Magnitudes and Natures of Nicotine Residues on and in Field-Treated Texas Mustard Greens

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The residual behavior of five nicotine formulations applied on Texas mustard greens was studied in detail. Contrary to the generally held opinion that volatilization of the nicotine results in only negligible persistence of residues, persistence half lives averaged 4.5 days, with initial deposits from 10 to 50 p.p.m. Because the cyanogen bromide colorimetric procedure used is nonspecific for parent nicotine, chromatographic and spectrophotometric procedures established that nicotine, cotinine, nornicotine, and anabasine were present in aged nicotine residues.

THE USE OF nicotine as such or as an acid extract from tobacco for the control of various insect pests on fruits, vegetables, and ornamental plants has a long and useful history. Despite the recent influx of the many potent synthetic organic insecticides, some nicotine products are still being recommended and used in most of the agricultural areas of the world.

Although nicotine has been studied extensively from an entomological viewpoint, surprisingly few studies of residues are reported in the literature, as summarized in Table I. There have been no reports in the available literature on the residual behavior of nicotine-containing sprays or dusts on vegetable crops. The investigations cited above were concerned only with the surface residues obtained by stripping the substrates with aqueous acid or alkaline solutions. Only a few stripping and determinative procedures have been reported in analytical detail (*7, 20, 23*).

Although these meager residue data indicate that nicotine spray residues are of intermediate persistence as compared with those of other pesticidal materials (*13, 14*), the consensus has been that even nicotine from nicotine sulfate applications volatilizes within a very short time and leaves only negligible residues. This belief has been supported by the vapor tension data in Table II, which show that free nicotine is volatile in relation to some other pesticide chemicals. In general support of this contention, Norton and Billings (*21*) reported average losses of 72% of the nicotine from spray residues on apples during the short period of drying after application, and Carman, Gunther, Blinn, and Garmus (*3*) found volatilized nicotine in the air of a treated citrus grove during the first 30 minutes after application. Another cogent reason for believing that nicotine residues on foodstuffs are negligible has been the long history of usage without serious injury due to the ingestion of aged nicotine-treated products.

Table I. Previous Investigations Demonstrating Persistence of Nicotine Residues

Crop	Interval after Spraying	Residue Behavior, Half Life, Days	Detection Method	Literature Cited
Apple foliage	"Aged"	Detected[a]	Chemical	(*24*)
	5 weeks	Detected[a]	Chemical and bioassay	(*17*)
	30 days	Detected[a]	Chemical	(*6*)
Apple fruit	Various	10–13	Chemical	(*21*)
	2 weeks	"Appreciable"[a]	Chemical	(*5*)
Citrus fruit	Various	5	Chemical	(*2*)
Rose foliage	Various	28	Chemical	(*4*)

[a] Too few data to permit calculation of half-life value (*13, 14*).

Because of the apparent contradiction of previous residue data with the belief that nicotine residues are transient and minimal, and because of a general interest in magnitude, locale, and fate of pesticide residues, the present study was undertaken to determine the magnitudes of total nicotine residue on and in a typical leafy vegetable crop (Texas mustard greens) as treated commercially, and to determine the persistence, locale, and chemical fate of any alkaloid-type residues found.

The analytical method employed for most previous studies was a modification of Markwood's (*20*) cyanogen bromide colorimetric method. This well known procedure is not specific for nicotine and it responds to most 3-substituted pyridine compounds. Compounds of this type may be congeners of nicotine or they may be among the metabolic or other degradation products in aged and penetrated nicotine residues. Thus Frankenburg, Gottscho, Vaitekunas, and Zachanius (*8–12*), in extensive work on the metabolism of nicotine during the fermentation of cigar tobacco leaves, have shown that under these conditions several degradation products of nicotine are 3-substituted pyridine compounds.

Table II. Vapor Tensions of Some Pesticide Chemicals

Chemical	Vapor Tension at 25° C., μ	Literature Cited
Nicotine	20	(*25*)
Parathion	3×10^{-2}	(*13, 14*)
Lindane	1×10^{-2}	(*13, 14*)
Chlordan	1×10^{-2}	(*13, 14*)
Aldrin	6×10^{-3}	(*13, 14*)
DDT	3×10^{-4}	(*13, 14*)
Dieldrin	1.8×10^{-4}	(*13, 14*)

Because of the nonspecific nature of the Markwood analytical procedure generally used for nicotine residue investigations, establishment of the identity of the compounds responding to this cyanogen bromide method in aged nicotine residues is important. The present report contains information demonstrating that cotinine is a degradation product from such residues on and in Texas mustard greens. In the original nicotine spray, anabasine and nornicotine were present as 4 and 6% impurities, respectively. The magnitudes and persistence behavior of nicotine residues on other

vegetables will be reported in a later paper (*1*).

