FACTORS AFFECTING THE EFFICACY OF DIPHENYLAMINE FOR APPLE SCALD CONTROL

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Lorenzo George Wilson

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ABSTRACT

FACTORS AFFECTING THE EFFICACY OF DIPHENYLAMINE FOR APPLE SCALD CONTROL

By

Lorenzo George Wilson

This work concerns the postharvest application of diphenylamine (DPA) to apples for the control of storage scald, an important physiological disorder affecting the quality of the stored fruit. Investigations were conducted to determine the need for renewing DPA solutions, to relate the DPA content of the treatment solutions to residues and scald control, and to ascertain the effects of applying DPA at elevated hydrostatic pressures for various time durations. Factors studied included solution age, temperature and contamination, plus the effect of fruit submergence at several depths and durations for two formulations of DPA on three apple varieties. DPA residues on the fruit, scald control and fruit injury were evaluated.

Reliable residue values of 0-25 ppm DPA for whole apples could not be obtained by gas chromatographic procedures. A colorimetric analytical procedure was adapted whereby DPA extracted from blended apple tissue with acetone was reacted with vanadium pentoxide and quantitatively determined spectrophotometrically at 605 mμ.

This basic procedure was employed also for estimating the DPA content of solutions. A simplified modification of the method for monitoring the DPA content of commercial applicator systems was examined.

Satisfactory scald control was obtained for all varieties with 1000 ppm DPA, which gave residues of approximately 2 ppm. There was no significant improvement in control by treatment with 2000 ppm when employed for the Delicious variety, even though fruit residues were doubled. Untreated McIntosh apples developed 95% scald, Delicious 59%, and Rome Beauty 79%. No fruit injuries from DPA treatments were observed.

DPA solutions contaminated with organic matter yielded greater DPA residues on apples than clean solutions. Aging of clean solutions yielded greater residues upon aging up to the maximum of four weeks, especially on McIntosh as compared to Delicious. Apples treated in solutions regulated to temperatures of 50, 60, or 70°F had similar DPA residues. Residues were increased by submerging the apples to depths of 6 ft and by prolonging the submergence treatment from 0 to 8 minutes. Although residues varied by fruit treatments, mean values remained within the 10 ppm tolerance established by the FDA.

Autoradiography of Delicious apples submerged for 10 minutes at 4.0 psi hydrostatic pressure in ^{14}C-DPA

solutions showed the DPA residues to be primarily confined to, or adjacent to the epidermal areas of the fruit.

It was concluded that the variable practices used for commercial scald control on apples with DPA yield fruit residues within an acceptable range and result in good scald control.

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vii
LIST OF APPENDICES	viii
INTRODUCTION	1
LITERATURE REVIEW	3
MATERIALS AND METHODS	15
DPA Analyses For Residues on Apples	15
Solution Analyses in the Laboratory	17
Solution Analyses in the Field	18
Aging Solutions	19
Artificial Scald Induction	21
Submersion (Hydrostatic Pressure)	22
Commercial DPA Applications	23
Synthesis of Radioactive Diphenylamine	24
Autoradiography	25
Statistical Procedures	26
RESULTS	27
DPA Analytical Technique	27
DPA Residues on Apples as Affected by Aging Solutions and Hydrostatic Pressure	27
Factors Affecting the DPA Content of Solutions	38
Storage Scald Control as Influenced by DPA Solution Age, Sanitation and Hydrostatic Pressure	43
Autoradiography of Apples Submerged in ^{14}C-DPA	46

Page

DISCUSSION 51

CONCLUSIONS. 61

SUMMARY 63

LITERATURE CITED 66

APPENDIX. 72

LIST OF TABLES

Table		Page
1.	Analysis of variance of DPA fruit residues in the 1967 aging solution study.	28
2.	Analysis of variance of DPA fruit residues in the 1968 aging solution study.	29
3.	Mean DPA residues (ppm) for apples as affected by organic matter.	30
4.	Mean DPA residues (ppm) for apples as affected by solution age	32
5.	Analysis of variance of DPA residues in the 1967 submersion study	37
6.	Analysis of variance of DPA residues in the 1968 submersion study	37
7.	Mean DPA residues (ppm) for apples as affected by duration of submersion in DPA in 1967	39
8.	Mean DPA residues (ppm) for apples as affected by submersion in DPA solution (hydrostatic pressure)	39
9.	Analysis of variance for DPA solution content in the 1967 aging solution study . . .	41
10.	Analysis of variance for DPA solution content in the 1968 aging solution study . . .	42
11.	Mean DPA content (ppm) of solutions as affected by solution age in 1967.	43
12.	Analysis of variance of scald reduction data from the 1968 aging solution study	44

Table Page

13. Analysis of variance of apple scald in the 1968 submersion study 45

14. Apple scald control in submersion solutions in 1968 as affected by DPA concentration . . 46

15. Commercial DPA solutions used in Michigan--1968 49

LIST OF FIGURES

Figure		Page
1.	DPA residues (ppm) as affected by the interaction between solution sanitation and technical formulation for apples dipped in 1000 ppm DPA solution for one minute in 1967	31
2.	DPA residues (ppm) as affected by the interaction between solution age and apple variety for apples dipped in 1000 ppm DPA solution for one minute in 1967 . .	33
3.	DPA residues (ppm) as affected by the interaction between solution age and technical formulation for apples dipped in 1000 ppm DPA solution for one minute in 1967.	34
4.	DPA residues (ppm) as affected by the interaction between solution age and sanitation for apples dipped in 1000 ppm DPA solution for one minute in 1967.	35
5.	DPA residues (ppm) as affected by the interaction between solution age and sanitation for apples dipped in 1000 ppm DPA solution for 30 seconds in 1968.	36
6.	DPA residues (ppm) as affected by the interaction between hydrostatic pressure and DPA solution concentration for apples submerged in DPA solutions for 0, 2 or 4 minutes in 1968	40
7.	Apple scald control in submersion solutions as affected by the interaction between hydrostatic pressure and DPA treatments for 0, 2 or 4 minutes' duration	47
8.	Autoradiograph of cross section of Delicious apple submerged in ^{14}C-DPA solution for 10 minutes at 4 psi pressure.	48

LIST OF APPENDICES

Table		Page
I.	DPA residues (ppm) on McIntosh and Delicious apples as influenced by DPA formulations used over a 4 week period in 1967.	73
II.	DPA residues (ppm) on McIntosh and Delicious apples as influenced by the presence of organic matter and the age of the DPA treatment solution in 1967. . .	74
III.	DPA residues (ppm) on McIntosh, Delicious and Rome Beauty apples as influenced by the presence of organic matter and treatment solution age in 1968.	75
IV.	DPA residues (ppm) on apples resulting from treatment in a simulated submersion system. 1967.	76
V.	Effect of DPA solution concentration, duration of treatment and depth of submersion of DPA residues on Delicious apples in 1968.	77
VI.	Apparent diphenylamine residues (ppm) observed on Delicious apples treated in a submersion system containing only water - 1968	77
VII.	DPA (ppm) in clean and contaminated treatment solutions of various ages in 1967 . .	78
VIII.	DPA (ppm) in clean and contaminated treatment solutions of various ages in 1968 . .	78
IX.	Effect of DPA solution age and sanitation on scald reduction for McIntosh, Delicious and Rome Beauty apples in 1968	79

Table Page

X. Effect of DPA concentration, submergence depth and duration of treatment on scald development on Delicious apples in 1968 . . 80

INTRODUCTION

Scald, a physiological disorder of apples which develops during storage, is controlled by post-harvest treatment of the fruit with diphenylamine (DPA). Label recommendations for use of this material encourage operators to change solutions as they become dirty or at least once every 48 hours, but there is widespread reluctance to comply with these precautions. There is a lack of experimental evidence supporting the need for frequently renewing the solutions. Therefore, experiments were designed to ascertain the effects of DPA solution age and the presence of organic matter in the applicator systems on DPA residues on apples. Furthermore, there is a need for information on the relationship of DPA content of treatment solutions to residues, and of both solution concentration and residue on scald control.

There also exists the possibility of incorporating the DPA application process into apple hydrohandling systems so as to provide savings in time and reduce the requirements for space and equipment in pre-storage operations. Such a procedure would possibly be hazardous

because the increased pressure may enhance penetration of the DPA solutions. Fruit residues, possible injury, and scald control under these circumstances of commercial usage of DPA were evaluated.

LITERATURE REVIEW

Storage scald of apples was recognized as physiological in origin and nature as early as 1903 (Powell and Fulton). It has been studied by many workers and the subject has been thoroughly reviewed by Pentzer and Heinze (1954), Ulrich (1958) and Smock (1961), in communications from The World Meteorlogical Organization (1963), and by the USDA Agricultural Research Service (1965). The following factors are recognized as tending to enhance scald: immaturity, hot or dry weather during harvest (particularly warm nights), delayed cold storage or slow cooling of fruit, high storage temperatures, large fruit, poorly colored fruit, fruit high in nitrogen and poor storage ventilation.

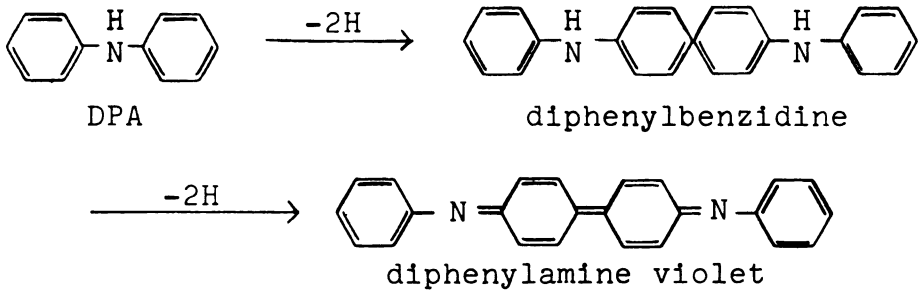
Methods for its control have been studied for many years and discussed extensively by Smock and Southwick (1945), Smock (1961) and Lutz and Hardenburg (1968). An important factor for effective control is fruit treatment within the first four weeks after harvest. Since Smock's original findings on the scald inhibiting properties of diphenylamine (DPA) in 1955, others (Hardenburg and Anderson, 1960 and 1962; Mattus, 1963; Smock, 1961) have

confirmed that this chemical provides dependable control when properly used. It is now extensively used as a liquid dip or drench for direct application to the apples, but Ginsburg (1968a) reports that DPA-impregnated wraps are equally effective.

Recent ideas for hydrohandling of apples in bulk containers (Dewey, et al., 1966; Stout, et al., 1966; Ginsburg, 1967) suggest that chemicals may be incorporated into the handling liquids. Although very little has been reported on the subject (Daines, 1967; Porritt and Meheriuk, 1968), bulk handling of apples in water could conceivably lead to the consolidation of sorting, sizing and grading operations with pre-storage treatment with a suitable storage scald inhibitor. Considering fungicides, Daines (1967) reports increased rot control due to increased hydrostatic pressures.

Diphenylamine, because of its wide use for controlling storage scald in Michigan, was of particular interest in this study. The Merck Index (1952) shows its formula as $C_{12}H_{11}N$ with a molecular weight of 169.22. It is made by heating aniline with hydrochloride. DPA crystals possess a floral odor and have a density of 1.16, a melting point of 53-4°C, a boiling point of 302°C and a flash point of 153°C. Diphenylamine discolors in light and is insoluble in water, but freely soluble in a variety of organic solvents. As a weak base, diphenylamine forms

salts with strong acids. Yatsu (1956) explains that in combination with a strong oxidizer (i.e. vanadium pentoxide in 10N sulfuric acid) DPA will form a blue oxidation product; first being oxidized to diphenylbenzidine and then to diphenylamine violet, as follows:



Snell and Snell (1949) described a technique for making nitrate estimations using DPA in sulfuric acid. The Merck Index (1952) states that this chemical is used in the manufacture of dyes, stabilizing nitrocellulose explosives and celluloid and the detection of several oxidizing substances, with which, in the presence of sulfuric acid, it gives a deep blue color. Incorporated into the Dische reagent, DPA is used in an improved method for the estimation of DNA in plant tissue (Giles and Myers, 1965) and in the diagnosis of leafroll infection in young potato leaves (Wenzl, 1965).

