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METABOLISM DEPARTMENT
AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
GREENSBORO, NORTH CAROLINA

UPTAKE AND METABOLISM OF METALAXYL IN GREENHOUSE ROTATIONAL CROPS FOLLOWING TARGET TOBACCO GROWN IN SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL

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Section 158.290
Confined Accumulation Studies on
Rotational Crops

Report No.: ABR-91084 Project No.: 409925

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Date: January 27, 1992 Approval Date: Jan 29, 1992

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Study Initiation Date: September 13, 1989

TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

CERTIFICATION OF GOOD LABORATORY PRACTICES

This study was initiated and a portion of the biological phase was conducted prior to the effective date of the EPA Good Laboratory Practice Standards (40 CFR Part 160) in accordance with good and acceptable scientific practices. To the best of my knowledge, the remaining portion of the study that was conducted after October 16, 1989, was conducted in accordance with the EPA Good Laboratory Practice Standards, 40 CFR Part 160 with the following exceptions: contracted soil analysis and chemical characterization of test substances and standards synthesized prior to March 4, 1991.

5.1.2.e Woo 1/29/92
Date

Study Director

5.1.2.e Woo 1/29/92
Date

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CIBA-GEIGY CORPORATION
AG DIVISION
QUALITY ASSURANCE UNIT

QUALITY ASSURANCE STATEMENT

Report Title: UPTAKE AND METABOLISM OF METALAXYL IN GREENHOUSE ROTATIONAL CROPS FOLLOWING TARGET TOBACCO GROWN IN SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL

Study Director: 5.1.2.e Woo Project No.: 409925

Protocol No.: 203-89 with Amendments

Final Report No.: ABR-91084

Pursuant to Good Laboratory Practice Regulations, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

INSPECTION/AUDIT TYPE	INSPECTION/AUDIT DATE	REPORTING DATE
Protocol Audit	9/11/89	9/11/89
Protocol Audit Part A	4/30/90	4/30/90
Protocol Audit Part B	3/5/91	3/5/91
In-Progress Inspection	9/13/89	11/1/89
In-Progress Inspection (Part A)	7/17/90	8/22/90
In-Progress Inspection (Part A)	12/2/91	12/3/91
In-Progress Inspection (Part B)	12/6/91	12/9/91
Interim Report	12/11/91	12/11/91
Interim Report (Part A)	12/16/91	12/16/91
Interim Report (Part B)	12/20/91	1/3/92
Final Report (ABR)	1/3, 7-9, 13-14, 21-22/92	1/22/92

Prepared by: 5.1.2.e Woo Date: 1/22/92

GENERAL INFORMATION

Study Participants: 5.1.2.e Woo [redacted]

Test Substance: [Phenyl-^{14}C]-Metalaxyl
Code: ^{14}C-CGA-48988
Common Name: Metalaxyl
Trade Name: Ridomil®
Specific Activity: 29.8
29.8 µCi/mg
Lot No.: CL-XX-34
Radiochemical Purity: 98.4%
Chemical Purity: 99.7%

Carrier: Acetone - HPLC Grade
Fisher Scientific
Lot No.: 891019

Testing Facilities: CIBA-GEIGY Corporation
Agricultural Division
Metabolism Department
Post Office Box 18300
410 Swing Road
Greensboro, NC 27419

Testing Periods: Tobacco Biology Phase (Target Crop)

Protocol Approval - 9/11/89
Soil Treatment - 9/13/89
Tobacco Transplant - 9/13/89
Mature Tobacco Harvest - 4/27/90

Rotational Crop Biology Phase

Protocol Approval - 5/3/90
Crop Planting - 5/3/90
Final Harvest - 11/19/90

Protocol Number:	203-89[1] with Amendment Lists 1, 2 and 3 (Tobacco), 203-89-Part A with Amendment List 1 (Rotational Crops), and 203-89-Part B with Amendment List 1 (Non-extractable Residues)
Archives:	The protocol, raw data, biology reports, and final report are stored at CIBA-GEIGY Corporation, Metabolism and Residue Chemistry Department's Archives, Agricultural Division, Greensboro, North Carolina. Specimens, if not depleted during the study, shall be retained at CIBA-GEIGY Corporation, Greensboro, North Carolina, for as long as the quality of the preparation affords evaluation, at which time the specimens will be discarded.

ABSTRACT

Chemical

Metalaxyl, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester, the active ingredient in Ridomil®, Apron® and Subdue® fungicides, is used in numerous food and non-food crops for the control of diseases, such as late blight, downy mildew and seedling diseases caused by oomycetes.

The structure of metalaxyl is included in Figure 1. The uptake and metabolism of [phenyl-^{14}C]-metalaxyl was investigated in four rotational crops grown in soil previously used for growing tobacco. The tobacco target crop was treated with one soil application of [phenyl-^{14}C]-metalaxyl at the rate of 3 lbs. a.i./A which is the maximum treatment rate used in tobacco[2]. The active ingredient was mixed with acetone prior to incorporation into the soil.

Maintenance of Treatment Area

The rotational crops grown in treated and control soil were housed in separate 10' x 10' air conditioned greenhouse cubicles. The cubicles were continuously monitored daily for temperature and relative humidity throughout the test period. The average low temperatures were 70°F and 72°F and the average high temperatures were 86°F and 87°F in the treated and control cubicles, respectively. Artificial lighting was used throughout the study to provide a 13-14 hour photoperiod. The crops received nutrient and pesticide treatments as needed.

Additional details concerning the biological phase of this study are documented in Report Nos. BIOL-90016 (rotational crops)[3] and BIOL-90017 (target tobacco)[4].

Residue Table

Spring wheat, leaf lettuce, sugar beets and soybeans were planted 232 days after the soil treatment with [phenyl-^{14}C]-metalaxyl. The preemergent soil application was at a rate of 3.0

lbs. a.i./A incorporated into the top 1 inch of soil.

Radioactive Residues Equivalent to ^{14}C-Metalaxyl in Greenhouse Rotational Crops Following Application to a Tobacco Target Crop

Samples	Days After Soil Treatment	Total ^{14}C Residues Equivalent to PPM of ^{14}C-CGA-48988	Days Post Planting
Lettuce			
50% Mature Foliage	261	0.877	29
100% Mature Foliage	292	0.564	60
Spring Wheat			
25% Mature Stalks	254	5.117	22
50% Mature Stalks	279	2.578	47
100% Mature Stalks	323	7.171	91
100% Mature Grain	323	0.593	91
100% Mature Hulls	323	7.762	91
Soybeans			
25% Mature Stalks	261	2.424	29
50% Mature Stalks	292	2.702	60
100% Mature Stalks	432	3.612	200
100% Mature Pods	432	1.061	200
100% Mature Beans	432	0.398	200
Sugar Beets			
25% Mature Foliage	271	1.125	39
50% Mature Foliage	307	0.856	75
50% Mature Roots	307	0.291	75
100% Mature Foliage	411	1.102	179
100% Mature Roots	411	0.275	179

As expected, residue levels were higher in greenhouse rotational crops than residues in rotational crops grown in the field under normal agricultural practices. The extractable radioactivity in soil samples and in immature and mature samples from the tobacco target crop and the four rotational crops was characterized by HPLC, normal phase 2D-TLC and reversed phase 2D-TLC. Enzyme hydrolyses were performed on selected samples from target tobacco, from each of the four rotational crops, on six -

glucose conjugates isolated from rotational lettuce, and on eight glucose conjugates isolated from rotational wheat. To determine the nature of metalaxyl residues in rotational crops, extensive characterization was conducted on a leafy vegetable crop (lettuce), a root crop (sugar beets) and a small grain crop (spring wheat). Isolation and identification efforts concentrated on spring wheat metabolites not only because wheat contained the highest levels of residues, but also because wheat had the most complex metabolic pathway of rotational crops. Mass spectral identifications were obtained on six intact glucose conjugates isolated from wheat and on one intact glucose conjugate isolated from 50% mature lettuce. The major metabolite in wheat (glucose conjugate of CGA-94689B) was also identified by NMR. A total of 95% of the extractable radioactivity (79% of the total ^{14}C) in mature spring wheat stalks, 91% of the total ^{14}C in 50% mature lettuce and 57% of the total ^{14}C in mature sugar beet roots, was characterized and/or identified.

The metabolite pathway of metalaxyl in rotational crops was similar to the metabolism in target crops but greater levels of aqueous soluble polar metabolites and conjugates were formed in rotational crops. The metabolism of metalaxyl progressed via a series of reactions including the oxidation of the benzylic carbons, demethylation of the ether and ester moieties with subsequent oxidation of the alcohols and hydroxylation of the phenyl ring. The hydrolysis, oxidation and hydroxylation reactions produced a complex mixture of acids, alcohols and phenols which are subsequently conjugated to glucose. A comprehensive metabolic pathway for the metabolism of metalaxyl in rotational crops is shown in Figure 62.

Problems

No problems occurred that would adversely affect the results of the study.

Contact Person

5.1.2.e Woo

INTRODUCTION

This study was initiated to support the reregistration of metalaxyl. Metalaxyl, N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester, the active ingredient in Ridomil®, Apron® and Subdue® fungicides, is used to control specific fungi in several crops. The objectives of this study were to investigate the uptake and metabolism of [phenyl-^{14}C]-metalaxyl in rotational crops to support the data requirements in the Environmental Fate Chemistry Section 158.290, Guideline No. 165-1 (Confined Accumulation Studies on Rotational Crops).

This study was conducted according to Metabolism Protocol No. 203-89[1] (203-89, 203-89 Part A, and 203-89 Part B). All phases of the study were conducted in the Metabolism Department, CIBA-GEIGY Corporation in Greensboro, North Carolina. Details on the biological phase are documented in Report Nos. BIOL-90017 (target tobacco)[4] and BIOL-90016[3] (rotational crops).

The objective of this study was to investigate the uptake and metabolism of [phenyl-^{14}C]-metalaxyl in spring wheat, lettuce, sugar beets and soybean rotational crops grown in the greenhouse following a target crop of tobacco. The tobacco was grown in soil treated with a 3.0 lbs. a.i./A preemergent application.

Data on the uptake and characterization of metabolites in the tobacco target crop treated prior to the planting of the greenhouse rotational crops are also included in this report. The identification of metabolites of [phenyl-^{14}C]-metalaxyl and a proposed metabolic pathway of metalaxyl in target tobacco has been reported from results of previous studies[5,6].

The rotational crops were planted 232 days after the application of [phenyl-^{14}C]-metalaxyl to the tobacco soil. Rotational crop samples were taken at 25% and/or 50% maturity and at 100% maturity. Soil samples were taken at the target crop planting, at each tobacco harvest, at the rotational crop planting and at the mature harvest of each rotational crop. Mature rotational crop samples were harvested 292 days (lettuce), 323 days

(spring wheat), 411 days (sugar beets) and 432 days (soybeans) after the preemergent soil treatment.

MATERIALS AND METHODS

Chemical

The test substance is described in the General Information Section of this report and the chemical structure is shown in Figure 1. Earlier studies[5,6,7,8,9] have shown the phenyl ring of metalaxyl to be stable in plant systems and therefore ring-labeled metalaxyl was appropriate for this study. The following amounts of [phenyl-^{14}C]-metalaxyl was used for the treatment of the tobacco target crop.

Preemergent Treatment: (3.0 lbs. a.i./A)

3.0 lbs. a.i./A x 453,600 mg/lb. x A/43,560 ft.^{2} x 0.69 ft.^{2}/pail = 21.56 mg/pail

21.56 mg/pail x 20 pails = 431.1 mg

The [phenyl-^{14}C]-metalaxyl was synthesized in the CIBA-GEIGY Laboratories, Greensboro, North Carolina. The 431.1 mg of [phenyl-^{14}C]-metalaxyl were dispensed into a 20 ml glass scintillation vial.

Site

Test Pails

Test pails were located in the greenhouse facility at CIBA-GEIGY Corporation, Greensboro, North Carolina.

Approximately 13 kg of GM-4 Georgia sandy soil were placed into each of 28 aluminum-lined plastic 14-quart capacity pails. Twenty pails were amended with an additional two kilograms of [phenyl-^{14}C]-metalaxyl treated soil distributed evenly on top of the 13 kg untreated soil. The remaining eight pails received an additional 2 kg of untreated GM-4 soil. All pails contained a total of 15 kg of soil with a depth of approximately 8 inches. Three or four Coker 319 tobacco seedlings (41 days old) were transplanted into each pail to a depth of 1-2 inches. The mature tobacco was harvested 226 days after soil treatment. Rotational crops were

planted 232 days after soil treatment in the same pails used for growing the tobacco.

James Spring Wheat, Giant Western 4189 Sugar Beets, Royal Oak Leaf Lettuce and Corsoy Soybean seeds were planted into pails which contained the Georgia sandy soil. There were 5 treated and 2 control pails for each crop.

The soil was analyzed by the United Agricultural Services, Inc. (Memphis, Tennessee). The results of the soil analyses of the Georgia sandy soil follow:

SOIL ANALYSIS

Laboratory: United Agricultural Services, Inc.
3765 Premier Cove
Memphis, Tennessee 38118
(901) 794-8800

Report Number: 030007-251-19
Report Date: 9/8/89
Receipt Date at CIBA-GEIGY: 9/19/89

Sample Number: GM-4

Soil pH (water):	5.3
Soil pH (buffer):	6.5
Percent Organic Matter:	2.3
C.E.C. (meq/100g):	5.38
Percent Sand:	90.0
Percent Clay:	2.0
Percent Silt:	8.0
Soil Texture:	Sand
Percent Field Moisture @ 1/3 Bar:	6.17
Bulk Density (gm/cm^3):	1.33

The treated and untreated control tobacco plants were separately housed in 10' x 10' temperature controlled greenhouse cubicles. Temperature (°F) and relative humidity (%) were continuously monitored throughout the test period.

The average low temperatures were 65°F and 67°F and the average high temperatures were 82°F and 78°F in the treated and control cubicles, respectively, throughout the growing period of the target crop. During the rotational crops growing periods, the

average low temperatures were 70°F and 72°F in the treated and control cubicles, respectively, while the average high temperatures were 86°F and 87°F, respectively.

The relative humidity ranged from 25% to 100% throughout the biological portions of the study. Overhead artificial lighting was used to supplement the natural sunlight and to extend the photoperiod to approximately 14 hours.

The crops received scheduled nutrient and pesticide applications as needed.

TEST METHODS

General Methods

Target Crop Treatment: The stock solution was prepared by dissolving 431.1 mg of [phenyl-^{14}C]-metalaxyl in 50 ml acetone (Fisher Scientific Lot No. 891019) on 9/13/89. The stock solution and 20 ml of an acetone rinse (Fisher Scientific Lot No. 881801) were quantitatively added to 40 kg of GM-4 soil and thoroughly mixed in a 60-quart Hobart mixer (Model H600T). Two kilograms of the [phenyl-^{14}C]-metalaxyl amended soil was evenly distributed on the top of the 13 kg soil in each of 20 pails resulting in a rate equivalent to 3.0 lbs. a.i./A in each pail. Eight control pails received 2 kg of untreated GM-4 soil plus 3.5 ml of acetone.

Coker 319 tobacco seedlings (41 days old) were transplanted into each pail and grown to maturity. The treated and control pails of soil were stored in their respective cubicles until the rotational crops were planted.

Crop Maintenance

Crop maintenance and major actions taken during the course of the target crop portion of the study are listed in Table I of BIOL-90017[4].

The tobacco target crop (Coker 319) was transplanted into the treated soil on the same day the soil was treated with [phenyl-^{14}C]-metalaxyl (9/13/89). Soil samples were taken on the day of treatment; plant and soil samples were taken 34 days after treatment (4 week samples), 170 days

after treatment (75% maturity) and at tobacco maturity (226 days after treatment).

Rotational Crops

The tobacco target crop was grown to maturity and harvested on 4/27/90. The treated and control pails of soil were stored in their respective cubicles until the rotational crops were planted.

James Spring Wheat, Giant Western 4189 Sugar Beets, Royal Oak Leaf Lettuce and Corsoy Soybeans were planted into pails which contained Georgia sandy soil. All four rotational crops were planted on 5/3/90, 232 days after the soil application of [phenyl-^{14}C]-metalaxyl.

Spring wheat, sugar beets and soybeans were harvested at 25%, 50% and 100% maturity. Leaf lettuce was sampled at 50% and 100% maturity. Harvests of immature spring wheat, leaf lettuce and soybeans consisted of above ground portions of plants. The mature harvest of spring wheat was divided into stalks, hulls and grain. The mature harvest of soybeans was divided into stalks, pods and beans. The foliage of mature lettuce was harvested. The harvests of 25% mature sugar beets consisted of foliage while the 50% and 100% mature harvests consisted of foliage and roots (beets).

Crop Maintenance

Crop maintenance and the major actions taken during the course of the rotational crop study are listed by crop in Table I of BIOL-90016[3].

Soil Sampling

Soil samples were taken at the time of planting the rotational crops and at the mature harvest of each of the rotational crops. Soil samples were taken with a Hoffler coring tool. Soil samples from treated pails were divided into 0-3", 3-6" and 6-8" segments.

Sample Handling

All samples were frozen immediately after collection (-20°C) for storage. Samples remained in freezers throughout the study, and were removed

long enough to take subsamples for analyses. Samples remained frozen throughout the study except during one three-day weekend (11/30/90 - 12/3/90) when a freezer containing some of the samples malfunctioned and the freezer temperature reached 14.5°C. The samples involved in the freezer malfunction included the target tobacco plant and soil samples and the mature rotational wheat and lettuce plant samples. The partial thawing that occurred did not affect the results of the study. The initial characterization of the wheat and lettuce samples was conducted prior to the freezer malfunction.

Analytical Methods

Homogenizations: Treated and control plant samples were homogenized in a Wiley Mill in accordance with AG-223[10]. Mature lettuce and mature tobacco leaves were rehomogenized after the freezer malfunction by allowing to thaw slightly at room temperature, transferring to new labelled plastic bags, and pounding with a rubber mallet. Four-week post treatment and 75% mature tobacco leaves were rehomogenized after the freezer malfunction by adding dry ice to the sample bag, shaking to mix, let stand a few minutes to allow sample to freeze, and pounding with a rubber mallet to homogenize.

Treated and control soil samples were homogenized in an Osterizer blender in accordance with either AG-223[10] or SOP 7.8[11].

Moisture Analysis: Soil moisture was determined by the difference in weight before and after drying approximately 2.0 gram samples using a Max 50 moisture analyzer, in accordance with SOP 4.60[12].

Combustion of Samples: Triplicate aliquots (~2 g) of soil samples and quadruplicate aliquots (~0.2 g) of plant samples were combusted in a Harvey Oxidizer according to either SOP 4.40[13] or SOP 4.67[14]. Combustion efficiencies were obtained using control plant or soil samples spiked with ^{14}C-mannitol. Radioassays were performed by liquid scintillation. The scintillation cocktail used for radioanalysis was Oxosol-^{14}C (National Diagnostics). Combustions of sucrose served as backgrounds. If any replicate of the multiple analyses was greater or less than 10% of the mean value for a given

sample, then additional aliquots were combusted and analyzed.

Liquid Scintillation Radioassays: Liquid scintillation radioassays were performed on a Beckman Instruments LS3801, Beckman Instruments LS5000TD or LKB Wallac 1217 Rackbeta scintillation counter. Counting efficiencies of the instruments were established by an external standard.

Data Evaluation and Calculations: Data reported as dpm/g and ppm were processed by the VAX Metabolism Data Base System using the following calculations:

PPM Calculations from the VAX Metabolism Data Base System:

1A. If DPM of sample - Bkg DPM ≤ Bkg DPM, then

$$\text{DPM/g} = \frac{(2 \times \text{Bkg DPM}) - \text{Bkg DPM}}{\text{Combustion efficiency} \times \text{Aliquot amount}}$$

$$\text{PPM} = \frac{\text{DPM/g}}{2220 \times \text{Soil moisture factor} \times \text{Specific activity}}$$

1B. If DPM of sample - Bkg DPM > Bkg DPM, then

$$\text{DPM/g} = \frac{(\text{DPM of sample} - \text{Bkg DPM})}{\text{Combustion efficiency} \times \text{Aliquot amount}}$$

$$\text{PPM} = \frac{\text{DPM/g}}{2220 \times \text{Soil moisture factor} \times \text{Specific activity}}$$

Histogram Calculations: Data reported from histograms processed by the VAX Metabolism Data Base System were calculated using the following equation:

$$\text{\% radioactivity in a peak or region} = 100 \times \frac{\Sigma\ (\text{quantifiable dpm values from selected fractions in peaks or regions})}{\Sigma\ (\text{quantifiable dpm values in histogram})}$$

The quantifiable dpm values (DPM of sample - background dpm) were those dpms that were greater than the background dpm value.

<u>Extraction and Partition Calculations</u>: Additional quantitative data reported was hand calculated from dpm values on the VAX Metabolism Data Base printouts or from direct printouts from the liquid scintillation counters. The following equations were used for the hand calculations of normalized values.

$$\text{\% extractable radioactivity} = \frac{100 \times \text{dpm in extract}}{\text{dpm in extract} + \text{dpm in non-extractable residues (filtercake)}}$$

$$\text{\% non-extractable radioactivity} = \frac{100 \times \text{dpm in non-extractables}}{\text{dpm in non-extractables} + \text{dpm in extract}}$$

$$\text{\% organosoluble radioactivity} = \frac{100 \times \text{dpm in organic fraction}}{\text{dpm in organic fraction} + \text{dpm in aqueous fraction}}$$

$$\text{\% aqueous soluble radioactivity} = \frac{100 \times \text{dpm in aqueous fraction}}{\text{dpm in aqueous fraction} + \text{dpm in organic fraction}}$$

The following equations were used for the hand calculations of actual experimental values:

$$\text{\% extractable radioactivity} = \frac{100 \times \text{dpm in extract}}{\text{dpm in original sample}}$$

$$\text{\% non-extractable radioactivity} = \frac{100 \times \text{dpm in non-extractables (filtercake)}}{\text{dpm in original sample}}$$

$$\text{\% organosoluble radioactivity} = \frac{100 \times \text{dpm in organic fraction}}{\text{dpm in extract}}$$

$$\text{\% aqueous soluble radioactivity} = \frac{100 \times \text{dpm in aqueous fraction}}{\text{dpm in extract}}$$

Standards: Table I presents information on standards used in this study. Radiolabeled and non-radiolabeled structures of metalaxyl and possible metalaxyl metabolites are shown in Figure 1. Standard stock solutions were made in acetonitrile at an approximate concentration of 6.7 to 10 mg/ml and were stored at approximately -20°C. Selected standards were chromatographed either in combination or separately without sample metabolites or chromatographed with sample metabolites in HPLC and TLC. Approximately 10 µg of each standard were used in HPLC and 30 µg of each standard were used in TLC analyses.

Treatment Solution Stability: Two aliquots of the treatment solution were diluted in methanol and were analyzed by thin layer chromatography in two one-dimensional (1D) systems. The stationary phase was a precoated (250 µm) silica gel plate (F-254, Merck Corporation). One mobile phase was toluene/dioxane/ligroine/acetic acid 4:2:2:1 and the other was chloroform/methanol 95:5. Radioactivity was visualized with a Raytest BERTA, Beta Emission Radio Thin Layer Analyzer and recorded on Polaroid 667 film. Radioactivity was quantitated with an Ambis Radioanalytic Imaging System (AMBIS, San Diego, California).

Thin Layer Chromatography: Normal phase one-dimensional (1D) or two-dimensional (2D) TLC separations on a stationary phase of silica gel were performed on 20 x 20 cm precoated (250 µm) silica gel plates (F-254, Merck Corporation) in saturated chambers. The mobile phases used were:

SS1: Chloroform/Methanol/Formic Acid/Water (166/26/2/2)
SS2: Ethyl Acetate/Ethanol/Acetic Acid (180/19/1)
SS7: Chloroform/Methanol/Formic Acid/Water (150/40/8/4)
SS8: Ethyl Acetate/Isopropanol/Water (128/48/24)
SS9: Chloroform/Ethyl Acetate/Formic Acid (160/30/30)

Radioactive zones were detected using an Ambis Radioanalytic Imaging System (AMBIS, San Diego, California) or a Raytest BERTA, Beta Emission Radio Thin Layer Analyzer and recorded on Polaroid 667

film. Quantitation of radioactive zones was conducted by the Ambis Radioanalytic Imaging System. Results were normalized to 100% recovery.

Reversed phase 2D-TLC separations were performed on 20 x 20 cm precoated (250 µm) reversed phase F plates (Analtech, Inc., Newark, Delaware) in saturated chambers. The mobile phases used were:

SS5: Water/Acetonitrile (170/30)
SS6: Acetonitrile/Ethyl Acetate/Ethanol/Formic Acid (80/80/20/10)

High Performance Liquid Chromatography: HPLC separations were performed using the following equipment, conditions and solvent systems:

Solvent Delivery System: Perkin-Elmer 410 LC Pump
Column: Whatman Partisil 10 ODS-2 reverse phase Magnum 9 semi-preparative column (25 cm x 9.4 mm ID)
Detector: Perkin-Elmer LC-95 UV/Visible Spectrophotometer
Data Output: LINEAR 1200 Chart Recorder or a UV signal interfaced through an IN/US β-RAM to a HYUNDAI Super 286E Plus computer equipped with a Panasonic KX-1180 multimode printer
Sample Collector: ISCO Foxy, ISCO Foxy II or ISCO Foxy 200 fraction collector

UV Detector Wavelength: 266 nm
Solvent Flow Rate: 2 ml/min

HPLC System 49:

Time Minutes	% Buffer*	% Acetonitrile	% 1-Propanol
0	77.6	19.4	3
20	63	34	3
5	63	34	3
5	0	97	3
10	0	97	3

*Buffer: 0.1% TEAA (triethylamine acetate) adjusted to pH 4 with acetic acid

HPLC System 50:

Time Minutes	% H_2O	% Acetonitrile
0	80	20
20	65	35
5	65	35
5	0	100
10	0	100

Twenty additional HPLC solvent systems used in the characterization, isolation and identification of metabolites in rotational crops are listed in Table II. The following system was used in the isolation of a polar glucose conjugate in wheat stalks (Figure 8).