Magnitudes

Nicotine Applications. Triplicated plots consisting of six 50-foot rows of Texas mustard green plants about 8 inches high were treated on December 12, 1955, in a conventional manner by means of a John Bean sprayer or a Niagara Crop Master duster with the formulations and dosages shown in Table III. Plots were guarded against drift contamination during treatment by means of a canvas shield placed around the application equipment.

For analysis, 2-kg. samples of the aerial portions of entire plants cut 1 inch above the ground were collected in Pliofilm bags 0, 1, 2, 4, 8, 15, and 22 days after treatment, and processed in the laboratory the same day.

Processing. The 2-kg. samples were ground in a food grinder, and 500-gram subsamples obtained by quartering were placed in 2-quart jars containing 20 grams of crystalline barium hydroxide and 500 ml. of *n*-hexane each. These mixtures were equilibrated for an hour in equipment previously described (*13*). The *n*-hexane solutions were separated from the aqueous and solid matter and stored at 10° C. until analyzed.

An *n*-hexane stripping solution was extracted with 0.32% acetic acid solution in three 70-ml. portions, which were combined in a 250-ml. volumetric flask. The acid was just neutralized to phenolphthalein with 4% sodium hydroxide solution; three drops of 0.32% acetic acid solution were added, and the volume was then adjusted to 250 ml. with a 0.44% sodium acetate solution.

Analysis. The colorimetric procedure used was a modification of the Markwood (*20*) cyanogen bromide method. Aliquots of 25 ml. each of the above acetate-diluted solution in 8-ounce amber bottles were placed for 1 hour in a water bath maintained at 20° ± 1° C. Then exactly 25 ml. of a 0.2% alcoholic solution of 2-naphthylamine was added with swirling, followed immediately by the addition of exactly 5 ml. of freshly prepared cyanogen bromide solution with mixing. The bottle was replaced in the water bath and protected from light for exactly 60 minutes. The transmittance was then determined at 470 mμ. Three standards were run concurrently with each daily set of samples to eliminate day-to-day variations in the standard curve.

Wax Studies. A replicate of each of the 1/24- and 7-day unground plant samples was carefully rinsed in a mild detergent solution, dried, and rubbed gently with fine glass wool to remove a portion of the cuticular wax on the leaf. The wax was extracted from the glass wool with chloroform. Alkaline substances in the extract were transferred into dilute hydrochloric acid solution and then reextracted with chloroform after this acid solution had been made basic. The final chloroform solution was concentrated in a Kuderna-Danish evaporative concentrator. The actual nicotine content was determined by a paper chromatographic technique selective for the alkaloid nicotine (see later section).

Results. Residue values for nicotine on and in Texas mustard greens and the formulations and dosages used are presented in Table III. These values, when plotted in the usual manner (*13, 14*), give the persisting half-life values listed in Table IV.

Qualitative analyses of the wax from the 1/24- and 7-day samples demonstrated the presence of nicotine and of related alkaloids residing within the wax layer of the mustard greens. The nicotine appeared in greater concentration in the 1/24-day sample than in the 7-day sample.

Table III. Nicotine Residues, P.P.M., on and in Field-Treated Texas Mustard Greens[a]