Small quantities of diphenylamine can be estimated using potassium dichromate in sulfuric acid as an oxidizer (Barnes, 1944). Snell and Snell (1949) suggest that an

aqueous solution of DPA produces a violet color with vanadium, detecting 2.5 ppm. Bruce, et al. (1958) blended apple samples with 90% methanol and extracted the slurry with petroleum ether. They then determined DPA by coupling with diazotized 2,4-dinitroaniline in phosphoric acid and measuring the absorption at three wavelengths in the ultraviolet or at 530 mμ on a spectrophotometer. Kennett (1961) refluxed the tissue sample with water and continuously extracted the DPA from the aqueous condensate with hexane. DPA was then partitioned into 18N sulfuric acid, reacted with a vanadium pentoxide reagent and read at 600 mμ on the spectrophotometer. Yatsu (1956) extracted the DPA from apple tissue with 70% acetone and partitioned it into pentane, adding KOH and alcohol and finally reacting it with a vanadium pentoxide reagent and reading absorbance on a spectrophotometer at 600 mμ within 4 minutes. Harvey (1958) extracted the peeling tissue with petroleum ether and eluted the DPA from a magnesium oxide column with 1:1 benzene-petroleum ether. The pulp was extracted with 70% acetone and the DPA partitioned into petroleum ether. Both were reacted with the sulfuric acid-vanadium pentoxide reagent and read at 590 mμ within 5 to 7 minutes. Even though Bache, et al.(1962) extracted with 50% methanol, partitioned into petroleum ether and then acidified with HCl, their reaction and wavelength used were the same as Harvey's. Padfield and Clark (1963)

also used the same method as Harvey, except peelings were placed in petroleum ether, brought to boiling under reflux, cooled and then an aliquot of the petroleum ether was chromatographed. In 1963, Gutenmann and Lisk described a gas chromatographic technique in which they used direct bromination of an acetone-hexane extract of apple tissue containing DPA to yield, presumably, the ortho-, para-hexabromo derivative of diphenylamine. This electrophilic compound was chromatographed and determined by electron affinity detection

Huelin and Coggiola (1968) presented evidence for a role of α-farnesene in scald and showed that DPA reduced its production in apples. Bull's eye rot (Lopatecki and Burdon, 1966), core flush (Padfield, 1959) and internal breakdown (Padfield, 1959 and Stevenson and Blake, 1961) are diseases reportedly inhibited by DPA. Daines (1962) showed that DPA markedly reduced soft rot or blue mold rot (Penicillium expansum) and controlled Stayman Spot (Daines, 1967). Ginsburg (1968b) found that DPA wrappers reduced apple bitter pit significantly and that penicillium rot of pears was controlled in storage by these wrappers (Ginsburg, 1968).

Chemical injury of apples by DPA has been reported by Dilley and Dewey (1963) on McIntosh and Rome Beauty with this damage occurring on the non-cavity surface and

in the stem and calyx cavities, respectively. Similar injury was observed on the cheeks and shoulders of Golden Delicious (Pierson and Schomer, 1968). Daines (1962), Rep. East Malling Res. Sta. (1967), Padfield (1959, 1961), Padfield and Smock (1960) and Smock (1961) showed DPA accumulations at the bottoms of containers and on fruits could result in injury to the skin of several apple varieties. Padfield (1961) suggested that complete drainage from the fruit is necessary to avoid chemical burning. Hardenburg and Anderson (1962) related similar injury to alcohol solutions of DPA. Damage to the lenticels on the exposed side of the fruit that received the impact of spray jets increased in severity with increased duration of spraying (Rep. East Mall Res. Sta., 1967). Padfield and Clark (1963) observed that a naturally occurring browning of lenticels on Rome Beauty was aggravated by DPA treatment. Hall, et al. (1961) reported a similar response on Granny Smith with accompanying injury appearing as dense, shiny, black areas on the skin. They showed that dipping in DPA caused senescent blotch to be more severe and that DPA-treated fruit appeared greener than untreated fruits after storage. Field applications by spray caused early leaf drop and some stronger preparations caused some marginal leaf scorching and brown spotting of apples on the tree.

Hall, et al. (1961) reported that DPA has a very limited mobility in apples and residues are low after cold storage. When DPA-impregnated sheets are placed between layers of apples in a container, DPA is effective at a distance from the sheets, but this effective distance depends on the amount of DPA available in the paper. They also observed, "no apparent movement of DPA from treated to untreated areas of the apple" and found a significant difference in scald between treated and untreated halves. DPA penetrates the fruit skin, since approximately equal amounts were found on the surface and in the pulp (Bruce, et al.,1958). Ginsburg observed in 1961 that the amount of DPA remaining on the surface was negligible and the following year (Ginsburg, 1962) he noted that the amount in the pulp was consistently higher than in the peel. However, Harvey and Clark (1959) indicated that about 84% of the residual DPA on apple fruits was removed from the surface with solvents and 94% was found in the outer 2-4 mm of tissue. According to Denmead, et al. (1961) DPA disappeared from the apple fruit surface at an exponential rate which differs by variety. These loss rates were sufficiently high to require that estimations be made very soon after treatment to avoid errors. Bache, et al. (1962) stated that if they are to be analyzed within 7 days it was acceptable to store samples at 32°, but if stored for longer periods they

should be frozen at 0°F or lower to avoid excessive loss of DPA by volatilization. Accordingly, the blended samples should be analyzed immediately or stored in vapor-tight containers, but for no longer than one week without serious loss of DPA.

The method of application of DPA affects residues (Hall, et al., 1969). Apraying fruit in crates gave relatively high residues (Bache, et al., 1962a). Flooding with DPA suspensions for 30 minutes in a hydrocooling system (Yatsu, 1956) resulted in very high residues, therefore he suggested that hydrocooler applications should be limited to solution concentrations of 100-200 ppm. Dipping apples in DPA solutions resulted in immediate values of 7-8 ppm, but after 3-7 months of storage, residues were 3 ppm or smaller. Ginsburg (1961, 1962) found that dip treatments of 1 min duration resulted in the apples taking up far less DPA than from wraps which were constantly in contact with the fruit. The higher residues found with wraps were due to vaporization onto the fruit (Bache, et al., 1962a). Wiping treated apples with a dry cloth resulted in 50% more DPA being adsorbed by the fruits (Denmead, et al., 1961), but the mechanical removal of excess DPA deposit left by wettable powder sprays did not impair scald control (Padfield and Smock, 1960). Insufficient residues for effective control were observed by Martin (1959) when DPA was vaporized with

steam and applied to apples in a tunnel. Preharvest sprays resulted in low residues and poor scald control (Bache, et al., 1962a). Harvey and Clark (1959) reported residues of one ppm which dropped to 0.5 ppm with a delay of 3 days between spraying and harvesting.

As the duration of DPA post-harvest spray treatments increased for all varieties, residues increased almost linearly up to a high of 18 ppm for Dougherty sprayed for 4 minutes (Denmead, et al., 1961). They postulated that this may have been due to permanent changes occurring in the apple's wax coating and reported 1-2 ppm residues for all varieties after 1 minute of treatment. Padfield and Clark (1963) also observed considerable increase in DPA uptake with increase of treatment time and suggested that 1-2 minutes is adequate.

Diphenylamine residues are related to the amount of the chemical applied (Hall, et al., 1961). Denmead, et al. (1961) observed that DPA residues on the fruit increased about 9 ppm over a 500-3000 ppm range of DPA solution concentration, but uptake was not proportional to the concentration of the emulsion, which gradually fell off as a result of uptake by apples and dilution of the emulsion. They also pointed out that the oil emulsion agent alone, without DPA, afforded some protection against scald. This would be expected with varieties known to respond to oiled wraps.

Varieties of apples vary widely in DPA uptake (Harvey and Clark, 1959; Padfield, 1961; Padfield and Clark, 1963 and Smock, 1961). Denmead, et al. (1961) reported that the level of DPA uptake from a particular treatment appeared to be negatively correlated with scald susceptibility; for instance, Granny Smith showed the lowest residues, but was rated as the most susceptible to scald of those examined. Other varieties, not considered to be susceptible, were found to have exceptionally high residues that ranged up to 14.5 ppm. In stating that varieties influence inhibitor coverage, Mattus (1963) reported that the best coverage was obtained on Stayman, Winesap and York; lesser, but still good coverage occurred on Rome Beauty; and the least on Delicious, Jonathan and Grimes Golden, which were about equal.

Fruit maturation increased DPA coverage (Mattus, 1963); however, Padfield and Clark (1963) reported that residues differ in relation to maturity by variety, with no general trend toward greater residues with either immature or senescent fruits. Denmead, et al. (1961) observed that storing apples at room temperature before treatment increased DPA uptake by about 100% after four weeks. In this period of time there was a noticeable increase in the "greasy" feel of the apple surface and an expected increased absorption of oil from the DPA emulsion.

The effectiveness of scald inhibitors is readily and easily evaluated using Martin and Grassia's (1965) half-fruit method. Hall, et al. (1961) reported that about 0.1 mg of DPA would be needed per average sized apple to control scald, but observed that some residual scald was present even with the use of DPA, especially at badly bruised areas and in the calyx cavity. They also reported that dipping in DPA gave commercial scald control of fruits from early harvests and showed a quantitative relationship between severity of scald and the concentration of DPA required for control. For instance, 1500 ppm was adequate for severe scald, but less was required in years of slight scald. Hilkenbaumer (1961) showed a marked decrease in scald after DPA treatment, the effect being proportional to the strength of the solution. The initial DPA concentration on the apple's surface was positively correlated with the extent of the scald protection during storage and holding apples at room temperature prior to DPA treatment reduced the susceptibility to scald (Denmead, et al., 1960). Padfield (1961) indicated that reasonable control was achieved with 250 and 500 ppm, and under all conditions, 1000 ppm DPA gave effective scald control. He also stated that 5 ppm residual DPA on the fruit surface gave effective control. Smock (1961) suggested that 3 to 5 ppm on the fruit at harvest time was adequate and Huelin

(1968) indicated that 0.2 $\mu g/cm^2$ in apple peelings appeared to be the minimum required to inhibit scald-inducing reactions.

Artificial induction of scald symptoms was effected by holding apples in an unsealed, 150 gauge polyethylene liner (Smock, 1961). This method served as a rough prediction procedure, but tended to underestimate the amount of scald developing in storage. Brooks, et al. (1919) and Huelin (1964) promoted typical scald development by exposure to fruit volatiles. Dilley, et al. (1963) proposed scald to be a two stage phenomenon, the first induced by anaerobiosis (as for example, 72 hours in a 100% nitrogen atmosphere) the second by actual symptom development under aerobic conditions at 70°F.

MATERIALS AND METHODS

DPA Analyses For Residues on Apples. The gas chromatographic technique outlined by Gutenmann and Lisk (1963) for the rapid determination of DPA in apples seemed suitable to the requirements of these experiments. DPA was extracted from chopped apple tissue with acetone and the extracts stored at -10°C by this procedure. Great difficulties were encountered in obtaining consistent and accurate results. Standardized concentrations of DPA finally were accurately analyzed after the halogenation step was altered by the use of chlorine instead of bromine. Analysis of all fruit samples from the 1967-68 harvest season treatments were completed using this modified technique. However, the determined DPA residues did not agree with the projected values according to the applied treatments. Additional extensive and prolonged efforts to accurately detect DPA by this method did not yield results of acceptable reliability.

