Naphthaleneacetic Acid Disappearance and Residue Studies in Citrus

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In a series of disappearance studies the quantity of NAA in Kinnow mandarin fruits 1 day after treatment was relatively high, decreased rapidly for a period of 2 to 3 weeks, and was present in amounts that were not significantly different from zero by the end of 3 to 6 weeks. Residue studies were conducted on nine varieties of citrus. In fruits that were young at treatment time but had matured by analysis time, residues were less than 0.07 ppm. However, the peel of fruits that were mature at treatment time contained appreciable residues (0.58 to 0.52 ppm) 10 to 26 days after treatment. The loss of NAA from dry deposits of the acid and the ammonium salt was studied. Susceptibility of NAA to photodecomposition was confirmed and losses from sublimation were demonstrated for the first time. Sublimation and photodecomposition rates from deposits of the acid were faster than from deposits of the ammonium salt.

Following the development of a method suitable for the determination of 1-naphthaleneacetic acid (NAA) residues in citrus fruits (Jolliffe and Coggins, 1970), experiments were initiated to examine the rate of disappearance of NAA from Kinnow mandarin fruits starting 1 day after application of the compound as a fruit-thinning agent. Residues at or near harvest time also were evaluated for Kinnow mandarin and a number of other varieties of citrus where fruit thinning may be important.

Another objective was to determine the influence of light and temperature on losses of NAA from dry deposits of the acid and ammonium naphthaleneacetate. There is no doubt that photodecomposition of NAA occurs (Crosby and Tang, 1969; Leeper *et al.*, 1962; Luckwill and Lloyd-Jones, 1962; Watkins and Woodcock, 1968; Watkins, 1969), but we are not aware of comparisons of the two forms mentioned. Although Luckwill and Lloyd-Jones (1962) concluded that sublimation would be an insignificant factor in the loss of NAA from dry deposits, we suspected otherwise and elected to reexamine the question. Photodecomposition and sublimation may be important in the environmental dissipation of the compound, in understanding the highly variable thinning action obtained, and in avoiding losses of NAA during analysis.

MATERIALS AND METHODS

Disappearance Study. Two groves in the Coachella Valley, a desert region, and one grove in the coastal region near Fallbrook, Calif. (Experiment III), were selected. In each location 12 trees received a thorough coverage spray of NAA and eight trees served as untreated controls. Analysis times and additional details concerning treatment conditions are shown in Table I.

The 12 treated trees were divided into three replications of four trees each. The number of fruits per sample varied from 160, when they were small, to a minimum of 16 as fruit size increased. Fruits from all untreated trees were combined and used for duplicate background and recovery determinations at each sampling date. After fruits were washed, a longitudinal slice from each fruit was used to obtain two 25-g samples for duplicate determinations. Results were subjected to statistical evaluations, with time as the independent variable.

Residue Study. The Kinnow mandarin experiments mentioned above were used for residue determinations on fruits that were approaching marketable stages of maturity. In addition, residue experiments were established in four Kinnow mandarin groves and in one of each of the following: Encore, Fremont, and Satsuma mandarin; Minneola and Orlando tangelo; Dancy tangerine; Temple orange (a tangor); and Valencia orange.

Treatments were applied in a randomized complete block design. Three of the Kinnow mandarin experiments were located in Arizona (two near Phoenix and one near Yuma). They consisted of four trees per plot and three replications. The Valencia orange experiment consisted of four trees per plot and ten replications. All other experiments consisted of single tree plots with 6 to 12 replications. In all cases, three residue replications and one composite untreated sample were collected for duplicate determinations. The residue replications were identical with the field replications for the three Arizona experiments and in all other cases each residue replication consisted of composite samples of fruit from two to four field replications. Samples were collected from Valencia orange trees that had been sprayed with 1000 ppm, and from Minneola tangelo and Encore mandarin trees that had been sprayed with 800 ppm. All other samples were obtained from trees that had received applications of 400 ppm of NAA. Spray mixtures contained X-77, a wetting agent, at the rate of 50 ml per 100 gal. Acid equivalent amounts of NAA were obtained by use of the commercial formulation listed in Table I.

The number of fruits per sample varied from 12 to 24 among the various experiments. The amount of NAA-washed from fruit surfaces was determined in most samples. Residues in the rind and pulp were determined separately and the NAA content of seeds was determined in cases where 10 g of seed were available. Rind and pulp samples consisted of 25 g of tissue obtained from longitudinal wedges of tissue. Samples were processed within a few days of collection.