Solvent Delivery System: DIONEX Advanced Gradient Pump/Quat, Model AGP-1
Column: DIONEX CarboPac PA1 (250mm x 9mm ID) column, P/N 39686
Detector: DIONEX Variable Wavelength Detector, Model VDM-2
Output: DIONEX - Bausch & Lomb Model D5216R-1BA Recorder
Sample Collector: ISCO Foxy 200 fraction collector

UV Detector Wavelength: 266 nm
Solvent Flow Rate: 2 ml/min

DIONEX System 1:

Time Minutes	% H2O	% 1N NaOH
0	100	0
18	100	0
7	0	100
20	0	100

Quantitation of radioactive metabolites separated by HPLC was achieved by collecting 0.5 minute fractions with a Foxy fraction collector (Isco Foxy, Lincoln, Nebraska) and assayed for radioactivity after the addition of 5 ml of Ready-Safe (Beckman) scintillation cocktail.

DEAE Sephadex Anion Exchange Chromatography: To determine if acidic metabolites were present, subsamples of the aqueous soluble fraction of mature sugar beet roots were chromatographed on a 2.5 x 27 cm column prepared with diethylaminoethyl

(DEAE) Sephadex® A-25 anion exchange packing (Pharmacia Fine Chemicals, Piscataway, NJ). The DEAE resin was preswollen in water for at least 12 hours prior to use. A linear gradient of 250 ml H_2O to 250 ml of 1M KBr was used to elute the radioactivity at a flow rate of approximately 1.5 ml/min. At least 82 fractions of 5 mls each were collected from the column with a Foxy fraction collector (Isco Foxy, Lincoln, Nebraska) and assayed for radioactivity after the addition of 10 ml Ready-Gel (Beckman) scintillation cocktail. For preparative purposes, larger quantities of the aqueous soluble fraction from 50% mature lettuce foliage was applied to a 2.5 x 41 cm column and the aqueous soluble fraction from mature wheat stalks was applied to a 2.5 x 24.5 cm column. The radioactivity was eluted successively with 400 mls of water, 200 mls of 0.2M KBr, 200 mls of 0.4M KBr, and 400 mls of 1M KBr. Multiple fractions of 10 mls each were collected from each column with a Foxy fraction collector. Aliquots of each fraction were assayed for radioactivity after the addition of Beckman Ready-Safe.

Sephadex LH Size Exclusion/Adsorption/ Partition Chromatography: For preparative purposes, the aqueous soluble fractions from 50% mature lettuce foliage and the extractable fraction from mature wheat stalks were chromatographed on columns prepared with Sephadex® LH-20 packing from Pharmacia Ltd., Piscataway, NJ (2.5 x 50 cm for lettuce and 2.5 x 41.5 cm for wheat). Four hundred fifty (450) to 600 mls of H_2O were used to elute the radioactivity at a flow rate of approximately 1 ml/min. Multiple fractions of 10 ml each were collected from each column with a Foxy fraction collector (Isco Foxy, Lincoln, Nebraska). Aliquots of each fraction were assayed for radioactivity after the addition of Beckman Ready-Safe.

Amberlite XAD-4 Chromatography: Amberlite XAD-4 Resin (Rohm and Haas) was prepared by refluxing the resin at least 16 hours with methanol in a soxhlet apparatus. The methanol from the resin prepared for the mature sugar beet roots was decanted and HPLC grade water was added to the resin. The resin was poured into a glass column (3.8 x 54 cm) and the column was equilibrated with water. The aqueous fraction of the mature sugar beet roots was applied to the column and eluted with 700 ml of

water. Radioactivity that was retained on the column with water was eluted with methanol. Radioactivity that was not retained on the column with water was acidified with HCl and applied on another column (7 x 20 cm) and eluted with 400 ml of acidified water. Radioactivity that was retained on the column with acidified water was eluted with methanol. The methanol eluates were collected in 50-100 ml fractions and aliquots were assayed for radioactivity after the addition of Beckman Ready-Safe.

The aqueous fractions of 50% mature lettuce foliage and mature wheat stalk were applied to Amberlite® XAD-4 for preparative purposes. After methanol reflux, the resin was equilibrated with acetonitrile followed by water. The resin was poured into a glass column (3.8 x ~40 cm for neutral 50% mature lettuce foliage metabolites, 3.8 x 44 cm for neutral mature wheat stalk metabolites, and 3.8 x 42 cm for acidic mature wheat stalk metabolites) and equilibrated with water. The neutral and acidic samples were applied to the column and eluted with 500 to 700 mls of water at approximately 1 ml/min. Radioactivity that was retained on the column was eluted with acetonitrile followed by methanol. Fractions from the acetonitrile and methanol eluates were collected in graduated cylinders and aliquots were assayed for radioactivity after the addition of Beckman Ready-Safe.

C-18 Column Chromatography: C-18 column chromatography was performed with 145 to 150 grams of dry octadecyl (C-18) 40 µm bulk packing for flash chromatography (J. T. Baker, Inc., Phillipsburg, New Jersey). The stationary phase was slurried in methanol, sonicated for ~15 minutes and was poured into the columns (2.5 x 50 cm for neutral 50% mature lettuce foliage and 2.5 x 56 cm for extractable mature wheat stalks). The columns were equilibrated initially with methanol and flushed with linear gradients from methanol to acetonitrile and then to the initial conditions of the mobile phase, either 95/5 (v/v) or 80/20 (v/v) water/acetonitrile. Radioactivity from lettuce and wheat samples were eluted with water/acetonitrile mixtures at a flow rate of 2-3 mls/min. Fractions were collected with a Foxy fraction collector (Isco Foxy, Lincoln, Nebraska) and aliquots were assayed

for radioactivity after the addition of Beckman Ready-Safe.

β-Glucosidase Enzyme Hydrolysis: Aqueous fractions were incubated in a shaker bath with 7-10 mg of β-glucosidase enzyme (Sigma, 4.0-5.5 units/mg) in 0.1 ml of 0.1M NaOAc buffer, adjusted to pH 4.6 with acetic acid, for at least 12 hours at 37°C. Larger hydrolyses were performed, keeping the enzyme to buffer ratio at approximately 7-10 mg enzyme to 0.1 ml buffer. Control reactions were incubated in the same manner without the enzyme. Products of enzyme hydrolysis were analyzed directly by HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2, 5 and 6, and 7 and 8.

Subsamples of non-extractable residues (filter-cakes) from 9/1 (v/v) methanol/water extractions of rotational crop samples (0.15-3.0 g) were incubated in a shaker bath with approximately 10-300 mg β-glucosidase enzyme (Sigma, 4.0-5.5 units/mg) in 5-25 mls of 0.1M NaOAc buffer, adjusted to pH 4.6 with acetic acid, for at least 16 hours at 37°C. Control reactions were incubated in the same manner without the enzyme.

α-Glucosidase Enzyme Hydrolysis: Aqueous fractions were incubated in a shaker bath with 7 mg of α-glucosidase enzyme (Sigma, 9.4 units/mg) in 0.1-0.2 ml of 0.05M KH_2PO_4 buffer, adjusted to pH 6.8 with potassium hydroxide, for at least 12 hours at 37°C. Control reactions were incubated in the same manner without the enzyme. Products of enzyme hydrolysis were analyzed directly by 2D-TLC in solvent systems 1 and 2.

Cellulase Enzyme Hydrolysis: Aqueous fractions were incubated in a shaker bath with approximately 10 mg of cellulase enzyme (Sigma, 5.1 units/mg) in 0.2 ml of 0.1M NaOAc buffer, adjusted to pH 4.6 with acetic acid, for at least 12 hours at 37°C. Control reactions were incubated in the same manner without the enzyme. Products of enzyme hydrolysis were analyzed directly by HPLC in solvent system 49 and by 2D-TLC in solvent systems 7 and 8.

Subsamples of non-extractable residues (filter-cakes) from 9/1 (v/v) methanol/water extractions of rotational crop samples (0.15-3.0 g) were incubated in a shaker bath with approximately 10-300 mg

cellulase enzyme (Sigma, 5.1 units/mg) in 5-25 mls of 0.1M NaOAc buffer, adjusted to pH 4.6 with acetic acid, for at least 16 hours at 37°C. Control reactions were incubated in the same manner without the enzyme.

Protease Enzyme Hydrolyses: Subsamples of non-extractable residues (filtercakes) from 9/1 (v/v) methanol/water extractions of rotational crop samples (0.15-3.0 g) were incubated in a shaker bath with approximately 10-300 mg protease enzyme (Sigma, 5.4 units/mg) in 5-40 mls of 0.2M TRIS-hydroxyl methyl aminomethane (THAM) buffer, adjusted to pH 7 with hydrochloric acid, for at least 16 hours at 37°C. Control reactions were incubated in the same manner without the enzyme.

Acid Hydrolysis: Aqueous fractions were acidified with HCl and brought to a final concentration of 0.1M HCl or 6M HCl in a volume of 100 µl. Hydrolyses reactions were incubated in a shaker bath for at least 12 hours at 37°C.

Acid hydrolyses with 0.1M HCl were also conducted on 8-20 g subsamples of non-extractable residues (filtercakes) from 9/1 (v/v) methanol/water extractions of mature wheat stalks, wheat grain and sugar beet roots by refluxing for 6 hours in 100 ml of 0.1M HCl. Subsamples of non-extractable residues from mature sugar beet roots (20 g) and mature wheat grain (5 g) were hydrolyzed by refluxing for 17 hours in 100 mls of 6M HCl. Concentrated nitric acid hydrolyses were conducted on 5 g subsamples of non-extractable residues from mature wheat stalks and grain by refluxing for 2.5 hours in 50 ml of 16M HNO_3 and refluxing an additional two hours after dilution with water to 8M HNO_3. A 10 g subsample of non-extractable residues from mature sugar beet roots was refluxed for 17 hours in 100 ml of 6M HNO_3.

Mass Spectrometry: All mass spectral data were obtained on a Finnigan TSQ-700 mass spectrometer. To obtain CI spectra, methane was used as the reagent gas with an ionizing energy of 70 eV and a source temperature of 150°C. To obtain ± thermospray (TSP) analyses, 50:50 MeOH/H_2O (0.1N NH_4OAc) at 1 ml/min was utilized as the carrier. Samples were introduced in acetylated form by a direct inlet probe. Tandem MS analyses for

structural elucidation of selected ions were also performed on the Finnigan TSQ-700 mass spectrometer.

Nuclear Magnetic Resonance Spectrometry: Proton NMR spectra were obtained on a Bruker AMX-400 NMR spectrometer equipped with an inverse detection probe set at 400.13 MHz. Spectra were acquired at room temperature in deuterated acetonitrile (99.96% deuterated, MSD Isotopes, Montreal, Canada). The residual protic solvent resonances at 1.93 ppm were used as internal references relative to tetramethylsilane. Chemical shifts are expressed in parts per million. Multiplicities are reported as s=singlet, d=doublet, dd=doublet of doublets, t=triplet, q=quartet, b=broad, and m=multiplet.

Derivatization of Metabolites: Derivatization was used in this study for two purposes: (1) as an aid in mass spectral identifications and (2) to confirm the presence or absence of sugar conjugates by chemical reaction leading to products readily separated by HPLC and TLC. Acetylated derivatives were prepared by reaction with an excess of acetic anhydride/pyridine (9/1, v/v) at room temperature for at least 12 hours. The reaction mixtures were brought to near dryness with a stream of nitrogen and resuspended in acetonitrile/water. Products of the acetylation experiments were analyzed by HPLC in solvent system 49 and 1D-TLC in solvent systems 1 and 5 or isolated by HPLC in solvent system 57.

Characterization of Radioactivity in Soil Samples: A flow diagram for the general procedure used to extract and characterize metalaxyl metabolites in soil samples is shown in Figure 2. The radioactivity in soil (10-61 g subsamples) was extracted twice by mechanically shaking with a volume of 9/1 (v/v) methanol/water equivalent to four times the weight of the subsample. Mixtures were shaken for 15 minutes on a reciprocal shaker (Eberbach Corporation, Ann Arbor, Michigan) and filtered through a Whatman glass fiber filter (Grade 934AH). After the second extraction, the glassware and filtercakes were rinsed with a volume of 9/1 (v/v) methanol/water equivalent to the weight of the subsample and combined with the original extracted filtrates. The volumes of each filtrate were recorded and duplicate aliquots were taken for radioanalysis of the extractable residues. The

filtercakes were weighed and triplicate subsamples (0.1 to 1 g) were taken for combustion and radioanalysis of the non-extractable residues.

The filtrates were concentrated on a Buchi rotary evaporator and filtered through a Cameo IIS Nylon 1.2 µm syringe filter (Micron Separation, Inc.). The filters and round bottom flasks from rotary evaporation were rinsed with methanol, combined as a rinse, and duplicate aliquots were taken for radioanalysis. Subsamples of the filtered filtrates were considered representative of the extractable radioactivity and were analyzed directly by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2 and in solvent systems 5 and 6.

Characterization of Radioactivity in Greenhouse Target and Rotational Crop Samples: Flow diagrams for the general procedures used to characterize the radioactivity in target tobacco and rotational crop plant samples are shown in Figures 3 and 4. The radioactivity in 10 to 100 gram subsamples were extracted two times by blending in a volume of 9/1 (v/v) methanol/water equivalent to 2 to 10 times the weight of the subsample using either a Tekmar or Brinkmann polytron for 3 minutes. The mixtures were filtered via vacuum filtration through glass fiber filter paper (Whatman grade 934AH) after each extraction and the filtrates were combined. The volumes of the dilute filtrates were recorded and duplicate aliquots were radioassayed to obtain the values that represented the percentages of extractable radioactivity.

The filtercakes were stored frozen and at the time of analysis weighed and triplicate subsamples (0.1 to 0.3 g) were taken for combustion and radioanalysis of the non-extractable residues.

The filtrates were concentrated to approximately 1-14 mls on a Buchi rotary evaporator and filtered through either a Gelman Sciences Acrodisc 0.45 µm syringe filter and/or a Cameo IIS Nylon 1.2 µm syringe filter (Micron Separation, Inc.) or a Millipore Ultrafree MC 0.45 µm centrifuge filter. The volumes of the filtered filtrates were recorded and duplicate aliquots were taken for radio-analysis. The glassware and the residues in the syringe and/or centrifuge filters were rinsed with

methanol, combined as one rinse, and aliquots of the filter rinses were radioassayed. Subsamples of the filtered filtrates and selected filter rinses were analyzed by 2D-TLC in solvent systems 1 and 2 and in solvent systems 5 and 6 and/or by HPLC in solvent system 49. Filtered filtrates were considered representative of the extractable radioactivity. Analysis of the remaining radioactivity in selected rinses yielded profiles qualitatively similar to the concentrated/filtered filtrates. The filtered filtrates were thereby used to quantitate metabolites in the extractable radioactivity.

The filtered filtrate and filter rinse for the top and bottom leaves for the 4 week target tobacco leaves were combined to form one sample and the filtered filtrate and filter rinse for the mature tobacco leaves were combined to form another sample (Figure 3). Each of the two combined samples were concentrated by a Buchi rotary evaporator and then partitioned three times with chloroform. Subsamples of the two organic and two aqueous samples were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2 and in solvent systems 5 and 6.

Enzyme hydrolyses with β-glucosidase were conducted on subsamples of the two aqueous fractions from the combined tobacco samples and on selected subsamples of filtered filtrates from rotational crops. The products from the treated and control samples were analyzed by HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2 and in solvent systems 5 and 6.

In addition, a subsample of the mature sugar beet root filtered filtrate was partitioned three times with ethyl acetate and the organosoluble fraction was concentrated by Meyers Model 111 N-Evap and analyzed by 2D-TLC in solvent systems 1 and 2. The mature soybean bean concentrated filtrate was partitioned with hexane and ethyl acetate instead of filtration.

Characterization and Identification of Radioactivity in Mature Sugar Beet Roots: A flow diagram for the general procedure used to extract, characterize and identify metalaxyl metabolites in mature sugar beet roots is shown in Figure 5. Two subsamples

(100 g and 200 g) of mature sugar beets roots were extracted separately two times by blending in 9/1 (v/v) methanol/water with a Tekmar Tissumizer Mark II polytron for three minutes. Each mixture was filtered via vacuum filtration through a glass fiber filter (Whatman grade 934AH) after each extraction and the corresponding filtrates for each subsample were combined. The volumes of the filtrates were recorded and duplicate aliquots were radioassayed to obtain the value that represented the percentage of extractable radioactivity.

The filtercakes were stored frozen and at the time of analysis weighed and triplicate subsamples (~0.2 gm) were taken for combustion and radioanalysis of the non-extractable residues.

The filtrates were concentrated on a Buchi rotary evaporator. A subsample of the filtrate from the 200 g extraction was analyzed by HPLC in solvent system 49. The filtrate corresponding to the 100 g extract was filtered through an MSI Cameo IIS Nylon 1.2 µm syringe filter, analyzed by HPLC and 2D-TLC and was combined with the concentrated filtrate from the 200 g extraction. The combined concentrated/filtered filtrate was considered representative of the extractable radioactivity. The combined filtrate was partitioned three times with chloroform. Phases were separated between partitions and the corresponding chloroform phases were combined. The volumes of aqueous and organic phases were recorded and duplicate aliquots were radioassayed to obtain the percentage of aqueous and organosoluble radioactivity in each extract.

A subsample of the organic fraction was analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6. Identification was accomplished by comparison of chemical standards using both 2D-TLC and HPLC. Quantitation was accomplished by HPLC.

A subsample of the aqueous fraction was analyzed by HPLC in solvent system 49 and the remainder of the aqueous fraction was applied to an XAD column. The radioactivity was eluted with ~700 mls of water and ~650 mls of methanol. Approximately 79.5% of the radioactivity eluted with methanol. The water fraction, which comprised 20.5% of the radioactivity, was acidified with concentrated HCl to pH 3

and applied to a second XAD column and eluted with 400 mls of acidified water (pH 3) and 650 mls of methanol. The methanol fraction from the second column was combined with the methanol fraction of the first column for a combined total of 92.6% of the aqueous soluble radioactivity. The combined methanol fraction was concentrated by rotary evaporation and redissolved in water.

A subsample of the methanol fraction was analyzed by A-25 DEAE Sephadex® anion exchange chromatography to determine if acidic metabolites were present in the aqueous fraction. Additional subsamples of the methanol fraction were characterized by enzyme hydrolyses with β-glucosidase and cellulase. The control and enzyme treated samples were characterized by HPLC in solvent system 49, and 2D-TLC in solvent systems 1 and 2 and solvent systems 7 and 8.

A 0.05 ml subsample of the methanol fraction was incubated with 0.05 ml of 0.2M HCl for at least 12 hours at 37°C and was characterized by HPLC in solvent system 49.

An additional 0.05 ml subsample of the methanol fraction was incubated with 0.05 ml of 12M HCl for at least 12 hours at 37°C and was characterized by HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2 and solvent systems 7 and 8.

Characterization and Identification of Radioactivity in 50% Mature Lettuce Foliage: A flow diagram for the general procedure used to extract, characterize and identify metalaxyl metabolites in 50% mature lettuce foliage is shown in Figure 6. Two subsamples (100 g and 184 g) of 50% mature lettuce foliage were extracted separately two times by blending in 9/1 (v/v) methanol/water with a Tekmar Tissumizer Mark II polytron for three minutes. Each mixture was filtered via vacuum filtration through a glass fiber filter (Whatman grade 934AH) after each extraction and the corresponding filtrates for each subsample were combined. The volumes of the filtrates were recorded and duplicate aliquots were radioassayed to obtain the value that represented the percentage of extractable radioactivity.

The filtercakes were stored frozen and at the time of analysis weighed and triplicate subsamples (~0.2 gm) were taken for combustion and radioanalysis of the non-extractable residues.

The filtrates were concentrated on a Buchi rotary evaporator and filtered through either an MSI Cameo IIS Nylon 1.2 µm syringe filter or a Gelman Science 0.45 µm G Prep centrifugal filtration device. The corresponding syringe and/or centrifuge filters and the round bottom flask from rotary evaporation were rinsed with either chloroform or methanol and the respective rinses were combined. Subsamples of the filtered filtrates were analyzed by HPLC in solvent system 49. The concentrated filtered filtrates were considered representative of the extractable radioactivity.

The filtered filtrates were individually partitioned three times with chloroform. Phases were separated between partitions by centrifugation and the corresponding chloroform phases were combined. The volumes of the aqueous and organic phases were recorded and duplicate aliquots were radioassayed to obtain the percentage of aqueous and organosoluble radioactivity in each extract.

Subsamples of each organic fraction were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6. Identification was accomplished by comparison of chemical standards using both 2D-TLC and HPLC. Quantitation was accomplished by HPLC.

The aqueous fractions were combined and applied to an A-25 anion exchange column and eluted with 400 mls of water followed by 200 mls of 0.2M KBr, 200 mls of 0.4M KBr, and 400 mls of 1M KBr.

The neutral aqueous radioactivity (N) eluted from the A-25 column with water and was applied to an XAD-4 column and eluted with 400 mls of water and 600 mls of acetonitrile. The column was further rinsed with 600 mls of methanol. The acetonitrile and methanol eluates were concentrated by rotary evaporation, resuspended in water, applied to an LH-20 size exclusion column and eluted with water, and applied to a C-18 column and eluted with linear gradients from 95/5 water/acetonitrile to 100% acetonitrile. Vials containing radioactivity were

combined, concentrated by rotary evaporation and lyophilization, resuspended in water/acetonitrile (80/20) and separated into seven regions (N1 - N7) by prep HPLC in gradient 50. Quantitation was accomplished by normalizing the amounts of radioactivity recovered in each of the seven regions.

Neutral aqueous metabolites N1, N2, N3, N5, N6, and N7 were characterized by enzyme hydrolysis with β-glucosidase. The control and treated samples from the enzyme hydrolyses were analyzed by HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6. Identification was accomplished by comparison of chemical standards with the enzyme treated sample using both 2D-TLC and HPLC.

For the isolation and identification of metabolite N1b, one separation by preparative 1D-TLC and one separation by preparative HPLC was performed prior to acetylation. After acetylation, acetylated N1b was isolated with two separations by preparative HPLC and identified by + TSP mass spectroscopy.

The acidic aqueous radioactivity (LA) eluted from the A-25 column with 0.2 - 0.4N KBr and was concentrated by lyophilization and resuspended in water. Subsamples were characterized by HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2 and solvent systems 7 and 8. A subsample of the acidic aqueous fraction was separated by preparative HPLC in solvent system 49 to form 4 regions, LA1, LA2, LA3, and LA4. Each of the four regions were concentrated by rotary evaporation, resuspended in water, and analyzed by 2D-TLC in solvent systems 7 and 8.

Characterization and Identification of Radioactivity in Mature Wheat Stalks: A flow diagram for the general procedure used to extract, characterize and identify metalaxyl metabolites in mature wheat stalks is shown in Figures 7, 8, and 9. A 101 g subsample of mature wheat stalks was extracted two times by blending in methanol/water (9/1) with a Tekmar Tissumizer Mark II polytron for 3 minutes. The mixture was filtered via vacuum filtration through a glass fiber filter (Whatman grade 934AH) after each extraction and the filtrates were combined. The volume of the filtrate was recorded and duplicate aliquots were radioassayed to obtain

the value that represented the percentage of extractable radioactivity.

The filtercake was stored frozen and at the time of analysis weighed and triplicate subsamples (~0.2 gm) were taken for combustion and radioanalysis of the non-extractable residues.

The filtrate was concentrated on a Buchi rotary evaporator and filtered through an MSI Cameo IIS Nylon 1.2 µm syringe filter. The syringe filter and round bottom flask from rotary evaporation were rinsed with methanol and combined as a rinse. Subsamples of the filtered filtrate and round bottom flask/syringe filter rinse were analyzed by HPLC in solvent system 49. The filtered filtrate was considered representative of the extractable radioactivity.

The filtered filtrate was applied to an LH-20 size exclusion column and eluted with water, applied to a C-18 column twice and eluted with a mixture of water and acetonitrile, and partitioned twice with chloroform. Phases were separated between partitions by centrifugation and the chloroform phases were combined. The volumes of the aqueous and organic phases were recorded and duplicate aliquots were radioassayed to obtain the percentage of aqueous and organosoluble radioactivity in the extract.

Subsamples of the organic fraction were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6. Identification was accomplished by comparison of chemical standards using both 2D-TLC and HPLC. Quantitation was accomplished by HPLC.

The aqueous fraction was applied to an A-25 anion exchange column and eluted with 400 mls of water followed by 200 mls of 0.2M KBr, 200 mls of 0.4M KBr, and 400 mls of 1M KBr.

The neutral aqueous radioactivity (N) eluted from the A-25 column with water and was applied to an XAD-4 column and eluted with 600 mls of water and 450 mls of acetonitrile. The acetonitrile eluate was concentrated by rotary evaporation and lyophilization, resuspended in water/acetonitrile (80/20) and separated into 8 regions (N1 - N8) by prep HPLC in gradient 50.

A flow diagram for the identification and/or characterization of metabolites in regions N1 to N7 is shown in Figure 8. For the isolation and identification of metabolites N1a, N2, N4, and N5, 3-5 separations by prep HPLC and 1 separation by either prep 1D or 2D-TLC were performed prior to acetylation. After acetylation, each acetylated metabolite was isolated with 2-3 separations by prep HPLC and identified by DIP/CI, ± TSP MS (mass spectroscopy) or by tandem MS on selected ions. Acetylated N5 was further characterized by ^{1}H NMR.

For the isolation and identification of metabolites N6a and N6b, 3 separations of region N6 by prep HPLC and 2 separations by prep 1D-TLC gave two metabolites, N6a and N6b, which were acetylated. Each acetylated metabolite was isolated with 2-3 additional separations by preparative HPLC and identified by ± TSP MS or MS/MS.

