyl) carbonate	.0005 –.002		O-chlorophenoxyacetic acid		10–100
O-chlorocinnamic acid	.0001 –.001		Parahydroxycinnamic acid	.0005	
Ethionine	.0006		Shikimic acid	.00001 –.001	
D-galacturonic acid	.0001 –.001		Na salt cinnamic acid	.0005 –.001	
4,hydroxy-3, methoxycinnamic acid (4)[x]	.00005–.0001		Sorbitol	.01 –.1	
Indole acetic acid		25–50	Soluble starch	.001 –.005	
Iso-butyl carbonate	.001		Sucrose octyl acetate	.001	
Isopropyl cinnamate	.0005 –.001		Transcinnamic acid	.00005	
Methyl cinnamate	.0001 –.001		Beta-d-xylose acetate	.0001 –.001	

[x]Literature citation.

Table 2. Compounds that reduced the coloration of 'McIntosh' apples.

Compound	Concentration range		Color reduction
	M	ppm	%
Alpha methyl D xyloside	.0001 –.001		5 to 17*
Alpha methyl cinnamic acid	.0005 –.001		8 to 17*
Symdimethyldiphenyl urea	.000001–.001		8 to 41**
Calcium D-galactoside	.0005 –.001		12
3(3,4-dichlorophenyl)-1,1-dimethyl urea	.0001 –.001		25* to 45**
Chloro-isopropyl N phenyl carbamate		12–1,000	13* to 39**
Methyl beta D-galactoside	.0001 –.001		15 to 16*
Quercetin	.00002 –.0016		19* to 35**

* = significant at .05 and ** = significant at .01.

Table 4. Ethylene carbonate and calcium carbonate responses with 'Milton' apples.

Treatment	Conc.	Anthocyanins[x]	Increase
	M	μg/cm²	%
H_2O	—	3.04 a	—
Calcium carbonate	.001	3.60 b	18
Ethylene carbonate	.001	3.62 b	19

[x]Means identified by the same letter are not significantly different at the .05 level.

The CO_2 released from the carbonates may have increased coloration of apples. This theory is based on the finding that CO_2 increased red color development (2 and 7). Diethyl pyrocarbonate breaks down in water to form CO_2 and ethanol. Hence, this compound was tried. The results in Table 5 are typical for those found with this compound. Though the increases were significant in 3 experiments, they were not large.

Table 5. Influence of diethyl pyrocarbonate on coloration of 'McIntosh' apples.

Treatment	Conc.	Anthocyanins[x]	Increase
	ppm	μg/cm²	%
H_2O	—	4.26 a	—
Diethyl pyrocarbonate	50	5.28 b	24
Diethyl pyrocarbonate	100	5.40 b	27
Diethyl pyrocarbonate	200	5.34 b	25
$CaCO_3$	100	5.10 a	20

[x]Means identified by the same letter are not significantly different at the .05 level.

To further check the influence of CO_2 on coloration, potassium carbonate-potassium bicarbonate buffers (10) were studied. These solutions presumably released CO_2 during the 2 hr moist chamber period after dipping. Table 6 gives the results of 1 of 4 experiments with these buffers. The data show that a significant increase in coloration occurred with the buffer solution that presumably gave the quickest CO_2 release. In 3 other experiments .001 *M* materials were used in different percentage combinations. In some cases there were just as great increases with the bicarbonate alone as with the mixtures. It was concluded that while there were significant increases with the buffers in 2 of the 4 experiments that they were not enough better than carbonates alone to justify their use in orchard trials.

Table 6. Effect of carbonate buffers on red color development of 'McIntosh' apples.

Treatment	Conc.	Mixture composition	Anthocyanins[x]	Increase
	M	%	μg/cm²	%
H_2O	—	—	6.96 a	—
K_2CO_3	.001	25		
$KHCO_3$	.001	75	6.80 a	0
K_2CO_3	.001	5		
$KHCO_3$	.001	95	8.72 b	25
$KHCO_3$	.001	100	8.02 a	15
$CaCO_3$	.001	—	7.68 a	10

[x]Means identified by the same letter are not significantly different at the .05 level.