Spray Treatment	Replicate	Days after Treatment: 0[b]	1	2	4	8	15	22
Black Leaf 40, 1 pt./50 gal. water at 50 gal./acre	A	..	19.4	..	14.8	7.4	3.3	0.8
	B	31.4	36.3	..	14.6	3.3	3.0	0.9
	C	22.0	23.9	..	16.2	5.1	3.1	0.8
	D	24.8	23.1	24.7	..	8.6	1.7	1.1
	E	23.1	18.2	26.4	17.6	7.9	2.2	0.7
	Average	25.3	24.2	25.6	15.8	6.5	2.7	0.9
Black Leaf 40, 1 pt. + 2 lb. Ivory soap/50 gal. water at 42.5 gal./acre	F	18.2	11.2	..	..	8.4	1.2	0.4
	G	12.1	18.8	20.9	11.0	6.4	1.4	0.6
	H	11.3	22.0	16.5	13.1	7.6	1.1	1.1
	I	15.4	18.2	11.3	10.1	5.1	1.1	0.2
	J	12.3	15.4	10.6	9.1	3.6	0.5	0.1
	Average	13.9	17.1	14.8	10.8	6.2	1.1	0.5
Black Leaf 40, 1 pt. + 1% Dreft/50 gal. water at 42.5 gal./acre	K	14.8	8.3	..	8.7	3.7	0.5	0.2
	L	18.7	14.9	10.7	8.5	3.3	0.5	0.1
	M	21.4	19.3	13.5	8.6	5.7	0.5	0.0
	N	23.8	18.0	15.6	10.7	5.6	0.5	0.2
	O	19.2	24.2	12.9	11.0	3.6	0.6	0.2
	Average	19.6	16.9	13.2	9.5	4.4	0.5	0.2
Black Leaf 40, 2 pt. + 3 lb. Ivory soap/50 gal. water at 51 gal./acre	P	49.0	34.1	..	33.4	12.8	6.2	2.7
	Q	38.5	50.3	35.7	31.1	12.6	7.7	2.2
	R	46.7	42.0	38.5	34.4	15.9	6.4	2.4
	S	46.2	39.6	54.5	32.7	12.2	6.6	3.0
	T	41.8	48.6	51.5	35.2	11.9	4.8	2.2
	Average	44.4	42.9	45.1	33.4	13.1	6.3	2.5
3% nicotine dust at 30 lb./acre	U	17.2	6.1	..	6.1	2.3	1.2	0.8
	V	10.4	8.3	4.9	4.4	2.6	0.9	0.5
	W	14.1	12.7	8.0	4.7	3.3	0.7	0.7
	X	17.8	11.0	9.1	4.6	3.2	1.1	0.7
	Y	11.3	8.8	8.3	5.5	2.9	1.5	0.5
	Average	14.1	9.4	7.6	5.1	2.9	1.1	0.6
Untreated control	1	0.0	0.8	..	0.8	0.7	0.3	0.3
	2	0.0	0.8	..	0.6	0.3	0.4	0.1
	3	0.4	1.3	1.1	1.0	0.3	0.1	0.1
	4	1.2	1.7	1.1	1.6	0.5	0.2	0.1
	5	0.2	0.0	0.8	0.2	..	0.1	0.1
	Average	0.4	0.9	1.0	0.8	0.4	0.2	0.1

[a] All values for treated samples are corrected for background and for fortification recovery (50%).
[b] Samples were collected 1 hour after treatment, or 1/24 day.

Table IV. Persisting Half-Life Values for Residues of Nicotine on Field-Sprayed Texas Mustard Greens

Replicate[a]	Half-Life Value, Days
A-E	4.2
F-J	4.0
K-O	3.2
P-T	4.2
U-Y	2.4,[b] 6.3

[a] See Table III for treatment data.
[b] Degradation half-life value (*13, 14*).

Natures

Nicotine Application. Plots of Texas mustard greens about 8 inches high were sprayed in the above manner with 1.5 pints of Black Leaf 40 (containing 4 and 6% each of anabasine and nornicotine) per 50 gallons of water, at the rate of 50 gallons per acre.

Replicated 2-kg. analytical samples of entire plants, and roots, were collected 1/24, 1, 7, and 14 days after treatment. The weight of each plant composing a sample was determined, and the roots removed, weighed, and discarded. All samples were frozen in Pliofilm bags until processed.

Preliminary Processing. One of the 7-day samples was ground in a food grinder, and a 500-gram subsample obtained by quartering was mixed with 500 ml. of 95% ethyl alcohol in a 2-quart jar. This mixture was adjusted to pH 10 with dilute ammonium hydroxide solution and equilibrated for an hour in equipment previously described (*13*). The resulting alcohol extract was filtered successively through cheesecloth, cotton, and filter paper. All subsequent operations on the filtrate were performed under an atmosphere of nitrogen.