Enzyme hydrolysis with β-glucosidase was conducted on subsamples of each metabolite (N1a, N1b, N2, N4, N5, N6a, and N6b) prior to acetylation to support the MS identification results. The control and treated samples from each enzyme hydrolysis were analyzed by cochromatographing with chemical standards using both HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6.

Metabolite N3 was isolated and characterized by 3 separations by preparative HPLC, 1 separation by preparative 1D-TLC, and enzyme hydrolysis with β-glucosidase. The control and treated samples from the enzyme hydrolysis were analyzed by HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6. Identification was accomplished by comparison of chemical standards with the enzyme treated sample using both HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6.

Metabolite N7 was characterized by enzyme hydrolysis with β-glucosidase. The control and treated samples from the enzyme hydrolysis were analyzed by HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6. Identification was accomplished by comparison of chemical standards with the enzyme treated sample using both HPLC solvent system 49 and 2D-TLC in

solvent systems 1 and 2 and solvent systems 5 and 6.

A flow diagram for the characterization of acidic metabolites is shown in Figure 9. The acidic aqueous radioactivity (B) eluted from the A-25 column with 0.2 - 0.4M KBr and was applied to an XAD-4 column and eluted with 400 mls of water and 600 mls of acetonitrile. The column was further rinsed with 720 mls of methanol. The acetonitrile and methanol eluates were concentrated by rotary evaporation and resuspended in water separately. The concentrated acetonitrile eluate was separated into three regions (TLCB-A, TLCB-B, and TLCB-C) by prep 1D-TLC in solvent system 8.

Subsamples of region TLCB-A, renamed B-I, were characterized by HPLC in solvent system 49 and 2D-TLC in solvent systems 1 and 2. Identification was accomplished by comparison of chemical standards using both HPLC solvent system 49 and 2D-TLC in solvent systems 1 and 2 and solvent systems 5 and 6. Quantitation was accomplished by HPLC.

Regions TLCB-B and TLCB-C and the concentrated methanol eluate were separated 1-2 times by preparative HPLC and vials with radioactivity were combined after the last common separation to form two fractions, B-II and B-III.

Subsamples of region B-II were characterized by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2, 5 and 6, and 7 and 8. Metabolites B1 and B2 were isolated from region B-II by two separations by prep 2D-TLC. Subsamples of B1 and B2 were characterized by cochromatography with standards by 2D-TLC in solvent systems 7 and 8 and by cochromatography with and without known metalaxyl hen metabolites P1 and P2[15] by HPLC in solvent system 49. Identification was accomplished by comparison of relative retentions of B1, B2, P1, and P2 with chemical standards on independent TLC plates as well as cochromatography with the corresponding hen metabolite by HPLC. Quantitation was accomplished by HPLC of a subsample of region B-II.

Subsamples of region B-III were characterized by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2, 5 and 6, and 7 and 8. Identification was accomplished by comparison of chemical

standards using both 2D-TLC and HPLC as well as comparison of retention and cochromatography with metalaxyl rat metabolite M9[16] by both 2D-TLC and HPLC. Quantitation was accomplished by HPLC of a subsample of region B-III.

Characterization of Non-Extractable Radioactivity in Greenhouse Rotational Crop Samples: Enzyme hydrolyses with β-glucosidase, cellulase, and protease were conducted on 0.15-3.0 gram subsamples of non-extractable residues (filtercakes) from 9/1 (v/v) methanol/water extractions of mature wheat stalks, mature wheat grain, and mature sugar beet roots. The products from the treated and control samples were either centrifuged or filtered via vacuum filtration through glass fiber filter paper to separate the supernatants from the residues. The volumes of the supernatants were recorded and duplicate aliquots were radioassayed to obtain the values that represented the percentages of non-extractable radioactivity released by enzyme hydrolyses.

The residues from the enzyme hydrolyses were air dried and at the time of analysis weighed and either the entire residue or subsamples of the residues were taken for combustion and radioanalysis.

Acid hydrolyses were conducted on 5-20 g subsamples of non-extractable residues (filtercakes) from 9/1 (v/v) methanol/water extractions of mature wheat stalks, wheat grain and sugar beet roots. Dilute acid hydrolyses were conducted with 0.1M HCl (stalks, grain and roots). Concentrated acid hydrolyses were conducted with 6M HCl (grain and roots), 6M HNO_3 (roots), and 16M HNO_3 (stalks and grain).

Except for the 16M HNO_3 hydrolyses, the reaction products were centrifuged/decanted and/or filtered to separate the hydrolysates from the residues. The 16M HNO_3 reaction products were completely solubilized in the concentrated acid and were considered 100% released. The volumes of the 6M HCl and 6M HNO_3 hydrolysates were recorded and duplicate aliquots were radioassayed to obtain the values that represented the percentages of non-extractable radioactivity released by 6M HCl and 6M HNO_3 acid hydrolyses.

The 0.1M HCl hydrolysates were partitioned several times with ethyl acetate. Phases were separated between partitions and the corresponding ethyl acetate phases were combined. The volumes of aqueous and organic phases were recorded and duplicate aliquots were radioassayed and summed to obtain the percentages of non-extractable radioactivity released by 0.1M HCl acid hydrolyses.

The residues from the 0.1M HCl, 6M HCl, and 6M HNO_3 acid hydrolyses were either air dried or kept frozen and at the time of analysis weighed and either the entire sample or triplicate subsamples of the residues were taken for combustion and radioanalysis. There were no residues for the 16M HNO_3 hydrolyses.

Mature Wheat Stalks - Non-Extractable Residues: The radioactivity in the non-extractable residue (filtercake) from the 9/1 (v/v) methanol/water extraction of mature wheat stalks was further characterized by hydrolyzing an approximate 1.7 g subsample of mature wheat stalk filtercake with 120 mg protease in 40 mls of 0.2M THAM buffer at pH 7 for at least 16 hours in a shaker bath at 37°C. The products from the enzyme hydrolysis were filtered via vacuum filtration through glass fiber filter paper (grade 934AH) to separate the supernatant and the residue. The volume of the supernatant was recorded and duplicate aliquots were radioassayed to obtain the value that represented the percentage of non-extractable radioactivity released by enzyme hydrolysis. The residues from the enzyme hydrolysis were air dried and at the time of analysis weighed and duplicate subsamples of the residues were taken from combustion and radioanalysis.

The supernatant from the protease hydrolysis of mature wheat stalk non-extractables was partitioned once with a volume of ethyl acetate equivalent to twice the volume of the supernatant. The volumes of the protease treated organic and aqueous fractions were recorded and duplicate aliquots were radioassayed to obtain the percentage of aqueous and organosoluble radioactivity released from the protease hydrolysis.

Subsamples of the mature wheat stalk organic fraction of the non-extractable residues released with

protease were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2. Characterization was accomplished by comparison of chemical standards using 2D-TLC and HPLC. Quantitation was accomplished by HPLC.

The aqueous fraction of the non-extractable residues released with protease was concentrated by lyophilization, resuspended in 15 mls of 0.1M NaOAc buffer at pH 4.6, and hydrolyzed with 120 mg β-glucosidase for at least 16 hours in a shaker bath at 37°C. After incubation with β-glucosidase, the aqueous fraction was partitioned once with a volume of ethyl acetate equivalent to two times the volume of the aqueous fraction. The volumes of the organic and aqueous fractions were recorded and duplicate aliquots were radioassayed to obtain the percentage of aqueous and organosoluble radioactivity released from the β-glucosidase hydrolysis of the protease aqueous fraction.

Subsamples of the mature wheat stalk protease/β-glucosidase organic fraction were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2. Characterization and quantitation was accomplished by comparison of chemical standards using HPLC.

Mature Wheat Grain - Non-Extractable Residues: The radioactivity in the non-extractable residue (filtercake) from the 9/1 (v/v) methanol/water extraction of mature wheat grain was further characterized by hydrolyzing a ~3 g subsample of non-extractable residues with 300 mg cellulase in 12 mls of 0.1M NaOAc buffer at pH 4.6 for at least 16 hours in a shaker bath at 37°C. The products from the enzyme hydrolysis were filtered via vacuum filtration through glass fiber filter paper (grade 934AH). The volume of the supernatant was recorded and duplicate aliquots were radioassayed to obtain the value that represented the percentage of non-extractable radioactivity released by enzyme hydrolysis. The residues from the enzyme hydrolyses were air dried and at the time of analysis weighed and triplicate subsamples of the residue were taken for combustion and radioanalysis.

The supernatant from the cellulase hydrolysis of mature wheat grain non-extractables was partitioned

three times with equal volumes of ethyl acetate. Phases were separated between partitions and the ethyl acetate phases were combined. The volumes of the cellulase treated organic and aqueous fractions were recorded and duplicate aliquots were radioassayed to obtain the percentage of aqueous and organosoluble radioactivity released from the cellulase hydrolysis.

Subsamples of the organic fraction from the mature wheat grain treated with cellulase were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2. Characterization was accomplished by comparison of chemical standards using 2D-TLC and HPLC. Quantitation was accomplished by 2D-TLC.

A subsample of the aqueous fraction from the mature wheat grain treated with cellulase was separated by preparative HPLC in solvent system 49 to form regions A and B. Each of the regions were concentrated by rotary evaporation, resuspended in water, and analyzed by 2D-TLC in solvent systems 1 and 2. Quantitation of the metabolites in region B was accomplished by 2D-TLC.

The radioactivity in the non-extractable residue (filtercake) from the 9/1 (v/v) methanol/water extraction of mature wheat grain was further characterized by refluxing a larger subsample (~10 g) in 100 ml of 0.1M HCl for 6 hours. The products from the acid hydrolysis were filtered via vacuum filtration through glass fiber filter paper to separate the supernatant and the residue. The volume of the supernatant was recorded and duplicate aliquots were radioassayed to obtain the value that represented the percentage of non-extractable radioactivity released by acid hydrolysis. The residues from the acid hydrolysis were air dried and at the time of analysis weighed and triplicate subsamples of the residue were taken for combustion and radioanalysis.

The supernatant from the acid hydrolysis of mature wheat grain non-extractables was partitioned several times with ethyl acetate. Phases were separated between partitions and the ethyl acetate phases were combined. The volumes of the acid hydrolyzed organic and aqueous fractions were recorded and duplicate aliquots were radioassayed to obtain the percentage of aqueous and

organosoluble radioactivity released from the acid hydrolysis.

The organic fraction from the acid hydrolysis of the mature wheat grain was separated twice by preparative 1D-TLC in solvent system 2 to yield two bands, labelled Region 2 (upper band) and Region 1 (lower band). Subsamples of the two bands were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2. Characterization was accomplished by comparison of chemical standards using 2D-TLC and HPLC. Quantitation was accomplished by HPLC.

The mature wheat grain acid hydrolyzed aqueous fraction was applied to an XAD-4 column and eluted with water followed by methanol and ethyl acetate. The methanol and ethyl acetate fractions were combined and concentrated for further analyses by HPLC in solvent system 49 and by 2D-TLC and solvent systems 1 and 2 and solvent systems 7 and 8. Characterization was accomplished by comparison of chemical standards using 2D-TLC and HPLC. Quantitation was accomplished by HPLC.

Mature Sugar Beet Root - Non-Extractable Residues: The radioactivity in the non-extractable residue (filtercake) from the 9/1 (v/v) methanol/water extraction of mature sugar beet root was further characterized by refluxing a larger subsample (~20 g) in 100 ml of 6M HCl for 17 hours. The products from the acid hydrolysis were filtered via vacuum filtration through glass fiber filter paper (grade 934AH) to separate the supernatant and the residue. The volume of the supernatant was recorded and duplicate aliquots were radioassayed to obtain the value that represented the percentage of non-extractable radioactivity released by acid hydrolysis. The residues from the acid hydrolysis were stored frozen and at the time of analysis weighed and triplicate subsamples of the residue were taken for combustion and radioanalysis.

The supernatant from the acid hydrolysis of mature sugar beet root non-extractable residues was concentrated to dryness by rotary evaporation, resuspended in HPLC grade water, and partitioned three times with equal volumes of ethyl acetate. Phases were separated between partitions by centrifugation and the ethyl acetate phases were

combined. The volumes of the acid hydrolyzed organic and aqueous fractions were recorded and duplicate aliquots were radioassayed to obtain the percentage of aqueous and organosoluble radioactivity released from the acid hydrolysis.

Subsamples of the mature sugar beet root acid hydrolyzed organic fraction were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2. Characterization was accomplished by comparison of chemical standards using 2D-TLC and HPLC. Quantitation was accomplished by 2D-TLC.

Subsamples of the mature sugar beet root acid hydrolyzed aqueous fraction were analyzed by HPLC in solvent system 49 and by 2D-TLC in solvent systems 1 and 2 and solvent systems 7 and 8. Characterization was accomplished by comparison of chemical standards using 2D-TLC and HPLC. Quantitation was accomplished by HPLC.

RESULTS AND DISCUSSION

The uptake, distribution, characterization, isolation and identification of metalaxyl metabolites in greenhouse rotational crops are presented in this report. In addition, the characterization data on metabolites in soil and in target tobacco are also presented in this report to support the metabolic pathway.

Chromatography of Standards

Separations of metalaxyl standards and possible metabolites of metalaxyl by HPLC in solvent system 49 (SS49), 1D-TLC in solvent system 1 (SS1), 2, 5, 6, 7 and 8 and 2D-TLC in solvent systems 1 and 2 are shown in Figures 10 and 11.

Treatment Solution Integrity

A subsample of the preemergent treatment solution was separated by 1D-TLC in two different solvent systems. All radioactivity visualized with the Raytest BERTA Beta Emission Radio Thin Layer Analyzer comigrated with metalaxyl. Quantitation by the Ambis Radioanalytic Imaging System indicated that >99% of the radioactivity was [phenyl-^{14}C]-metalaxyl.

Distribution and Characterization of Radioactivity in Soil Treated with [Phenyl-^{14}C]-Metalaxyl

The distribution of radioactivity in target crop soil is summarized in Table III. Radioactive residues at the time of planting were 4.487 ppm in the 0-3", 0.955 ppm in the 3-6", and 0.887 ppm in the 6-8" soil layers. Residue levels decreased to 1.759 ppm in the 0-3" soil layer at the mature tobacco harvest and increased to 1.584 ppm and 1.021 ppm in the 3-6" and 6-8" soil layers, respectively.

The distribution of soil radioactivity at the rotational crop plantings and at the mature harvests of each rotational crop is summarized in Table IV. At the rotational crop plantings (232 days after treatment), the levels in the soil were 1.054 ppm, 0.672 ppm and 0.594 ppm in the 0-3", 3-6" and 6-8" depths, respectively. Soil samples were taken at the mature harvest of each rotational crop. The final rotational crop to reach maturity was soybeans (432 days after treatment). Residues in the soil at the mature soybean harvest had decreased to 0.788 ppm in the 0-3", 0.693 ppm in the 3-6" and 0.486 ppm in the 6-8" soil layers. Residues in control samples were less than 0.001 ppm throughout the target crop (Table III) and rotational crop (Table IV) growing periods.

The 0-3" and 6-8" soil samples at the target crop planting, at the mature target crop harvest, at the rotational crop plantings and at the final rotational crop harvest (soybeans) were extracted. The extractable radioactivity was characterized by HPLC and TLC to determine the nature of soil residues in the upper and lower 3 inches of soil (Table V). The percentages of extractable radioactivity in the 0-3" soil layers represented 95.1% of the total radioactivity on the day of treatment (at the target tobacco planting), 35.4% at the mature tobacco harvest, 32.9% at the rotational crop plantings and 29.6% at the mature soybean harvest. The radioactivity in the 6-8" soil layer was 91.6% extractable at the target tobacco planting, 30.3% at the mature tobacco harvest, 38.5% at the rotational crop plantings and 20.7% extractable at the mature rotational soybean harvest. These results indicate that extensive metabolism and/or soil binding had

occurred in the soil by the time the rotational crops had been planted.

The major degradation of metalaxyl in the soil proceeded via the cleavage of the ester bond yielding the metabolite, CGA-62826 (N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine). HPLC quantitation of the metabolites present in the extractable radioactivity from these soil samples is shown in Table VI. Separations of the radioactivity by normal phase 2D-TLC and reversed phase 2D-TLC with synthetic standards supported the HPLC quantitation and aided in the characterization of some of the metabolites which comigrate by HPLC. The HPLC and normal phase 2D-TLC separations of the extractable radioactivity in the tobacco and rotational crop soils are shown in Figures 12 and 13, respectively. Metalaxyl represented 99% of the extractable radioactivity in the 0-3" soil layer at the target tobacco planting. In the 0-3" soil layer at the rotational crop plantings, metalaxyl represented 53% of the extractable radioactivity and the major metabolites were CGA-62826 (28% of extractable) and CGA-108905 (7%). Minor metabolites included CGA-107955, CGA-37734, CGA-79353, CGA-78532 and CGA-108906. Each represented 1-3% of the extractable radioactivity in the 0-3" soil layer. In the 0-3" soil layer at the mature harvest of rotational soybeans, metalaxyl had decreased to 40% of the extractable radioactivity and the major metabolites were CGA-62826 (28%), CGA-108906 (10%) and CGA-108905 (8%). Minor metabolites at the mature harvest included the minor metabolites detected at the time of planting.

The mixture of metabolites of metalaxyl in the 6-8" layer was qualitatively similar to the 0-3" layer but quantitatively different (Table VI). At the rotational crop planting, the major soil metabolite, CGA-62826 represented a larger percentage (37% vs. 28%) and metalaxyl represented a smaller percentage (39% vs. 53%) of the extractable radioactivity in the 6-8" layer when compared with the 0-3" layer. At the mature harvest of rotational soybeans, the 6-8" soil layer also contained a larger percentage of CGA-62826 (48% vs. 28%) and a smaller percentage of metalaxyl (20% vs. 40%) than the respective 0-3" soil layers. In addition, the 6-8" soil layer at the mature soybean harvest contained a metabolite (9% of the extractable

radioactivity) with chromatographic properties of the glucose conjugate of CGA-62826 which may have been a contribution from soybean root tissue.

The major soil metabolite was CGA-62826, which has been previously identified as the major metabolite in a field study on the degradation of [phenyl-^{14}C]-metalaxyl in soil[17].

The characterization of metalaxyl metabolites in the soil indicated that the soil used in this study represented the mixture of metalaxyl metabolites that could be found in soil treated in the field under normal agricultural practices. As expected, however, the levels of radioactivity in the sandy soil used in this greenhouse study were approximately 3X higher than previously reported in field rotational crop studies[18,19,20,21,22,23]. Due to the higher residue levels in soil, the uptake in rotational crops grown in this greenhouse study were also higher than previously reported under normal agricultural conditions, which aided the elucidation of the metabolic pathways of metalaxyl in rotational crops.

Uptake, Distribution and Characterization of Radioactivity in Target Tobacco

The uptake and distribution patterns of radioactive residues in the target tobacco crop at each harvest are shown in Table VII. Residues equivalent to [phenyl-^{14}C]-metalaxyl were 8.8 ppm in the bottom leaves and 2.8 ppm in the top tobacco leaves, 34 days after treatment. By the time of the mature tobacco harvest, 226 days after treatment, levels had decreased to 2.7 ppm in the bottom leaves and 1.5 ppm in the top leaves.

Four weeks after treatment, the radioactivity was 93% extractable in both the top and bottom leaves (Table VIII). The extractable radioactivity from these two samples were combined and partitioned with chloroform. Approximately 68% of the radioactivity was organosoluble and 32% remained in the aqueous fraction (Table IX). Analysis of the organosoluble radioactivity by normal phase 2D-TLC, reversed phase 2D-TLC and HPLC indicated that approximately 80% was metalaxyl, and the major metabolites were CGA-107955 (7%) and N7, the glucose conjugate of CGA-107955 (10%) (Table IX and

Figure 14). Treatment of the aqueous fraction with β-glucosidase and subsequent analyses by 2D-TLC and HPLC resulted in the release of several metabolites of metalaxyl including CGA-94689A, CGA-94689B/N1a aglycone, CGA-100255, CGA-37734, CGA-62826 and CGA-107955 indicating that extensive conjugation with glucose had occurred (Figure 15).

The radioactivity in both the top and bottom leaves of the mature tobacco were approximately 91% extractable (Table VIII) and 57% of the extractable radioactivity in the combined top and bottom leaf sample was organosoluble (Table IX). Analysis of the organosoluble radioactivity and the aqueous soluble radioactivity after hydrolyses with β-glucosidase showed a metabolic pathway qualitatively similar to the metabolites in the 4 week samples but with further transformations to polar metabolites (Table IX and Figures 14, 15 and 16). Analysis of the organosoluble radioactivity and the products released with β-glucosidase account for 72% of the total radioactivity in 4 week tobacco and 62% of the mature tobacco. Previous data showed that less specific hydrolyses released more radioactivity in tobacco than β-glucosidase, indicating that additional phase I metabolites were probably present in conjugated forms[5]. The characterization of radioactivity in target tobacco supports the metabolic pathway previously reported for target tobacco treated with [phenyl-^{14}C]-metalaxyl[5,6].

Uptake and Distribution of Radioactivity in Rotational Crops

The levels and distribution of ^{14}C-residues equivalent to [phenyl-^{14}C]-metalaxyl in greenhouse rotational crops are shown in Table X. As expected, radioactive residues were higher in the rotational crops grown in the greenhouse in sandy soil than residues of metalaxyl and/or its metabolites in rotational crops grown under normal agricultural conditions in the field (Table XI)[18,19,20,21,22,23]. Soil analyses indicated that the qualitative mixture of metabolites taken up by the plants in this study would be representative of uptake under field conditions with the exception of higher quantities of ^{14}C-residues in the soil and subsequent higher uptake by the rotational crops.

Radioactive levels were lower in the greenhouse sugar beets than in the other rotational crops and decreased from 0.291 ppm in the 50% mature roots to 0.275 in mature sugar beet roots. Residues were also relatively low in lettuce and declined from 0.877 ppm at 50% maturity to 0.564 ppm at maturity. The levels in the greenhouse rotational sugar beets and lettuce were >10X higher than residues in rotational sugar beets and lettuce grown in the field[18,19] (Table XI).

Greenhouse soybean residue levels in 25% mature and 50% mature stalks were 2.424 and 2.702 ppm, respectively. At maturity, the residue levels were 3.612, 1.061 and 0.398 ppm in mature stalks, pods and beans, respectively. The 25% mature spring wheat stalks and the 50% stalks contained residues of 5.117 ppm and 2.578 ppm, respectively. The highest residues in the greenhouse rotational crops were detected in the mature spring wheat hulls (7.762 ppm) and mature stalks (7.171) while the mature wheat grain contained 0.593 ppm. Residues in control samples were less than quantifiable (<0.003 ppm) for all rotational crops except for mature wheat samples which had residues ≤0.007 ppm.

Under normal agricultural practices in the field, the residues of metalaxyl and/or its metabolites were also lower in rotational winter wheat, spring oats and soybeans than residues in this greenhouse study (Table XI). Mature rotational winter wheat grown in the field contained residues of 0.56 ppm in mature stalks and 0.11 ppm in mature grain[20] while field spring oats contained residues of 0.19 in the mature stalks and 0.09 ppm in the grain[23]. Residues in mature rotational soybeans grown in the field were 0.59 ppm in mature stalks and 0.17 ppm in mature beans[21].

Extraction Characteristics and Metabolic Profiles of Extractable Radioactivity in Immature and Mature Greenhouse Rotational Crops

Target crop studies of [phenyl-^{14}C]-metalaxyl on tobacco, potatoes, grapes and lettuce showed that metalaxyl is metabolized to several phase I alcohols, phenols and acids and corresponding phase II conjugates common to all target crops. Characterization of the radioactivity in the tobacco target crop grown in this study supported previously

reported metabolic pathways[5,6,7,8,9]. To characterize the radioactivity in rotational crops, immature and mature samples of rotational lettuce, wheat, sugar beets and soybeans were extracted in 9/1 (v/v) methanol/water (Table XII). The radioactivity in lettuce was greater than 90% extractable with less than 10% of the residues being bound. The radioactivity in immature and mature stalks and foliage from spring wheat, soybeans and sugar beets were between 79 and 98% extractable. A large percentage of the radioactivity in soybean pods and beans was also extractable (79-82%). High percentages of bound residues were present in sugar beet roots (37%), mature wheat grain (78%), and mature wheat hulls (39%).

The extractable radioactivity (concentrated/filtered filtrate) in immature and mature samples of rotational wheat, lettuce, sugar beets and soybeans was characterized by HPLC, normal phase 2D-TLC and reversed phase 2D-TLC. The preliminary HPLC histograms and the normal phase 2D-TLC separations are shown for lettuce (Figure 17), wheat (Figure 18), soybeans (Figure 19) and sugar beets (Figure 20). Reversed phase 2D-TLC analyses of extractable radioactivity separated conjugates from phase I metabolites and aided the interpretation of data. Mature samples of each rotational crop contained a higher percentage of polar aqueous soluble metabolites and phase II conjugates than the immature samples. Otherwise, the metabolic pathway appeared similar regardless of the development stage in stalks and foliage. The aqueous soluble polar metabolites migrated near the origin region by normal phase 2D-TLC and separated into regions F through L by HPLC. The letter assignments of the polar regions in the HPLC separations were based on additional isolation and/or characterization of metabolites. Due to sample complexity, some peaks in the preliminary profiles were not assigned letter regions. However, extensive efforts focused on the isolation, characterization and identification of radioactivity in selected rotational crop samples as described in the characterization and identification sections of this report.

The extractable radioactivity in immature and mature lettuce (Figure 17) separated by HPLC into 13 and 8 regions, respectively, and included

metalaxyl and several polar acidic and glucose conjugate metabolites. The major conjugates included the glucose conjugate of CGA-100255 and a glucose conjugate of an N-dealkylated derivative of CGA-100255 (N1b).