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Table I. Quantity of NAA Found in Kinnow Mandarin Fruits at Various Intervals Subsequent to Entire Tree Application of the Compound[a]

Days after treatment	μg NAA per fruit[b] Experiment number			Average wt/fruit (g) Experiment number			ppm NAA[b] Experiment number			Theoretical ppm NAA[c] Experiment number		
	I	II	III	I	II	III	I	II	III	I	II	III
1	1.99c	2.98c	1.49c	1.42	7.89	0.42	1.40c	0.38d	3.53d	1.40	0.38	3.53
8	0.67b	1.03b	0.46b	2.05	9.29	0.57	0.33b	0.11c	0.81c	0.97	0.33	2.61
17	0.27a	0.90b	0.24ab	4.95	15.13	0.84	0.05a	0.06b	0.28b	0.40	0.20	1.77
24	0.17a	0.79b	0.08a	7.83	17.59	1.11	0.02a	0.05b	0.07ab	0.25	0.17	1.34
42	0.16a	0.27a	0.06a	15.67	27.88	3.53	0.01a	0.01a	0.02ab	0.13	0.11	0.42
59			0.02a			8.33			0.00a			0.18
91	0.15a	0.06a	0.00a	46.27	54.60	17.90	0.00a	0.00a	0.00a	0.04	0.05	0.08

[a] Foliage and young fruit were thoroughly covered with aqueous sprays of NAA. Applications were made May 18, June 1, and June 15, 1970, in Experiments I, II, and III, respectively. Acid equivalent concentrations of 200 ppm were used in Experiments I and II and 400 ppm was used in Experiment III. The spray mixture contained X-77 (a wetting agent) at the rate of 50 ml/100 gal, plus an appropriate quantity of Fruit Fix Super Concentrate 800, a commercial formulation containing the ammonium salt of NAA. [b] Average of three replications with duplicate determinations in each. All values were corrected for background and recovery. Means in the same column followed by different letters are statistically different at the 0.05 level of probability. [c] Theoretical concentrations of NAA expected in fruits based on quantities found 1 day after treatment. These values were calculated to demonstrate that factors other than dilution during growth are operative.

All NAA treatments were applied in the spring of 1970 and fruits that were young at treatment time were collected as they approached the marketable stage of maturity during the fall and winter of 1970–71. When the Encore mandarin and Valencia orange trees were sprayed, mature fruits and young fruits were on the trees. Since this would usually be the case with these two varieties, the amount of residue in these fruits is important for the tolerance that needs to be established prior to registration of NAA for use on citrus. No further residue samples were collected from the Encore mandarin trees, but residue samples were collected from the Valencia orange trees as the young fruits approached maturity in February 1971.

Light and Temperature Study. The loss of NAA from dry deposits was determined for the conditions listed in Table II. All studies were conducted in a small growth chamber equipped with temperature controls and with Ken-Rad XHO-cool white fluorescent lamps. Tungsten lamps were not used.

Stock solutions containing acid equivalent concentrations of 5 ppm of NAA and ammonium naphthaleneacetate were prepared. Deposits in 20 Petri dishes (50-mm diameter) were prepared for each material by introducing 1 ml of stock solution and allowing the solvent to evaporate. Immediately after the solvent had evaporated, the quantity of NAA was determined in four dishes. This was considered as the quantity of NAA present at zero time. At various times, four dishes of each were processed and the quantity of NAA remaining was compared with values initially obtained. The time intervals that were used varied widely from condition to condition, depending on rates of loss found in preliminary studies, but in all cases four time intervals were evaluated.

From the resulting disappearance curves, the amount of time required for a 10% loss of NAA was calculated for each condition. This amount of loss was selected for comparison since at least 10% loss was obtained for all conditions and the rate of loss *vs.* time was a linear function over the range selected.

Analytical Procedures. For the disappearance study the fluorometric method developed by Jolliffe and Coggins (1970) was used, but the following minor modifications were employed to reduce losses and to increase sensitivity. The tissue was macerated directly in small-mouth Mason jars, thus no transfer was needed prior to the addition of sulfuric acid. Near the end of the cleanup procedure, after the methylene chloride containing the NAA was washed with distilled water,

