SPROUT INHIBITOR RESIDUES

Residue Determination of Naphthaleneacetic Acid and Its Methyl Ester in Potatoes by a Combination of Gas Chromatography and Ultraviolet Spectrophotometry

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A method for the determination of residues of naphthaleneacetic acid (NAA) and the corresponding methyl ester (MENA) in potatoes is based on the separation of MENA from interfering plant substances by gas-liquid chromatography and subsequent determination of absorbance of collected fractions at 281 or 224 mμ. The NAA is first esterified with diazomethane to form MENA prior to separation by gas-liquid chromatography. Several varieties of potatoes were treated with MENA, and a decline study of MENA and NAA residues was made for 0 to 90 days after the initial application. The disappearance of MENA residues followed a logarithmic decline curve. Only traces of MENA penetrated the skin of the potatoes. Evidence is presented for the partial hydrolysis of MENA residues to NAA during storage of potatoes.

THE first observation that the methyl ester of naphthaleneacetic acid inhibited bud growth in potatoes was reported by Guthrie in 1939 (*3*). Other investigators studied the effect of MENA vapors on sprouting inhibition (*2, 4*). Since then, a commercial formulation of MENA has been marketed as a "potato fix" and recommended as a sprouting inhibitor of potatoes placed in commercial channels.

Because of the new Federal Food and Drug legislation on plant growth regulators and other food additives, a sensitive and practical method had to be developed for the residue determination of MENA and its possible metabolite, NAA. The only available sensitive assay for this compound was the biological test utilizing the hyponastic response of MENA on the first well developed leaves of young potato plants (*1*).

Recently, a method for the residue analysis of the insecticide Thiodan by a combination of gas-liquid chromatography and infrared spectrophotometry (*7*) was developed. An analogous technique has now been developed for trace determinations of NAA and its methyl ester in potatoes. Briefly, a crude, concentrated chloroform extract of potatoes was fractionated by gas chromatography at 220° C. The effluent fraction was collected at a predetermined retention time and analyzed in the ultraviolet at 281 or 224 mμ.

Equipment and Materials

Gas Chromatograph Apparatus. Aerograph Model A-100C, equipped with heated fraction collector, terminating in a 7/15 ⅀ outer joint; hot-wire tungsten filaments katharometer detector; Brown 1-mv. recorder with a 1-second pen speed response.

Column and Packing. Dow-11 high-vacuum silicone grease (30% w./w.) on Chromosorb 30/60 mesh, 6-foot (1/4-inch diameter) spiral stainless steel column.

Pipet. A 100-μl. Hamilton syringe-type pipet was used for the quantitative work.

Collector. The collector consisted of a tapered 1.0 × 14 cm. glass tube fitted with a 7/15 ⅀ outer joint and was identical to the one described by Zweig *et al.* (*7*).

Sample Tube. A McNaught and MacKay-Shevky-Stafford sedimentation tube (6.5 ml.) was used for the final concentration of plant extracts.

Spectrophotometer. Beckman, Model DU.

Stock Solutions. Double-distilled methyl ester naphthaleneacetic acid (Thompson Chemicals Corp., Los Angeles, Calif.). Standard solutions in mixed pentanes contained 2.0 mg. per ml. for readings at 281 mμ and 0.2 mg. per ml. at 224 mμ.

Diazald. Stabilized form of diazomethane (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) was obtained from the Aldrich Chemical Co. Diazomethane was freshly prepared by reacting Diazald (251 mg. in 8 ml. of dry ethyl ether) with potassium hydroxide (112 mg. of potassium hydroxide in 0.5 ml. of water and 15 ml. of diethylene glycol monoethyl ether–Carbitol) at 70° to 75° C., and the mixture was distilled. Diazomethane distils with the ethyl ether and is condensed in an icebath. *Because diazomethane is very poisonous, all reactions must be carried out in fume hood (6).*

Experimental Methods

Extraction. Whole potatoes, skins, or flesh, 1 to 2 kg., were placed in the bowl of a Hobart food chopper and chopped for about 25 seconds to reduce to appropriate size. Five hundred grams of the chopped potatoes were weighed into a tared, 1-gallon paint can. Three hundred grams of anhydrous sodium sulfate powder were mixed into the pulp, and 1 liter of technical chloroform was placed in the can. Each can contained a stainless-steel baffle to improve the extraction efficiency. The cans were sealed and rolled for 1 hour with a gentle and continuous rolling action at approximately 35 r.p.m. on a U. S. Stoneware ball-mill roller. The resultant chloroform mixture was dried with anhydrous sodium sulfate, decanted, filtered, and the solution was then stored in glass containers.