Profiles of the extractable radioactivity in immature wheat stalks (Figure 18) showed phase I metabolites and included metalaxyl, CGA-62826, CGA-67868 and several polar metabolites. The extractable radioactivity in mature wheat stalks separated into 13 regions by HPLC. The mature wheat hull profile contained regions common with mature stalks although it was not as complex. By maturity, the level of metalaxyl had decreased in wheat stalks and the major metabolites were the polar acidics and glucose conjugates. The major conjugates in stalks and hulls included the glucose conjugates of CGA-94689A/B. The major metabolite in the extractable radioactivity in mature grain was free CGA-108905.

Profiles of the extractable radioactivity in immature soybean stalks showed 10 regions of radioactivity by HPLC separation and included metalaxyl, minor phase I metabolites and several polar metabolites (Figure 19). By maturity, the levels of metalaxyl and phase I metabolites had decreased significantly while the percentages of polar metabolites increased. The major conjugate in stalks and pods included the glucose conjugates of CGA-62826. The major phase I metabolite in mature soybean pods was the acid metabolite, CGA-62826.

The HPLC profile of the extractable radioactivity in sugar beet foliage at 25% maturity contained 3 major regions of phase I metabolites, including metalaxyl, CGA-67868 and a mixture of CGA-94689A/B and CGA-107955 (Figure 20). The mature foliage and root profiles showed greatly reduced levels of phase I metabolites and higher levels of polar metabolites than immature foliage. Mature roots contained higher levels of metalaxyl, CGA-62826 and CGA-67868 than mature foliage. Although the extractable radioactivity of the mature sugar beet roots contained an excess of coextractants which prevented separation on TLC, a 2D-TLC analysis of an ethyl acetate partition of the extractable

radioactivity confirmed the presence of the phase I metabolites in roots.

β-Glucosidase Hydrolyses of the Extractable Radioactivity in Mature Target and Rotational Crop Samples

To confirm the presence of polar neutral conjugates and to further characterize the glucose conjugates of metalaxyl metabolites present in target tobacco and rotational crops, subsamples of the extractable filtered filtrate from mature tobacco bottom leaves, mature lettuce foliage, mature wheat stalks, mature soybean stalks, mature wheat hulls and mature soybean pods were treated with β-glucosidase and the corresponding hydrolyses products were analyzed by 2D-TLC and HPLC (Table XIII and Figures 21-26). The radioactivity in sugar beet roots was subjected to enzyme hydrolyses after additional cleanup and results are discussed in the characterization and identification section.

β-Glucosidase hydrolyses of mixtures of metabolites in the extractable radioactivity does not release all of the aglycones conjugated to glucose and is not as efficient in the release of aglycones conjugated by sugar ester linkages as sugar alcohol linkages. However, analysis of the enzyme products gave an indication of the nature of some of the polar metabolites.

The results confirmed the presence of glucose conjugates of CGA-94689 isomer B in all crops and tissue types analyzed and the isomer A of CGA-94689 was released in all crops except for lettuce. The target tobacco released the CGA-94689 isomers, CGA-100255, CGA-37734 and CGA-62826. CGA-94689 A and B were the major products released in the mature spring wheat stalks and hulls while CGA-62826 was the major product hydrolyzed from soybean stalks and pods. Additional products released by wheat and soybeans included CGA-37734 and CGA-108905. A small amount of CGA-107955 was also released from wheat hulls. Lettuce released CGA-100255, an N-dealkylated derivative of CGA-100255 (N1b aglycone), CGA-62826 and CGA-94689B.

Characterization and Identification of Metabolites in Rotational Crops

To determine the nature of metabolites in all rotational crops, additional characterization, isolation and identification of metabolites was conducted on a leafy vegetable (50% mature lettuce foliage), a root crop (mature sugar beet roots), and a small grain crop (mature spring wheat stalks). The 50% mature leaf lettuce was chosen for further analysis because the immature lettuce contained approximately 1.5X higher residues and the radioactive profile was more complex than the mature lettuce. The major glucose conjugate in 50% mature lettuce was isolated and analyzed by mass spectrometry. Extensive mass spectral identification of polar metabolites common to all rotational crops concentrated on metabolites isolated from mature spring wheat because levels were higher in wheat stalks than in other rotational crop samples and also because the metabolic profile of metalaxyl in wheat was more complex than the other crops. The major glucose conjugate in mature wheat stalks was also identified by NMR.

Characterization and Identification of Organosoluble Radioactivity in Rotational Crops

The analysis of the organosoluble radioactivity in mature sugar beet roots, 50% mature lettuce foliage and mature wheat stalks supported data previously reported for phase I metabolites in target crops and supported data generated in this report for target tobacco (Table IX). The identification of the organosoluble radioactivity was confirmed by HPLC retention time comparisons and by cochromatography with standards by normal phase 2D-TLC and reversed phase 2D-TLC. Multiple phase I metabolites were formed in the three crops via oxidation of the ring methyl moieties (CGA-94689A and CGA-94689B), hydroxylation of the phenyl ring (CGA-100255), subsequent N-dealkylation of CGA-100255 to yield the N1b aglycone, and hydrolysis/demethylation of the ester (CGA-62826). Demethylation of the ether yielded CGA-67869 and subsequent demethylation of the ester formed the alcohol acid CGA-107955. N-dealkylation of CGA-67869 yielded the hydroxyacetamide, CGA-37734. N-dealkylation of metalaxyl resulted in CGA-67868 and oxidation of the aromatic methyl moieties

yielded the N1a aglycone (benzylic alcohol of CGA-67868). The CGA-94689 metabolites oxidized to the acid ether, CGA-108905.

Approximately 24% of the extractable radioactivity in mature sugar beet roots was organosoluble with the major metabolites being CGA-62826 (37%), metalaxyl (22%), and CGA-108905 (16%) (Table XIV and Figure 27). A mixture of CGA-94689A/B and CGA-107955 represented 7% of the organosoluble radioactivity and N7, the glucose conjugate of CGA-107955, represented 6% of the organosoluble radioactivity.

The extractable radioactivity in 50% mature lettuce foliage was 23% organosoluble (Table XVI). Metalaxyl comprised 72% of the organosoluble radioactivity (15% of the total ^{14}C) in 50% mature lettuce. A mixture of twelve additional metabolites each represented between 1 and 6% of the organosoluble radioactivity (0.2-1.3% of the total ^{14}C) in the immature lettuce (Table XVI and Figure 29). Eight of the 12 organosoluble metabolites were identified and four were characterized.

The extractable radioactivity in mature wheat stalks was 10% organosoluble and only 0.7% of the organic fraction (0.1% of the total ^{14}C) in mature wheat stalks was parent metalaxyl (Table XVII). The major phase I metabolites were CGA-94689 A/B, which represented 25% of the organosoluble radioactivity (1.9% of the total ^{14}C) (Table XVII and Figure 30). Other major organosoluble metabolites each representing between 9 and 16% of the organic fraction (0.7-1.2% of the total ^{14}C) were the glucose conjugates of CGA-94689A/B, CGA-108905, CGA-37734, N1a aglycone (benzyl alcohol of CGA-67868) and CGA-62826. Five additional metabolites identified in the organic fraction each represented less than 0.5% of the total radioactivity in mature spring wheat stalks. One unknown metabolite represented <0.1% of the total ^{14}C.

Characterization and Identification of the Aqueous Soluble Radioactivity in Rotational Crops

Aqueous Soluble Radioactivity in Sugar Beets

Enzyme and acid hydrolyses on mature sugar beet roots were conducted on the aqueous fraction after the extractable filtered filtrate was partitioned with chloroform and some of the coextractants were removed by separation on XAD resin.

The aqueous fraction of the mature roots was treated with β-glucosidase, cellulase, 0.1M HCl and 6M HCl. The β-glucosidase, cellulase, and 0.1M HCl hydrolyses released small amounts of phase I metabolites. Analyses of the three hydrolysates appeared similar by normal phase 2D-TLC. The cellulase results are presented in Table XV and Figure 28. Small amounts of CGA-62826 (6%) and CGA-108906 (3%) were released with enzyme hydrolysis but other metabolites appeared unchanged. The major metabolite identified in the aqueous fraction of sugar beet roots was CGA-79353 which represented 17% of the radioactivity in the control aqueous fraction. Other metabolites included non-conjugated CGA-62826, CGA-108906 and CGA-108905 as well as four additional regions of unresolved radioactivity. After acid hydrolysis with 6M HCl, the amount of CGA-62826 increased dramatically from 2% to 43% of the aqueous radioactivity (20.1% of the total ^{14}C) (Table XV and Figures 27 and 28). The amount of CGA-79353 remained 17% of the aqueous (8% of the total) and CGA-108905 remained a minor metabolite. Unresolved regions of radioactivity each represented residue levels less than or equal to 0.02 ppm. Due to the complex nature of the conjugates and/or the presence of coextractants, the 6M HCl hydrolysis resulted in a greater release of phase I metabolites than enzyme or dilute acid hydrolyses.

Aqueous Soluble Radioactivity in Lettuce and Wheat

The aqueous soluble radioactivity from mature wheat stalks and 50% mature lettuce was separated into neutral and acidic polar compounds by DEAE Sephadex® A-25 anion exchange chromatography. Approximately 69% of the aqueous soluble radioactivity in wheat and 48% of the aqueous soluble radioactivity in lettuce had little retention on

the anion exchange column and eluted with water, which is indicative of primarily neutral metabolites. The neutral and acidic metabolites were isolated, characterized and identified as described below.

Identification of Neutral Polar Metabolites in Lettuce and Wheat

The polar neutral metabolites in both lettuce and wheat were separated into 7 and 8 regions (N1-N8) of radioactivity, respectively, by HPLC (Figures 30, 6, 7). Acetylation experiments and enzyme hydrolyses on 8 of the neutral metabolites in wheat and enzyme hydrolyses on 6 of the neutral metabolites in lettuce indicated that the neutral metabolites in rotational crops were glucose conjugates of phase I metabolites. The analysis of the enzyme hydrolysis products by HPLC, reversed phase 2D-TLC and normal phase 2D-TLC indicated that the neutral metabolites N1-N7 were similar in both wheat and lettuce (Figures 31-45). The metabolite N4 isolated from 50% mature lettuce and N8 isolated from mature wheat stalks were not analyzed because they represented only 0.2% and <0.1% of the total radioactivity, respectively. Mass spectral identifications were obtained from 6 of the intact glucose conjugates (N1, N2, N4, N5, N6a, N6b) isolated from mature rotational wheat stalks and an NMR spectrum was obtained for the major metabolite N5 in wheat. In addition, mass spectral identification of the intact glucose conjugate N1b isolated from 50% mature lettuce was obtained.

The characterization and identification of the neutral metabolites in HPLC regions N1-N7 are presented below. The quantitation of metabolites in lettuce and wheat is shown in Table XVI (lettuce) and Table XVII (wheat).

1. N1 is a mixture of two glucose conjugates designated N1a and N1b that eluted with the void region of the HPLC in solvent system 49 (Figures 31, 37, 38, 45). The majority of region N1 in wheat stalks was N1a (Figures 38 and 45). The aglycone released with β-glucosidase did not comigrate in multiple systems with available standards. The mass spectral analysis by DCI and thermospray of the peracetylated N1a metabolite isolated from wheat yielded a

protonated ion peak of m/z 540 $(M+H)^+$, a 331 fragment (glucose tetracetate ion) and a 192 fragment related to the aglycone (loss of peracetylated glucose and oxygen) (Figure 46). The spectral analyses support the postulated identification of N1a as the benzylic alcohol of CGA-67868.

The majority of region N1 in immature lettuce was N1b. Enzyme hydrolysis with β-glucosidase released an aglycone that did not comigrate with the aglycone released from N1a or with available standards (Figures 31 and 37). Mass spectral analyses of the peracetylated N1b isolated from 50% mature lettuce by TSP yielded a protonated ion peak of m/z 540 $(M+H)^+$ indicating that the molecular weights of N1a and N1b are equal (Figure 47). However, the protonated aglycone fragment of N1b was 210 (loss of the peracetylated glucose) which supported the identification of a phenyl ring hydroxy metabolite of CGA-67868 versus the 192 fragment of N1a which supported the identification of a benzyl alcohol metabolite. The spectral analyses support the postulated identification of N1b as the glucose conjugate of a phenyl ring hydroxy metabolite of CGA-67868.

Both N1a and N1b have been postulated to be N-dealkylated derivatives of CGA-94689 and CGA-100255, respectively, with the loss of the ester side chain rather than the loss of the ether side chain, although the mass spectral analyses could also support identifications of N1a and N1b being N-dealkylated with a loss of the ether side chain and with an ester acid side chain intact.

2. N2 has been identified as the glucose conjugate of CGA-37734 by TSP mass spectroscopy of the peracetylated metabolite isolated from wheat stalks yielding a protonated ion $(M+H)^+$ peak of m/z 510 and a negative ion spectrum with a $(M-H)^-$ peak of m/z 508 (Figure 48). The identification was supported by β-glucosidase hydrolysis of this metabolite isolated from wheat and lettuce resulting in the release of CGA-37734 as confirmed by HPLC, normal phase 2D-TLC and reversed phase 2D-TLC (Figures 32, 37, 39, 45).

3. N3 has been identified as the glucose conjugate of CGA-100255 by analysis of the products released with β-glucosidase hydrolysis of the N3 metabolite isolated from both wheat and lettuce (Figures 33, 37, 40, 45). The release of CGA-100255 was confirmed by HPLC, reversed phase 2D-TLC and normal phase 2D-TLC.

4. N4 has been identified as the glucose conjugate of CGA-94689 isomer A by TSP mass spectroscopy of the peracetylated metabolite isolated from rotational wheat stalks yielding a protonated ion peak $(M+H)^+$ of m/z 626 and a negative ion spectrum with a $(M-H)^-$ peak of m/z 624 (Figure 49). A weak fragment at m/z 296 is consistent with the protonated aglycone (CGA-94689). The identification was supported by β-glucosidase hydrolysis of this metabolite isolated from wheat resulting in the release of CGA-94689A as confirmed by HPLC, normal phase 2D-TLC and reversed phase 2D-TLC (Figures 41 and 45).

5. N5 has been identified as the glucose conjugate of CGA-94689B by both proton NMR and TSP mass spectrometry of the peracetylated metabolite isolated from rotational wheat (Figures 50 and 51). The NMR data are consistent with an approximate 7 to 3 mole ratio of isomers of the peracetylated glucose conjugate of CGA-94689B. The chemical shift assignments for the major isomer are shown in Figure 51 (2nd page). The TSP spectrum showed the protonated ion peak at m/z 626 $(M+H)^+$ with a weak fragment at m/z 296 which is consistent with the protonated aglycone (CGA-94689) (Figure 50). The negative ion TSP spectrum showed the $(M-H)^-$ fragment at m/z 624. The identification was supported by β-glucosidase hydrolysis of this metabolite isolated from both wheat and lettuce resulting in the release of CGA-94689B as confirmed by HPLC, normal phase 2D-TLC and reversed phase 2D-TLC (Figures 34, 37, 42, 45).

6. N6 has been identified as the glucose conjugate(s) of CGA-62826. The N6 isolated from wheat was a mixture of two glucose conjugates, designated N6a and N6b, and were separated by preparative TLC in solvent system 8 after three preparative HPLC separations. Both N6a and N6b were analyzed in the peracetylated form by TSP

MS. The TSP spectra of N6a showed the protonated ion peak at m/z 596 $(M+H)^+$ and the ammonium adduct ion $(M+NH_4)^+$ at m/z 613 (Figure 52). A parent ion spectrum of the 266 fragment (protonated aglycone) of N6b showed a fragmentation pattern similar to the TSP spectrum of N6a. Daughter ion spectra of the 596 fragments from N6a and N6b yielded major fragments of 536 $(M+H - HOAc)^+$, 331 (glucose tetraacetate), 266 (protonated aglycone) and 169 (Figure 53). The spectral data on N6a and N6b were consistent with the protonated molecular ion of 596. The ability to separate N6a from N6b by TLC and differences in enzyme hydrolyses indicate that N6a is conformationally different than N6b. Enzyme hydrolyses with β-glucosidase of a mixture of N6a and N6b isolated from wheat resulted in the partial release of CGA-62826 as confirmed by reversed phase 2D-TLC, normal phase 2D-TLC and HPLC (Figures 43 and 45). Enzyme hydrolysis with β-glucosidase of N6b isolated from wheat partially released CGA-62826 (40%) as confirmed by normal phase 2D-TLC and HPLC. The N6a isolated from wheat was only minimally susceptible to β-glucosidase (4.6% release of CGA-62826 as confirmed by HPLC and reversed phase 2D-TLC). Treatment of N6a with α-glucosidase released approximately 12% of the radioactivity which comigrated with CGA-62826 as confirmed by HPLC and normal phase 2D-TLC. The N6 isolated from immature lettuce was susceptible to β-glucosidase and resulted in the release of >90% of CGA-62826 as confirmed by HPLC, reversed phase 2D-TLC and normal phase 2D-TLC (Figures 35, 37).

7. N7 has been identified as the glucose conjugate of CGA-107955 by analyses of the products released with β-glucosidase of N7 isolated from both wheat and lettuce (Figures 36, 37, 44, 45). The release of CGA-107955 was confirmed by HPLC, reversed phase 2D-TLC and normal phase 2D-TLC. Small amounts of CGA-67869 (9-15%) were also released from both the wheat and the lettuce isolates. The wheat isolate of N7 also appeared to have small amounts of free CGA-37734 (4%) and the enzyme treated sample contained small amounts of CGA-94689A/B (3-5.5%) as confirmed by 2D-TLC.

Characterization and Identification of Acidic Polar Metabolites in Lettuce and Wheat

The characterization and identification of the aqueous soluble radioactivity that was acidic in nature based on retention on the Sephadex® A-25 DEAE anion exchange column is described below. The acidic radioactivity in 50% mature lettuce separated into four polar regions designated as LA1-LA4 by HPLC (Figure 54). The majority of the analyses were conducted on the acidic metabolites isolated from wheat because residue levels were higher in wheat than in other crops. The acidic radioactivity (Fraction B), in mature wheat stalks was separated by TLC and HPLC into three subfractions, designated B-I, B-II and B-III. The quantitation of the acidic polar metabolites is shown in Table XVI for lettuce and Table XVII for wheat. The acidic metabolites in lettuce (LA1-LA4) had similar retentions by HPLC to the wheat subfractions B-II and B-III.

B-I was a mixture of six metabolites separated by HPLC and normal phase 2D-TLC and identified by comigration and comparison with chemical standards (Figure 55). The major metabolites in this subfraction were CGA-94689A/B (78% of B-I and 3.6% of the total ^{14}C), while minor metabolites representing 4-12% of the radioactivity in B-I included the aglycone of N1a (benzyl alcohol of CGA-67868), CGA-37734, and CGA-100255. Two unknown metabolites represented less than 1% of the subfraction.

B-II was a mixture of two major metabolites, each representing 48 and 49% of the radioactivity in the subfraction (a total of 4.6% of the total ^{14}C) and one minor metabolite representing 3% of the subfraction. The two major metabolites were isolated by two 2D-TLC separations and designated B1 and B2. B1 and B2 were chromatographically similar to two isomers designated P1 and P2 in the hen metalaxyl study[15]. The hen metabolites were isolated from excreta and identified by NMR and mass spectrometry as isomers of the benzylic alcohol of CGA-107955 (*N*-[2-(hydroxymethyl)-6-methylphenyl]-*N*-(hydroxyacetyl)-alanine).[15] B1 was characterized as P2 and B2 was characterized as P1 by cochromatography in HPLC and by comparison of relative retentions with chemical standards by normal phase 2D-TLC (Figure 56).

B-III was a mixture of 10 metabolites (Figure 57). The major metabolite, P0, represented 34% of the subfraction (2.6% of the total ^{14}C) and was chromatographically similar to a metabolite isolated from hens treated with ^{14}C-metalaxyl designated P0[15] and a rat metabolite designated M9[16]. M9 was previously isolated from rat urine and identified by NMR and mass spectrometry as the benzylic alcohol of CGA-62826 (N-[(2-hydroxymethyl)-6-methylphenyl]-N-(methoxyacetyl)alanine).[16] The P0 metabolite in mature wheat stalks was characterized as M9 by comparison of chemical standards using both 2D-TLC in two systems and HPLC as well as comparison of retention and cochromatography with the ^{14}C-M9 metabolite isolated from the rat. Other metabolites in the acidic subfraction B-III included CGA-108905 (13.2%), CGA-107955 (9.7%), CGA-62826 (6.8%), CGA-119857 (4.8%), and CGA-94689B (0.8%). Four unknowns representing a combined total of 2.3% of the total ^{14}C were also present in subfraction B-III.

Characterization and Identification of Bound Residues

To characterize the nature of the non-extractable residues in rotational crops grown in soil treated with [phenyl-^{14}C]-metalaxyl, additional experiments were conducted on mature wheat grain, mature sugar beet roots and mature wheat stalks. The radioactivity in mature wheat grain was 78.0% non-extractable, mature sugar beet roots was 37.5% non-extractable and mature wheat stalks was 20.8% non-extractable after extractions with methanol/water (9/1, v/v) (Table XII). Table XVIII shows that initial enzyme hydrolyses of the non-extractable radioactivity in the three tissue samples treated with protease, β-glucosidase and cellulase released between 31 and 64% of the non-extractable radioactivity. The control buffered solutions without enzyme released similar amounts (28-60%), which indicated that the pH, temperature, and/or salt effects in the buffer were more important than the enzyme activity in releasing radioactivity. Reflux hydrolyses with dilute acid (0.1M HCl) released 66% of the non-extractable radioactivity in mature wheat stalks and grain and only 26% of the bound residues in mature sugar beet roots. When the concentration of acid was increased to 6M HCl, 59% of the non-extractable radioactivity was released from

mature sugar beet roots and 74% was released from mature wheat grain. A reflux digestion with 6M nitric acid solubilized 95% of the radioactivity from mature sugar beet roots while 16M nitric acid solubilized all of the radioactivity in mature wheat stalks and grain.

To characterize the nature of the bound residues, the products from the mild hydrolyses of mature wheat stalks and grain with enzyme and dilute acid and the products from the harsher hydrolysis of mature sugar beet roots with 6M HCl were analyzed by 2D-TLC and HPLC. The products released with protease digestion of the mature wheat stalk non-extractable residues was partitioned with ethyl acetate and the aqueous fraction was subsequently hydrolyzed with β-glucosidase and partitioned with ethyl acetate. Analyses of the organosoluble products from these hydrolyses identify 89-97% of the organosoluble residues as phase I metabolites which represent a total of 33.5% of the non-extractable radioactivity in wheat stalks (Figure 58, Table XIX). The major metabolites released from the non-extractable residues were CGA-94689A/B which were also the major metabolites conjugated to glucose in the extractable radioactivity from mature wheat stalks (Table XVII and Figure 18).

The major metabolite characterized in the non-extractable radioactivity released with cellulase hydrolysis or 0.1M HCl hydrolysis of mature wheat grain was CGA-108905 which represented 19% or 24% of the non-extractable radioactivity, respectively (Figures 59 and 60, Table XX). The metabolite CGA-108905 was also the major metabolite in the extractable radioactivity from mature wheat grain (Figure 18). The acid hydrolysis also released an additional 7.1% of the non-extractable radioactivity that comigrated with CGA-79353 and CGA-119857 in 2D-TLC.

The major metabolites released from the 6M HCl hydrolysis of the non-extractable radioactivity in mature sugar beet roots were also the major metabolites present in the extractable radioactivity. Metabolites CGA-62826 and CGA-108905 represented 14% of the non-extractable radioactivity. Metalaxyl, CGA-67868 and CGA-67869 represented 12% and CGA-79353 and CGA-108906 represented 5% (Figure 61 and Table XXI).

Characterization of the non-extractable metabolites in the wheat stalks, wheat grain and sugar beet roots indicated that the bound non-extractable residues remaining after methanol/water extractions are occluded metabolites or complex conjugates of the metabolites identified previously in the extractable radioactivity.

Nature of the Residues of [Phenyl-^{14}C]-Metalaxyl in Rotational Crops

Radioactive residues equivalent to ^{14}C-metalaxyl in mature spring wheat, lettuce, sugar beets and soybean rotational crops grown in the greenhouse following a tobacco target crop ranged from a low of 0.275 ppm in mature sugar beet roots, to a high of 7.762 ppm in mature spring wheat hulls.

Radioactive residues in the 0-3" soil samples were 1.054 ppm at the time of the rotational crop plantings and decreased to 0.788 ppm at the final mature harvest of the rotational crops. Characterization of the extractable radioactivity showed that the soil at the target crop planting was mainly metalaxyl (94% of the total ^{14}C), but at the time of the rotation crop plantings, metalaxyl represented only 17% of the total ^{14}C (0.182 ppm). The major extractable metabolites in the soil at the rotational crop plantings were the acid metabolites CGA-62826 which represented 28% of the extractable radioactivity (9% of the total ^{14}C) and CGA-108905 which represented 7% of the extractable radioactivity (2% of the total ^{14}C).

The extractable radioactivity in immature and mature samples from the tobacco target and four rotational crops was characterized by HPLC, normal phase 2D-TLC and reversed phase 2D-TLC. Enzyme hydrolyses were performed on selected samples from each of the four rotational crops, on six glucose conjugates isolated from rotational lettuce and on eight glucose conjugates isolated from wheat. Spectral identification efforts focused on the mature wheat stalks because levels were higher in wheat than in other rotational crops. Mass spectral identifications were obtained on six intact glucose conjugates isolated from wheat and one intact glucose conjugate isolated from the 50% mature lettuce. The major metabolite in wheat was also identified by NMR. The majority of bound

residues released with enzyme and acid hydrolyses in mature wheat stalks, mature wheat grain and mature sugar beet roots were phase I metabolites and conjugates present in the extractable radioactivity.