The procedure was further modified to use the spectrophotometric technique described by Yatsu (1956) as modified by Bruce, et al. (1957) and Harvey (1958). Since acetone absorbs light at similar wavelengths to DPA, it

was necessary to transfer the DPA into hexane. One hundred milliliters of a mixture of hexane isomers were added to 100 ml of acetone extract of apple tissue in a 1000 ml separatory funnel. A highly purified grade of hexane was needed to reduce the interference caused by the impurities found in most grades. Five ml of 1.0 M calcium chloride was added along with 600-700 ml of deionized water to break the emulsion formed in partitioning the DPA into the hexane. These liquids were shaken for two minutes then allowed to stand for two minutes before drawing off the lower layer containing acetone, water and $CaCl_2$. The hexane phase, containing the DPA was then transferred to a second 1000 ml separatory funnel and excess water and 5 ml of $CaCl_2$ were again added. Shaking for two minutes was followed by a 2-minute standing period before the DPA·hexane layer was drawn off into a 125 ml separatory funnel. Five ml of vanadium pentoxide reagent (100 µg/ml of vanadium pentoxide in 10 M sulfuric acid) were added to the DPA·hexane phase and this mixture was shaken for 2 minutes, allowed to stand 2 minutes and then the reacted product (bottom layer) was vacuum-filtered through a Teflon-coated fiber glass disc into a small test tube. This filtered product was transferred to a cuvette and placed in the light path of a Beckman, Model DU spectrophotometer. The absorbancy of this product was read using a tungsten light source with a

wavelength setting of 605 mμ. The color of this product was very unstable so all readings were made at exactly 6 minutes from the time the chemicals were first combined. The reaction of the DPA extracted from apple tissue plus the vanadium pentoxide reagent resulted in the formation of an opaque, non-uniform sludge. Passing this viscous product through the filter cleared the solution and increased the accuracy of this procedure. Experimental samples were read on the spectrophotometer using acetone, which was partitioned in the manner described, as a blank. Known concentrations of DPA in acetone were partitioned into hexane and reacted with the vanadium pentoxide reagent to prepare a standard curve.

Solution Analyses in the Laboratory. Solutions prepared from liquid emulsion formulations of DPA can be analyzed with greater uniformity than those made from wettable powders. For both, technical grade DPA was mixed with water to prepare treating solutions according to manufacturers' specifications. Before sampling, the solution was well mixed in order to insure a representative 1 ml aliquot which was diluted with 9 ml of absolute ethanol. From this diluted solution 0.1 ml was removed with a micro-syringe and reacted in a test tube with the vanadium pentoxide reagent. The degree of blue color development indicated the amount of DPA. The stability of the color of this product was greater than that of DPA

residue analyses, but still had to be read on the spectrophotometer within 5 to 7 minutes after the initial combination of the chemicals. These colored products were compared with the vanadium pentoxide reagent combined only with ethanol and read at 585 mμ wavelength (Kraght, 1966). Standard curves were prepared from known concentrations of technical grade DPA dissolved in absolute ethanol.

Solution Analyses in the Field. A test was devised for relatively simple estimations of DPA content in solutions in use in storages and packing houses. The same vanadium pentoxide reagent, previously described for DPA residue and DPA solution analyses, was carefully taken to the field in a tightly-sealed, glass bottle. A plastic or glass eyedropper calibrated to 1 ml was used to take an aliquot from a well-mixed solution. Preferably a pipette or syringe should be used for greater accuracy, but for coarse estimations the eyedropper suffices. One ml of the solution was diluted with 9 ml of alcohol, if available, or water and shaken well. The same sampling device could be used to remove a 1 ml aliquot of the diluted sample for reaction in a separate vessel (test tube) with 5 ml of the vanadium pentoxide reagent. The intensity of blue color development was directly correlated with the amount of DPA (up to 100 μg) which, upon reaction with vanadium pentoxide, formed a blue color

complex that was near the upper limits of the spectrophotometer at the wavelength used. However, for field estimations, this deep blue color was readily distinguished from the less intense blue color of solutions which had become weakened by usage. Two solutions were compared for field estimations; therefore, an unused sample of the solution was taken and held for this purpose. The solution in question was treated as described above and at the same time a fresh solution, which was prepared according to the manufacturers' specification, was also diluted and reacted with the reagent. By comparison of colored products the relative concentration of DPA in solution could be adequately estimated. Such a test should indicate whether or not an operator should replace his treating solution. The test could be further improved by preparing in advance solutions of known concentrations of DPA and removing 1 ml aliquots for reaction and comparison with unknowns. This would provide a range of concentrations to fit the solution in question.

Aging Solutions. In 1967-68 treatments were delayed while DPA analytical techniques were studied in an effort to improve their reliability. Delicious and McIntosh apples were harvested and stored until February, when this study was conducted. The samples consisted of randomly selected fruit representing all harvests and all

trees from which the apples were obtained. At the start of the experiment, 12 solutions were prepared. Two formulations of liquid emulsions were provided by commercial sources; "A," Deccoscald 25,[1] and "B," No-Scald DPA Liquid,[2] both of which were prepared to 1000 ppm according to label directions. About 6 grams of chopped organic matter consisting of orchard trash, soil and rotten fruits, were added to half of the solutions.

At 4 weekly intervals, 10-fruit samples of McIntosh and Delicious apples were dipped for one minute in each of the above solutions and allowed to dry at room temperature. Once dried, all apples were chopped and the DPA extracted and quantitatively determined by previously described procedures.

In 1968 the treating solutions were prepared at intervals prior to the harvest of each variety. For example, McIntosh apples harvested at the Graham Experiment Station on September 12th were treated within 48 hours in solutions prepared 1, 2 or 4 weeks earlier or in a solution freshly prepared the day of treatment. As in the previous year, there was a corresponding solution that contained orchard trash, rotting apples and soil for each clean solution. All solutions were held at room temperature in covered plastic containers of 10 gallons capacity.

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[1]Product of Wallace and Tiernan, Inc., Monrovia, Calif.

[2]Product of Chemley Products, Chicago, Ill.

Fruits were harvested earlier than of optimum maturity in order to be favorable for the development of storage scald. A sample consisted of randomly selected apples encompassing all the trees included in the harvest of each variety. Two 50-apple replicates of each of the eight treatments were dipped for 30 seconds each. After allowing the apples to dry at room temperature, 10 fruits were randomly selected for DPA residue analysis, placed in a plastic bag and frozen immediately. When time permitted, these were thawed slightly, chopped and extracted in the manner described previously. The remaining 40 apples of each sample were carefully placed in an open plastic bag (2 samples per field crate) and held at 32°F. After all varieties had been treated and stored, they were transferred on October 22 to 38°F and held until it was established that scald could be artificially induced by nitrogen treatment in the laboratory.

Artificial Scald Induction. The apples with storage scald potential were artificially induced to scald by the procedure described by Dilley, et al. (1963). A sample of 20 apples was placed in a closed container through which nitrogen gas was passed continuously at the rate of 250 to 300 ml per minute. The apples were maintained in this oxygen-free atmosphere for 72 hours at 20°C, then transferred to air and held another 72 hours at 20-25°C. Apple scald was classified as (a) none (non-existent or only in

association with bruising), (b) slight if present but not obvious to the average consumer, or (c) severe if it was obvious to even the untrained person that the fruit was defective. To place McIntosh, Delicious and Rome Beauty varieties on common ground, scald reduction values were used: the difference in the number of scalded apples between DPA-treated and untreated fruits.

Submersion (Hydrostatic Pressure). In 1967 the physical factors involved in treating apples under water were examined, whereas in 1968, both physical and physiological effects of hydrostatic pressure were studied. In 1967, hydrostatic pressures up to 4 psi were developed using a 6-quart pressure cooker vessel containing 1000 ppm DPA treating solution and 10 apples. Treatment time varied from 0 to 8 minutes, plus one minute which was required to place the apples in solution, establish the desired pressure (via application of compressed air to vessel) and remove the fruits from the treating system. Each sample was prepared randomly (as in the aging solution studies) and handled following treatment in a similar manner to previous tests. Both Delicious and McIntosh apples were studied.

In 1968, Delicious apples were treated with two concentrations of DPA (1000 and 2000 ppm) plus one treatment of water alone. Hydrostatic pressures of 0, 1.5 and 3.0 psi were developed by submerging the apples just below the

surface of the solution, 2.5 feet and 5 feet beneath the surface, respectively. A 100-gallon treatment tank was constructed from two 55 gallon drums welded together end to end. Each 50-apple sample was lowered to the specified depth in a weighted metal container. Total treatment time, including submersion and removal from the system, was 30 seconds plus the designated treatment time. The fruits were drained before packing 10 apples and 40 apples in previously described containers for residue analyses or physiological determinations, respectively. The treating solutions were sampled and analyzed for DPA content.

Commercial DPA Applications. The concentration of DPA in commercial treating systems, both fresh and after varying periods of usage, and as affected by varying amounts of orchard trash accumulation were obtained from seven cooperators. Each operator was asked to collect a 70 ml sample of his solution at the start and finish of each day's run. It was also suggested that samples be taken whenever the solution was fortified or altered in any other way. The screw top vials containing these samples were stored in a cool place at the storage until collected by MSU Horticulture Department personnel near the end of the season.

Synthesis of Radioactive Diphenylamine. Aniline hydrochloride-^{14}C(UL)[1] was converted to aniline-^{14}C(UL) and the synthesis of Mueller (1959) for DPA was subsequently followed, since yields of 65% were reported, using aniline as the precursor. Labeled aniline hydrochloride in ethanol was combined with 0.4 M NaOH and shaken thoroughly to remove all the radioactive material from its ampoule. Aniline hydrochloride was then partitioned into chloroform and this fraction transferred to a 100 ml boiling flask to which a condenser was attached. Heating proceeded very slowly until the chloroform was distilled off. A capillary tube attached to a nitrogen cylinder was placed in the condenser during the final two hours of distillation and for the remaining steps in the synthesis. This nitrogen atmosphere was used to reduce the possibility of oxidation of the diphenylamine. Refluxing was discontinued after 8 hours and the resulting liquid had a total volume of 2-3 ml to which 0.3 gram of iodine was added to act as a catalyst for the final reaction. Heat was applied continuously until only a white crystalline product and black char (iodine) remained in the boiling blask. All reamining materials were dissolved in chloroform and steam distilled (Sigal et al., 1966) collecting a total volume of 800 ml distillate,

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[1]Lot No. 212-266 from New England Nuclear, Boston, Mass.

which was dried in a separatory funnel with excess anhydrous sodium sulfate. The remaining 600 ml of pink-colored distillate, was very slowly distilled on a spinning band column to a volume of 5 ml. This product, diphenylamine, was diluted in 100 ml absolute ethanol and used in tracer studies. The A_{max} of this product was the same as that for analytical grade DPA.

Autoradiography. Apple tissue discs (1-4 mm thick) for autoradiography were cut from the center of the ^{14}C-DPA treated apple with a sharp knife. Each free-hand section was then placed on a no. 3 Buchner funnel mounted on a 500 ml vacuum filter flask and connected to a Virtis freeze-drying system. A 12 cm disc of Teflon-coated fiberglass filter membrane was placed beneath and adjacent to the apple disc. Between this and the funnel was a 9.5 cm Whatman No. 1 filter paper. To increase the vacuum on the tissue, a sheet of Saran film was laid over the top of these materials and pulled tightly against the apple. Within 24 hours, the apple disc was dehydrated, but leathery and pliable. This was separated from the membrane and Saran film sheets and placed in a desiccator until used in autoradiography. Kodak Royal Blue X-ray film (Wang & Willis, 1965) provided good sensitivity and acceptable resolution upon exposure of the discs for 28 days.

Statistical Procedures. Analysis of variance was applied to the data of these experiments. Means of factors found to be significant by the F test were further compared by Tukey's w procedure (Tukey's test) at the 5 percent level (Steel and Torrie, 1960).

RESULTS

DPA Analytical Technique. The technique described for analyzing for DPA residues in apple tissue was periodically checked to ascertain its reliability as a method of recovering maximum residues. In one such test, known quantities of DPA were added to the blended apple tissue and recovered by extraction. Fortification with quantities of DPA which would produce theoretical recoveries of 3.2 and 6.4 ppm resulted in average recoveries of 103% and 96.6%, respectively. Apparent residues averaged 3.37 ppm higher than the values which could be attributed to DPA. This interference was probably due to preharvest spray materials remaining on the apples and the chemical components of the apples themselves. Corrected DPA residues are reported as total apparent DPA minus values representing interference from non-DPA sources.

DPA Residues on Apples as Affected by Aging Solutions and Hydrostatic Pressure. The analyses of variance of data obtained from the aging solution studies in 1967 and 1968 are summarized in Tables 1 and 2, respectively. The effect of varieties was significant in 1967, with the mean for McIntosh being 3.93 ppm and Delicious, 2.84 ppm.