Preparation of Extract for Gas-Liquid Chromatography. A suitable aliquot of the stripping solvent corresponding to as much as 100 grams of sample was concentrated to dryness in vacuo at 50 to 60° C. The residue was dissolved in 2 ml. of mixed pentanes (b.p. 30–60° C.), filtered quantitatively through Whatman No. 1 filter paper into a 6.5-ml. sedimentation tube, and washed several times with the pentanes. The solution and washes were combined to an approximate volume of 6 ml. The sedimentation tube was immersed in a warm water bath, and the solvent was evaporated under a stream of dry air to approximately 200 μl. The inner wall of the sedimentation tube was rinsed with two washes of 1 ml. of mixed pentane, and the solvent was again

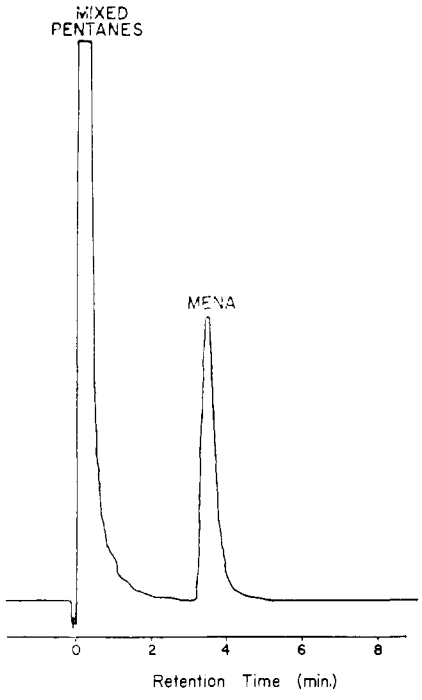

Figure 1. Representative curve for MENA obtained on the gas chromatographic apparatus

Temperature	220° C.
Column	Dow 11
Helium flow	50 ml./min.
Filament current	250 ma.
Attentuation	none
Sample	60 μg.

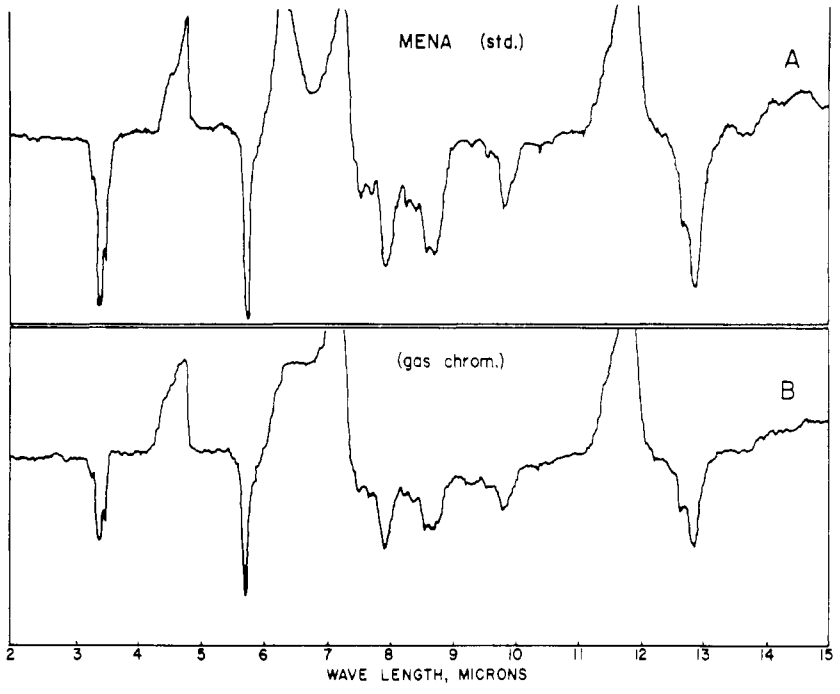

Figure 2. Infrared spectra for MENA before gas chromatography (A) and after gas chromatography (B)

evaporated to a final volume of 200 μl.