Acidic Materials. The filtered alcohol solution was diluted threefold with water and adjusted to pH 3 with crystalline tartaric acid. This acidified solution was extracted with 200 ml. and then with 100 ml. of chloroform. The combined chloroform extracts were extracted with 150 ml. of 2*N* ammonium hydroxide solution. This ammonia solution was strongly acidified with 6*N* sulfuric acid solution and extracted with two 70-ml. portions of chloroform. The combined chloroform extracts were then extracted with 75 ml. of 2*N* ammonium hydroxide solution, which in turn was acidified as above and extracted with two 37-ml. portions of chloroform. This extraction procedure was repeated with decreasing volumes until the combined volume of the final chloroform extracts amounted to only 1 ml.

Neutral Materials. The chloroform solution remaining above from the initial extraction with ammonium hydroxide solution was dried over anhydrous sodium sulfate and the volume was reduced to near dryness in a Kuderna-Danish evaporative concentrator.

Basic Materials. The acidic aqueous alcohol solution remaining above from the initial chloroform extraction was adjusted to pH 10 with dilute ammonium hydroxide solution and extracted with 200 ml. and then with 100 ml. of chloroform. These combined chloroform extracts were extracted with 150 ml. of dilute sulfuric acid solution, which was then made basic with 2*N* ammonium hydroxide solution and extracted twice with 75-ml. portions of chloroform. This extraction procedure was continued with decreasing portions, as above, until the volume of the final chloroform extract was only 1 ml.

Qualitative Analysis. Aliquots of each of the above extracts were placed upon filter-paper circles, air-dried, sprayed with a 1% alcoholic solution of *p*-aminobenzoic acid, and exposed to cyanogen bromide vapors. Only the fraction containing the basic substances afforded a positive cyanogen bromide color.

Final Processing. Each remaining 2-kg. sample was ground and equilibrated for 1 hour with 2 liters of 5% sulfuric acid solution as above. This mixture was filtered through cheesecloth, then through tightly packed cotton, and the volume recovered was recorded. After adjustment to pH 10 with concentrated ammonium hydroxide solution, this filtrate was extracted successively with 200-, 200-, 100-, and 100-ml. portions of chloroform. The combined chloroform solutions were extracted with three 100-ml. portions of 5% sulfuric acid solution, which were combined and washed repeatedly with 100-ml. portions of chloroform until the chloroform washings were colorless (usually three washings were sufficient). This acid solution was then made basic with concentrated ammonium hydroxide solution and extracted with three 20-ml. portions of chloroform, and the combined chloroform solutions were extracted with three 20-ml. portions of 5% sulfuric acid solution; the acid extract was washed several times with chloroform, then made basic with concentrated ammonium hydroxide solution and extracted with three 10-ml. portions of chloroform. These chloroform extracts were combined, dried over anhydrous sodium sulfate, and concentrated to 1 ml. in a Kuderna-Danish evaporative concentrator for semiquantitative analysis by the colorimetric and chromatographic procedures described below.

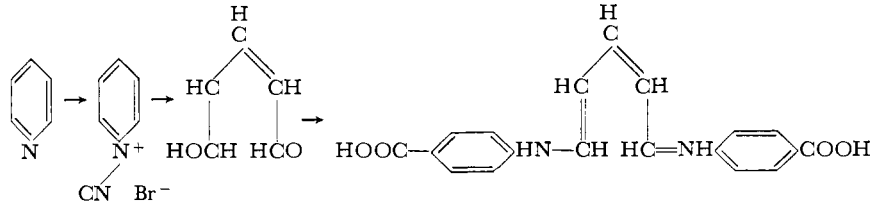
For comparative fate studies on impervious surfaces, 75-mg. quantities of 99% nicotine were pipetted onto 1.5-inch aluminum foil dishes and placed uncovered in a greenhouse room at 27° C. After 1, 2, 5, 7, and 12 days, the contents of replicate dishes were analyzed. Each residue was dissolved in acetone and its total nicotine-type alkaloid content was determined on triplicated aliquots by the colorimetric procedure. Other aliquots of each acetone solution were then separated by the paper chromatographic procedure described below, and the comparative percentages of the fractions resolved on each chromatogram were determined with the aid of a densitometer, as described below.

Colorimetric Procedure. To a low-actinic glass test tube containing 3.0 ml. of a solution composed of 24.6 grams of sodium acetate trihydrate, 1.1 ml. of glacial acetic acid, and 5 grams of *p*-aminobenzoic acid per 1000 ml. of solution was added an amount of final chloroform extract calculated to contain from 1 to 30 γ of nicotine-type alkaloids. To this mixture was added 0.6 ml. of a 1% aqueous cyanogen bromide solution with swirling. Exactly 15 minutes after the addition of the cyanogen bromide, the absorbance of the solution was determined at 470 mμ, using distilled water to set the colorimeter. A set of standard solutions was run with each set of determinations.