TABLE 1.--Analysis of variance of DPA fruit residues in the 1967 aging solution study (see Appendix Table I or II for data).

Source	df	F
Blocks	2	
Total for variety	5	
Blocks	2	
Variety	1	42.78 *
Error a	2	
Total for formulation	11	
Total for variety	5	
Formulation	1	71.18 **
Variety x formulation	1	6.24
Error b	4	
Total for sanitation[1]	23	
Total for formulation	11	
Sanitation	1	31.75 **
Sanitation x variety	1	3.28
Sanitation x formulation	1	16.85 **
Sanitation x formulation x variety	1	4.22
Error c	8	
Total for age	119	
Total for sanitation	23	
Age	4	8.07 **
Age x variety	4	3.43 *
Age x formulation	4	6.61 **
Age x sanitation	4	3.31 *
Error d	80	

[1]Clean DPA solutions vs. those containing organic matter (OM).

TABLE 2.--Analysis of variance of DPA fruit residues in the 1968 aging solution study (see Appendix Table III for data).

Source	df	F
Blocks	1	
Total for variety	5	
Blocks	1	
Variety	2	6.00
Error a	2	
Total for sanitation	11	
Total for variety	5	
Sanitation[1]	1	30.37 *
Sanitation x variety	2	1.64
Error b	3	
Total for age	47	
Total for sanitation	11	
Age	3	6.35 **
Age x variety	6	1.28
Age x sanitation	3	5.50 **
Error c	24	

[1]Clean DPA solutions vs. those containing organic matter (OM).

No varietial differences occurred in the 1968 trials. The source of technical DPA was a factor, with mean residue values of 4.59 ppm for formulation A[1] and 2.18 ppm for formulation B[2] in 1967. Only one material (Formulation A) was used in the 1968 studies. The addition of organic matter to DPA solutions increased fruit residues in both years, being significant at the 1% level in 1967 and at the 5% level in 1968 (Table 3). In 1967 there was

TABLE 3.--Mean DPA residues (ppm) for apples as affected by organic matter.

	Control	Organic Matter
1967	2.33	4.43
1968	1.38	8.07

a highly significant interaction between the sanitation of the DPA solution and DPA formulation (Figure 1). A rather gradual increase in fruit residues occurred with aging of the solutions in 1967, whereas, residues almost doubled between the 2- and 4-week old solutions in 1968 (Table 4). Tukey's test revealed differences between the

[1]Deccoscald 25, product of Wallace and Tierman, Inc., Monrovia, Calif.

[2]No-Scald DPA Liquid, product of Chemley Products, Chicago, Ill.

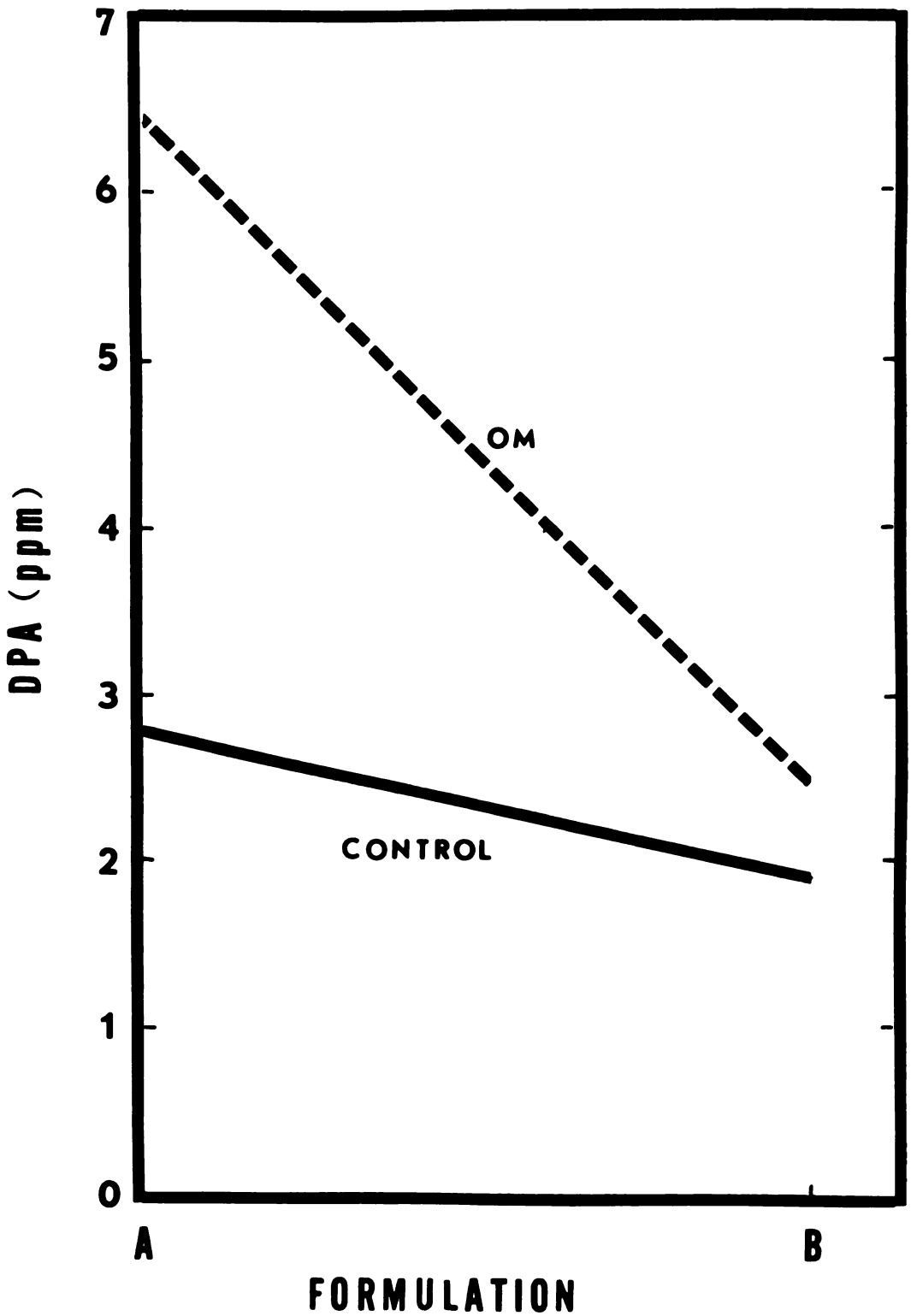

Figure 1. DPA residues (ppm) as affected by the interaction between solution sanitation and technical formulation for apples dipped in 1000 ppm DPA solution for one minute in 1967.

TABLE 4.--Mean DPA residues (ppm) for apples as affected by solution age.[1]

	Age (weeks)				
	0	1	2	3	4
1967	2.11 a	3.55 bc	3.81 bc	3.05 ab	4.39 c
1968	1.77 a	3.84 b	4.49 b	---	8.80 c

[1]Means not followed by the same letter are significantly different by Tukey's test.

means of DPA residues for apples treated in solutions of different ages in 1967, especially when fresh and 4-week old solutions were compared. Similar differences between means were detected in the 1968 trials. Figure 2 illustrates the significant interaction between apple varieties and solution age in the 1967 trials. The interaction between DPA solution age and the technical formulation of DPA in 1967 was significant at the 1% level (Figure 3). Results in both seasons revealed significant interactions (1967, P=.05; 1968, P=.01) between DPA solution age and the presence of organic matter in the solutions, as shown in Figures 4 and 5 for 1967 and 1968, respectively.

The analyses of variance of data obtained in the submersion studies conducted in 1967 and 1968 are summarized in Tables 5 and 6, respectively. Apple varieties were significantly different in 1967 with residues for McIntosh being 4.29 ppm and Delicious being 5.63 ppm of

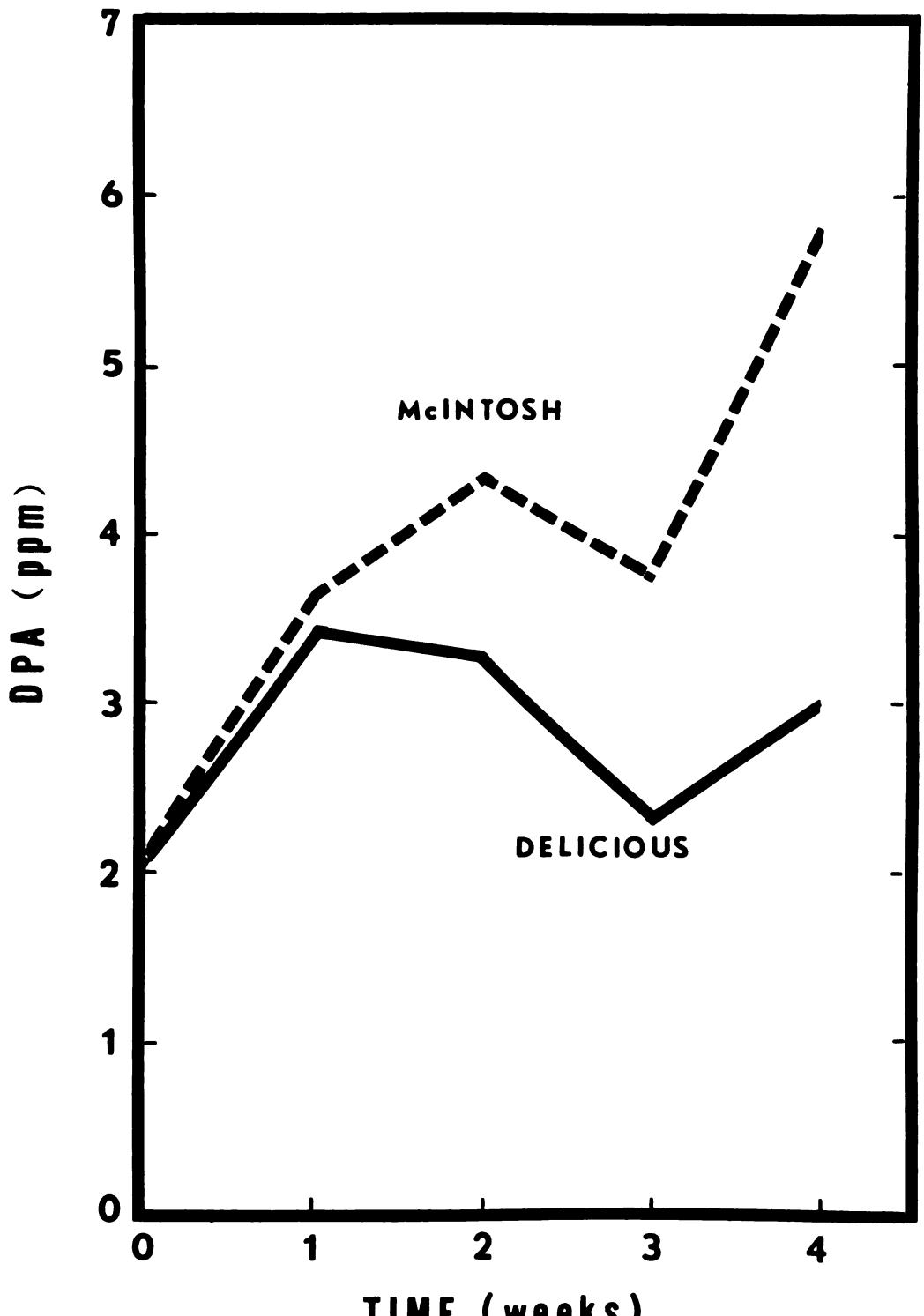

Figure 2. DPA residues (ppm) as affected by the interaction between solution age and apple variety for apples dipped in 1000 ppm DPA solution for one minute in 1967.