A summary of the identification of metabolites in mature wheat stalks, 50% mature lettuce and mature sugar beet roots is shown in Table XXII and a comprehensive pathway for the metabolism of metalaxyl in rotational crops is shown in Figure 62.

Ninety-five percent of the extractable radioactivity and 33% of the non-extractable radioactivity in mature wheat stalks was identified, accounting for 79.2% of the total radioactivity (Table XXII). In 50% mature lettuce, 91% of the total radioactivity was characterized and/or identified. Although the accountability of total ^{14}C residues identified in mature sugar beet roots was lower (57%), sugar beets grown in the field under normal agricultural practices contained total ^{14}C residues of only 0.02 ppm in mature roots (Table XI).

The metabolic pathway for metalaxyl and the soil metabolites taken up by rotational crops proceeded via branches previously described for metalaxyl in target crops but greater levels of polar conjugates were formed. The metabolic pathway of metalaxyl in rotational crops is shown in Figure 62.

Metalaxyl and its soil metabolites were rapidly metabolized in rotational crops via four branches of the pathway to form Phase I metabolites.

1. Oxidation of the aromatic methyl of metalaxyl forms the benzylic isomers of CGA-94689 (isomers A and B). Demethylation of the methyl ester of CGA-94689 yields the benzylic alcohol of CGA-62826 (designated P0 in plants and hens and M9 in rats. Sequential demethylation of the ether moiety of P0 gives metabolites B1 and B2 (isomers of N-[2-(hydroxymethyl)-6-methylphenyl]-N-(hydroxyacetyl)-alanine). N-dealkylation of CGA-94689 yields the benzyl alcohol of CGA-67868 which is the postulated aglycone of the glucose conjugate designated N1a. Oxidation of CGA-94689 yields the benzoic acid CGA-108905 which can then be demethylated on the methyl ester to form CGA-108906.

2. Oxidation at the meta position of the aromatic ring of metalaxyl forms the phenol, CGA-100255. N-dealkylation of CGA-100255 yields the phenyl ring hydroxy metabolite of CGA-67868 which is the postulated aglycone of the glucose conjugate designated N1b.

3. Hydrolysis resulting in the demethylation of the methyl ester forms the acid metabolite, CGA-62826. Subsequent demethylation of the ether yields the alcohol acid, CGA-107955. N-dealkylation of CGA-107955 gives the hydroxyacetamide, CGA-37734.

4. Hydrolysis resulting in the demethylation of the methyl ether gives the alcohol CGA-67869. Stepwise demethylation of the ester gives the alcohol acid, CGA-107955. Hydrolysis of CGA-67869 forms the acid ether, CGA-79353, while N-dealkylation of CGA-67869 gives the hydroxyacetamide, CGA-37734.

The four branches of the overall metabolic pathway were active to some extent in all rotational crops but the major branch of the pathway varied by crop. The major metabolic route for spring wheat proceeded via branch 1 by oxidizing the aromatic methyl and forming the isomers of CGA-94689 and the subsequent metabolites in the pathway. A major metabolic branch for lettuce proceeded via branch 2 yielding the glucose conjugate of the phenol metabolite, CGA-100255 and the glucose conjugate of the N-dealkylated CGA-100255, designated N1b and postulated to be the phenyl ring hydroxy metabolite of CGA-67868. The major metabolic route for metalaxyl in mature soybeans proceeded via branch 3 by demethylation of the methyl ester and yielding the acid metabolites CGA-62826 and CGA-107955. The major metabolic route for metalaxyl in sugar beets involved branches 3 and 4 via demethylation of the methyl ester and methyl ether resulting in the formation of the acid metabolites CGA-62826 and CGA-79353. The predominance of branch 3 for both sugar beets and soybeans could be due in part to differences in the uptake of CGA-62826 from the soil. Both sugar beets and soybeans had longer maturation times than wheat or lettuce and therefore had greater exposure to the major soil metabolite, CGA-62826.

The metabolites, CGA-94689A, CGA-94689B, CGA-37734, CGA-100255, CGA-62826, the benzylic alcohol of CGA-67868, CGA-107955, CGA-108905 and the phenyl ring hydroxy of CGA-67868 also underwent Phase II reactions and were present in the rotational crops as glucose conjugates. Further metabolism of Phase II conjugates to disaccharides or oligosaccharides and non-extractable residues is expected for metalaxyl due to the presence of multiple oxidation sites that are possible substrates for several enzyme systems present in higher plants.

The metabolic pathway of metalaxyl in rotational crops is similar to the metabolism in target crops.[5,6,7,8,9] The phase I metabolism of metalaxyl in rotational crops is also similar to the metabolism of metalaxyl in the rat[16], goat[24] and hen.[15] Many of the free metabolites identified in animals also occur in free and conjugated forms in rotational crops.

CONCLUSIONS

Rotational crops of lettuce, spring wheat, sugar beets and soybeans were grown to maturity in the greenhouse following a tobacco target crop grown in soil treated with [phenyl-^{14}C]-metalaxyl at a rate of 3 lbs. a.i./A. Residues in the soil at the time of rotational crop planting were 1.05 ppm and the major extractable residues were metalaxyl and CGA-62826.

Residues in the greenhouse rotational crops at maturity were 0.275 ppm in sugar beet roots, 0.564 ppm in lettuce, 3.612 ppm in soybean stalks and 7.171 ppm in spring wheat stalks. Residues in mature soybean beans and mature wheat grain were 0.398 ppm and 0.593 ppm, respectively. Residues in the greenhouse grown rotational crops were higher than those found in field grown rotational crops, which aided the identification of metabolites.

The nature of residues has been defined in 3 crop groupings: leafy crop (lettuce), small grain crop (spring wheat) and a root crop (sugar beets). The phase I residues in rotational crops are qualitatively similar to residues previously identified in target crops and animals. However, metabolism proceeded further in rotational crops than in target

crops and higher percentages of phase II metabolites (glucose conjugates) were formed.

A total of 79% of the total radioactivity in mature spring wheat stalks, 91% of the total ^{14}C in 50% mature lettuce and 57% of the total ^{14}C in mature sugar beet roots was characterized and/or identified. Seven intact glucose conjugates were isolated from rotational crops and identified by mass spectral analyses. One conjugate was also analyzed by NMR. Other metabolites were identified by analysis of enzyme/hydrolysis products, cochromatography with standards, comparison to standards and/or cochromatography and comparison to metabolites isolated from animals.

The metabolism of [phenyl-^{14}C]-metalaxyl is complex. Fourteen phase I metabolites and 9 glucose conjugates were identified in rotational crops. Several complex conjugates were also characterized. The metabolic pathway of metalaxyl proceeded through a series of hydrolysis, oxidation and hydroxylation reactions which involved the oxidation of the benzylic carbon, demethylation of the ether and ester moieties with subsequent oxidation of the alcohols and hydroxylation of the phenol ring. A complex mixture of acids, alcohols and phenols were formed in rotational crops and subsequently conjugated to glucose. A comprehensive pathway of the metabolism of metalaxyl in rotational crops is presented in Figure 62.

TABLE I. SYNTHESIS REFERENCE NUMBERS AND PURITY INFORMATION FOR METALAXYL STANDARDS

Standard	Biochemistry Inventory Control No.	Synthesis Ref. No.	Purity[1]	Information Reanalysis Date	Activity
^{14}C-CGA-48988	B06486	CL-XX-34	98.9	3/91	29.8
	B06607	JAK-VI-18	99.0	12/91	49
^{14}C-CGA-62826	B06487	WS-I-30	QS	QS	1.1
^{14}C-CGA-67866	B06488	GAN-XIV-91	QS	QS	176.9
CGA-78532	B05983	JAK-IV-15-1	>99	9/91	--
	B06328	JAK-IV-15-1	>99	9/91	--
	B06498	JAK-IV-15-1	99	9/91	--
	B06692	JAK-IV-15-1	>99.9	10/93	--
CGA-68124	B05987	BPM-X-31	99.7	6/91	--
	B06329	BPM-X-31	99.7	6/91	--
	B06497	BPM-X-31	99.9	5/93	--
	B06693	BPM-X-31	99.9	5/93	--
CGA-108906	B05979	GB-XLIV-85	99.0	4/92	--
	B06330	GB-XLIV-85	99.0	4/92	--
	B06677	WFH-VII-96	99.9	10/93	--
	B06694	WFH-VII-96	>99.9	10/93	--
CGA-119857	B05985	GB-XLIV-1	99.9	11/91	--
	B06331	GB-XLIV-1	99.9	11/91	--
	B06503	GB-XLIV-1	99.9	11/91	--
	B06695	GB-XLIV-1	>99.9	10/93	--
CGA-62826	B05984	BPM-I-4B	>99	4/92	--
	B06332	BPM-I-4B	>99	4/92	--
	B06492	BPM-I-4B	99	4/92	--
	B06696	BPM-I-4B	>99	4/92	--
CGA-37734	B05989	BPM-I-8	98.9	5/91	--
	B06333	BPM-I-8	98.9	5/91	--
	B06491	BPM-I-8	97.7	5/93	--
	B06697	BPM-I-8	97.7	5/93	--
CGA-67867	B05990	RAF-IX-58	99.4	4/91	--
	B06334	RAF-IX-58	99.4	4/91	--
	B06495	RAF-IX-58	99.9	5/93	--
	B06698	RAF-IX-58	>99.9	5/93	--
CGA-94689B	B05992	JAK-II-50(B)	97.6	5/92	--
	B06335	JAK-II-50(B)	97.6	5/92	--

[1] QS = Qualitative Standard. No purity available.

TABLE I. SYNTHESIS REFERENCE NUMBERS AND PURITY INFORMATION FOR METALAXYL STANDARDS (Continued)

Standard	Biochemistry Inventory Control No.	Synthesis Ref. No.	Purity[1]	Information Reanalysis Date	Activity
CGA-100255	B05988	JAK-I-57	99.7	3/92	--
	B06336	JAK-I-57	99.7	3/92	--
	B06676	WFH-VII-86	99.5	10/93	--
	B06699	WFH-VII-86	99.5	10/93	--
CGA-94689A/B	B05991	JAK-II-21	96.1	5/92	--
	B06337	JAK-II-21	96.1	5/92	--
	B06500	JAK-II-21	96.1	5/92	--
	B06700	JAK-II-21	96.1	5/92	--
CGA-67868	B05986	BPM-X-26	99.5	7/91	--
	B06338	BPM-X-26	99.5	7/91	--
	B06494	BPM-X-26	99.4	5/93	--
	B06701	BPM-X-26	99.4	5/93	--
CGA-67869	B05980	RAF-XI-19	95.2	4/92	--
	B06339	RAF-XI-19	95.2	4/92	--
	B06496	RAF-XI-19	95.2	4/92	--
	B06702	RAF-XI-19	95.2	4/92	--
CGA-48988	B05570	S87-1208	95.8	5/93	--
	B05981	S85-0831	96.5	4/91	--
	B06340	S85-0831	96.5	4/91	--
	B06490	S87-1208	95.8	5/93	--
	B06903	S84-0577	99.6	6/92	--
CGA-67866	B05982	RAF-IX-91	99.4	4/91	--
	B06341	RAF-IX-91	99.4	9/91	--
	B06493	RAF-IX-91	99.4	9/91	--
	B06704	RAF-IX-91	>99.9	10/93	--
CGA-107955	B06375	GB-XLVI-7	>99	9/92	--
	B06501	GB-XLVI-7	99	9/92	--
	B06705	GB-XLVI-7	>99	9/92	--
CGA-79353	B06376	JAK-IV-14-1	>99	10/91	--
	B06499	JAK-IV-14-1	99	10/91	--
	B06706	JAK-IV-14-1	99.3	10/93	--
CGA-108905	B06377	GB-XLIV-78	QS	QS	--
	B06502	GB-XLIV-78	98.5	4/92	--
	B06707	GB-XLIV-78	QS	QS	--

[1] QS = Qualitative Standard. No purity available.

TABLE II. HPLC SOLVENT SYSTEMS USED FOR METALAXYL METABOLITE ISOLATIONS

	Time (Mins.)	% H_2O	% ACN
Solvent System 51	0	95	5
	15	95	5
	10	80	20
	5	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 52	0	95	5
	10	80	20
	15	70	30
	5	65	35
	5	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 53	0	95	5
	10	95	5
	10	75	25
	10	65	35
	5	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 54	0	80	20
	5	60	40
	10	25	75
	15	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 55	0	45	55
	10	45	55
	18	10	90
	2	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 56	0	80	20
	10	70	30
	20	65	35
	5	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 57	0	80	20
	5	60	40
	10	25	75
	5	25	75
	15	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 58	0	55	45
	15	55	45
	18	20	80
	2	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 59	0	55	45
	15	55	45
	18	30	70
	2	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 60	0	95	5
	13	80	20
	17	70	30
	3	65	35
	2	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 61	0	80	20
	30	70	30
	3	65	35
	2	0	100
	10	0	100

	Time (Mins.)	% H_2O	% ACN
Solvent System 62	0	95	5
	5	95	5
	10	75	25
	15	65	35
	5	0	100
	10	0	100

TABLE II. HPLC SOLVENT SYSTEMS USED FOR METALAXYL METABOLITE ISOLATIONS (Continued)

Solvent System 63

Time (Mins.)	% H_2O	% ACN
0	90	10
20	90	10
10	75	25
5	65	35
5	50	50
5	0	100
10	0	100

Solvent System 64

Time (Mins.)	% H_2O	% ACN
0	55	45
20	55	45
20	35	65
2	0	100
10	0	100

Solvent System 65

Time (Mins.)	% H_2O	% ACN
0	90	10
15	90	10
25	65	35
5	50	50
5	0	100
10	0	100

Solvent System 66

Time (Mins.)	% H_2O	% ACN
0	90	10
15	90	10
10	75	25
10	65	35
10	50	50
5	0	100
10	0	100

Solvent System 67

Time (Mins.)	% H_2O	% ACN
0	80	20
30	65	35
5	60	40
5	0	100
10	0	100

Solvent System 68

Time (Mins.)	% H_2O	% ACN
0	55	45
15	50	50
25	25	75
2	0	100
10	0	100

Solvent System 69

Time (Mins.)	% H_2O	% ACN
0	95	5
15	95	5
15	75	25
5	65	35
2	0	100
10	0	100

Solvent System 70

Time (Mins.)	% H_2O	% ACN
0	80	20
10	70	30
15	65	35
10	60	40
3	50	50
2	0	100
10	0	100

TABLE III. DISTRIBUTION OF RADIOACTIVITY IN TOBACCO SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL

Sample	Days After Soil Treatment	Total ^{14}C-Residues Equivalent to PPM of [Phenyl-^{14}C]-Metalaxyl	Sample Date
Soil at Tobacco Planting			
0-3"	0	4.487	09/13/89
3-6"	0	0.955	09/13/89
6-8"	0	0.887	09/13/89
Soil at 4 Weeks Post Treatment			
0-3"	34	1.530	10/17/89
3-6"	34	0.811	10/17/89
6-8"	34	0.454	10/17/89
Soil at 75% Mature Harvest			
0-3"	170	1.278	03/02/90
3-6"	170	1.177	03/02/90
6-8"	170	1.016	03/02/90
Soil at Mature Tobacco Harvest			
0-3"	226	1.759	04/27/90
3-6"	226	1.584	04/27/90
6-8"	226	1.021	04/27/90
Control Soil (0-8")			
Soil at Tobacco Planting	--	<0.001	09/13/89
Soil at 4 Weeks Post Treatment	--	<0.001	10/17/89
Soil at 75% Mature Harvest	--	<0.001	03/02/90
Soil at Mature Tobacco Harvest	--	<0.001	04/27/90

TABLE IV. DISTRIBUTION OF RADIOACTIVITY IN ROTATIONAL CROP SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL

Sample	Days After Soil Treatment	Total ^{14}C-Residues Equivalent to PPM of [Phenyl-^{14}C]-Metalaxyl	Sample Date
Soil at Rotational Crop Planting			
0-3"	232	1.054	05/03/90
3-6"	232	0.672	05/03/90
6-8"	232	0.594	05/03/90
Soil at Mature Lettuce Harvest			
0-3"	292	1.153	07/02/90
3-6"	292	1.036	07/02/90
6-8"	292	0.424	07/02/90
Soil at Mature Wheat Harvest			
0-3"	323	0.992	08/02/90
3-6"	323	0.783	08/02/90
6-8"	323	0.340	08/02/90
Soil at Mature Soybean Harvest			
0-3"	432	0.788	11/19/90
3-6"	432	0.693	11/19/90
6-8"	432	0.486	11/19/90
Soil at Mature Sugar Beet Harvest			
0-3"	411	0.728	10/29/90
3-6"	411	0.527	10/29/90
6-8"	411	0.451	10/29/90
Control Soil (0-8")			
Soil at Rotation Crop Planting	--	<0.001	05/03/90
Soil at Mature Lettuce Harvest	--	<0.001	07/02/90
Soil at Mature Wheat Harvest	--	<0.001	08/02/90
Soil at Mature Soybean Harvest	--	<0.001	11/19/90
Soil at Mature Sugar Beet Harvest	--	<0.001	10/29/90

TABLE V. CHARACTERIZATION OF RADIOACTIVITY IN TARGET TOBACCO AND ROTATIONAL CROP SOIL SAMPLES[a]

Sample	PPM[b]	% Extractable	% Non-Extractable
Soil at Target Tobacco Planting			
0-3"	4.487	95.1 (97.3)	4.9 (5.0)
6-8"	0.887	91.6 (94.1)	8.4 (8.7)
Soil at Mature Tobacco Harvest			
0-3"	1.759	35.4 (29.8)	64.6 (54.3)
6-8"	1.021	30.3 (30.0)	69.7 (69.2)
Soil at Rotational Crop Planting			
0-3"	1.054	32.9 (36.7)	67.1 (74.9)
6-8"	0.594	38.5 (38.9)	61.5 (62.2)
Soil at Mature Soybean Harvest			
0-3"	0.788	29.6 (29.3)	70.4 (69.7)
6-8"	0.486	20.7 (19.5)	79.3 (74.7)

[a]Values in parentheses are actual experimental values and are not normalized.

[b]PPM equivalent to [phenyl-^{14}C]-metalaxyl.

TABLE VI. HPLC QUANTITATION OF EXTRACTABLE RADIOACTIVITY IN GREENHOUSE TARGET AND ROTATIONAL CROP SOIL SAMPLES

	% CGA-48988	% CGA-67869	% CGA-62826[1]	% CGA-107955	% CGA-108905	% CGA-37734	% CGA-62826 conjugate	% CGA-79353	% CGA-108906	% CGA-78532	% S-1
0-3" Soil at Target Tobacco Planting											
% of extractable	98.7		1.2								
% of total 14C	93.9		1.1								
total PPM	4.212		0.051								
6-8" Soil at Target Tobacco Planting											
% of extractable	98.3	1.0									
% of total 14C	90.0	0.9									
total PPM	0.799	0.008									
0-3" Soil at Mature Tobacco Harvest											
% of extractable	62.7		25.7	1.0	8.5						
% of total 14C	22.2		9.1	0.4	3.0						
total PPM	0.390		0.160	0.006	0.053						
6-8" Soil at Mature Tobacco Harvest											
% of extractable	54.8		27.8	2.6	9.1				2.3		1.5
% of total 14C	16.6		8.4	0.8	2.8				0.7		0.5
total PPM	0.170		0.086	0.008	0.028				0.007		0.005
0-3" Soil at Rotation Crop Planting											
% of extractable	52.6		27.8	2.8	7.2	2.5		1.0	2.6	1.5	1.9
% of total 14C	17.3		9.1	0.9	2.4	0.8		0.3	0.9	0.5	0.6
total PPM	0.182		0.096	0.010	0.025	0.009		0.003	0.009	0.005	0.007
6-8" Soil at Rotation Crop Planting											
% of extractable	38.9		36.7	3.6	9.9	3.5		2.0	2.2		1.7
% of total 14C	15.0		14.1	1.4	3.8	1.3		0.8	0.8		0.7
total PPM	0.089		0.084	0.008	0.023	0.008		0.005	0.005		0.004
0-3" Soil at Mature Soybean Harvest											
% of extractable	39.9		28.2	2.7	7.6	3.0		3.0	10.1	2.0	1.6
% of total 14C	11.8		8.3	0.8	2.2	0.9		0.9	3.0	0.6	0.5
total PPM	0.093		0.066	0.006	0.018	0.007		0.007	0.024	0.005	0.004
6-8" Soil at Mature Soybean Harvest											
% of extractable	20.3		48.0	4.3	5.1		8.8	4.1	5.6	1.3	1.8
% of total 14C	4.2		9.9	0.9	1.1		1.8	0.8	1.2	0.3	0.4
total PPM	0.020		0.048	0.004	0.005		0.009	0.004	0.006	0.001	0.002

[1]A small percentage (<2%) of the extractable radioactivity comigrating with CGA-62826 by HPLC could be CGA-67868 as quantitated by reversed phase 2D-TLC. Exceptions include the 6-8" soil at the mature soybean harvest (4%) and the 0-3" soil at rotational crop planting (2.5%).

TABLE VII. RADIOACTIVE RESIDUES IN GREENHOUSE TOBACCO GROWN IN SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL

Harvest Interval/ Plant Part	Days After Soil Treatment	Total ^{14}C-Residues Equivalent to PPM of [Phenyl-^{14}C]-Metalaxyl	Sample Date
4 Weeks Post Treatment			
Bottom Leaves	34	8.789	10/17/89
Middle Leaves	34	3.810	10/17/89
Top Leaves	34	2.784	10/17/89
75% Mature Harvest			
Bottom Leaves	170	1.238	03/02/90
Middle Leaves	170	0.885	03/02/90
Top Leaves	170	0.386	03/02/90
Mature Tobacco Harvest			
Bottom Leaves	226	2.739	04/27/90
Middle Leaves	226	2.230	04/27/90
Top Leaves	226	1.487	04/27/90

TABLE VIII. DISTRIBUTION OF RADIOACTIVITY AND EXTRACTION DATA FROM TOBACCO TREATED WITH [PHENYL-^{14}C]-METALAXYL[a]

Sample	PPM[b]	% Extractable	% Non-Extractable
4 Weeks Post Treatment			
Bottom Leaves	8.789	93.0 (97.8)	7.0 (7.3)
Top Leaves	2.784	92.9 (95.8)	7.1 (7.4)
Mature Harvest			
Bottom Leaves	2.739	91.5 (81.1)	8.5 (7.5)
Top Leaves	1.487	91.1 (92.3)	8.9 (9.1)

[a]Values in parentheses are actual experimental values and are not normalized.

[b]PPM equivalent to [phenyl-^{14}C]-metalaxyl.

TABLE IX. CHARACTERIZATION OF RADIOACTIVITY IN THE TOBACCO TARGET CROP

	4 Week Post Treatment Tobacco Combined Top and Bottom Leaves	Mature Tobacco Combined Top and Bottom Leaves
Average PPM	6.223	1.651
Average % Extractable	93.0	91.2
% Organosoluble	67.8	56.7
% Aqueous Soluble	32.2	43.3

2D-TLC Quantitation of the Organic Fraction

Metabolite	% Organic	% Extractable	% Total	ppm[a]	% Organic	% Extractable	% Total	ppm[a]
CGA-48988	79.7	54.0	50.3	3.127	69.9	39.6	36.1	0.596
CGA-107955	7.0	4.7	4.4	0.274	8.3	4.7	4.3	0.071
N7[b]	10.0	6.8	6.3	0.392	17.6	10.0	9.1	0.150
CGA-94689B	1.2	0.8	0.8	0.047	--	--	--	--
N4/N5[c]	--	--	--	--	1.5	0.9	0.8	0.013
CGA-119857	--	--	--	--	1.0	0.6	0.5	0.009
Total Organic	97.9	66.3	61.8	3.840	98.3	55.8	50.8	0.839

2D-TLC Quantitation of Release Products in the Aqueous Fraction after β-glucosidase Enzyme Hydrolyses

Aglycone Released	% Aqueous	% Extractable	% Total	ppm[a]	% Aqueous	% Extractable	% Total	ppm[a]
CGA-94689A	5.8	1.9	1.7	0.106	4.5	1.9	1.8	0.030
CGA-94689B/N1a aglycone	11.0	3.5	3.3	0.205	8.4	3.6	3.3	0.054
CGA-100255	8.8	2.8	2.6	0.162	4.0	1.7	1.6	0.026
CGA-37734	1.6	0.5	0.5	0.031	3.5	1.5	1.4	0.023
CGA-62826	1.4	0.5	0.4	0.025	3.6	1.6	1.4	0.023
CGA-107955	2.7	0.9	0.8	0.050	1.6	0.7	0.6	0.010
CGA-107955[d]	(6.5)	(2.1)	(1.9)	(0.118)	(4.2)	(1.8)	(1.7)	(0.028)
Total Free + Released in Aqueous	35.1	11.3	10.4	0.647	28.2	12.1	11.2	0.184

[a]PPM are expressed in ppm equivalent to [phenyl-^{14}C]-metalaxyl.
[b]N7 is the glucose conjugate of CGA-107955.
[c]N4/N5 are the glucose conjugates of CGA-94689A/B.
[d]Numbers in parentheses represent the total, free + released, percentages and ppm of CGA-107955 in the aqueous fraction treated with β-glucosidase.