Gas-Liquid Chromatography of MENA. Just prior to the analyses of plant samples, appropriate amounts of known standards (1.0 to 100 μg.) were chromatographed at 220° C. with a helium gas flow of 50 ml. per minute. The signal could be detected with a conventional hot-wire katharometer at the higher concentrations (25 μg. and up), and the retention time for collection ranged between 3.0 to 7.5 minutes (Figure 1).

Appropriate aliquot volumes of residue extracts warmed in hot water were chromatographed under identical conditions, and fractions were trapped in the collector between 3.0 and 7.5 minutes over absorbent cotton saturated with 95% ethanol. Fractions thus collected were eluted from the collector with several portions of 95% ethanol, and the final volume was adjusted to 5.0 ml. in a volumetric flask. These solutions were analyzed by ultraviolet spectrophotometry at 281 or 224 mμ.

After each analysis, the chromatographic column was backflushed with helium for 20 minutes by introducing the gas stream into the collector and removing the rubber diaphragm of the inlet port.

Ultraviolet Spectrophotometry. Absorbances of the final ethanolic solutions of collected fractions were measured in 1-cm. cells at 224 mμ and if the absorbance were above 1.0, at 281 mμ. Suitable column blanks were obtained in the manner described above, and their absorbance ranged from 0.03 to 0.08. Higher blanks were noted if the packing in the glass collector were not thoroughly washed with diethyl ether and ethanol before collecting another fraction.

Straight-line standard curves for MENA were obtained at 281 and 224 mμ before and after gas-liquid chromatography.

Residue Determination of NAA. Standards of NAA and unknown samples, suspected to contain NAA, were first esterified with diazomethane prior to separation by gas-liquid chromatography.

Standard solutions of NAA or potato extracts were evaporated to apparent dryness in vacuo at 50° to 60° C. and redissolved in about 1 ml. of anhydrous ethyl ether. The diazomethane solution was added until the solution remained yellow; usually 1 to 2 ml. was sufficient. The solution was taken to dryness with a stream of warm air and redissolved in a small amount of mixed pentane (0.2 to 1.0 ml.). Aliquots of this final solution were processed as before by GLC and ultraviolet spectrophotometry. All absorbance readings of treated samples were reported as net absorbance by subtracting the absorbance obtained from the extractives of an equivalent weight of untreated samples analyzed by the same experimental procedures.

Treatment of Potatoes. Experiment 1A. Russet Burbank potatoes were washed with water and sprayed to run-off using a fine nozzle with 1 volume emulsifiable solution of MENA (50% active ingredient) and 5 volumes of water. This represented about 11 times the recommended concentration for commercial use.

Experiment 1B. Russet Burbank potatoes were treated with 1.8 times the amount of MENA as in Experiment 1A (2 volumes 50% MENA formulation plus 5 volumes of water).

Experiment 2. Four varieties of potatoes (Russet Burbank, Pontiac, Katahdin, and White Rose) were washed with water and placed on a roller-type conveyor in a commercial packing house. The potatoes were thoroughly sprayed to run-off from a stationary nozzle with 1 quart of an emulsifiable solution, (36.4% MENA as active ingredient) and diluted with 10 gallons of water; this was the recommended concentration for commercial use (*5*).

Storage and Sampling of Potatoes. After treatment, the potatoes were stored in a constant-temperature room at 50° F. and 80 to 90% relative humidity. At time intervals varying from 0 to 90 days after treatment, the potatoes were sampled and analyzed for MENA and NAA as described above. Several batches of potatoes were peeled, and the peeling, flesh, and unpeeled whole potatoes were analyzed separately.