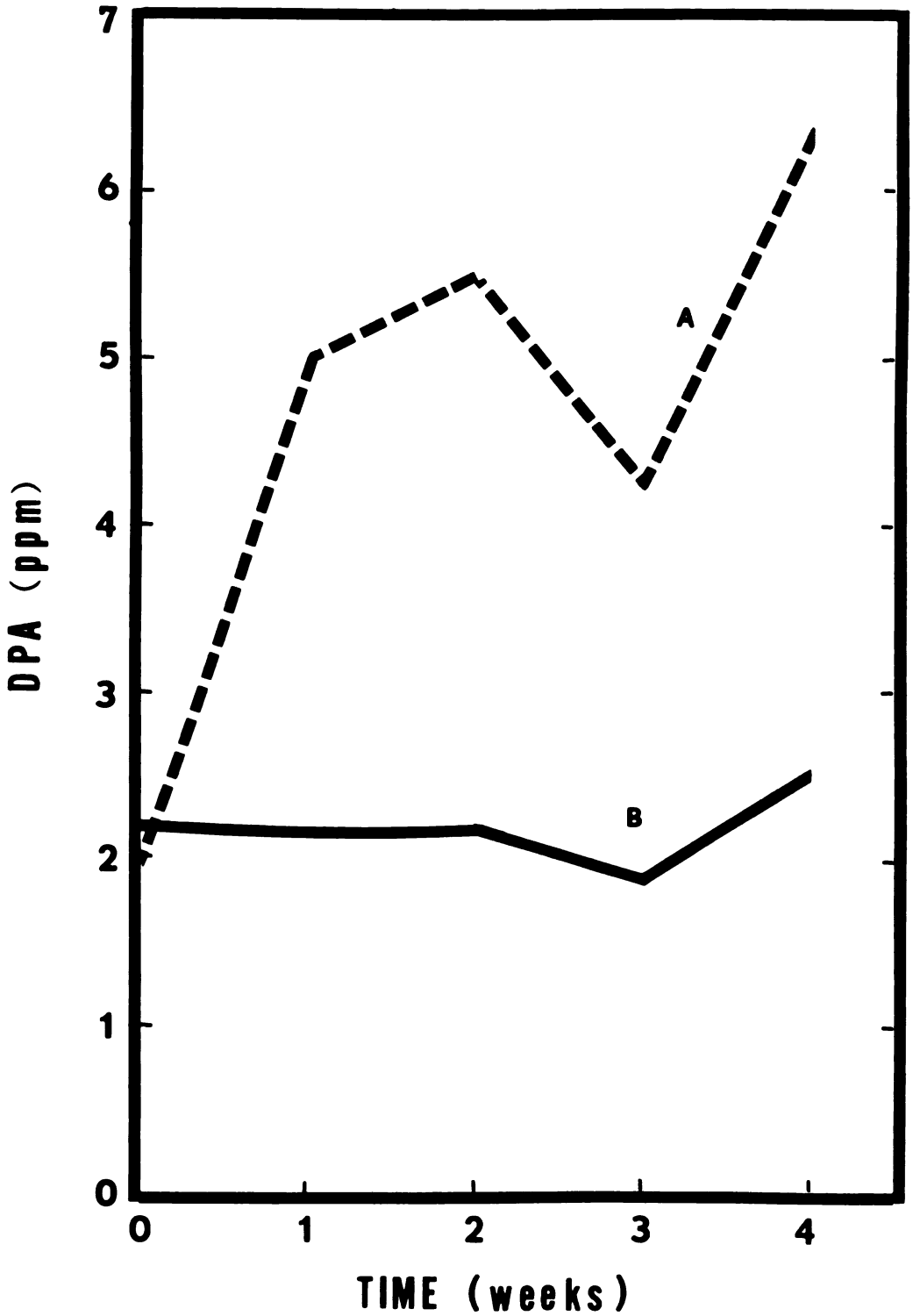

Figure 3. DPA residues (ppm) as affected by the interaction between solution age and technical formulation for apples dipped in 1000 ppm DPA solution for one minute in 1967.

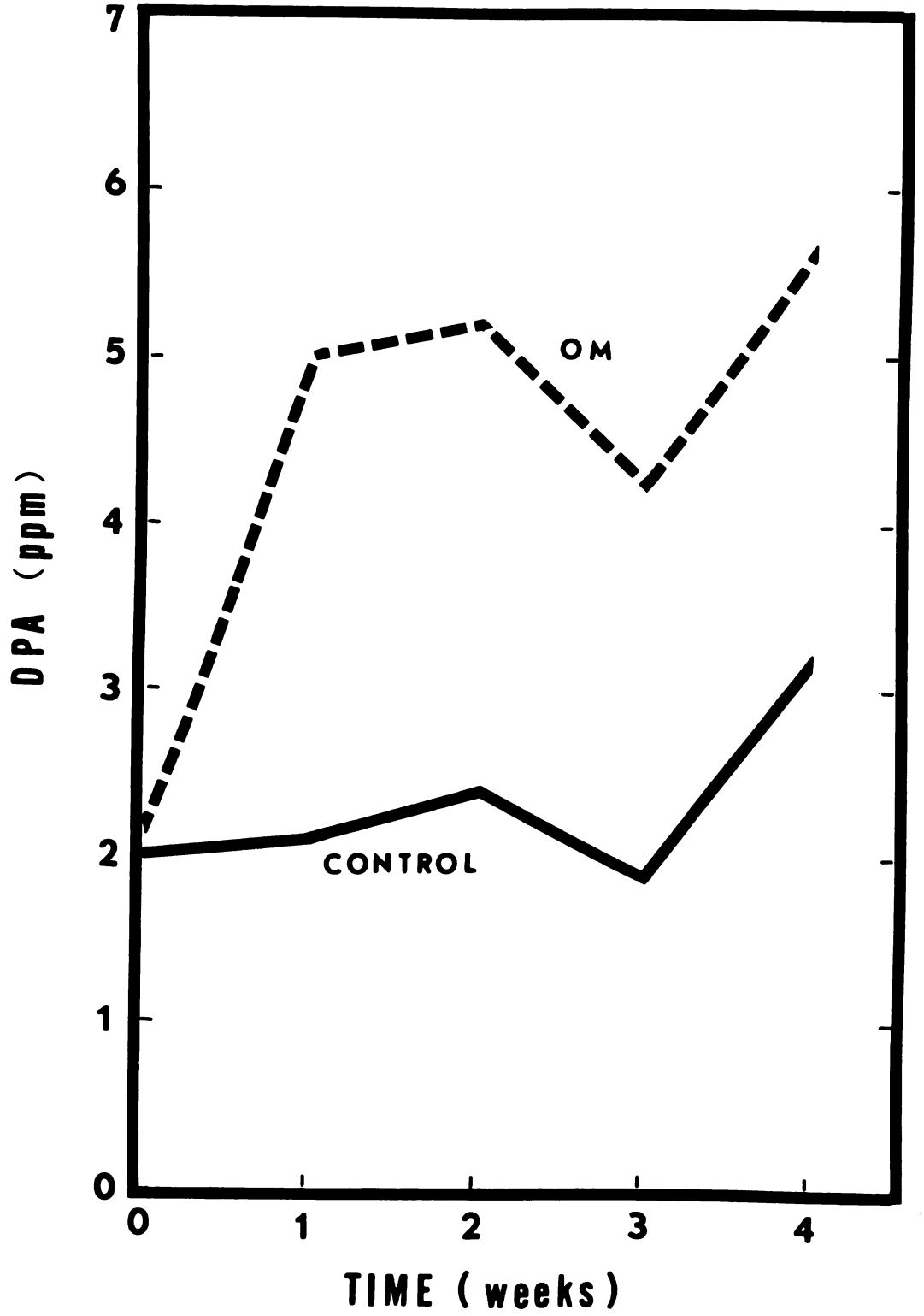

Figure 4. DPA residues (ppm) as affected by the interaction between solution age and sanitation for apples dipped in 1000 ppm DPA solution for one minute in 1967.

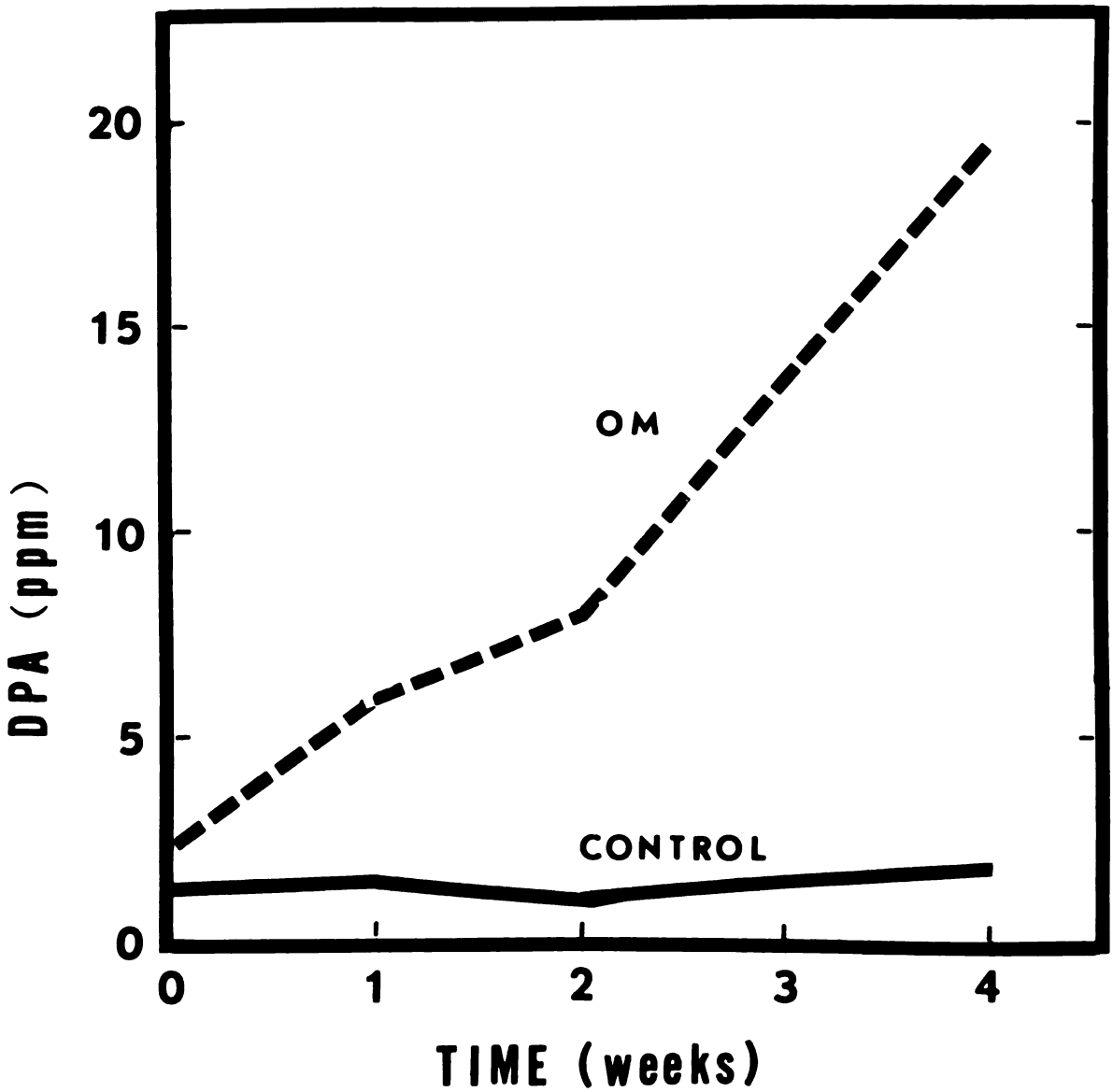

Figure 5. DPA residues (ppm) as affected by the interaction between solution age and sanitation for apples dipped in 1000 ppm DPA solution for 30 seconds in 1968.

TABLE 5.--Analysis of variance of DPA residues in the 1967 submersion study (see Appendix Table IV for data).

Source	df	F
Total	39	
Varieties	1	7.78 *
Time (Duration)	3	13.46 **
Pressure	4	12.18 **
Time x Pressure	12	---
Error	19	

TABLE 6.--Analysis of variance of DPA residues in the 1968 submersion study (see Appendix Table V for data).

Source	df	F
Blocks	1	
Total for concentration	3	
Blocks	1	
Concentration	1	7036.20 **
Error a	1	
Total for time	11	
Total for concentration	3	
Time (Duration)	2	3.82
Time x concentration	2	1.49
Error b	4	
Total for pressure	11	
Total for concentration	3	
Pressure	2	35.45 **
Pressure x concentration	2	14.85 *
Error b	4	

DPA. The duration of the hydrostatic pressure application had a significant effect on DPA residues of apples in 1967 (Table 7), but not in 1968. The data presented in Table 8 shows for both seasons that as the hydrostatic pressure on the apples in DPA solution increased the DPA residues on the apples increased.