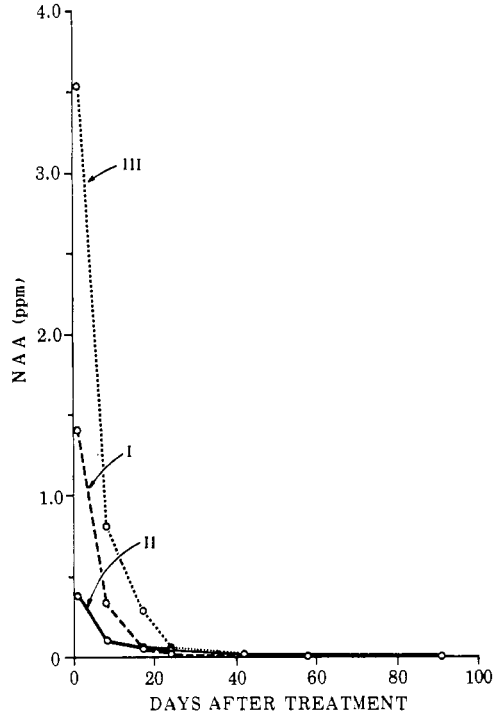

Figure 1. Rate of disappearance of NAA from Kinnow mandarin fruits. Applications were made May 18, June 1, and June 15, 1970, to young fruitlets in Experiments I, II, and III, respectively. Acid equivalent concentrations of 200 ppm were used in Experiments I and II and 400 ppm was used in Experiment III

Table II. Influence of Light and Temperature on the Loss of NAA from Dry Deposits of the Acid and the Ammonium Salt

Treatment conditions[a] Light (μW/cm²)[b] Visible	uv	Temperature, °C	Hours required for 10% loss Acid	Salt
0	0	34	9.2	32.0
0	0	42	1.2	16.0
630	15	34	4.3	10.3
1270	70	34	2.0	7.6
2530	150	38	1.3	5.0
5060	350	42	0.8	0.8
8540	510	43	0.2	0.5

[a] Refers to temperature of deposit as measured by thermocouples. [b] One foot-candle = 1.582 μW/cm².

the solvent was evaporated under reduced pressure, the NAA was dissolved in the desired volume of 2% potassium bicarbonate, and the resulting solution was subjected to fluorescence analysis. This allowed the use of assay volumes as small as 5 ml and the sensitivity of the method was thereby increased.

In the residue study the modifications mentioned above were used. Otherwise the procedures reported by Jolliffe and Coggins (1970) were used for analysis of peel and pulp. Prior to collecting slices of tissue for peel and pulp, intact fruits were washed with water and the quantity of NAA recovered from fruit surfaces was determined. The wash water was acidified, NAA was partitioned into methylene chloride, and then the sample was subjected to the same cleanup procedure used for pulp tissue. Where a sufficient quantity of seed was available, NAA content was determined by using the pulp extraction and cleanup procedure.

Each set of determinations included evaluations of apparent NAA content of tissue from untreated trees (background) and determinations of efficiency of recovery from samples fortified with known quantities of NAA (% recovery).

In the study of NAA loss from dry deposits, NAA in the deposit was dissolved in 5 ml of 2% potassium bicarbonate and the quantity of NAA was calculated from comparison of fluorescence intensity with known quantities of NAA.

RESULTS AND DISCUSSION

Disappearance Study. Since biological as well as analytical sources of variability were involved, a multiple range test was used as an evaluation guide in each experiment. Results are presented in Table I. Concentrations higher than 0.07 ppm and quantities higher than 0.3 μg per fruit were clearly distinguishable from zero levels. Thus, it appears that the experimental design and the analytical procedures were adequate for determinations of rates of disappearance of NAA from Kinnow mandarin fruits.

Whether results (Table I and Figure 1) are evaluated as quantity per fruit or as concentration, relatively high amounts of NAA were found 1 day after treatment. Rapid rates of loss occurred over a period of 2 to 3 weeks. At the end of 3 to 6 weeks, and for the duration of the experiment, amounts of NAA that were not significantly different from zero were found.

A rapid decrease in NAA concentration should occur due to dilution during growth. The observed rate of decrease in concentration, however, was considerably faster than can be accounted for on the basis of dilution. The differences between theoretical decreases due to growth dilution and amounts actually found were substantial (Table I). Thus, it is logical to assume that translocation out of the fruit and/or metabolism of the NAA molecule contributed to the observed decreases. When data are evaluated on the basis of quantity of NAA per fruit, dilution due to growth is not a factor. Since a substantial decrease in quantity per fruit was observed, the same conclusion regarding translocation and/or metabolism prevails.

Residue Study. In view of the results obtained in the disappearance study on Kinnow mandarin, little or no NAA residue was expected in mature fruits that were in the fruitlet stage of development at the time NAA was applied. Nevertheless, analyses were made for all experiments that were established.