Results and Discussion

Reproducibility and Sensitivity of Method for MENA. Samples of MENA were collected after gas-liquid chromatography at 220° C., and examined by infrared spectrophotometry (Perkin-Elmer 221) using the mull technique,

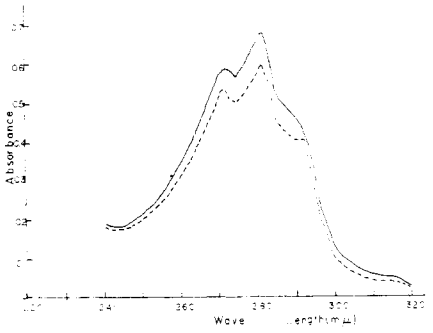

Figure 3. Ultraviolet spectra for MENA before and after gas chromatography in such concentration as to show optical absorbance in the regions of 271 and 281 mμ

MENA (20.0 μg./ml. 95% ethanol)
1-cm. cell path length
——— before GLC
..... after GLC

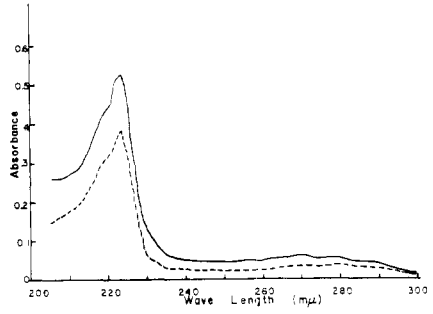

Figure 4. Ultraviolet spectra for MENA before and after gas chromatography in such concentration as to show optical absorbance in the region of 224 mμ

MENA (1.0 μg./ml. 95% ethanol)
1-cm. cell path length
——— before GLC
..... after GLC

and by ultraviolet spectrophotometry in 95% ethanol over the range 220 to 320 mμ. The infrared spectrum of the recovered fraction was similar to that of the double-distilled standard at the major absorption bands where comparisons were made. Such similarity indicated no decomposition (Figure 2). The ultraviolet spectra were qualitatively identical before and after GLC, exhibiting characteristic absorption maxima at 224, 271, and 281 mμ (Figures 3 and 4). A comparison of the absorbance of a given solution of MENA (1 μg. per ml.), however, indicated a loss of 34% based on 281 mμ and 24% based on 224 mμ as a result of gas chromatography. This discrepancy may be explained by the fact that at this concentration of MENA the experimental precision at 281 mμ is subject to much greater deviations than at 224 mμ. The loss of MENA on the gas chromatograph was entirely reproducible at concentrations ranging from 0 to 20 μg. per ml. (Figure 5). Each point represented duplicate runs with a precision of ±5.5%, and lay on a straight line which extrapolated through the origin. Residue determinations were calculated by this standard curve which takes into account losses inherent in the method.

The sensitivity of the method was limited by the readability of the absorbance with available instruments as well as by the extent to which naturally occurring plant substances surviving the separation process interfered with the analytical method. For example, an average background for 10 grams of untreated potatoes had an absorbance of 0.238 at 224 mμ with individual values ranging from 0.202 to 0.270. For 5 grams of potato extractives, the absorbance ranged from 0.130 to 0.270, and for 2.0 grams or less from 0.042 to 0.082. These latter values were in the neighborhood of the column blanks ranging from 0.030 to 0.080. The relationship between the amount of potato extractives applied to the gas chromatographic column and the absorbance of the collected fractions was not linear. This might be due to materials other than plant extracts bleeding off the column and contributing to the absorbance. Thus, setting twice the absorbance from a 10-gram check sample as the lower limit of significance,

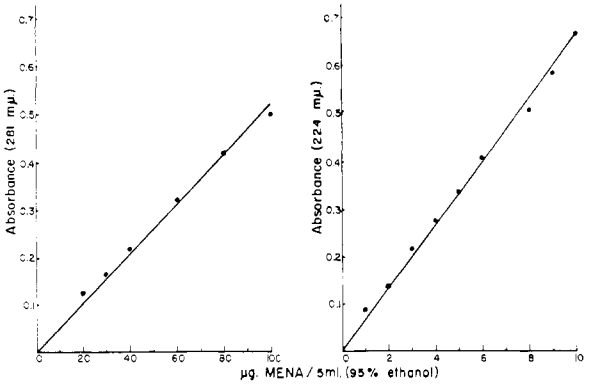

Figure 5. MENA standard curves after gas chromatography measured at 281 mμ and 224 mμ