It may be observed (Table 6) that residues were significantly affected by DPA solution concentration in 1968, having means of 2.10 and 4.08 ppm DPA residues for apples treated in 1000 and 2000 ppm DPA solutions, respectively. Figure 6 illustrates the significant interaction observed between hydrostatic pressure and DPA solution concentration in the 1968 trials. In addition to submersion treatments in 1000 and 2000 ppm DPA solutions, a third treatment of submerging apples in water alone resulted in apparent DPA residue values of 1.28 to 3.04 ppm (Appendix Table VI). The mean interference value of 2.10 ppm was subtracted from the treatment values to give the corrected DPA residues.

Factors Affecting the DPA Content of Solutions. The analyses of variance for the DPA concentrations of solutions used in the aging solution studies are summarized in Tables 9 and 10. The presence of organic matter significantly reduced the amount of measurable DPA in solution in 1967, but not in the 1968 trials. In the absence of organic matter the solutions contained an

TABLE 7.--Mean DPA residues (ppm) for apples as affected by duration of submersion in DPA solution in 1967.[1]

Time (min.)[2]			
1	2	3	4
2.87 a	4.40 ab	5.51 bc	6.70 c

[1]Means not followed by the same letter are significantly different by Tukey's test.

[2]Treatment times included an additional 1 min. for preparation.

TABLE 8.--Mean DPA residues (ppm) for apples as affected by submersion in DPA solution (hydrostatic pressure).[1]

	Pressure (psi)					
	0	1	2	3	4	5
1967	3.76 a	2.62 a	--	4.82 ab	6.29 bc	7.31 c
1968	2.76 a	--	2.57 a	--	3.95 b	--

[1]Means not followed by the same letter are significantly different by Tukey's test.

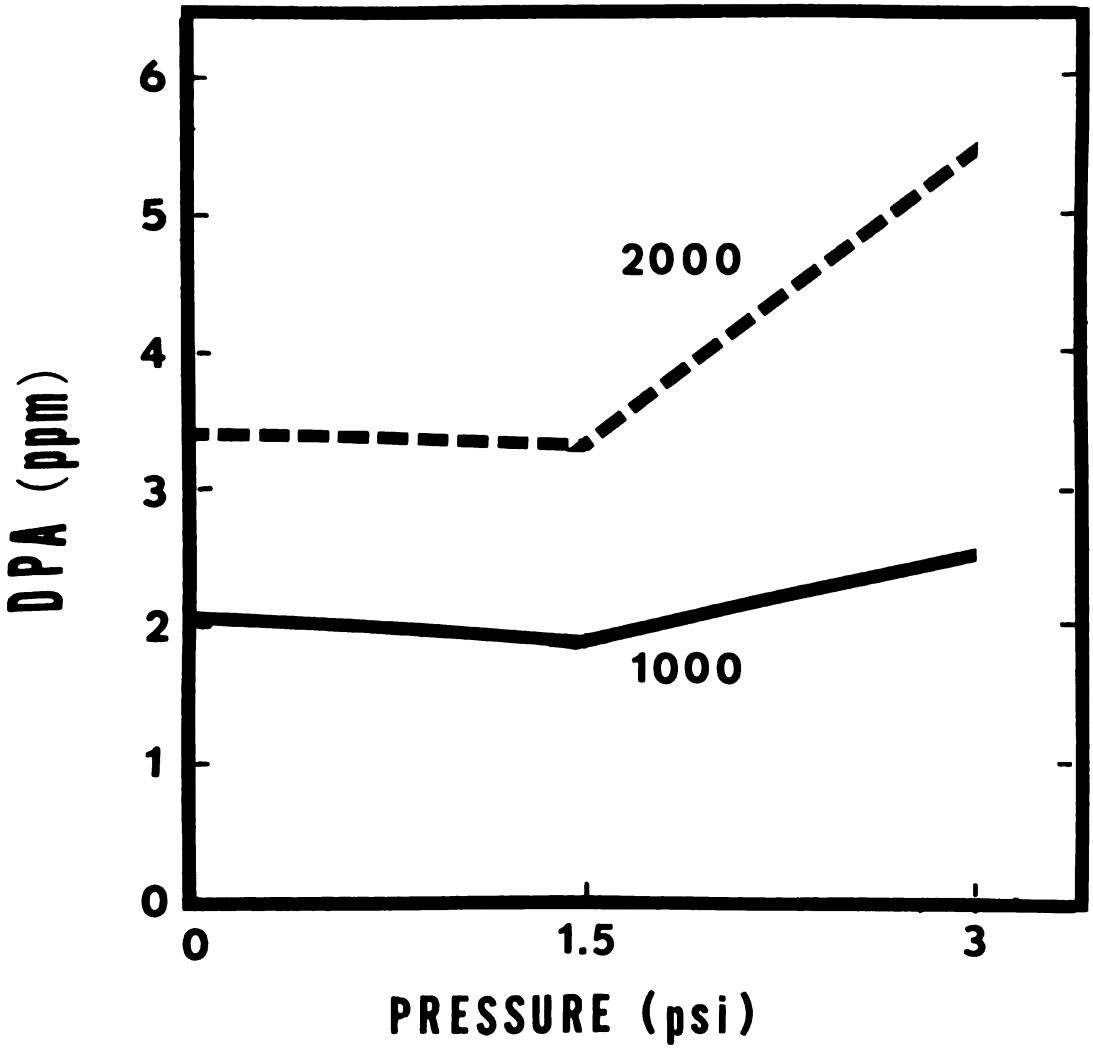

Figure 6. DPA residues (ppm) as affected by the interaction between hydrostatic pressure and DPA solution concentration for apples submerged in DPA solutions for 0, 2, or 4 minutes in 1968.

TABLE 9.--Analysis of variance for DPA solution content in the 1967 aging solution study (see Appendix Table VII for data).

Source	df	F
Blocks (Formulations)	1	
Total for temperature	5	
Blocks (Formulations)	1	
Temperature	2	0.63
Error a	2	
Total for sanitation	11	
Total for temperature	5	
Sanitation[1]	1	16.14 *
Sanitation x temperature	2	2.40
Error b	3	
Total for age	59	
Total for sanitation	11	
Age	4	7.86 **
Age x temperature	8	1.01
Age x sanitation	4	1.89
Age x sanitation x temperature	8	0.18
Error c	24	

[1]Clean DPA solutions vs. those containing organic matter (OM).

TABLE 10.--Analysis of variance for DPA solution content in the 1968 aging solution study (see Appendix Table VIII for data).

Source	df	F
Blocks	2	
Total for sanitation	5	
Blocks	2	
Sanitation[1]	1	7.95
Error a	2	
Total for age	23	
Total for sanitation	5	
Age	3	1.12
Age x sanitation	3	--
Error b	12	

[1]Clean DPA solutions vs. those containing organic matter.

average of 899 ppm of DPA, but with organic matter, an average of only 733 ppm of DPA was measured. The age of the solutions significantly affected the amount of measurable DPA only in 1967. The influence of solution age is presented in Table 11. The mean values were significantly different but not consistent with age.

Submersion solutions made up according to label recommendations were analyzed for DPA content in 1968. The 1000 ppm solution contained an average of 1046 ppm DPA. The 2000 ppm solution contained an average of 1912 ppm during the treatment period.

TABLE 11.--Mean DPA content (ppm) of solutions as affected by solution age in 1967.[1]

Age (weeks)				
0	1	2	3	4
1021 a	886 ab	748 bc	826 abc	600 c

[1]Means not followed by the same letter are significantly different by Tukey's test.

Storage Scald Control as Influenced by DPA Solution Age, Sanitation and Hydrostatic Pressure. Scald control was evaluated in 1968 to determine the efficacy of the chemical scald inhibitors. It was recorded as the total number of scalded apples for three solution ages compared with the No DPA treatment, and analyzed as a randomized block. There were significant differences between treatments and between varieties for scald control. Considering the scald reduction comparisons for the 1968 aging solution study (Table 12), the only significant effect was attributed to varietal differences, being 11.69, 9.75 and 14.88 scald reduction units[1] for McIntosh, Delicious and Rome Beauty, respectively.

Scald control differences between the three 1968 submersion treatments were evaluated and the analysis of variance of this data is shown in Table 13. The only

[1]Scald reduction units are the difference between untreated and DPA-treated apples in the number of scalded fruits per 20.

TABLE 12.--Analysis of variance of scald reduction[1] data from the 1968 aging solution study (see Appendix Table IX for data).

Source	df	F
Blocks	1	
Total for variety	5	
Blocks	1	
Variety	2	29.36 *
Error a	2	
Total for sanitation	11	
Total for variety	5	
Sanitation	1	--
Sanitation x variety	2	--
Error b	3	
Total for age	47	
Total for sanitation	11	
Age	3	--
Age x variety	6	--
Age x sanitation	3	1.52
Error c	24	

[1]Scald reduction units are the difference between untreated and DPA-treated apples in the number of scalded fruits per 20.

TABLE 13.--Analysis of variance of apple scale in the 1968 submersion study (see Appendix Table X for data).

Source	df	F
Blocks	1	
Total for concentration	5	
Blocks	1	
Concentration	2	229.58 **
Error a	2	
Total for time	17	
Total for concentration	5	
Time	2	---
Time x concentration	4	
Error b	6	
Total for pressure	53	
Total for time	17	
Pressure	2	---
Pressure x concentration	4	---
Pressure x time	4	3.03 *
Error c	26	

factor which significantly affected scald control was DPA concentration of the solutions (Table 14). Scald control observed in the 1968 DPA submersion studies was affected by the significant interaction between hydrostatic pressure and duration of DPA treatment. As the pressure increased from 0 to 3 psi and as the duration of exposure to this pressure increased from 0 to 4 minutes, scald was reduced, as illustrated in Figure 7.

TABLE 14.--Apple scald control in submersion solutions in 1968 as affected by DPA concentration.[1]

DPA Concentration (ppm)		
0	1000	2000
(Average number of scalded apples / 20 fruits)		
15.06 a	2.17 b	1.38 b

[1]Means not followed by the same letter are significantly different by Tukey's test.

Autoradiography of Apples Submerged in ^{14}C-DPA. Exposure of apples to 4 psi pressure for 10 minutes in 1000 ppm DPA solution fortified with ^{14}C-DPA revealed that most of the DPA remained at or near the Delicious apple surface, with only a trace in the seed cavity. It appeared that some DPA was forced through lenticels beyond the hypodermal area (Figure 8).