Chromatographic Procedure. Several of the chromatographic procedures, for nicotine and related alkaloids, reported in the literature (*16*, *18*, *22*) were evaluated. The procedure used for the present study incorporates features from all three.

Aliquots of solutions containing a mixture of the extracted alkaloids in acetone or chloroform were spotted 1.5 cm. from the bottom of a Whatman No. 1 filter paper (23 × 23 cm.) which had previously been sprayed with a buffer of pH 6.5 and air-dried. After the spots had dried, the vertical edges of the paper were stapled together to form a cylinder which was placed on one end in a Petri dish containing the developing solvent, *n*-butyl alcohol saturated with buffer of pH 6.5. Cylinder and dish were inserted into a 1-gallon jar with a tightly fitted lid.

The developing solvent usually reached within 1 to 2 cm. of the top edge of the paper after 8 hours at room temperature, whereupon the paper cylinder was air-dried, sprayed with a 1% solution of *p*-aminobenzoic acid in ethyl alcohol, air-dried again, and then placed for 6 minutes in a covered 1-gallon jar containing crystals of cyanogen bromide for color development. This color reaction is sensitive to less than 0.25 γ of each of the tested alkaloids, according to the following model reaction:



In Table V are shown typical R_f values, determined in this manner, for some of the compounds (nicotine alkaloids) of obvious interest in the present investigation.

Evaluation of Chromatograms. Two-dimensional color densities of developed chromatographic strips were determined with a Photovolt Model 52C densitometer and Model 501-A density

Table V. Typical R_f Values for Some Nicotine-Type Alkaloids

Compound	R_f
Nornicotine	0.14
Anabasine	0.28
Oxynicotine	0.41
Nicotine	0.61
Cotinine	0.84
N-Methylnicotinamide	0.87
3-Acetylpyridine	0.89
2,3′-Bipyridine	0.91

meter. The slit opening was adjusted to 15 × 1 mm. to cover the breadth of the broadest spots on the chromatograms.

In a typical measurement of paper-strip chromatograms containing known concentrations of nicotine, from 0.2- to 40.0-γ quantities of 99% nicotine were chromatographed, air - dried, sprayed with *p*-aminobenzoic acid, air-dried again, and developed in cyanogen bromide vapor for 6 minutes. The colored spots were scanned through the densitometer without filter, and the density for each unit length of paper was recorded. Areas under the resulting curves were calculated and plotted against log concentration to afford a straight line.

Developed strips containing the alkaloids, extracted from field samples of Texas mustard greens, were scanned through the densitometer also without filter. The area for a given spot and its percentage of the total colored area of the entire chromatographed strip were calculated. From these percentages, the concentrations of individual alkaloids (in parts per million) were obtained by multiplying by the corresponding concentration of the total mixture examined by the colorimetric procedure.

Infrared Identification. Characterization of the compounds responding to cyanogen bromide in the concentrated alkaloid mixtures from field-treated Texas mustard greens was obtained by infrared comparisons. These mixtures were streaked on Whatman No. 42 paper, previously treated with phosphate buffer, and developed vertically as described above. Upon drying of the paper and location of the various alkaloid zones on test strips cut from the vertical edges of the paper, the zones were separated by cutting. These zones were exhaustively extracted with methylene chloride and concentrated in Kuderna-Danish evaporative concentrators. An aliquot of each concentrate was re-chromatographed to establish its chromatographic purity, and this isolative procedure was repeated until chromatographic purity was achieved. The methylene chloride concentrate of a given zone was then carefully evaporated at room temperature, and the resulting

Table VI. Migration Values for Some Nicotine-Type Alkaloids

Compound	Travel toward Cathode, Cm.
Nicotine	22.5
Anabasine	19.0
Oxynicotine	11.1
Cotinine	8.0

residue was dissolved in 0.5 ml. of spectral grade carbon disulfide for infrared scrutiny from 2.5 to 15 microns on a Perkin-Elmer Model 21 instrument in a 1.0-mm. rock salt microcell. Instrument settings were resolution 927, response 1 to 1, gain 5.5, speed 2 minutes per micron, and suppression 2.