Table I. Recovery of MENA from Potatoes, Fortified with 1 P.P.M. of MENA

Sample	Grams Analyzed	Net Absorbance[a] (281 mμ)	MENA, P.P.M.	Recovery, %
A[b]	50	0.214	0.71	71
B-1[b]	25	0.140	0.93	93
B-2[c]	25	0.151	1.01	101
C-1[b]	50	0.242	0.81	81
C-2[c]	50	0.294	0.98	98

[a] Optical absorbance readings corrected for blank (50-gram blank, 0.123; 25-gram blank, 0.077).
[b] Samples A, B-1, C-1—1 p. p. m. MENA added prior to extraction.
[c] Samples B-2, C-2—1 p. p. m. MENA added after extraction.

Table II. The Decline of MENA Residues on Russet Burbank Potatoes

Sample Form	Days since Last Application	Experiment 1A: 1 Vol. 50% MENA + 5 Vol. Water — Grams analyzed	Net absorbance (224 mμ)	MENA p.p.m.	Experiment 1B: 2 Vol. 50% MENA + 5 Vol. Water — Grams analyzed	Net absorbance (224 mμ)	MENA, p.p.m.
Whole	0	0.025	0.217	132.0	0.025	0.361	220.0
Whole	3	0.025	0.230	140.0	0.025	0.407	248.0
Whole	7	0.025	0.263	160.0	0.025	0.408	248.0
Whole	30	0.10	0.181	43.0	0.025	0.325	198.0
Whole	60	1.0	0.572	8.6	0.25	0.673	40.8
Whole	90	5.0	0.926	2.8	1.0	1.017	15.1
Peel	0	0.005	0.353[a]	1080			
Flesh	0	1.0	0.218	3.3			
Peel	30	0.01	0.329	496			
Flesh	30	10.0	0.148	<0.4			
Peel	60	0.1	0.712	104			
Flesh	60	10.0	0.016	<0.4			

[a] Absorbance of appropriate checks ranged from 0.048 to 0.071 for the pee and 0.13 to 0.146 for the flesh.

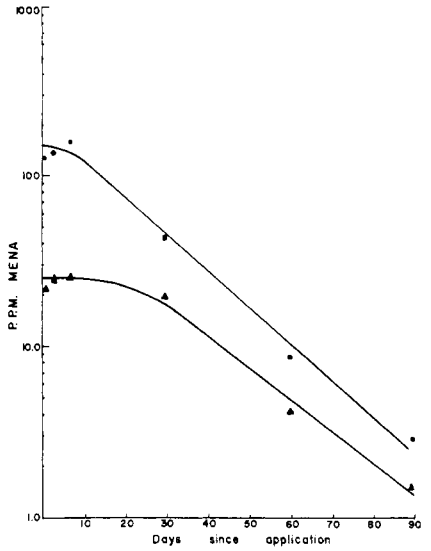

Figure 6. MENA residue decline curves for Russet Burbank potatoes (Expt. 1) using 10 and 18 times recommended dosage