TABLE X. RADIOACTIVE RESIDUES IN GREENHOUSE ROTATIONAL CROPS GROWN IN SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL

Samples	Days After Soil Treatment	Total ^{14}C-Residues Equivalent to PPM of [Phenyl-^{14}C]-Metalaxyl	Days Post Planting	Sample Date
Lettuce				
50% Mature Foliage	261	0.877	29	06/01/90
100% Mature Foliage	292	0.564	60	07/02/90
Spring Wheat				
25% Mature Stalks	254	5.117	22	05/25/90
50% Mature Stalks	279	2.578	47	06/19/90
100% Mature Stalks	323	7.171	91	08/02/90
100% Mature Grain	323	0.593	91	08/02/90
100% Mature Hulls	323	7.762	91	08/02/90
Soybeans				
25% Mature Stalks	261	2.424	29	06/01/90
50% Mature Stalks	292	2.702	60	07/02/90
100% Mature Stalks	432	3.612	200	11/19/90
100% Mature Pods	432	1.061	200	11/19/90
100% Mature Beans	432	0.398	200	11/19/90
Sugar Beets				
25% Mature Foliage	271	1.125	39	06/11/90
50% Mature Foliage	307	0.856	75	07/17/90
50% Mature Roots	307	0.291	75	07/17/90
100% Mature Foliage	411	1.102	179	10/29/90
100% Mature Roots	411	0.275	179	10/29/90

TABLE XI. UPTAKE AND DISTRIBUTION OF RADIOACTIVITY IN FIELD GROWN ROTATIONAL CROPS FOLLOWING A POTATO TARGET CROP TREATED WITH [PHENYL-^{14}C]-METALAXYL[a]

Rotation Crop	Days Since 1st Treatment at Planting	PPM[b] in 0-3" Soil at Rotational Crop Planting	Days Since 1st Treatment at Mature Harvests	PPM[b] in Mature Plant Sample
Sugar Beets	315	0.33	455	Foliage-0.02 Roots-0.02
Lettuce	315	0.30	392	Foliage-0.05
Corn	329	0.29	476	Stalks-0.06 Cobs-0.02 Grain-0.03
Soybeans	336	0.34	476	Stalks-0.59 Beans-0.17
Winter Wheat	84	0.74	385	Stalks-0.56 Grain-0.11
Spring Oats	315	0.33	413	Stalks-0.19 Grain-0.09

[a]Data were extrapolated from ABR-79005 (sugar beets)[18], ABR-78078 (lettuce)[19], ABR-79003 (soybeans)[21], ABR-78077 (winter wheat)[20], ABR-79004 (corn)[22], and ABR-79002 (spring oats)[23]. The potato target crop was sprayed postemergence six times with [phenyl-^{14}C]-metalaxyl at a rate of 0.40 lb. a.i./A at 14-day intervals for a total application rate of 2.4 lbs. a.i./A. The first spray treatment was 6 weeks after plant emergence. The plots were located at the CIBA-GEIGY Northeast Research Station in Livingston, New York and the soil was a silt loam.

[b]PPM are expressed in ppm equivalent to [phenyl-^{14}C]-metalaxyl.

TABLE XII. DISTRIBUTION OF RADIOACTIVITY AND EXTRACTION DATA FROM GREENHOUSE ROTATIONAL CROPS GROWN IN SOIL TREATED WITH [PHENYL-^{14}C]-METALAXYL[a]

Sample	PPM[b]	% Extractable	% Non-Extractable
Lettuce			
50% Mature Foliage	0.877	92.6 (91.0)	7.4 (7.3)
100% Mature Foliage	0.564	90.8 (78.1)	9.2 (7.9)
Spring Wheat			
25% Mature Stalks	5.117	88.8 (79.3)	11.2 (10.0)
100% Mature Stalks	7.171	79.2 (73.6)	20.8 (19.3)
100% Mature Grain	0.593	22.0 (21.1)	78.0 (74.6)
100% Mature Hulls	7.762	60.8 (55.5)	39.2 (35.7)
Soybeans			
25% Mature Stalks	2.424	93.6 (91.1)	6.4 (6.2)
100% Mature Stalks	3.612	88.2 (90.2)	11.8 (12.1)
100% Mature Pods	1.061	81.6 (78.3)	18.4 (17.6)
100% Mature Beans	0.398	79.4 (74.9)	20.6 (19.4)
Sugar Beets			
25% Mature Foliage	1.125	97.5 (116.1)	2.5 (3.0)
100% Mature Foliage	1.102	88.1 (92.8)	11.9 (12.5)
100% Mature Roots	0.275	62.5 (59.6)	37.5 (35.8)

[a]Values in parentheses are actual experimental values and are not normalized.
[b]PPM are expressed in ppm equivalent to [phenyl-^{14}C]-metalaxyl.

TABLE XIII. PERCENT OF EXTRACTABLE RADIOACTIVITY RELEASED AS HYDROLYSIS PRODUCTS OF GLUCOSE CONJUGATES OF [PHENYL-^{14}C]-METALAXYL METABOLITES AFTER ENZYME TREATMENTS WITH β-GLUCOSIDASE[1]

Released Aglycone	Mature Target Tobacco Bottom Leaves	Mature Lettuce Foliage	Mature Wheat Stalk	Mature Wheat Hull	Mature Soybean Stalk	Mature Soybean Pods
CGA-94689A	4.3	--	16.4	19.5	2.2	1.5
CGA-94689B/ N1a aglycone	8.0	6.6	27.4	18.4	8.2	2.5
CGA-100255	5.2	8.9	--	--	--	--
CGA-37734	2.0	--	7.2	6.7 (9.0)	1.7	--
N1b aglycone	--	11.9	--	--	--	--
CGA-62826	2.4	2.7	2.7	5.5	22.4	14.3 (33.2)
CGA-107955	-- (9.4)	--	--	1.4	--	--
CGA-108905	--	-- (2.0)	2.3	--	5.4	1.6 (10.7)

[1]Numbers in parentheses represent total (free + released) percentages of aglycone in the filtered filtrate fraction treated with β-glucosidase.

TABLE XIV. CHARACTERIZATION AND IDENTIFICATION OF [PHENYL-^{14}C]-METALAXYL METABOLITES IN THE ORGANIC FRACTION OF MATURE SUGAR BEET ROOTS

Total ^{14}C PPM[1]	0.275
% Total in Extractable	61.5
% Extractable in Organic	23.9
% Extractable in Aqueous	76.1

HPLC Quantitation of the Organic Fraction

Metabolite	% Organic	% Extractable	Total % ^{14}C	PPM[1]
CGA-62826	36.8[2]	8.8	5.4	0.015
Metalaxyl	22.2	5.3	3.3	0.009
CGA-108905	15.9	3.8	2.3	0.006
CGA-94689A/B/ CGA-107955	7.4	1.8	1.1	0.003
N7 (Glucose Conjugate of CGA-107955)[3]	6.5	1.6	1.0	0.003
Total	88.8	21.3	13.1	0.036

[1]PPM are expressed in ppm equivalent to [phenyl-^{14}C]-metalaxyl.

[2]Approximately 4% of the 36.8% quantitated as CGA-62826 is CGA-67868, as determined by 2D-TLC in solvent systems 9 and 9.

[3]N7 co-migrates with CGA-108905 by HPLC and was quantitated after separation by 2D-TLC in solvent systems 1 and 2.

TABLE XV. CHARACTERIZATION AND IDENTIFICATION OF [PHENYL-^{14}C]-METALAXYL METABOLITES IN THE AQUEOUS FRACTION OF MATURE SUGAR BEET ROOTS

Total ^{14}C ppm[1]	0.275
% Total in Extractable	61.5
% Extractable in Organic	23.9
% Extractable in Aqueous	76.1

2D-TLC Quantitation of Metabolites in Aqueous Fraction

Metabolite	Control		Cellulase		6M HCl			
	% Aqueous	% Extractable	% Aqueous	% Extractable	% Aqueous	% Extractable	% Total ^{14}C	ppm[1]
CGA-62826	1.8	1.4	8.1	6.2	43.0	32.7	20.1	0.055
CGA-108905	1.6	1.2	1.5	1.1	1.9	1.5	0.9	0.003
CGA-79353	16.9	12.9	17.8	13.6	17.3	13.2	8.1	0.022
CGA-108906	4.5	3.4	7.1	5.4	--	--	--	--
Region 1	31.0	23.6	35.8	27.2	15.7	12.0	7.4	0.020
Region 2	16.0	12.2	11.4	8.7	9.9	7.5	4.6	0.013
Region 3	14.2	10.8	15.4	11.7	6.9	5.3	3.3	0.009
Region 4	9.1	6.9	--	--	2.3	1.8	1.1	0.003
Total	95.1	72.4	97.1	73.9	97.0	74.0	45.4	0.125

[1]PPM are expressed in ppm equivalent to [phenyl-^{14}C]-metalaxyl.

TABLE XVI. CHARACTERIZATION AND IDENTIFICATION OF [PHENYL-^{14}C]-METALAXYL METABOLITES IN 50% MATURE LETTUCE FOLIAGE

Total ^{14}C PPM[1]	0.877
% Total in Extractable	91.1
% Extractable in Organic	23.4
% Extractable in Aqueous	76.6
% Aqueous in Neutrals-N	48.2
% Aqueous in Acidics-LA	51.8

HPLC Quantitation of Metabolites in the Organic Fraction

Organosoluble Metabolites	% Organic	% Extractable	% Total ^{14}C	ppm[1]
LU1 (unknown)	1.7	0.4	0.4	0.004
N1b aglycone (phenyl ring hydroxy of CGA-67868)	1.7	0.4	0.4	0.004
LU2 (unknown)	2.7	0.6	0.6	0.005
LU3 (unknown)	1.3	0.3	0.3	0.003
LU4 (unknown)	1.1	0.3	0.2	0.002
CGA-108905 + N7 (glucose conj. of CGA-107955)	4.4	1.0	0.9	0.008
CGA-107955	5.9	1.4	1.3	0.011
CGA-94689B	2.5	0.6	0.5	0.004
CGA-100255 + CGA-67868 + CGA-62826	4.2	1.0	0.9	0.008
CGA-48988	71.8	16.8	15.3	0.134
Total	97.3	22.8	20.8	0.183

HPLC Quantitation of Metabolites in the Neutral-N Fraction

Aqueous Soluble Neutral Metabolites	% Neutral	% Aqueous	% Extractable	% Total ^{14}C	ppm[1]
N1b (glucose conj. of phenyl ring hydroxy of CGA-67868)	46.1	22.2	17.0	15.5	0.136
N2 (glucose conj. of CGA-37734)	5.1	2.5	1.9	1.7	0.015
N3 (glucose conj. of CGA-100255)	32.9	15.9	12.1	11.1	0.097
N4 (post. glucose conj. of CGA-94689A)	0.5	0.2	0.2	0.2	0.002
N5 (glucose conj. of CGA-94689B)	5.6	2.7	2.1	1.9	0.017
N6 (glucose conj. of CGA-62826)	4.5	2.2	1.7	1.5	0.013
N7 (glucose conj. of CGA-107955)	5.3	2.6	2.0	1.8	0.016
Total	100.0	48.3	37.0	33.7	0.296

(Continued on next page)

TABLE XVI. CHARACTERIZATION AND IDENTIFICATION OF [PHENYL-^{14}C]-METALAXYL METABOLITES IN 50% MATURE LETTUCE FOLIAGE (Continued)

HPLC Quantitation of Metabolites in the Acidic-LA Fraction

Aqueous Soluble Acidic Metabolites	% Acidic-LA	% Aqueous	% Extractable	% Total ^{14}C	ppm[1]
LA1]2	5.0	2.6	2.0	1.8	0.016
LA2	42.5	22.0	16.9	15.4	0.135
LA3	36.8	19.1	14.6	13.3	0.117
LA4	15.7	8.1	6.2	5.7	0.050
Total	100.0	51.8	39.7	36.2	0.318
Summary					
Total (Organic)			22.8	20.8	0.183
Total (Neutral-N)			37.0	33.7	0.296
Total (Acidic-LA)			39.7	36.2	0.318
Total (Aqueous) (Neutrals + Acidics)			76.7	69.9	0.614
Total (Extractable) (Organic + Aqueous)			99.5	90.7	0.797

[1]PPM are expressed in ppm equivalent to [phenyl-^{14}C]-metalaxyl.

[2]HPLC retention times similar to aqueous soluble acidic metabolites in fractions B-II and B-III isolated from mature wheat stalks (Table XVII).

TABLE XVII. CHARACTERIZATION AND IDENTIFICATION OF [PHENYL-^{14}C]-METALAXYL METABOLITES IN MATURE SPRING WHEAT STALKS

Total ^{14}C PPM[1]	7.171
% Total Extractable	74.5
% Extractable in Organic	10.5
% Extractable in Aqueous	89.5
% Aqueous in Neutrals-N	69.0
% Aqueous in Acidics-B	25.3
% Aqueous in Acidics-C	5.7
% Acidics-B in Acidics-BI	27.4
% Acidics-B in Acidics-BII	27.7
% Acidics-B in Acidics-BIII	44.9

HPLC Quantitation of Metabolites in the Organic Fraction

Organosoluble Metabolites	% Organic	% Extractable	% Total ^{14}C	ppm[1]
U0 (unknown)	0.3	<0.1	<0.1	0.002
N1a aglycone (benzyl alcohol of CGA-67868)	10.4	1.1	0.8	0.057
N4/N5 (glucose conjs. of CGA-94689A/B)	15.9	1.7	1.2	0.086
CGA-37734	12.5	1.3	1.0	0.072
CGA-108905	12.3	1.3	1.0	0.072
CGA-107955	5.7	0.6	0.4	0.029
CGA-94689A/B	24.7	2.6	1.9	0.136
CGA-100255	3.8	0.4	0.3	0.022
CGA-67868	3.1	0.3	0.2	0.014
CGA-62826	8.6	0.9	0.7	0.050
CGA-67869	1.9	0.2	0.1	0.007
CGA-48988	0.7	0.1	0.1	0.007
Total	99.9	10.5	7.8	0.559

(Continued on Next Page)

TABLE XVII. CHARACTERIZATION AND IDENTIFICATION OF ^{14}C-METALAXYL METABOLITES IN MATURE SPRING WHEAT STALKS (Continued)

HPLC Quantitation of Metabolites in the Neutral-N Fraction

Aqueous Soluble Neutral Metabolites	% Neutral	% Aqueous	% Extractable	% Total ^{14}C	ppm[1]
N1a (glucose conj. of benzyl alcohol of CGA-67868)	13.8	9.5	8.5	6.3	0.452
N2 (glucose conj. of CGA-37734)	9.3	6.4	5.7	4.3	0.308
N3 (glucose conj. of CGA-100255)	2.9	2.0	1.8	1.3	0.093
N4 (glucose conj. of CGA-94689A)	26.5	18.3	16.4	12.2	0.875
N5 (glucose conj. of CGA-94689B)	39.9	27.5	24.6	18.4	1.319
N6a/N6b (glucose conjs. of CGA-62826)	4.3	3.0	2.7	2.0	0.143
N7 (glucose conj. of CGA-107955)	3.2	2.2	2.0	1.5	0.108
N8 (unknown)	0.1	0.1	0.1	<0.1	0.003
Total	100.0	69.0	61.8	46.0	3.299

HPLC Quantitation of Metabolites in the Acidic-B Fraction

Aqueous Soluble Acidic B-I Metabolites	% Acidic-BI	% Acidic-B	% Aqueous	% Extractable	% Total ^{14}C	ppm[1]
U1 (unknown)	0.2	0.1	<0.1	<0.1	<0.1	0.001
N1a aglycone (benzyl alcohol of CGA-67868)	11.7	3.2	0.8	0.7	0.5	0.036
U2 (unknown)	0.7	0.2	<0.1	<0.1	<0.1	0.002
CGA-37734	5.4	1.5	0.4	0.3	0.2	0.014
CGA-94689A/B	77.8	21.3	5.4	4.8	3.6	0.258
CGA-100255	4.2	1.2	0.3	0.3	0.2	0.014
Subtotal	100.0	27.4	6.9	6.2	4.6	0.330

Aqueous Soluble Acidic B-II Metabolites	% Acidic-BII	% Acidic-B	% Aqueous	% Extractable	% Total ^{14}C	ppm[1]
U3 (unknown)	3.0	0.8	0.2	0.2	0.1	0.007
B1 (benzyl alcohol of CGA-107955)	48.2	13.4	3.4	3.0	2.3	0.165
B2 (benzyl alcohol of CGA-107955)	48.8	13.5	3.4	3.1	2.3	0.165
Subtotal	100.0	27.7	7.0	6.3	4.7	0.337

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TABLE XVII. CHARACTERIZATION AND IDENTIFICATION OF [PHENYL-^{14}C]-METALAXYL METABOLITES IN MATURE SPRING WHEAT STALKS (Continued)

Aqueous Soluble Acidic B-III Metabolites	% Acidic-BIII	% Acidic-B	% Aqueous	% Extractable	% Total ^{14}C	ppm[1]
U4 (unknown)	9.5	4.3	1.1	1.0	0.7	0.050
U5 (unknown)	10.7	4.8	1.2	1.1	0.8	0.057
P0 (benzyl alcohol of CGA-62826)	34.0	15.3	3.9	3.5	2.6	0.186
U6 (unknown)	4.9	2.2	0.6	0.5	0.4	0.029
U7 (unknown)	5.7	2.6	0.6	0.6	0.4	0.029
CGA-119857	4.8	2.2	0.5	0.5	0.4	0.029
CGA-108905	13.2	5.9	1.5	1.3	1.0	0.072
CGA-107955	9.7	4.4	1.1	1.0	0.7	0.050
CGA-94689B	0.8	0.4	0.1	0.1	0.1	0.007
CGA-62826	6.8	3.1	0.8	0.7	0.5	0.036
Subtotal	100.1	44.9	11.4	10.3	7.6	0.550
Total (Acidic-B)	--	100.0	25.3	22.8	16.9	1.212
SUMMARY						
Total (Organic)				10.5	7.8	0.559
Total (Neutral-N)				61.8	46.0	3.299
Total (Acidic-B)				22.8	16.9	1.212
Total (Aqueous) (Neutrals + Acidics)				84.6	62.9	4.511
Total (Extractable) (Organic + Aqueous)				95.1	70.7	5.070

[1]PPM are expressed in ppm equivalent to [phenyl-^{14}C]-metalaxyl. All ppm values, including totals, were calculated by multiplying the following: (% Total ^{14}C) X (7.171/100).

TABLE XVIII. CHARACTERIZATION OF NON-EXTRACTABLE RADIOACTIVITY RELEASED BY ENZYME AND ACID HYDROLYSES FROM SELECTED ROTATIONAL CROP SAMPLES

Non-Extractable Fraction (Filtercake)	% Total ^{14}C	ppm[1]
Mature Wheat Stalks	20.8	1.492
Mature Wheat Grain	78.0	0.463
Mature Sugar Beet Roots	37.5	0.103

	Mature Wheat Stalk			Mature Wheat Grain			Mature Sugar Beet Root		
Enzyme Hydrolysis Experiment	% Non-Extractable Released	% Total ^{14}C	ppm[1]	% Non-Extractable Released	% Total ^{14}C	ppm[1]	% Non-Extractable Released	% Total ^{14}C	ppm[1]
Protease Treated	52.9	11.0	0.789	61.4	47.9	0.284	33.2	12.4	0.034
Protease Control	50.3	10.5	0.753	60.0	46.8	0.278	34.0	12.8	0.035
Cellulase Treated	43.4	9.0	0.645	64.3[2]	50.2	0.298	31.3	11.7	0.032
Cellulase Control	32.9[2]	6.8	0.488	52.3	40.8	0.242	28.5	10.7	0.029
β-Glucosidase Treated	42.2[3]	8.8	0.631	57.5	44.9	0.266	33.4	12.5	0.034
β-Glucosidase Control	35.0	7.3	0.523	53.6	41.8	0.248	31.9	12.0	0.033
Dilute Acid Hydrolysis Experiment									
0.1M HCl	66.4	16.9[5]	1.212	65.6	51.2	0.304	26.0	9.8	0.027
Concentrated Acid Hydrolysis Experiment									
6.0M HCl	--	--	--	74.0	57.7	0.342	59.3	22.8[4]	0.063
6.0M HNO_3	--	--	--	--	--	--	94.9	36.5[4]	0.100
16.0M HNO_3	100.0	25.5[5]	1.829	100.0	78.0	0.463	--	--	--

[1]PPM are expressed in PPM equivalent to [phenyl-^{14}C]-metalaxyl.
[2]Value is not normalized but is the actual value calculated from the radioassay of the supernatant.
[3]Value is not normalized but is the actual value calculated from the combustion of the residue.
[4]The % total ^{14}C values were calculated using the % non-extractable value of 38.5% (0.106 ppm) obtained from the preparative (200 g) extraction of mature sugar beet roots vs. the % non-extractable value of 37.5% (0.103 ppm) obtained from the analytical (100 g) extraction.
[5]The % total ^{14}C values were calculated using the % non-extractable value of 25.5% (1.829 ppm) obtained from the preparative (101 g) extraction of mature wheat stalks vs. the % non-extractable value of 20.8% (1.492 ppm) obtained from the analytical (20 g) extraction.

TABLE XIX. CHARACTERIZATION AND IDENTIFICATION OF NON-EXTRACTABLE [PHENYL-^{14}C]-METALAXYL METABOLITES RELEASED BY ENZYME HYDROLYSES FROM MATURE WHEAT STALK RESIDUES

Non-Extractable Fraction	% Released Radioactivity	% Non-Extractable	% Total ^{14}C[1]	ppm[2]
Original Non-Extractable	---	---	25.5	1.829
PROTEASE HYDROLYSIS				
Released Radioactivity	---	71.5	18.2	1.305
Organosolubles Released	43.5	31.1	7.9	0.567
Aqueous Solubles Released	56.5	40.4	10.3	0.739
β-GLUCOSIDASE HYDROLYSIS OF PROTEASE AQUEOUS				
Organosolubles Released	5.1	3.6	0.9	0.065
Aqueous Solubles Released	51.4	36.8	9.4	0.674

HPLC Quantitation of Metabolites in the Non-Extractable/ Filtercake Fraction	Protease Organic Fraction			Protease β-Glucosidase Organic Fraction			Total		
	% Organic	% Non-Extractable	% Total ^{14}C[1]	% Organic	% Non-Extractable	% Total ^{14}C[1]	% Non-Extractable	% Total ^{14}C[1]	ppm[2]
CGA-48988	15.7	4.9	1.2	3.4	0.1	<0.1	5.0	1.3	0.093
CGA-67868	1.7	0.5	0.1	---	---	---	0.5	0.1	0.007
CGA-62826/CGA-100255	2.1	0.7	0.2	13.3	0.5	0.1	1.1	0.3	0.022
CGA-94689A/B	65.0	20.2	5.2	28.9	1.0	0.3	21.3	5.4	0.387
CGA-107955	---	---	---	12.5	0.5	0.1	0.5	0.1	0.007
CGA-108905	---	---	---	7.9	0.3	0.1	0.3	0.1	0.007
CGA-37734	8.9	2.8	0.7	12.9	0.5	0.1	3.2	0.8	0.057
CGA-119857	---	---	---	1.7	0.1	<0.1	0.1	<0.1	0.001
N1a/N1b aglycones	3.9	1.2	0.3	8.6	0.3	0.1	1.5	0.4	0.029
TOTAL	97.3	30.3	7.7	89.2	3.2	0.8	33.5	8.5	0.610

[1]The % total ^{14}C values were calculated using the % non-extractable value of 25.5% (1.829 ppm) obtained from the preparative (101 g) extraction of mature wheat stalks vs. the % non-extractable value of 20.8% (1.492 ppm) obtained from the analytical (20 g) extraction.

[2]PPM are expressed in PPM equivalent to [phenyl-^{14}C]-metalaxyl.

TABLE XX. CHARACTERIZATION AND IDENTIFICATION OF NON-EXTRACTABLE [PHENYL-^{14}C]-METALAXYL METABOLITES RELEASED BY ENZYME AND ACID HYDROLYSES FROM MATURE WHEAT GRAIN

Non-Extractable Fraction	% Released Radioactivity	% Non-Extractable	% Total ^{14}C	ppm[1]
Original Non-Extract.	---	---	78.0	0.463
CELLULASE HYDROLYSIS				
Released Hydrolysis	---	61.7	48.1	0.285
Organosolubles Released	24.1	14.9	11.6	0.069
Aqueous Solubles Released	75.9	46.8	36.5	0.216

Non-Extractable Fraction	% Released Radioactivity	% Non-Extractable	% Total ^{14}C	ppm[1]
Original Non-Extractable	---	---	78.0	0.463
0.1M HCl HYDROLYSIS				
Released Radioactivity	---	65.6	51.2	0.304
Organosolubles Released	40.9	26.9	21.0	0.125
Aqueous Solubles Released	59.1	38.8	30.3	0.180
Organic Upper Band	24.5	16.1	12.6	0.075
Organic Lower Band	16.4	10.8	8.4	0.050

2D-TLC Quantitation of Metabolites in the Non-Extractable/ Filtercake Fraction

	CELLULASE ORGANIC FRACTION				CELLULASE AQUEOUS FRACTION				TOTAL		
	% Organic	% Non-Extractable	% Total ^{14}C	ppm[1]	% Aqueous	% Non-Extractable	% Total ^{14}C	ppm[1]	% Non-Extractable	% Total ^{14}C	ppm[1]
CGA-108905	86.2	12.8	10.0	0.059	13.1	6.1	4.8	0.028	19.0	14.8	0.088
CGA-79353	---	---	---	---	1.2	0.5	0.4	0.002	0.5	0.4	0.002
TOTAL	86.2	12.8	10.0	0.059	14.2	6.7	5.2	0.030	19.5	15.2	0.090

HPLC Quantitation of Metabolites in the Non-Extractable/ Filtercake Fraction

	0.1M HCl HYDROLYSIS ORGANIC REGION 2				0.1M HCl HYDROLYSIS ORGANIC REGION 1				0.1M HCl HYDROLYSIS AQUEOUS FRACTION				TOTAL		
	% Organic	% Non-Extractable	% Total ^{14}C	ppm[1]	% Organic	% Non-Extractable	% Total ^{14}C	ppm[1]	% Aqueous	% Non-Extractable	% Total ^{14}C	ppm[1]	% Non-Extractable	% Total ^{14}C	ppm[1]
CGA-67866	--	--	--	--	--	--	--	--	1.8	0.7	0.5	0.003	0.7	0.5	0.003
CGA-48988/CGA-67869	--	--	--	--	--	--	--	--	3.1	1.2	0.9	0.005	1.2	0.9	0.005
CGA-62826	--	--	--	--	--	--	--	--	1.2	0.5	0.4	0.002	0.5	0.4	0.002
CGA-108905	59.3	16.0	12.5	0.074	--	--	--	--	21.1	8.2	6.4	0.038	24.1	18.8	0.112
CGA-119857/CGA-79353	--	--	--	--	13.4	3.6	2.8	0.017	9.1[2]	3.5	2.8	0.017	7.1	5.6	0.033
CGA-108906	---	---	---	---	7.2	1.9	1.5	0.009	---	---	---	---	1.9	1.5	0.009
TOTAL	59.3	16.0	12.5	0.074	20.5	5.5	4.3	0.026	36.3	14.1	11.0	0.065	35.6	27.7	0.164

[1]PPM are expressed in PPM equivalent to [phenyl-^{14}C]-metalaxyl
[2]PPM are [2]CGA-119857 and CGA-79353 were quantitated by normal phase 2D-TLC due to comigration of other metabolites with CGA-119857 and CGA-79353 by HPLC.