Paper Electrophoresis. Both paper electrophoresis and zone electrophoresis were examined, but only the former resulted in an adequate separation. A Reco Model E-800-2 electrophoresis migration chamber was used. A piece of Whatman No. 42 filter paper was cut to fit the electrophoresis chamber, and the migration zone of the paper was cut into strips 1 inch wide. An alkaloid solution was pipetted onto the anode side and allowed to dry. The entire paper was then sprayed with a buffer solution of pH 4.9 and placed in the electrophoresis chamber with the ends of the paper immersed in the buffer solution. After 6 hours at 600 volts the paper was removed from the chamber, air-dried, sprayed with *p*-aminobenzoic acid, air-dried again, and placed in cyanogen bromide vapor for color development. Migration values for some of the nicotine-type alkaloids of interest are listed in Table VI.

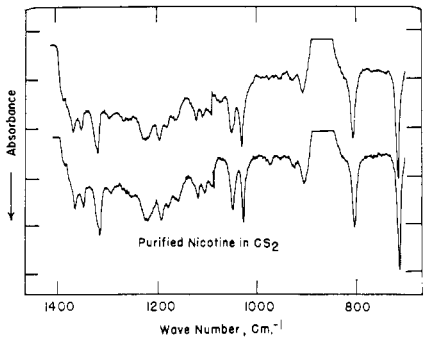


Figure 1. Comparative infrared characteristics of nicotine isolated from field-sprayed Texas mustard greens (upper spectrum) and purified nicotine

Results

Identity. In Figures 1 and 2 are presented infrared characteristics of nicotine regenerated from its picrate, an analytical sample of cotinine, and of the compounds as isolated from field-sprayed Texas mustard greens. Fractions tentatively characterized as anabasine and nornicotine were determined by their identical chromatographic behavior with known materials (see Tables V and VII) and by comparative ultraviolet absorption characteristics. The unknown fraction or mixture was not resolvable chromatographically or electrophoretically and did not appear to contain any of the compounds listed in Table V.

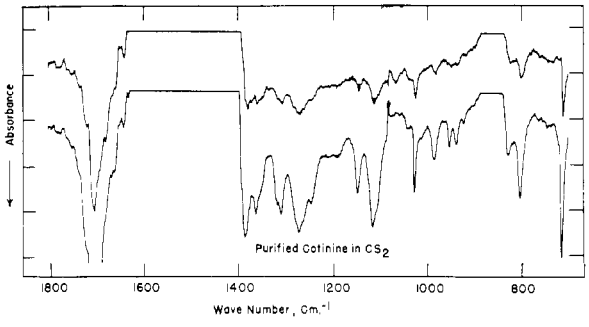


Figure 2. Comparative infrared characteristics of cotinine isolated from field-sprayed Texas mustard greens (upper spectrum) and purified cotinine

Table VII. Magnitudes of Residues of Nicotine-Type Alkaloids on and in Field-Sprayed Texas Mustard Greens[a]

Compound	R_f[b]	Residues, P.P.M., Days after Treatment				Residues (γ per Plant), Days after Treatment			
		0	1	7	14	0	1	7	14
Unknown[c]	0.92	3.2	2.4	0.45	0.15	12.3	13.9	6.6	5.1
Cotinine	0.81	3.2	3.0	0.85	0.14	12.3	17.3	12.5	4.7
Nicotine	0.59	41.2	14.3	2.20	0.37	153.5	82.5	32.5	12.5
Anabasine	0.28	4.2	0.7	0.12	0.02	15.7	4.1	1.8	0.7
Nornicotine	0.14	3.5	0.9	0.48	0.12	13.0	5.2	7.1	4.0
Total	..	55.3	21.3	4.10	0.80	206.8	123.0	60.5	27.0

[a] Values not corrected for recovery, but represent only material recoverable by the described extraction procedure.
[b] Compare with corresponding values in Table V.
[c] Paper chromatograms indicate that this fraction is a mixture of unknown compounds.