▲ Experiment 1A
● Experiment 1B

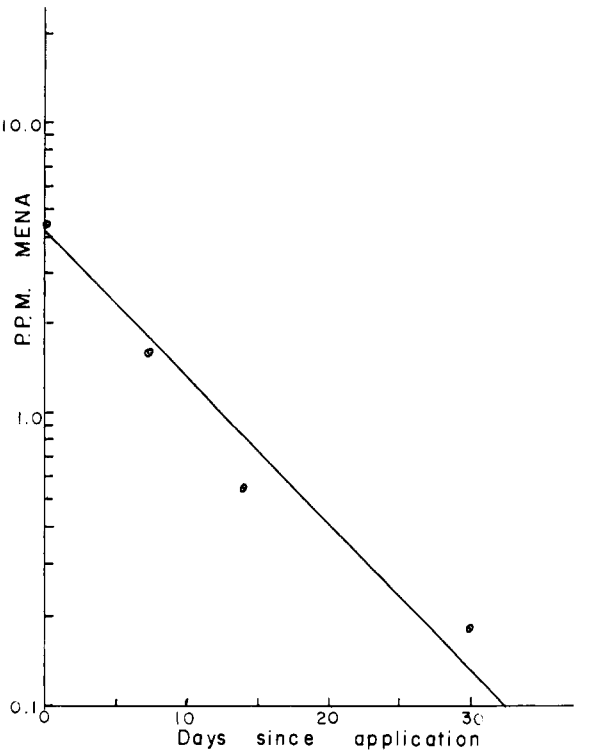

Figure 7. MENA residue decline curve for Russet Burbank potatoes (Expt. 2) using recommended dosage

Table III. The Decline of MENA Residues on Several Varieties of Whole Potatoes

Experiment 2. Samples treated with 1 liter of 36.4% MENA concentrate plus 40 liters of water

Variety	Days since Application	Grams Analyzed	Net Absorbance (224 mμ)	MENA, P.P.M.
Russet Burbank	0	1.0	0.295	4.5
Katahdin	0	1.0	0.272	4.2
Pontiac	0	1.0	0.391	5.9
White Rose	0	1.0	0.317	4.8
Russet Burbank	7	2.0	0.219	1.7
Katahdin	7	2.0	0.249	1.9
Pontiac	7	2.0	0.246	1.9
White Rose	7	2.0	0.275	2.1
Russet Burbank	14	4.0	0.143	0.6
Katahdin	14	4.0	0.121	0.5
Pontiac	14	4.0	0.063	<0.4
White Rose	14	4.0	0.348	1.3
Russet Burbank	30	10.0	0.117	<0.4
Katahdin	30	10.0	0.289	0.4
Pontiac	30	10.0	0.159	<0.4
White Rose	30	10.0	0.200	<0.4
Russet Burbank	60	10.0	0.000	<0.4
Katahdin	60	10.0	0.140	<0.4
Pontiac	60	10.0	0.040	<0.4
White Rose	60	10.0	0.055	<0.4

Table IV. Comparison of MENA Residues in Peel and Flesh with Residues from Whole Potatoes

Russet Burbank, Experiment 1A, initial residue

Sample	Weight of Tissue, Grams	MENA P. P. M.	MENA Total mg.
Whole potatoes	1028	132	135.70
Peel	120	1080	129.60
Flesh	894	3.3	2.95
Total peel + flesh	1014		132.55

a sensitivity of 4 μg. per 10 grams or 0.4 p.p.m. was achieved (Figure 5).

The reason for backflushing the chromatography column after potato sample injection was that a number of compounds continued coming off the column up to 1 hour after the initial introduction of the sample. These late components gave high absorbance readings at 224 mμ and consequently interfered with successive analyses. Backflushing eliminated this interference.

Esterification of NAA. Several 5-μg. samples of NAA were esterified with diazomethane, yielding theoretically 5.37 μg. of MENA. These samples, analyzed by the GLC-UV technique, gave an average of 4.75 μg. of MENA, an 88.5% recovery. As esterification of NAA was satisfactory, this method was suitable for residue determinations of possible MENA hydrolysis products in treated potatoes. Analyses of MENA formulations showed that no significant amounts of the free acid were present in the original material.

Recovery of MENA from Potatoes. Samples of Russet Burbank potatoes were fortified with 1 p.p.m. MENA, added by pipetting appropriate volumes of a standard solution in pentane. Twenty-five and fifty gram aliquot samples were analyzed by the GLC-UV method at 281 mμ. These results (Table I) show an average recovery of 81 and 100%, respectively, when MENA was added to the potatoes before and after extraction.

Decline of MENA Residues in Potatoes. Russet Burbank potatoes, treated with about 11 and 20 times the recommended concentration of MENA (Experiments 1A and 1B), showed little decline from the initial deposit in the first 7 days of storage at 50° F. and a relative humidity of 80%. From then to the end of the experiment—i.e., 90 days after treatment—the residue of MENA declined almost linearly (Table II and Figure 6).