TABLE XXI. CHARACTERIZATION AND IDENTIFICATION OF NON-EXTRACTABLE [PHENYL-^{14}C]-METALAXYL METABOLITES RELEASED BY ACID HYDROLYSIS FROM MATURE SUGAR BEET ROOTS

Non-Extractable Fraction	% Released Radioactivity	% Non-Extractable	% Total ^{14}C	ppm[2]
Original Non-Extractable	---	---	38.5	0.106
6M HCl HYDROLYSIS				
Released Radioactivity	---	59.3	22.8	0.063
Organosolubles Released	41.1	24.4	9.4	0.026
Aqueous Solubles Released	58.9	34.9	13.4	0.037

2D-TLC or HPLC Quantitation of Metabolites in the Non-Extractable/ Filtercake Fraction[1]	6M HCl HYDROLYSIS ORGANIC FRACTION				6M HCl HYDROLYSIS AQUEOUS FRACTION				TOTAL		
	% Organic	% Non-Extractable	% Total ^{14}C	ppm[2]	% Aqueous	% Non-Extractable	% Total ^{14}C	ppm[2]	% Non-Extractable	% Total ^{14}C	ppm[2]
CGA-48988/CGA-67869/ CGA-67868	38.5	9.4	3.6	0.010	8.4[3]	2.9	1.1	0.003	12.3	4.7	0.013
CGA-62826/CGA-108905	39.0	9.5	3.7	0.010	11.9[4]	4.2	1.6	0.004	13.7	5.3	0.015
CGA-107955/CGA-119857	3.3	0.8	0.3	0.001	3.8	1.3	0.5	0.001	2.1	0.8	0.002
CGA-108906/CGA-79353	8.2	2.0	0.8	0.002	7.9	2.8	1.1	0.003	4.8	1.8	0.005
TOTAL	89.0	21.7	8.4	0.023	32.0	11.2	4.3	0.012	32.9	12.7	0.035

[1] The organic fraction was quantitated by normal phase 2D-TLC while the aqueous fraction was quantitated by HPLC.
[2] PPM are expressed in PPM equivalent to [phenyl-^{14}C]-metalaxyl.
[3] CGA-67868 is quantitated with the CGA-62826/CGA-108905 metabolites.
[4] Some of the 11.9% quantitated may include CGA-100255 and CGA-67868.

TABLE XXII. SUMMARY OF THE CHARACTERIZATION AND IDENTIFICATION OF [PHENYL-^{14}C]-METALAXYL METABOLITES IN WHEAT, LETTUCE AND SUGAR BEET ROTATIONAL CROPS

	SMALL GRAIN CROP		LEAFY VEGETABLE CROP		ROOT CROP	
	Mature Wheat Stalk		50% Mature Lettuce Foliage		Mature Sugar Beet Root	
	% Total ^{14}C	ppm[1]	% Total ^{14}C	ppm[1]	% Total ^{14}C	ppm[1]
PHASE 1 METABOLITES						
CGA-48988	0.1	0.007	15.3	0.134	3.3	0.009
CGA-67869	0.1	0.007	---	---	---	---
CGA-62826	1.2	0.086	---	---	[6.2	[0.017
CGA-67868	0.2	0.014	[0.9	[0.008		
CGA-100255	0.5	0.036			---	---
CGA-94689A/B	5.6	0.402	0.5	0.004	[1.1	[0.003
CGA-107955	1.1	0.079	1.3	0.011		
CGA-108905	2.0	0.143	0.9[2]	0.008	3.0	0.008
CGA-37734	1.2	0.086	---	---	---	---
CGA-119857	0.4	0.029	---	---	---	---
CGA-79353	---	---	---	---	7.9	0.022
CGA-108906	---	---	---	---	2.1	0.006
N1b aglycone (phenyl ring hydroxy of CGA-67868)[3]	---	---	0.4	0.004	---	---
N1a aglycone (benzyl alcohol of CGA-67868)[3]	1.3	0.093	---	---	---	---
NEUTRAL AQUEOUS METABOLITES						
N7 (glucose conj. of CGA-107955)	1.5	0.108	1.8	0.016	1.0	0.003
N6a/N6b (glucose conjs. of CGA-62826)	2.0	0.143	1.5	0.013	---	---
N5 (glucose conj. of CGA-94689B)	19.6[4]	1.406	1.9	0.017	---	---
N4 (glucose conj. of CGA-94689A)	12.2	0.875	0.2	0.002	---	---
N3 (glucose conj. of CGA-100255)	1.3	0.093	11.[illegible]	0.097	---	---
N2 (glucose conj. of CGA-37734)	4.3	0.308	1.7	0.015	---	---
N1b (glucose conj. of phenyl ring hydroxy of CGA-67868)[3]	---	---	15.5	0.136	---	---
N1a (glucose conj. of benzyl alcohol of CGA-67868)[3]	6.3	0.452	---	---	---	---
ACIDIC AQUEOUS METABOLITES						
B1 (benzyl alcohol of CGA-107955)	2.3	0.165	---	---	---	---
B2 (benzyl alcohol of CGA-107955)	2.3	0.165	---	---	---	---
P0 (benzyl alcohol of CGA-62826)	2.6	0.186	---	---	---	---
LA1]5	---	---	1.8	0.016	---	---
LA2	---	---	15.4	0.135	---	---
LA3	---	---	13.3	0.117	---	---
LA4	---	---	5.7	0.050	---	---
COMPLEX CONJUGATES FROM EXTRACTABLES						
Complex conj. of CGA-62826	---	---	---	---	19.3	0.053
Complex conj. of CGA-108905	---	---	---	---	0.1	<0.001
Complex conj. of CGA-79353	---	---	---	---	0.2	0.001
COMPLEX CONJUGATES FROM NON-EXTRACTABLES						
Complex conj. of CGA-48988	1.3	0.093	---	---	[4.7[6]	[0.013
Complex conj. of CGA-67868	0.1	0.007	---	---		
Complex conj. of CGA-62826/CGA-100255	0.3	0.022	---	---	[5.3[7]	[0.015
Complex conj. of CGA-108905	0.1	0.007	---	---		
Complex conjs. of CGA-94689A/B	5.4	0.387	---	---	---	---
Complex conj. of CGA-107955	0.1	0.007	---	---	[0.8	[0.002
Complex conj. of CGA-119857	<0.1	0.001	---	---		
Complex conj. of CGA-37734	0.8	0.057	---	---	---	---
Complex conjs. of N1a/N1b aglycones	0.4	0.029	---	---	---	---
Complex conjs. of CGA-108906/CGA-79353	---	---	---	---	1.8	0.005

(Continued on next page)

TABLE XXII. <u>SUMMARY OF THE CHARACTERIZATION AND IDENTIFICATION OF [PHENYL-^{14}C]-METALAXYL METABOLITES IN WHEAT, LETTUCE AND SUGAR BEET ROTATIONAL CROPS</u> (Continued)

	SMALL GRAIN CROP		LEAFY VEGETABLE CROP		ROOT CROP	
	Mature Wheat Stalk		50% Mature Lettuce Foliage		Mature Sugar Beet Root	
	% Total ^{14}C	ppm[1]	% Total ^{14}C	ppm[1]	% Total ^{14}C	ppm[1]
CHARACTERIZED METABOLITES NOT IDENTIFIED[8]						
Characterized organosolubles	<0.1	0.002	1.5	0.013	---	---
Characterized neutral aqueous solubles	<0.1	0.003	---	---	---	---
Characterized aqueous solubles	2.5	0.179	---	---	---	---
TOTAL	79.2	5.677	90.7	0.796	56.8	0.157

[1]PPM are expressed in PPM equivalent to [phenyl-^{14}C]-metalaxyl.

[2]The 0.9% quantitated as CGA-108905 includes N7 as well as CGA-108905.

[3]Postulated identifications based on mass spectral analyses of metabolites isolated from wheat (N1a) and lettuce (N1b).

[4]1.2% of the 19.6% was a mixture of N4 and N5.

[5]Radioactivity was characterized but not identified. HPLC retention times were similar to aqueous soluble acid metabolites in fractions B-II and B-III isolated from mature wheat stalks (see Table XVII).

[6]CGA-67869 is quantitated with the CGA-48988/CGA-67868 metabolites. Some CGA-67868 is also quantitated with the CGA-62826/CGA-100255/CGA-108905 metabolites.

[7]Some CGA-67868 is included in the quantitation of the CGA-62826/CGA-100255/CGA-108905 metabolites.

[8]Metabolites separated by HPLC and/or 2D-TLC but were not identified.

FIGURE 1. CHEMICAL NAMES AND STRUCTURES

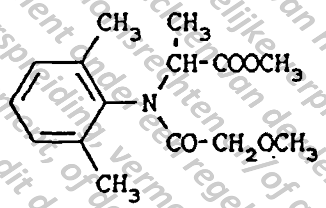
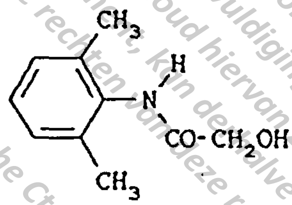
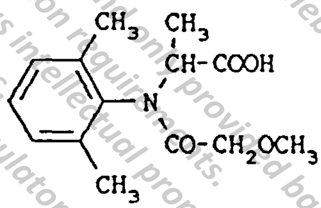
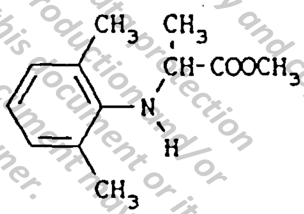
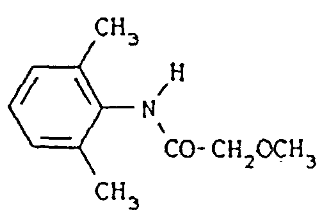
Company Code Chemical Name	Structure
CGA-48988 N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester	
CGA-37734 N-(2,6-dimethylphenyl)-2-hydroxyacetamide	
CGA-62826 N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine	
CGA-67866 N-(2,6-dimethylphenyl)-alanine methyl ester	
CGA-67868 N-(2,6-dimethylphenyl)-2-methoxyacetamide	

FIGURE 1. CHEMICAL NAMES AND STRUCTURES
(Continued)

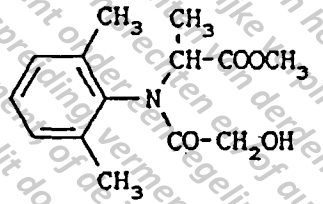
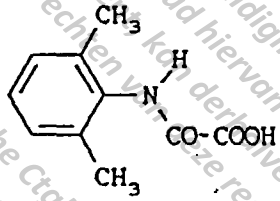
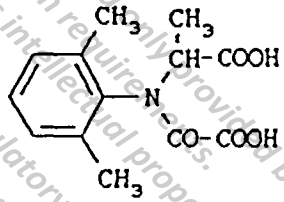
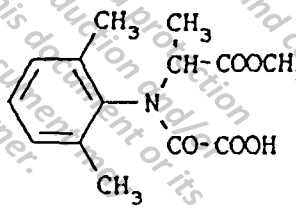
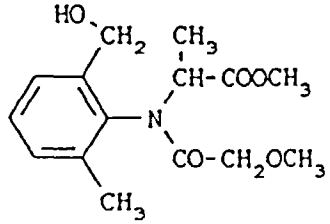
Company Code Chemical Name	Structure
CGA-67869 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine methyl ester	
CGA-68124 [(2,6-dimethylphenyl)-amino]oxoacetic acid	
CGA-78532 N-(carboxycarbonyl)-N-(2,6-dimethylphenyl) alanine	
CGA-79353 N-(carboxycarbonyl)-N-(2,6-dimethylphenyl) alanine methyl ester	
CGA-94689 N-[(2-hydroxymethyl)-6-methylphenyl]-N-(methoxy acetyl)-alanine methyl ester	

FIGURE 1. CHEMICAL NAMES AND STRUCTURES
(Continued)

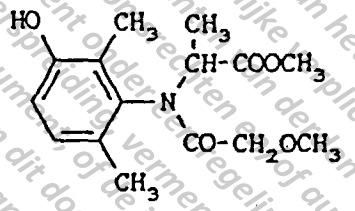
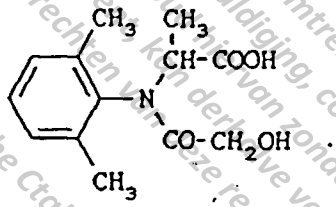
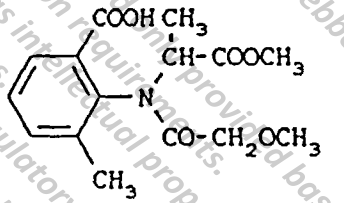
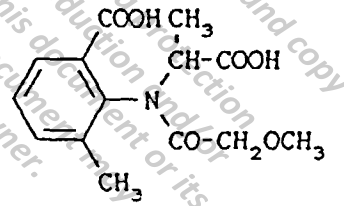
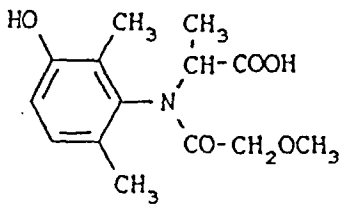
Company Code Chemical Name	Structure
CGA-100255 N-(3-hydroxy-2,6-dimethyl phenyl)-N-(methoxyacetyl) alanine methyl ester	
CGA-107955 N-(2,6-dimethylphenyl)-N-(hydroxyacetyl)-alanine	
CGA-108905 2-[(methoxyacetyl)(2-methoxy-1-methyl-2-oxoethyl)amino]-3-methylbenzoic acid	
CGA-108906 2-[(1-carboxyethyl)(methoxy acetyl)amino]-3-methyl benzoic acid	
CGA-119857 N-(3-hydroxy-2,6-dimethyl phenyl)-N-(methoxyacetyl)-alanine	

FIGURE 1. CHEMICAL NAMES AND STRUCTURES
(Continued)

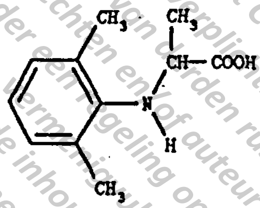
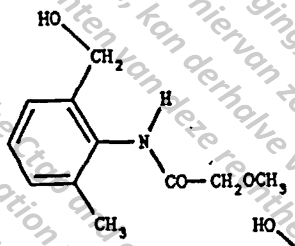
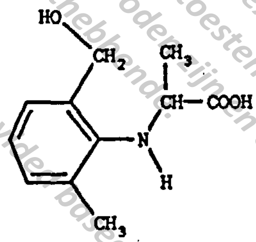
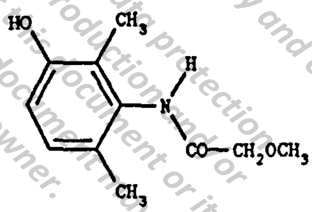
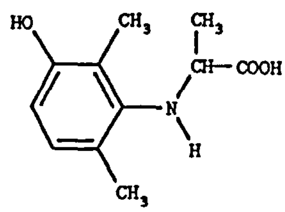
Company Code Chemical Name	Structure
CGA-67867 N-(2,6-dimethylphenyl- alanine	
N1a aglycone postulated N-[(2-hydroxymethyl)-6- methylphenyl]-2- methoxyacetamide MS could support N-[(2-hydroxymethyl)-6- methylphenyl]-alanine	 
N1b aglycone postulated N-(3-hydroxy-2,6- dimethylphenyl)-2- methoxyacetamide MS could support N-(3-hydroxy-2,6- dimethylphenyl)-alanine	 

FIGURE 1. CHEMICAL NAMES AND STRUCTURES
(Continued)

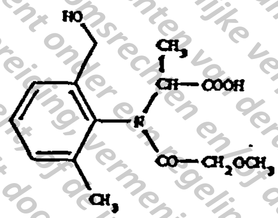
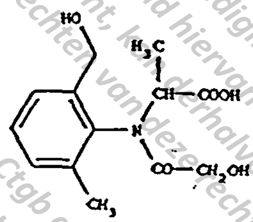
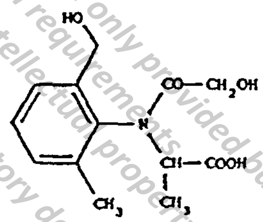
Company Code Chemical Name	Structure
PO (Rat M9) N-[(2-hydroxymethyl)-6-methylphenyl]-N-(methoxy acetyl)-alanine	
B2 (Hen P1) N-[2-(hydroxymethyl)-6-methylphenyl]-N-(hydroxyacetyl)alanine	
B1 (Hen P2) N-[2-(hydroxymethyl)-6-methylphenyl]-N-(hydroxyacetyl)alanine	

FIGURE 2. PROCEDURES FOR THE EXTRACTION AND CHARACTERIZATION OF RADIOACTIVITY IN GREENHOUSE TARGET CROP AND ROTATIONAL CROP SOILS

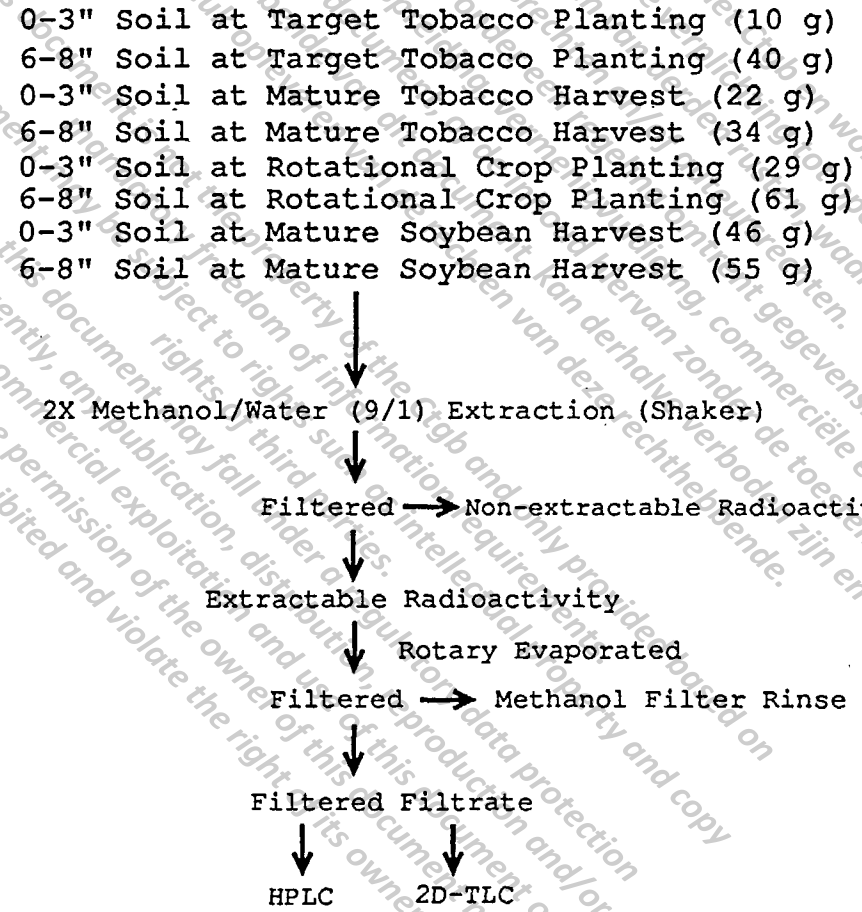


FIGURE 3. PROCEDURES FOR THE EXTRACTION AND CHARACTERIZATION OF RADIOACTIVITY IN GREENHOUSE TARGET TOBACCO

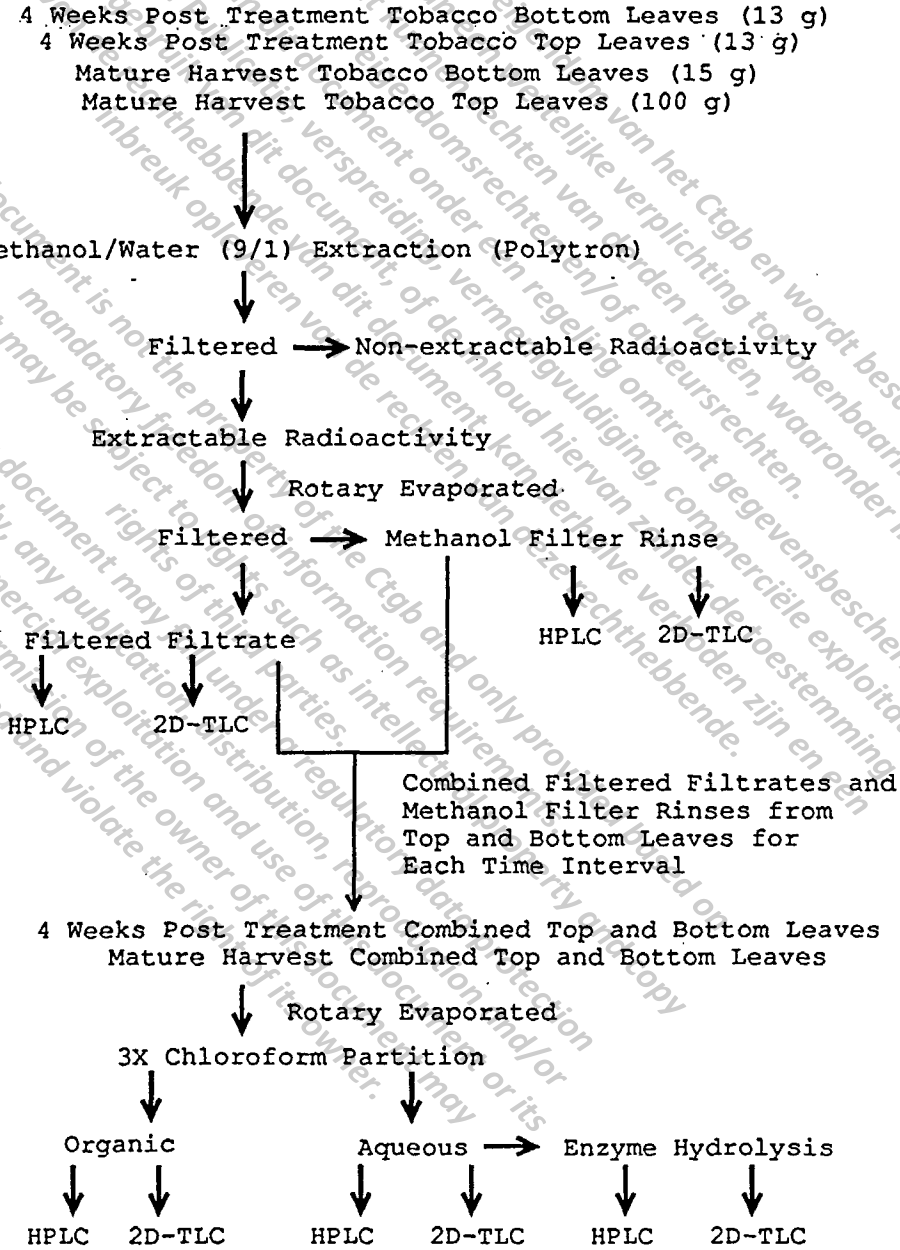
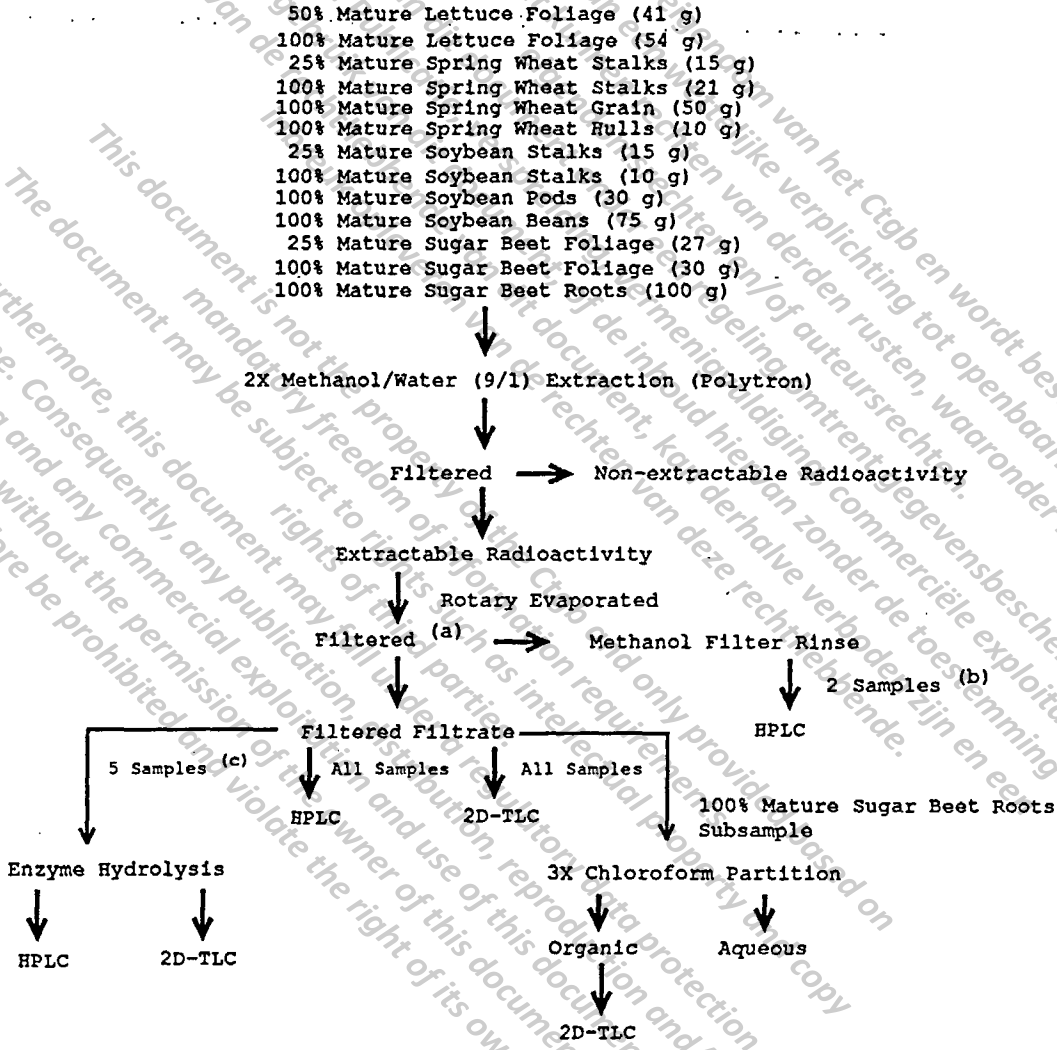


FIGURE 4. PROCEDURES FOR THE EXTRACTION AND CHARACTERIZATION OF RADIOACTIVITY IN GREENHOUSE ROTATIONAL CROPS



(a) Due to the nature of the sample, 100% mature soybean beans were not filtered at this point. Instead, the concentrated filtrate was partitioned 3X with hexane, followed by 3X with ethyl acetate.