Commercial Use of DPA. Table 15 summarizes the survey results from seven apple storage operators in Michigan during the 1968 harvest season. According to the solution analyses, none of the operators attained an average DPA content in his system which approximated the desired concentration. The average range between high and low concentrations was about 700 ppm. In 1000 ppm preparations, DPA concentrations dropped as low as one-tenth the desired level; for 2000 ppm preparations concentrations dropped to 60% of the initial content. The average concentrations

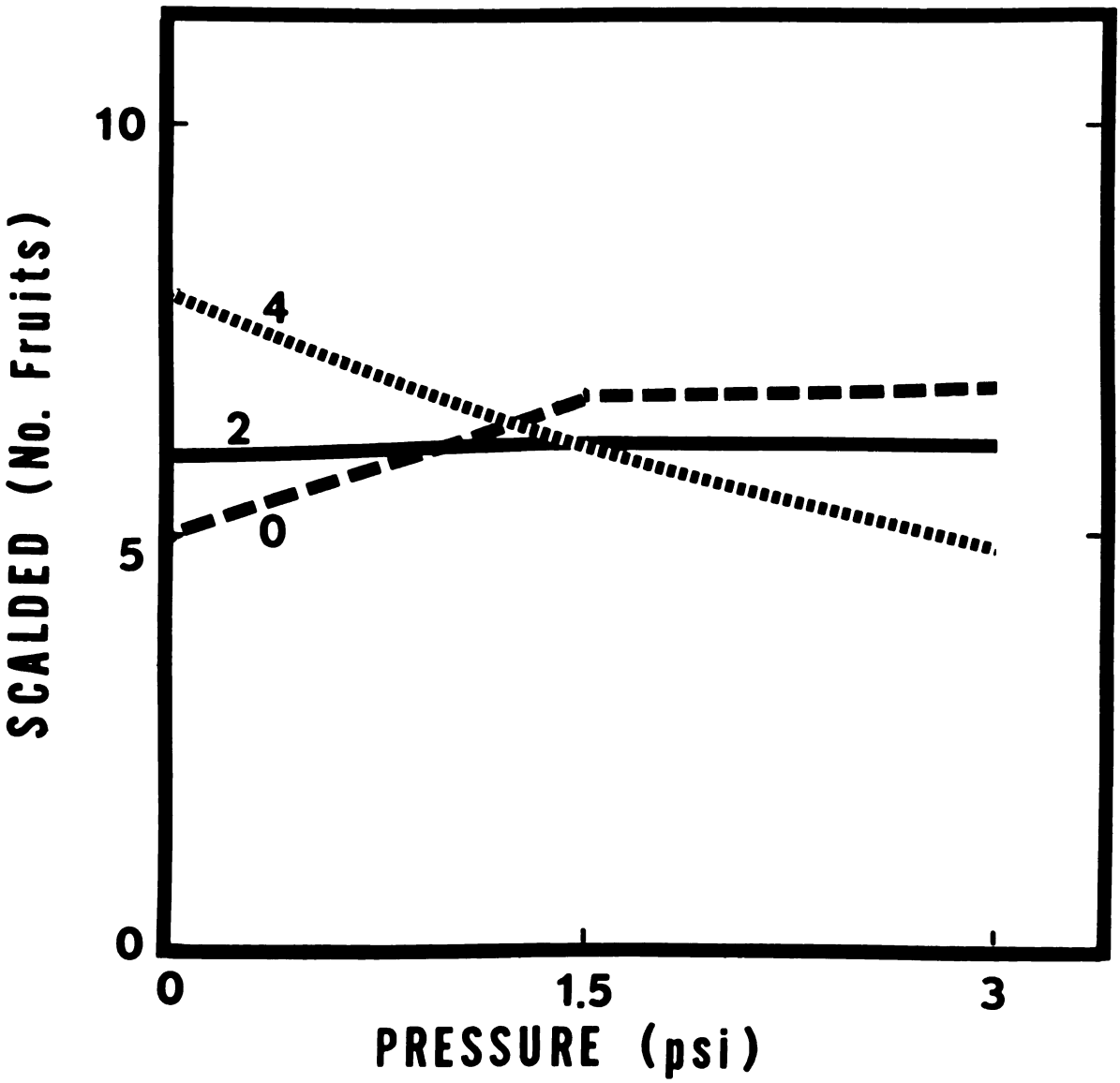

Figure 7. Apple scald control in submersion solutions as affected by the interaction between hydrostatic pressure and DPA treatments for 0, 2, or 4 minutes' duration.

Figure 8. Autoradiograph of cross section of Delicious apple submerged in ^{14}C-DPA solution for 10 minutes at 4 psi pressure.

TABLE 15.--Commercial DPA solutions used in Michigan--1968.

Operator	System	Varieties	No. Samples	Fungicide, etc.	DPA	DPA (ppm) in Solution			
						Desired	Low	High	Average
1	Drench (Truck)	McI, Del.	23	Captan, Triton	No Scald DPA (WP)	1000	97.5	943.0	399.2
2	Drench	McI, Del, Romes, Winesaps	18	Captan	Deccoscald	1000	476.3	1423.3	873.1
3	Drench	McI, Del, Romes	33	Glyodin	Deccoscald	1000	156.7	1024.5	499.1
4	Drench (2-High)	Del.	19	--	Deccoscald	2000	1251.6	2375.3	1669.6
		G. Del.	5	--	Deccoscald	1000	548.6	1074.0	880.9
5	Dip	Del.	17	--	No Scald DPA	2000	1269.0	2113.3	1616.2
6	Dip	Del.	22	Captan	No Scald DPA	1000	88.0	834.1	457.7
7	Dip	McI, Del.	24	Captan	Deccoscald	1000	348.5	734.8	537.3
					Average	1000 ppm	285.9	1005.6	608.2
						2000 ppm	1260.3	2244.3	1642.9
					Average (1000) ppm	Drench	319.8	1116.2	663.1
						Dip	218.3	784.5	497.5

of 1000 and 2000 ppm applications were about 60% and 80%, respectively, of the desired levels.

The average concentration of DPA in drench applicator systems was about 15% higher than for systems in which the apples were dipped in 1000 ppm of DPA solution.

DISCUSSION

Storage scald control is the ultimate test of DPA application methods, provided the resulting DPA residues on the apples do not exceed the established FDA tolerance of 10 ppm. Parameters of usage that neither impair scald control nor result in unacceptable DPA residues on apples should be of minimal concern to the industry and consumers.

McIntosh, Delicious and Rome Beauty apples in these tests developed varying amounts of scald without treatment, whereas, the application of DPA reduced scald incidence to commercially acceptable levels. The promotion of scald development by the artificial procedure of Dilley, et al.(1963) proved satisfactory, except for the development of severe external suboxidation symptoms on McIntosh which hindered the scald evaluations. Untreated Delicious apples evaluated for scald 9 weeks after harvest in the aging solution study developed 16 percent less scald than those similarly treated at 10 weeks after harvest in the submerging study. This response reflected Huelin's (1968) observation that untreated apples usually are stored about one-third of their normal storage life before there

is an onset of visible scald. Denmead, et al. (1961) found that DPA uptake was negatively correlated with varietal susceptibility to scald and, generally, Delicious is more susceptible to scald than McIntosh under Michigan conditions. Therefore, the greater residues observed on McIntosh after the initial two treatment periods (Figure 2) were expected and probably related to the more advanced maturity of McIntosh, a variety of relatively short storage life. Varietal differences in scald development and DPA uptake could also be expected in view of similar findings reported by Smock (1961) and Padfield and Clark (1963).

The significant interaction between variety and solution age observed in 1967, but not in 1968, may be attributed to the differences in experimental procedures followed in the two years. All solutions in 1967 were prepared at the same time and aging of these solutions occurred simultaneously with aging of the fruit which was held in storage at 32°F. In 1968, the solutions were aged prior to harvest so all apples could be treated at a comparable age within 48 hours of harvest. Fruit aging may have increased the uptake and retention of DPA since Mattus (1963) has shown that better DPA coverage was obtained as fruit maturity advanced. McIntosh approached senescence more rapidly than Delicious, a late storage variety, thereby accounting for the varietal differences

that resulted in the significant interaction found in 1967.

Delicious had greater DPA residues than McIntosh in the submergence tests. This may have been related to the open calyx, a characteristic more common to Delicious than to McIntosh. Hydrostatic pressures developed by submergence may have caused DPA to be forced into the cavities of Delicious apples. This was borne out by the consistently and progressively higher DPA residues in Delicious as the pressure increased and as the pressure was sustained longer. This suggests that problems may occur in practice when Delicious are subjected to hydrostatic pressure during handling operations.

Submerging Delicious apples in 1000 ppm DPA solution provided scald control equal to 2000 ppm. Scald developed on apples submerged in water, but not on those submerged in DPA, which accounted for scald control differences attributed to concentration (Table 13). Scald developed on 75 percent of the apples submerged in water and on about one-tenth of the apples submerged in DPA. These data do not support Hilkenbaumer's (1961) report that the effect of DPA treatment on scald was proportional to the DPA solution concentration. This may be due to the method of application, since increased DPA uptake in relation to greater hydrostatic pressures and increased duration of application (Table 8) resulted in

fewer scalded apples. DPA residues were twice as great on apples treated in 2000 ppm as on apples treated in 1000 ppm solutions. A similar effect was observed by Denmead, et al (1961) and Hall, et al (1961) in that DPA residues on apples were related to the amount of the chemical applied. Residues at both concentrations were acceptable, but since there was no improvement in scald control at 2000 ppm, DPA applications of 1000 ppm, as recommended by Dewey and Dilley (1964) for Michigan would appear to be adequate.

The gas chromatographic procedure for the determination of DPA residues in apples (Gutenmann and Lisk, 1963) proved unsatisfactory. This was apparently due to the acetone extraction of a wide spectrum of compounds from the blended apple tissue, possibly including other pesticide residues as well as naturally occurring compounds present in apples. Detection of the chlorinated derivative was reproducible and quantitative using standard preparations of DPA; however, the introduction of apple tissue resulted in unreliable results which could not be related to the fruit treatments. Extensive trials and modifications of this procedure to overcome these difficulties proved time consuming and inadequate. Even though this procedure apparently has provided satisfactory results in some laboratories, it was neither accurate nor dependable for the apples employed in this study.

Many hours of subsequent experimentation provided a modification of the colorimetric technique (Yatsu, 1956, Harvey, 1958) that enabled the salvation of experimental materials collected in the 1967 trials. This colorimetric procedure was used also to determine DPA residues in 1968. Recoveries of DPA were satisfactory and comparable to those reported by Yatsu (1956) and Harvey (1958).

Researchers or analysts desiring to detect DPA residues in limited quantities of apple tissue should find this modified colorimetric technique to be satisfactory. To minimize interference from impurities, however, it is essential to use dependably pure reagent grades of acetone and hexane. Furthermore, all DPA residue values should be corrected for interferences due to natural substances from the apple tissue and other spray residues readily extracted by acetone and diffusible into hexane.

Such factors as age of the DPA solution, presence of organic matter in the DPA solutions, depth of submergence of the apples in DPA solutions and the resulting hydrostatic pressures, and duration of treatment at the increased hydrostatic pressures did not affect scald control. However, all of these factors, as well as others, increased fruit residues of DPA above the effective minimum levels needed for scald control according to Huelin (1968), yet within the 10 ppm residue tolerance established as safe by the FDA.

One could expect that solution age and the presence of organic matter in the solution would make DPA less available so as to decrease fruit residues in comparison to those from freshly prepared or clean solutions. The combining of DPA with the organic matter, however, may have a partitioning effect and provide a better means of transfer of the chemical to the fruit surface. The surface tension of the fruit cuticle treated with old, contaminated solutions might be sufficiently reduced to increase the DPA uptake.

While at least partially responsible for increased DPA uptake, the aging and contamination of solutions also impaired the measurement of DPA in solution. Representative samples of the solution may not have been obtained for analysis; if true, the results would suggest that another effect of age and contamination was to promote the formation of a mixture rather than a colloidal suspension. This effect would make the DPA particles less available for detection by the colorimetric procedure. Although not investigated in these trials, the possibility of breakdown products of DPA forming upon aging and in the presence of organic matter should be considered. Fresh solutions and solutions free of contamination from orchard trash contained relatively consistent amounts of measurable DPA. Therefore, if DPA breakdown products are involved, they must be, apparently, less prevalent and less problematic under optimum conditions.

Crystals were occasionally observed on the apples treated with DPA solutions containing organic matter in 1967. In 1968, an oily deposition on fruits was sometimes associated with old, contaminated solutions. Therefore, even though treatment of apples in DPA solutions which had foul odors and appeared dirty provided acceptable residues and scald control, operators need to use good judgment in replacing solutions in order to avoid fruit rots and other problems.

DPA residue differences were noted in relation to technical formulation of the two materials used, yet both materials were acceptable in respect to scald control, freedom from injury and residue tolerance. The differences may be associated with oil base composition variations in their manufacture.

DPA treatment solution temperatures of 50, 60 and 70°F did not affect residues and this permitted temperatures to be used as replicates for analysis of the 1967 results. The lack of a statistically significant temperature effect should be of particular importance to fruit handlers since they cannot afford to control the temperatures within the DPA applicator systems. The results indicated that residue or scald problems from materials used in this temperature range should be of little practical concern.

Previously mentioned experimental fruit handling differences may have been responsible for the significant effect of duration of submergence on DPA residues which occurred in 1967, but not in 1968. Since duration of treatment affected the DPA residues in the studies of Denmead, et al.(1961) and Padfield and Clark (1963), they suggested DPA applications should be limited to less than 2 minutes. Longer durations were herein studied since fruit hydrohandling systems may purposely or inadvertantly expose apples to DPA solutions at 3- to 5-foot depths for at least several minutes.