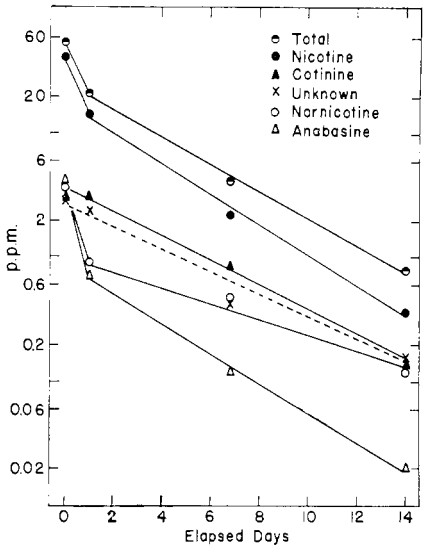


Figure 3. Residual behavior of nicotine-type alkaloids on and in field-sprayed Texas mustard greens

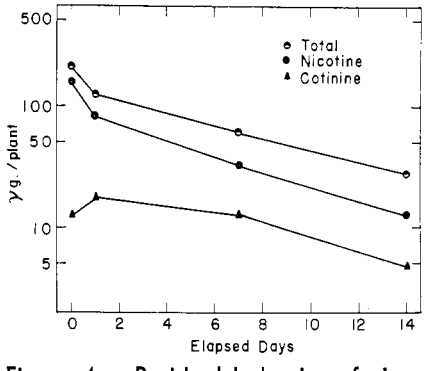


Figure 4. Residual behavior of nicotine and cotinine on and in field-sprayed Texas mustard greens with growth factor corrected

Magnitude. Magnitudes of the nicotine-type alkaloids found as residues on and in field-sprayed Texas mustard greens are collated in Table VII; these values are not absolute because of unknown manipulative losses during the complicated extraction operations.

In order to assess the effect of the rapid growth of mustard greens on the magnitudes of these residues, the same data are also presented in this table, in terms of micrograms per plant. The data in Table VIII demonstrate the behavior of nicotine residues on the impervious surface of aluminum foil, for contrast with the data in Table VII.

Persistence. To visualize the varying residual behavior patterns of these different materials more clearly, the data shown in Table VII are presented graphically in Figures 3 and 4. The persisting half-life values obtained from these plots and from a plot of the data from Table VIII are recorded in Table IX.

Discussion

Previous published data for nicotine surface or cuticular residues (see Table I) demonstrate generally less than 10 p.p.m. for initial deposits. In Table III it may be seen that after 4 days total residues of nicotine on and in Texas mustard greens were 10 p.p.m. or above, depending on the treatments utilized. This residue after 4 days is of course much higher than might be expected from a material which is as highly volatile as nicotine. The half-life values of 3 to 6 days, shown in Table IV, are also greater than might be expected from such a volatile material, because a correction for the growth of the mustard greens would approximately double these values.

These half lives are of the same order of magnitude as those of other pesticide chemicals (*13, 14*) on similar substrates. The fact that nicotine is quickly transported into, and resides in, the wax layer of the leaf indicates that nicotine residues follow a conventional penetration pattern, with both degradation and persistence behavior (Table IV). It is therefore conjectured that, with a conventional nicotine (sulfate) spray, free nicotine is released from the deposit. Some of the nicotine is dissipated rapidly in the form of vapors, but the rest largely dissolves in the wax layer and then migrates partially into the aqueous portions of the leaves. In these environments the nicotine is protected from the otherwise normal factors of physical dislodgement and ready volatilization, and is thus subject to metabolic attack.

Because the cyanogen bromide color reaction used for this residue study will respond to almost any 3-substituted pyridine nucleus, the primary metabolites of nicotine would be expected to be included as apparent nicotine in the residue data presented in Table III. The decrease in apparent nicotine obtained by prior workers does not necessarily indicate a loss in total alkaloid, but probably includes a conversion of nicotine to related compounds less completely included in the analytical procedures utilized.

As may be seen in Tables VII and IX, nicotine is the major component of aged spray residues on and in Texas mustard greens. This conclusion contradicts the commonly held belief that nicotine is rapidly and completely volatilized or otherwise dissipated from the leaf surface. However, as shown in Figure 1, nicotine, anabasine, and nornicotine are dissipated at a rapid rate during the first day after treatment. This rapid dissipation is undoubtedly due partly to volatilization as shown by the data in Table IX, where degradation and persistence half lives of deposits and residues of the compounds of interest on two types of surfaces are compared. Subsequent decay of the residues of these compounds follows the normal pattern for persisting residues, with later losses presumably attributable to biological and chemical alterations (see Table VIII). That cotinine was found to be a major metabolic or other degradative product from nicotine, as