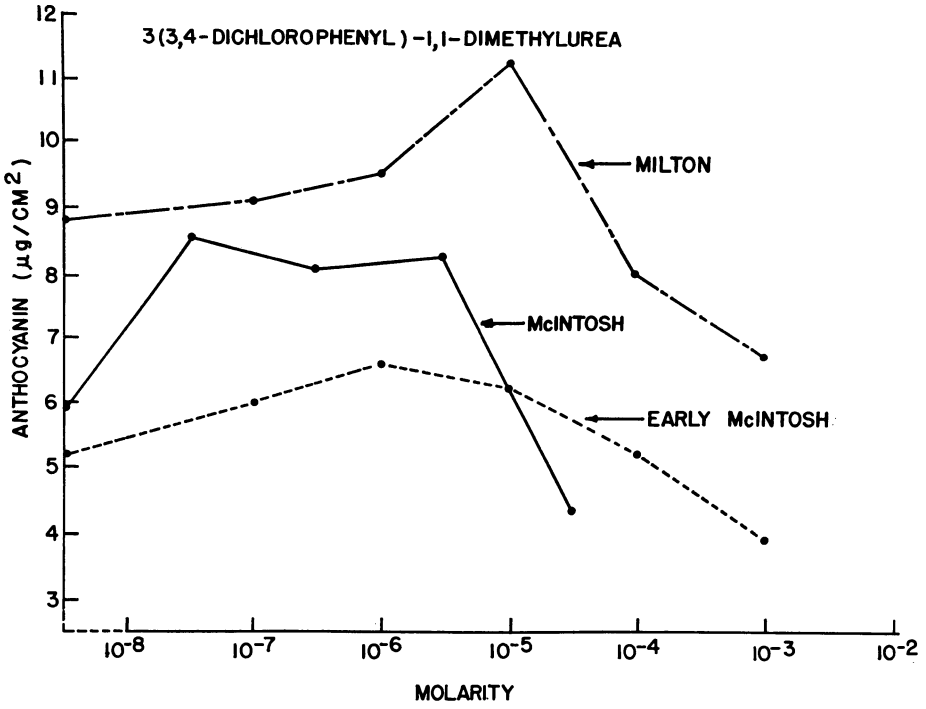

Fig. 1. Effect of 3(3,4-dichlorophenyl)-1,1-dimethyl urea, diuron, on color development of three varieties of apples.

It has been reported that rutin (quercetin-3-rutinoside) was present in the skin of 'Grimes Golden' apples (6). Table 7 shows that there were increases in coloration with this material although there was not a good concentration gradient. In 14 experiments with 'Cortland' there was no increase in coloration with rutin at .00005 *M*. It is concluded that 'McIntosh' was more responsive to rutin treatment than 'Cortland.'

A compound that showed both inhibition and stimulation of color formation was 3(3,4-dichlorophenyl)1,1-dimethyl urea (diuron). At relatively high concentrations this material is an inhibitor of photosynthesis and Creasy (2) showed that even at $10^{-6}M$ it inhibited anthocyanin development in strawberry leaf disks. Fig. 1 shows that a concentration of $10^{-3}M$ inhibited coloration of 3 varieties of apples. The data are not shown for 'Delicious' but this was also true for this variety. Lower concentrations such as 10^{-5} to $10^{-6}M$ caused significant increases in these 4 varieties.

Table 7. Effect of rutin on coloration of 'McIntosh' apples.

Treatment	Conc.	Anthocyanins[x]	Increase
	M	μg/cm²	%
H_2O	—	6.18 a	—
Rutin	.000125	6.92 a	12
Rutin	.0000625	7.23 b	17
Rutin	.000031	7.20 b	17
Rutin	.000015	7.62 b	23

[x]Means identified by the same letter are not significantly different at the .05 level.

A number of experiments were carried out with this material. Table 8 shows the variability of results that was experienced with this material. The results do show that 10 ppm of diuron would be worthy of trial in orchard experiments. Table 8 suggests, but does not prove, that higher concentrations such as 20 to 40 ppm were less inhibitory later in the summer than earlier in the season.

Table 8. Percentage increase in coloration of 'McIntosh' apples treated with 3(3,4-dichlorophenyl)-1,1-dimethyl urea.

Harvest date	Concentration in ppm				
	40	20	10	5	2
July 8	—	—	40**	—	36**
July 10	—	−25**	—	—	8*
July 11	—	−15**	—	—	14**
July 12	—	—	32**	30**	2
July 15	—	—	18*	—	—
July 16	—	—	0	—	—
July 23	—	—	—	32	—
July 24	—	—	26*	11	—
July 27	—	—	11	—	—
July 30	—	36**	33*	22	18
Aug. 1	−15	10	7	—	—
Aug. 2	−15	14	14	15	—
Aug. 6	—	—	62**	—	—
Aug. 7	—	—	36**	—	—
Aug. 9	—	—	38**	—	—
Aug. 12	—	—	11	—	—
Aug. 14	—	—	17	—	—
Aug. 24	—	—	30**	—	—
Aug. 26	—	31**	11	—	—
Aug. 27	—	—	17	—	—
Aug. 29	17	20*	28**	21*	—

* = significant at .05 level and ** = significant at .01 level.

Monuron, 3,(p-chlorophenyl)-1,1-dimethyl urea, which is similar to diuron except it has only 1 chlorine atom also gave color increases. Three experiments with this material with 'McIntosh' as various concentrations showed that 10 ppm gave the best response. When compared directly with diuron in 4 experiments, monuron gave increases comparable to diuron.