When four different varieties of potatoes, including smooth- and rough-skinned tubers, were treated with the recommended amount of MENA, the MENA residue declined from an initial deposit of about 5 p.p.m. to less than 0.4 p.p.m. after 60 days (Table III). No significant differences in the decline

Table V. Amount of NAA Resulting from Hydrolysis of MENA Applied to Russet Burbank Potatoes

Days since Appl.	Grams Analyzed	Absorbance, 224 mμ Before esterification	Absorbance, 224 mμ After esterification	MENA, P.P.M.	MENA +NAA, P.P.M.	NAA, P.P.M.
30 (Experiment 1A)	0.1	0.330	0.395	43.0	53.0	10.0
Check	0.1	0.049	0.049			
60 (Experiment 1B)	0.1	0.310	0.376	39.7	50.0	10.3
Check	0.1	0.049	0.049			
0	1.0	0.331	0.438	4.10	5.10	1.00
Check	1.0	0.064	0.103			
14	5.0	0.506	1.530	1.20	3.90	2.70
Check	5.0	0.130	0.238			
30	5.0	0.209	0.475	0.24	0.72	0.48
Check	5.0	0.130	0.238			
60	5.0	0.120	0.425	0	0.58	0.58
Check	5.0	0.130	0.238			

curves (Figure 7) for the different varieties of potatoes were noted. A straight-line relationship existed.

MENA Residues in Peel and Flesh of Potatoes. The possible penetration of MENA residues from the top surface of the peel into the flesh of the potatoes was studied. In one such study in Experiment 1A of Table II, the initial peel residue was 1080 p.p.m., while the flesh contained only 3.3 p.p.m. MENA. This residue was less than 0.4% of the total. Thirty days after treatment the percentage of MENA in the flesh declined to 0.05% of the total; after 60 days, there was no detectable MENA in the flesh.

This experiment could also be validated by summing up the MENA residues in peel and flesh and comparing these results with MENA residues from whole potatoes (Table IV).

Additional analyses of peel and flesh from the four varieties of potatoes, Experiment 2, showed a similar pattern. The initial MENA residue in the peel of Pontiac potatoes was 48.5 p.p.m., while the flesh contained no detectable residue. Katahdin and Russet Burbank peel had an initial residue of 46.5 and 45.5 p.p.m., respectively. No MENA was detected in the flesh then, or at any other sampling date, up to 37 days after application. Thus, the conclusion was that MENA did not penetrate appreciably through the skin of the potatoes even after 60 days from the date of treatment.

Determination of Residues of Naphthaleneacetic Acid. Dissipation of MENA residues could presumably follow two alternate routes—volatilization and hydrolysis. The latter pathway was investigated by determining residue concentrations of NAA in treated potatoes. Potato extracts were analyzed by GLC-UV before and after esterification, and the amount of NAA calculated by taking the difference of the absorbance at 224 mμ before and after esterification (Table V).

Two samples with high initial MENA residues were analyzed 30 and 60 days after initial application. In one sample, the MENA residue had dropped from 132 to 43 p.p.m. (Experiment 1A) in 30 days and from 220 to 39.7 p.p.m. (Experiment 1B) in 60 days. If hydrolysis had occurred, the MENA loss at the 30 to 60 days' samples presumably would show up as high NAA residues. Actual analysis of these samples, however, showed only a residue of 10 p.p.m. NAA for both samples (Table V). Potato samples which had a considerably lower initial MENA residue (Experiment 2) had NAA residues ranging from 1.00 to 0.58 p.p.m. at 0 to 60 days after application. Therefore, the decline of high initial MENA residues was probably due to volatilization rather than hydrolysis. At lower initial MENA residues, a larger percentage of the ester was hydrolyzed to the free acid. However, even the concentration of the free acid declined during storage, thus indicating three modes of dissipation of MENA—hydrolysis of MENA, volatilization of MENA, volatilization of NAA.

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