Table VIII. Magnitudes and Nature of Residues of Nicotine Aged on Aluminum Foil Surfaces

Compound	R_f[a]	Milligrams per 75-Mg. Sample, Days of Exposure: 1	2	5	7	12
Cotinine[b]	0.83	2.4	1.4	1.1	0.4	0.3
Nicotine	0.61	16.5	2.8	1.1	0.2	0.1
Anabasine	0.30	1.1	0.5	0.2	0.3	0.05
Unknown[c]	0.19	3.2	0.9	0.5	0.6	0.3
Total	..	23.2	5.6	2.9	1.5	0.8

[a] Compare with corresponding values in Tables V and VII.
[b] Mixture of cotinine and unknown alkaloids.
[c] Fraction also contains nornicotine, as indicated by chromatographic nonhomogeneity.

Table IX. Persistence Half-Life Values for Residues of Nicotine-Type Alkaloids on and in Field-Sprayed Texas Mustard Greens and on Aluminum Foil

Compound	Half Life, Days: Without Growth, Correction (from Table VII)		With Growth, Correction (from Table VII)		On Aluminum Foil (from Table VIII)	
Unknown[a]	..	3.0	..	10.0		
Cotinine	..	3.0	..	7.0	..	4.6
Nicotine	0.6[b]	2.6	2.2[b]	4.8	0.4[b]	1.8
Anabasine	0.5[b]	2.5	0.6[b]	5.1	0.8[b]	3.9
Nornicotine	0.4[b]	4.0	..	8.0	..	6.1[d]
Total alkaloids[c]	0.6[b]	3.0	1.4[b]	6.0	0.5[b]	3.2

[a] Paper chromatograms indicate mixture of unknown compounds.
[b] Degradation half-life values (*13*).
[c] Half-life value for unseparated alkaloid residue.
[d] Mixture of unknown alkaloid and nornicotine (see [c], Table VIII).

graphically illustrated in Figures 2 and 4, is not surprising, because Frankenburg found it among the products from the fermentation of tobacco, from the irradiation of nicotine with ultraviolet light, and from the autoxidation of nicotine (*12*). Recently, McKennis, Turnbull, Wingfield, and Dewey (*19*) found cotinine as a metabolic product of nicotine in mammals, and Guthrie, Ringler, and Bowery (*15*) reported cotinine as a metabolite of nicotine in insects.

Quantitative recovery from the plant tissues of any of the compounds mentioned is not claimed, and it is probable that differential losses of constituents occurred during the many partitioning steps utilized. In these procedures, for example, manipulative losses would be expected to involve nornicotine > anabasine > nicotine. Nicotinic acid was not included among the candidate degradation products.

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HERBICIDE RESIDUES

Effect of Higher Application Rates on Crop Residues of Isopropyl *N*-Phenylcarbamate and Isopropyl *N*-(3-Chlorophenyl)carbamate

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The expanding experimental use of isopropyl *N*-(3-chlorophenyl)carbamate and isopropyl *N*-phenylcarbamate as selective herbicides has created the need for further residue studies on a number of new crops. Residue analyses are reported for rice, celery, peas, pea forage, lima beans, green beans, soybeans, and soybean forage receiving treatment with CIPC. Residue analyses are also given for spinach, strawberries, and sugar beets treated with IPC. The analytical method of Gard and Rudd for determining micro quantities of CIPC in crops was successfully used for the measurement of both carbamate residues. Some of these crops received greater than normal treatment and no residue was found which exceeded 0.05 p.p.m., the lower limit of sensitivity of the analytical method.

OVER THE PAST SEVERAL years the increasing experimental use of isopropyl *N*-phenylcarbamate (IPC) and isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) as selective herbicides has emphasized the effectiveness of these compounds in controlling grasses and narrow-leaf weeds during the growth of edible food crops such as rice, celery, grapes, tomatoes, carrots, sweet potatoes, onions, spinach, strawberries, lettuce, peanuts, cottonseeds, peas, and sugar beets.

Smith's (*5*) results with rice crops showed that the average yield of harvested rice increased from about 56 to about 100 bushels of rice per acre when one technique was used for applying the CIPC, and from about 34 to about 86 bushels per acre in another experiment using another mode of application.

Residue analyses (*1, 2*) have shown that any residues remaining with the crops treated at recommended rates are below the sensitivity limits of the method—i.e., 0.05 p.p.m. of CIPC and IPC. As experimental use of these