SOME FACTORS AFFECTING VARIABILITY IN RESPONSE

As can be seen from Table 8, the results from experiment to experiment were variable. Some of the factors that might affect variability were studied.

Time of sampling. 'McIntosh' apples freshly harvested at 7 AM developed significantly more color than those harvested at 2 PM when both lots were given equal time periods under the lights. In studying apples freshly harvested from the tree it was shown that time of day of sampling could be an important factor (9). The 7 AM apples were picked at a core temperature of 58°F and the 2 PM samples were harvested at 73° on August 21, 1967. Both lots were given 48 hrs of light. This effect of time of sampling during the day might not show up if the nights were warm.

Effect of presence of leaves. During the growing season apples can be placed under the lights with or without spur leaves attached. Table 9 shows that when the leaves were present there was a response to sucrose. In another experiment with .001*M* $CaCO_3$ there was a significant response with leaves present but not without leaves.

Effect of partial cuticle removal. It was thought that some of the erratic responses might have been due to variable penetration of chemicals through the cuticle of the apple. When ether was used to "completely" remove the cuticle, there was excessive injury. Hence, partial removal of the cuticle was attempted. 'Cortland' apples out of storage were washed in warm water containing .8% Triton B 1956 emulsifier. They were then rinsed in distilled H_2O. Table 10 shows that there was no response to $CaCO_3$ when the cuticle was intact but there was a positive response when the cuticle was partially removed.

Table 9. Effect of presence of leaves on coloration response to sucrose with 'McIntosh' apples.

Treatment	Conc.	Plus or minus leaves	Anthocyanins[x]
	M		μg/cm²
H_2O	—	−	6.30 a
Sucrose	.1	−	6.62 a
H_2O	—	+	5.74 a
Sucrose	.1	+	7.54 b

[x]Means identified by the same letter are not significantly different at the .05 level.

Table 10. Effect of partial cuticle removal on response of 'Cortland' apples to calcium carbonate.

Treatment	Conc.	Anthocyanins[x]
	M	μg/cm²
Cuticle intact; H_2O	*p*	3.28 a
Cuticle intact; $CaCO_3$	.001	3.34 a
Cuticle removed; H_2O	—	3.20 a
Cuticle removed; $CaCO_3$	.001	3.80 b

[x]Means identified by the same letter are not significantly different at the .05 level.

DISCUSSION

This report and earlier ones (6 and 8) emphasize 2 problems. One is the variability in results from experiment to experiment. The second is that the level of response of dipped apples to various chemicals was not high. Variability is a characteristic of color experimentation. Apples vary in age, exposure on the tree, crop size, development of cuticle, amount of desiccation, weather conditions prior to harvest, amount of spray residue and possibly other factors.

Over 200 chemicals have now been screened. Some of them have been field tested (8) and others have not. When materials are found such as 3(3,4 - dichlorophenyl) - 1,1 - dimethyl urea that gave a rather consistent response, they must be field tested to evaluate all possible pomological factors such as yield, accelerated ripening, storage behavior and other factors. Why this material worked on apples is not known. At higher concentrations it was a color inhibitor. A related compound, sym-dimethyldiphenyl urea depressed color even at $10^{-6}M$.

LITERATURE CITED

1. ARNOLD, A. W. 1964. Factors affecting anthocyanin synthesis in the hypocotyl of Impatiens balsami. *Diss. Abstr.* 25: 772–773.
2. CREASY, L. L. 1968. The significance of carbohydrate metabolism in flavonoid synthesis in strawberry leaf disks. *Phytochem.* 7:1743–1749.
3. ECK, PAUL. 1968. Chemical color enhancement of cranberry fruit. *HortScience* 3:70–72.
4. HESS, D. 1967. Substratinduktion bei der Anthocyan-Synthese von Petunia. *Naturwiss.* 54:289–290.
5. SHAWA, AZMI. 1966. Stenographic report of the Ocean Spray Cranberry Research Seminar. Hanson, Mass., Dec. 14, 1966.
6. SIEGELMAN, H. W. 1955. Quercetin glycosides of Grimes Golden apple skin. *J. Biol. Chem.* 2:647–654.
7. SMOCK, R. M. 1963. Methods of study of red color enhancement on apples. *Proc. Amer. Soc. Hort. Sci.* 83:162–171.
8. ————. 1964. Red color study on apples; a second progress report. *Proc. New York State Hort. Soc.* 109:185–188.
9. ————. 1966. Laboratory studies of anthocyanin development in 'McIntosh' apples. *Proc. Amer. Soc. Hort. Sci.* 88:80–88.
10. UMBREIT, W. W., R. H. BURRIS, and J. F. STAUFFER. 1957. Manometric techniques. Burgess Pub. Co., Minneapolis, Minn.