Hydrostatic pressures developed by either air pressure or water head yielded similar effects, therefore, hydrostatic pressure must be responsible for physically forcing additional DPA into the apples. Even though such depths as employed in these tests exceeded those employed in apple hydrohandling systems, DPA residues were within FDA tolerances, fruit injuries were absent, and scald was controlled.

Both hydrostatic pressure and duration of treatment increased DPA residues on apples. Autoradiography revealed that ^{14}C-DPA was primarily deposited at the fruit surface with traces in the core cavity. One might expect the open-calyx of Delicious to permit more DPA to enter the interior of the apple when this variety is subjected to such extreme conditions of hydrostatic pressure. The

predominance of ^{14}C-DPA at the surface, where scald occurs, suggests a local effect of DPA in controlling scald. But, if the observations of Padfield (1959) and Stevenson and Blake (1961) that DPA reduces internal breakdown are true, the effect of DPA may be realized within the fruit in areas not directly in contact with the material during treatment.

The survey of DPA usage by fruit handlers illustrated the diversity of practices employed in Michigan. Apparently there is a great range in suitable concentrations that will control scald sufficiently. Dewey and Dilley (1964) considered that 1000 ppm DPA will control scald on apples in Michigan, but some growers still use 2000 ppm on some varieties to be certain of scald control. Also, there appeared to be considerable lattitude in the precautions required for the use of DPA on apples. Operators seem to be using very dirty appearing solutions which contain far less DPA than recommended, apparently without encountering scald control problems.

The higher concentrations of DPA found in drench than in dip systems may be attributed to the constant mixing which occurs in the former applicator due to the circulation of the liquid which is required for application. In the dip system, mixing is less certain, even with mechanical stirring or circulating equipment within the dip tanks.

The DPA analysis results for commercial solutions demonstrated the need of a means for rapidly testing the DPA content of commercially employed drench and dip solutions. Such a field test would provide the necessary information to adjust the DPA solution concentration to compensate for the daily variations that are constantly encountered.

CONCLUSIONS

1. The colorimetric technique as modified for these studies is a satisfactory method of determining DPA residues of 0-25 ppm in apple tissue, and suitable for the determination of DPA in relatively small quantities of material.
2. Under the conditions of these studies, scald control was influenced by apple variety and DPA concentration, but not by aging, the presence or organic matter, increased hydrostatic pressure, and duration of treatment at increased hydrostatic pressures of the solutions employed for treating the apples.
3. It was found that the following treatments increased DPA residues in apples:
   a. dipping in 2000 ppm compared with 1000 ppm solutions,
   b. dipping in progressively older DPA solutions,
   c. dipping in DPA solutions contaminated with orchard trash and soil,
   d. submersion to depths as great as 6.5 feet, and
   e. submersion periods prolonged up to 8 minutes.

4. Solution temperatures of 50, 60 or 70°F did not affect DPA residues on apples.
5. Within the limits of these trials, the amount of measurable DPA in solutions decreased as a result of the presence of organic matter and upon aging of the solution.
6. There is a need to monitor the DPA content of solutions in applicator systems. For this purpose an analytical procedure, useful to operators as a guide for adjustment of the DPA content of their solutions, was developed.

SUMMARY

DPA residues for apple fruit in the range of 0 to 25 ppm could not be satisfactorily determined by gas chromatographic procedures. A colorimetric method was adapted and satisfactorily employed. Further modification permitted the colorimetric method to be used for ascertaining the DPA content of solutions prepared according to label recommendations for treatment of apples with DPA for scald control. A simplified version of the colorimetric procedure was tested and found to be of limited value for the estimation of DPA content of solutions in commercial applicators.

McIntosh had greater residues than Delicious when treated in DPA solutions aged for up to 4 weeks, but in the submerging studies Delicious, commonly characterized by open-calyx, had greater residues than McIntosh.

Both technical formulations of DPA employed provided satisfactory DPA residues on apples.

The presence of organic matter in DPA solutions resulted in greater DPA residues on apples than when treated in relatively clean solutions, but storage scald control was not impaired nor were injuries attributed to

organic matter. This factor resulted in less measurable DPA in solutions. Aging DPA solutions maintained free of extraneous material for up to 4 weeks had no effect on DPA residues for apples treated in these solutions.

No difference in scald control was observed between apples submerged in 1000 ppm and 2000 ppm solutions, even though the latter produced DPA residues twice as great as at 1000 ppm.

The method of artificially producing scald symptoms was satisfactorily used for these experiments and revealed that DPA controlled scald in comparison to untreated apples, which developed commercially significant scald.

Although DPA residues on apples varied in response to treatment conditions, they were within the 10 ppm tolerance established as safe by the Food and Drug Administration.

Autoradiography of Delicious apples submerged in ^{14}C-DPA solution confirmed others' findings that the majority of DPA residues are in or adjacent to the apple peel.

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APPENDIX

APPENDIX TABLE I.--DPA residues (ppm) on McIntosh and Delicious apples as influenced by DPA formulations used over a 4 week period in 1967.

Age (wks)	Formulation A McI	Formulation A Del	Formulation B McI	Formulation B Del	$\bar{x}$
	DPA (ppm)		DPA (ppm)		
0	2.28	1.73	1.95	2.51	2.11
1	5.11	4.79	2.19	2.12	3.54
2	6.36	4.58	2.32	2.01	3.81
3	5.36	3.16	2.19	1.51	3.05
4	8.38	4.18	3.22	1.82	4.39
$\bar{x}$	5.50	3.69	2.37	1.99	3.38
	$\bar{x}$ = 4.59		$\bar{x}$ = 2.18		

APPENDIX TABLE II.--DPA residues (ppm) on McIntosh and Delicious apples as influenced by the presence of organic matter and the age of the DPA treatment solution in 1967.

Age (wks)	Control		Organic Matter		$\bar{x}$
	McI	Del	McI	Del	
	DPA (ppm)		DPA (ppm)		
0	2.09	1.97	2.14	2.27	2.11
1	2.07	2.19	5.22	4.71	3.54
2	2.56	2.28	6.12	4.32	3.81
3	2.16	1.61	5.39	3.06	3.05
4	3.83	2.43	7.76	3.40	4.39
$\bar{x}$	2.54	2.09	5.33	3.55	3.38
	$\bar{x}$ = 2.33		$\bar{x}$ = 4.43		

APPENDIX TABLE III. DPA residues (ppm) on McIntosh, Delicious and Rome Beauty apples as influenced by the presence of organic matter and treatment solution age in 1968.

Solution Age (wks.)	Free of Organic Matter			With Organic Matter			Age $\bar{x}$
	McI	Del	Rome	McI	Del	Rome	
	DPA (ppm)			DPA (ppm)			
0 (fresh)	1.17	0.01	1.16	2.32	2.15	1.96	
	4.12	0.37	0.55	3.39	2.30	1.79	1.7
1	0.58	1.75	2.10	4.78	7.52	12.98	
	1.82	2.15	0.59	3.18	4.87	3.73	3.8
2	0.95	0.82	0.17	13.86	7.66	1.97	
	0.84	1.23	2.21	14.15	6.78	3.22	4.4
4	2.87	0.33	1.58	34.83	15.63	7.58	
	2.66	0.53	2.48	11.22	18.00	7.85	8.8
Variety $\bar{x}$	1.87	0.89	1.36	10.97	8.11	5.14	4.72
Sanitation $\bar{x}$		1.38			8.07		

APPENDIX TABLE IV.--DPA residues (ppm) on apples resulting from treatment in a simulated submersion system. 1967.

Hydrostatic Pressure (psi)	Duration of Treatment (min)				
	1	2	4	8	$\bar{X}$
			McIntosh		
0	1.67	3.83	4.67	5.90	4.02
1	4.99	1.99	2.37	6.46	3.95
2	3.66	4.44	3.71	5.18	4.24
3	3.33	3.54	4.76	7.09	4.68
4	2.38	2.84	8.91	9.63	5.94
$\bar{X}$	3.21	3.33	4.88	6.85	4.29
			Delicious		
0	1.71	3.92	4.21	7.43	4.32
1	0.80	2.95	3.30	3.70	2.68
2	2.69	5.65	4.32	8.87	5.38
3	4.45	7.40	8.08	11.70	7.90
4	5.63	8.84	11.24	8.51	8.55
$\bar{X}$	3.05	5.75	6.23	8.04	5.63

APPENDIX TABLE V.--Effect of DPA solution concentration, duration of treatment and depth of submersion on DPA residues on Delicious apples in 1968.

Treatment Depth (ft.)	Hydrostatic Pressure (psi)	DPA Solution (ppm) Duration of treatment (min) 0	2	4	mean
		1000			
0	0	1.89	1.89	2.42	2.07
2.5	1.5	2.16	0.95	2.42	1.84
5	3	2.70	1.45	3.06	2.40
					2.10
		2000			
0	0	3.57	3.39	3.37	3.44
2.5	1.5	2.17	4.32	3.44	3.31
5	3	4.22	4.03	8.23	5.49
					4.08

APPENDIX TABLE VI.--Apparent diphenylamine residues (ppm) observed on Delicious apples treated in a submersion system containing only water - 1968.

Treatment Depth (ft.)	Hydrostatic Pressure (psi)	Duration of Treatment (min) 0	2	4	$\bar{X}$
0 (surface)	0	1.28	1.77	1.70	1.58
2.5	1.5	2.10	3.04	2.02	2.38
5.0	3.0	1.38	2.97	2.66	2.34
	$\bar{X}$	1.25	2.59	2.13	2.10

APPENDIX TABLE VII.--DPA (ppm) in clean and contaminated treatment solutions of various ages in 1967.

Age (wks)	Control	Organic Matter (OM)
	DPA (ppm)	DPA (ppm)
0 (fresh)	972	1069
1	1017	755
2	835	622
3	927	725
4	744	456
	$\bar{\chi}$ = 899 ppm	$\bar{\chi}$ = 733 ppm
	All Treatments $\bar{\chi}$ = 816 ppm	

APPENDIX TABLE VIII.--DPA (ppm) in clean and contaminated treatment solutions of various ages in 1968.

	Control			Organic Matter (OM)		
Age (weeks)	I McI	II Del	III Rome	I McI	II Del	III Rome
	DPA (ppm)			DPA (ppm)		
0 (fresh)	1068	1132	1068	949	325	494
1	830	1124	1045	534	525	905
2	789	1068	1094	724	643	405
4	1979	740	887	1994	489	869
	$\bar{\chi}$ = 1069 ppm			$\bar{\chi}$ = 738 ppm		
	All Treatments $\bar{\chi}$ = 816 ppm					

APPENDIX TABLE IX.--Effect of DPA solution age and sanitation on scald reduction[1] for McIntosh, Delicious and Rome Beauty apples in 1968.

Solution Age (wks)	McIntosh		Delicious		Rome Beauty	
	Control	OM	Control	OM	Control	OM
	(Scald reduction units)					
0	10	13	10.5	10.5	15.5	15.5
1	10	11	8.5	9	15.5	15.5
2	15	11	10.5	9	15	15
4	8	15.5	10.5	9.5	14.5	12.5

[1]Scald reduction units are the difference between untreated and DPA-treated apples in the number of scalded fruits per 20.

APPENDIX TABLE X.--Effect of DPA concentration, submergence depth and duration of treatment on scald development on Delicious apples in 1968.

Treatment Depth (ft.)	Hydrostatic Pressure (psi)	DPA Solution (ppm)									
		0			1000			2000			
		Duration of Treatment (min.)									
		0	2	4	0	2	4	0	2	4	$\bar{x}$
		Number of Scalded Apples[1]									
0	0	12	15.5	18	1.5	2	1	1.5	0.5	4.5	6.3
2.5	1.5	15.5	14.5	15	3	4	2	1.5	0	1.5	6.3
5	3	15.5	15.5	14	2.5	2.5	0.5	2.5	0.5	0	5.9
	$\bar{x}$	14.3	15.2	15.7	2.3	2.8	1.3	1.8	0.3	2	6.2
		$\bar{x}$	15.1			2.2			1.4		

[1]Average from 20 apples in each of 2 replicates.