The analytical modifications adopted for the studies reported in this paper increased the reliable quantitative range of the method to include concentrations at least as low as 0.02 ppm. When biological variability was introduced, however, this level of detection was not routinely realized. The best information available for citrus (Table I) indicates that when analytical plus biological sources of variability are considered, the minimum reliable detection level is 0.07 ppm.

In the 14 experiments where fruits were young at treatment time but had matured by analysis time, the concentration range, corrected for background and recovery, found in fruit from treated trees was 0.00 to 0.04 ppm. The range of apparent concentrations (uncorrected for background and recovery) of NAA in untreated tissue was 0.02 to 0.07 ppm for peel, 0.02 to 0.04 ppm for pulp, and 0.01 to 0.06 ppm for seed. Identical ranges of apparent concentrations of NAA for peel, pulp, and seed of treated fruits were obtained. No surface residues were found. Thus, we must conclude that if residues were present in any part of treated fruits, levels were below the reliable detection range.

Mature fruits were present on Valencia orange and Encore mandarin trees when NAA was applied. Residues of 0.58 and 0.52 ppm were found in peel tissue of samples collected 10 and 26 days after application of 1000 and 800 ppm of NAA to Valencia orange and Encore mandarin trees, respectively. No detectable residues were found in pulp tissue.

Light and Temperature Study. Our results show that sublimation is a factor in the loss of NAA from dry deposits and that losses from deposits of NAA are appreciably faster than losses from deposits of ammonium naphthaleneacetate (Table II). Losses due to sublimation are contrary to the conclusion by Luckwill and Lloyd-Jones (1962) that sublimation of NAA is insignificant. The study by Luckwill and Lloyd-Jones was conducted at a considerably higher temperature (80° C) and was based on loss of radioactivity from dry deposits of ^{14}C-carboxyl labeled NAA. We are unable to explain their results, especially if they studied a deposit of NAA rather than a nonvolatile salt.

Leeper *et al.* (1962) reported a correlation between photodecarboxylation of NAA and temperature. It is possible that the correlation they found was due to differences in sublimation losses. They trapped the vapor phase in a solution of barium hydroxide and assumed that the resulting radioactivity was due to evolution of $^{14}CO_2$ *via* photodecarboxylation. Although there is no doubt that photodecarboxylation occurs, it is clear that vapor phase NAA likewise would have been trapped in a solution of barium hydroxide.

The experimental design that we used to study losses at various light intensities did not distinguish between losses due to sublimation and photodecomposition. Since appreciably faster losses occurred in light than in darkness at similar temperatures, it is apparent that losses from photodecomposition are more important than losses from sublimation, and that high rates of loss are associated with high light intensities. Visible and uv light intensities used in these studies are presented in Table II. Since NAA absorbs essentially no light in the visible region and absorbs strongly in the uv region, it is obvious that uv light was responsible for photodecomposition. Although considerably faster losses occurred from acid deposits than from salt deposits at low light intensities, differences in loss from the two types of deposits were not large at the higher light intensities (5060 and 8540 $\mu W/cm^2$). These results suggest that rates of loss at the high light intensities that would exist in the field would not be appreciably different for the two forms of NAA.

It may be argued that photodecomposition products could introduce errors into the fluorometric analysis used in this

phase of the study. The first photodecomposition product of NAA is 1-methylnaphthalene (Crosby and Tang, 1969). Although this compound has a fluorescence emission spectrum that is almost identical to NAA, it could not be a source of error in this study because it is extremely volatile. For example, when we tried to prepare dry deposits of the compound, we were unable to recover any at time zero. Thus, we concluded that volatility of the first photodecomposition product would remove it as a source of error and that subsequent photodecomposition products would not be present in the dry deposit to contribute to analysis errors. All of the other known photodecomposition products possess fluorescence emission spectra that are different from NAA. In each photodecomposition study, the resulting deposit was evaluated for fluorescence intensity, to determine quantity of NAA, and the configuration of emission spectra was studied. In no case did we find an emission spectrum that was different from NAA. This provides conclusive evidence that photodecomposition products were not a source of error.

In NAA studies that involve biological performance, environmental dissipation or the development or use of analytical procedures, sublimation, and photodecomposition should be considered.

ACKNOWLEDGMENT

The authors acknowledge Ross Rodney, Lowell F. True, and Gilbert L. Henning for conducting the many field experiments that were necessary for these studies.

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Received for review May 24, 1971. Accepted July 6, 1971. This project received partial financial support from The California Citrus Advisory Board and from Arizona mandarin growers.