(b) Filter rinse samples analyzed by HPLC were: 100% mature lettuce foliage and 100% mature sugar beet roots.

(c) Samples characterized by enzyme hydrolysis were: 100% mature lettuce foliage, 100% mature spring wheat stalks, 100% mature spring wheat hulls, 100% mature soybean stalks and 100% mature soybean pods. In addition, the filtered filtrate from 100% mature tobacco bottom leaves was characterized by enzyme hydrolysis.

FIGURE 5. PROCEDURES FOR THE EXTRACTION AND CHARACTERIZATION OF RADIOACTIVITY IN MATURE SUGAR BEET ROOTS

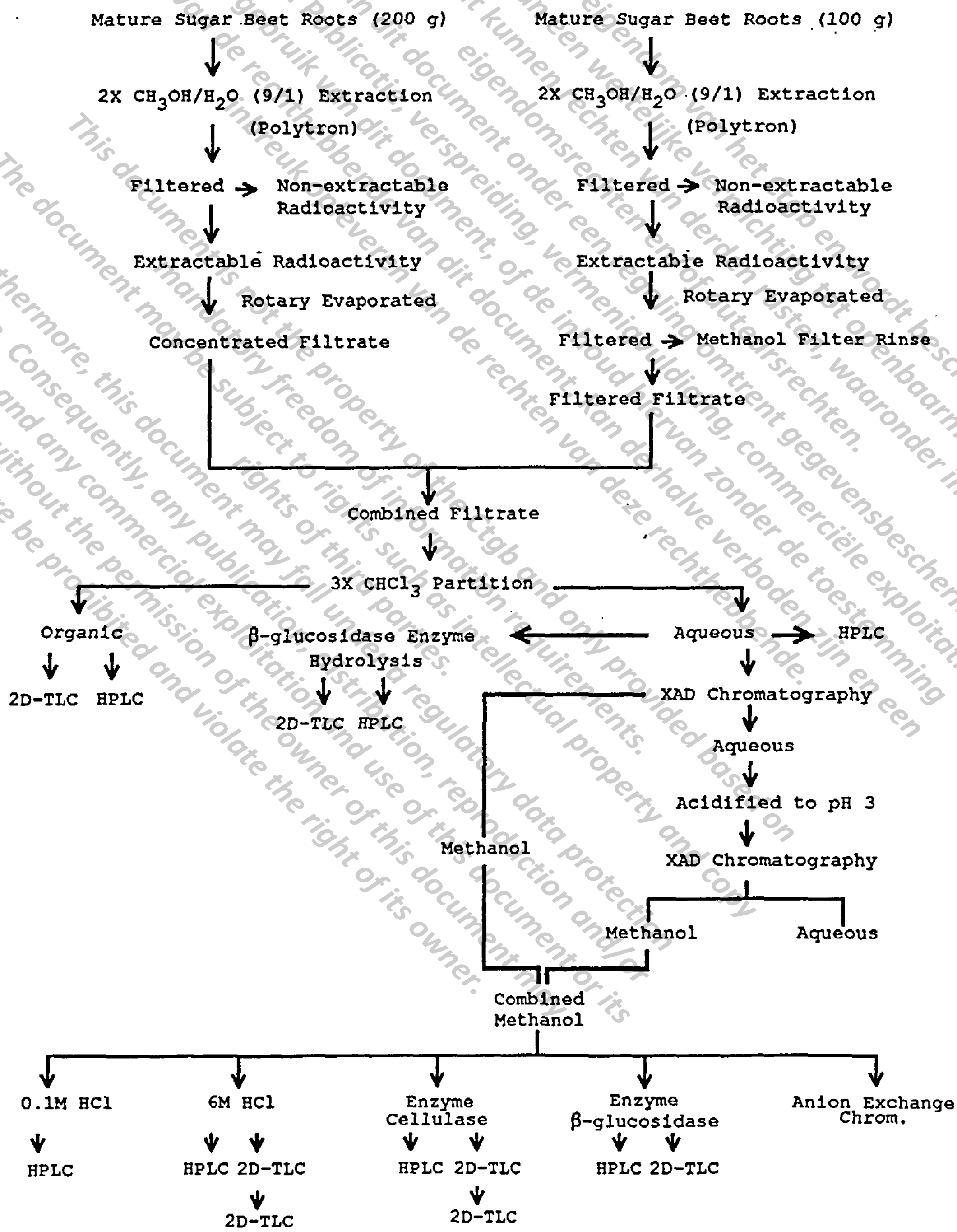


FIGURE 6. PROCEDURES FOR THE EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RADIOACTIVITY IN 50% MATURE LETTUCE FOLIAGE

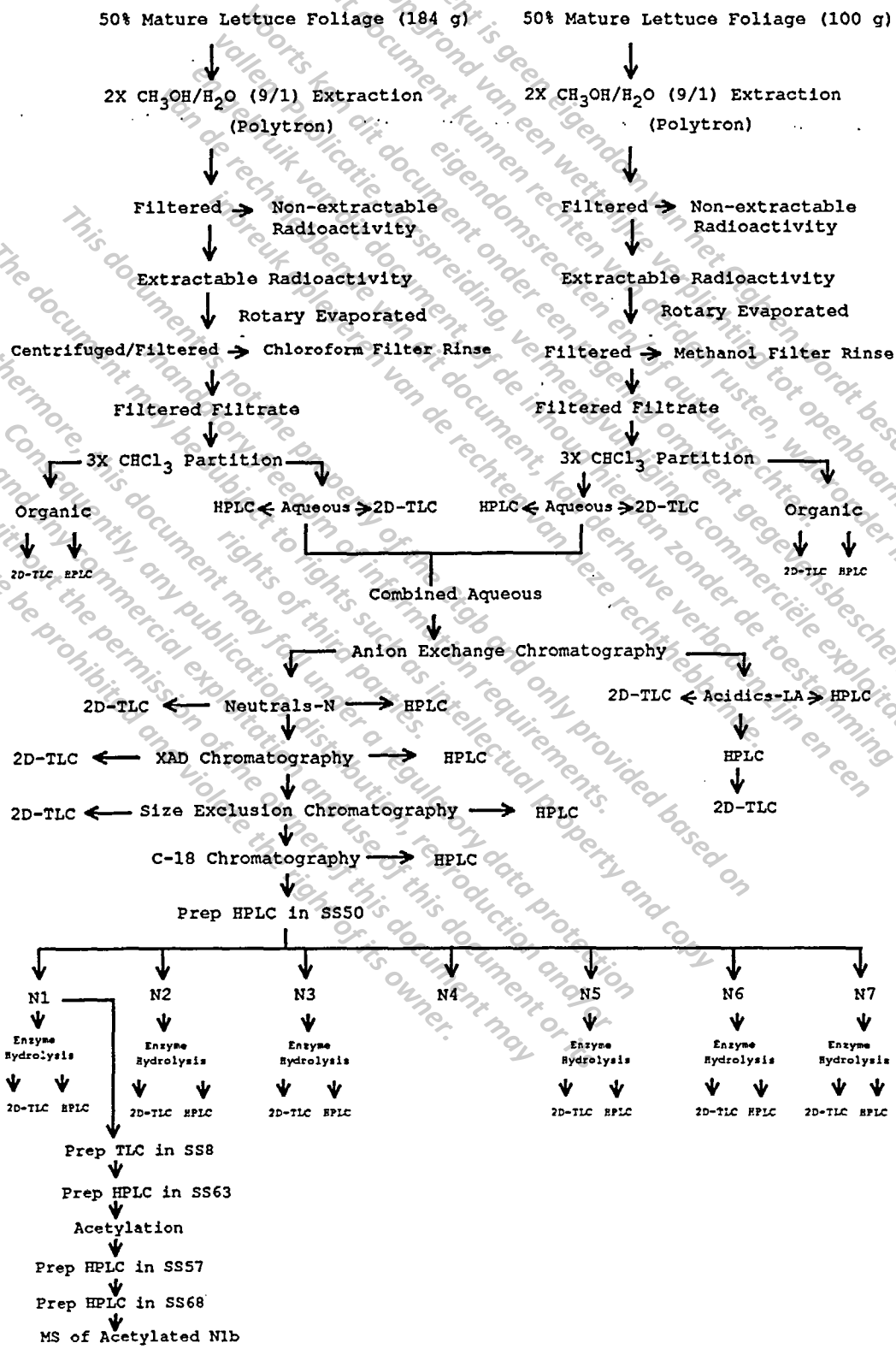


FIGURE 7. PROCEDURES FOR THE EXTRACTION AND CHARACTERIZATION OF RADIOACTIVITY IN MATURE WHEAT STALKS

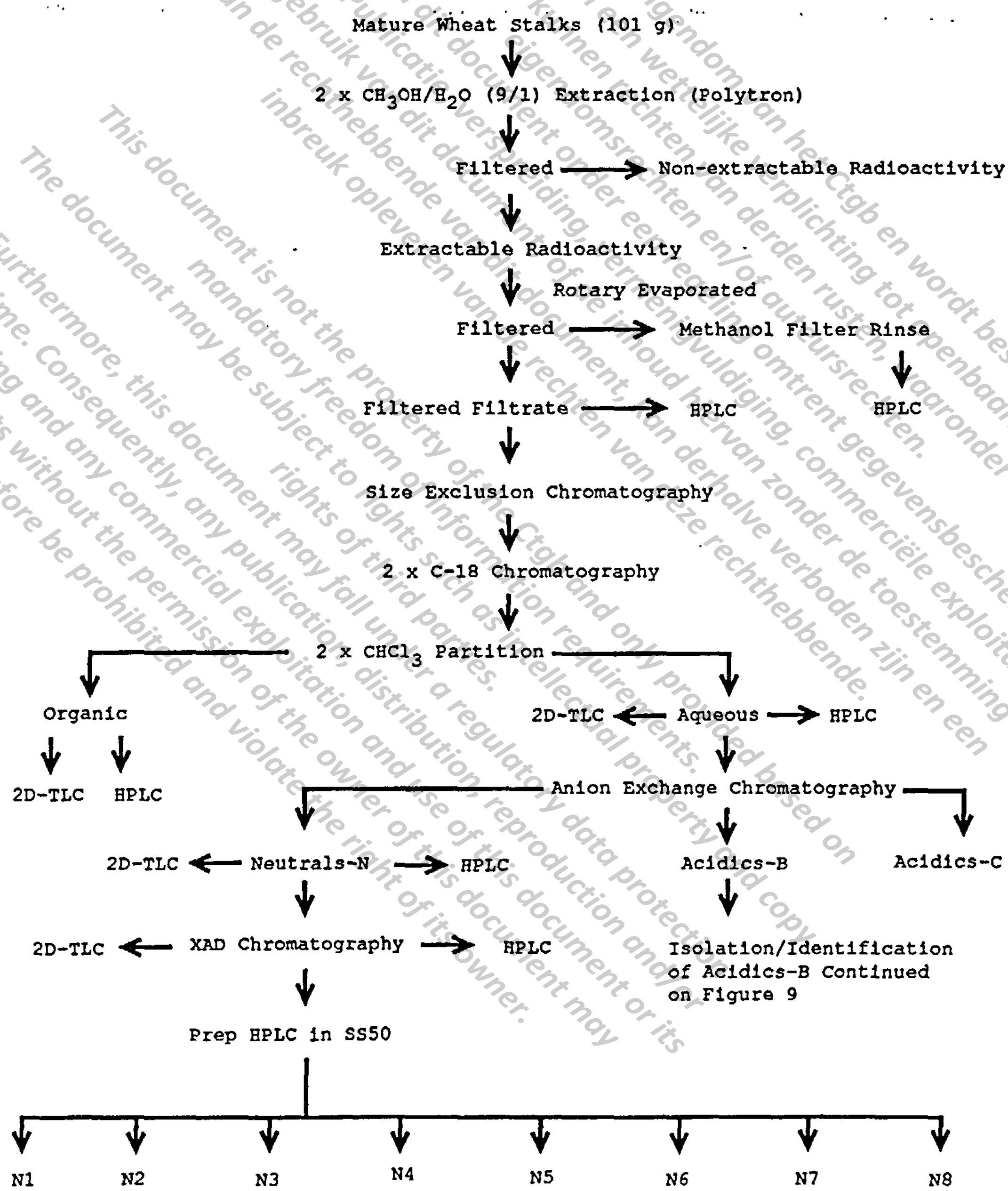


FIGURE 8. PROCEDURES FOR THE ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF NEUTRAL AQUEOUS METABOLITES OF [PHENYL-^{14}C]-METALAXYL IN MATURE WHEAT STALKS

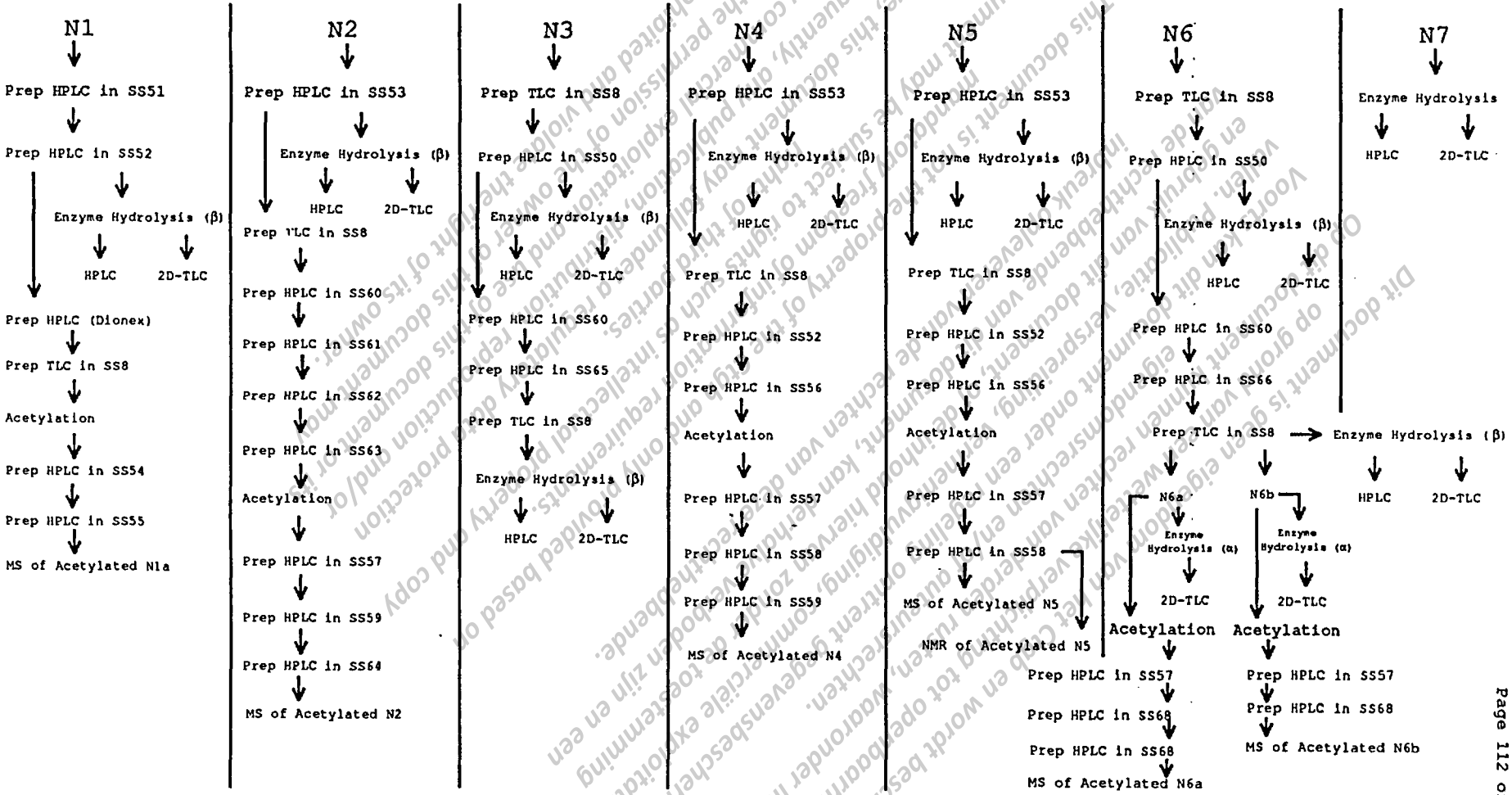


FIGURE 9. PROCEDURES FOR THE CHARACTERIZATION OF ACIDIC AQUEOUS METABOLITES OF [PHENYL-^{14}C]-METALAXYL IN MATURE WHEAT STALKS

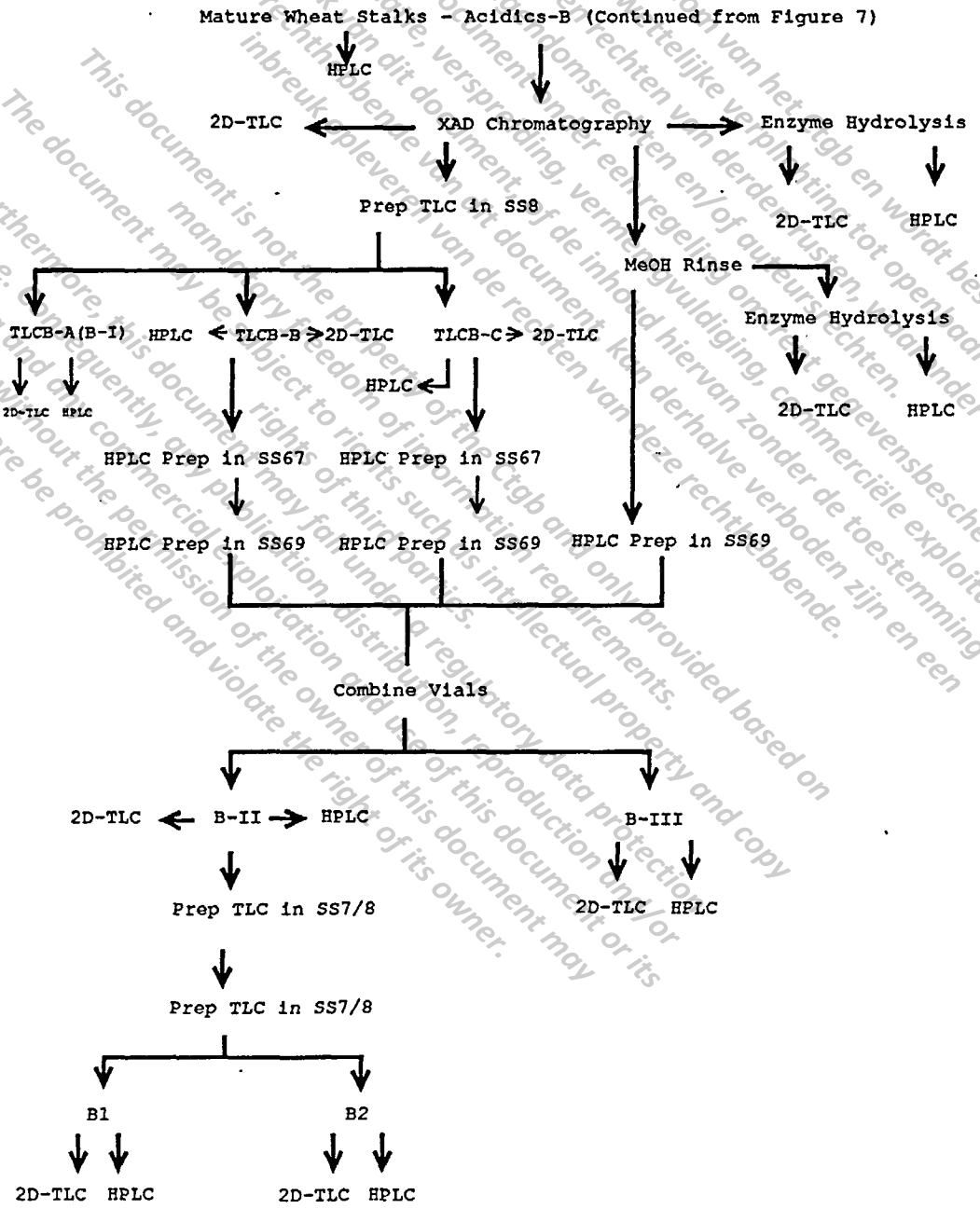
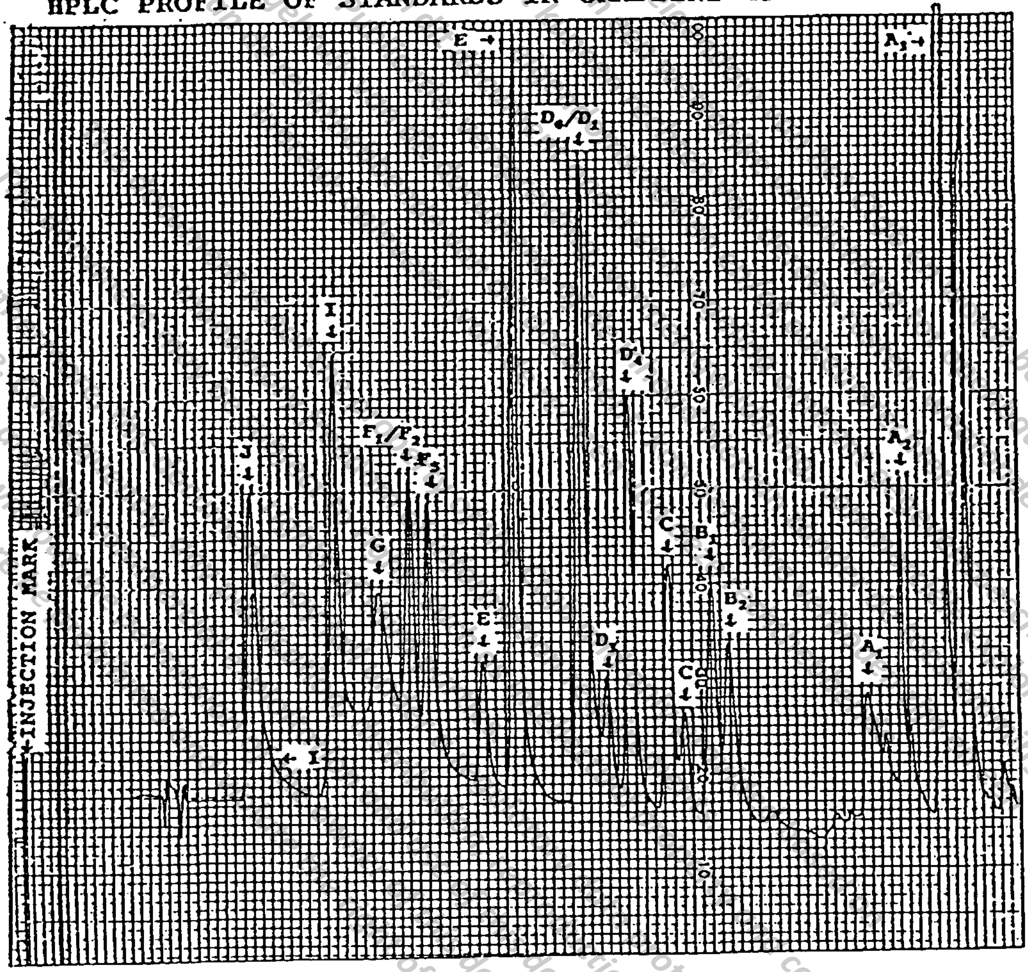


FIGURE 10. HPLC PROFILE OF STANDARDS

HPLC PROFILE OF STANDARDS IN GRADIENT 49



KEY

A_1 – CGA-67869
A_2 – CGA-48988
A_3 – CGA-67866
B_1 – CGA-67868
B_2 – CGA-62826

C – CGA-100255
D_0 – CGA-67867
D_7 – CGA-107955
D_3 – CGA-94689A

D_4 – CGA-94689B
E – CGA-108905
F_1 – CGA-68124
F_2 – CGA-119857

F_5 – CGA-37734
G – CGA-79353
I – CGA-108906
J – CGA-78532

FIGURE 11. THIN LAYER CHROMATOGRAMS OF STANDARDS

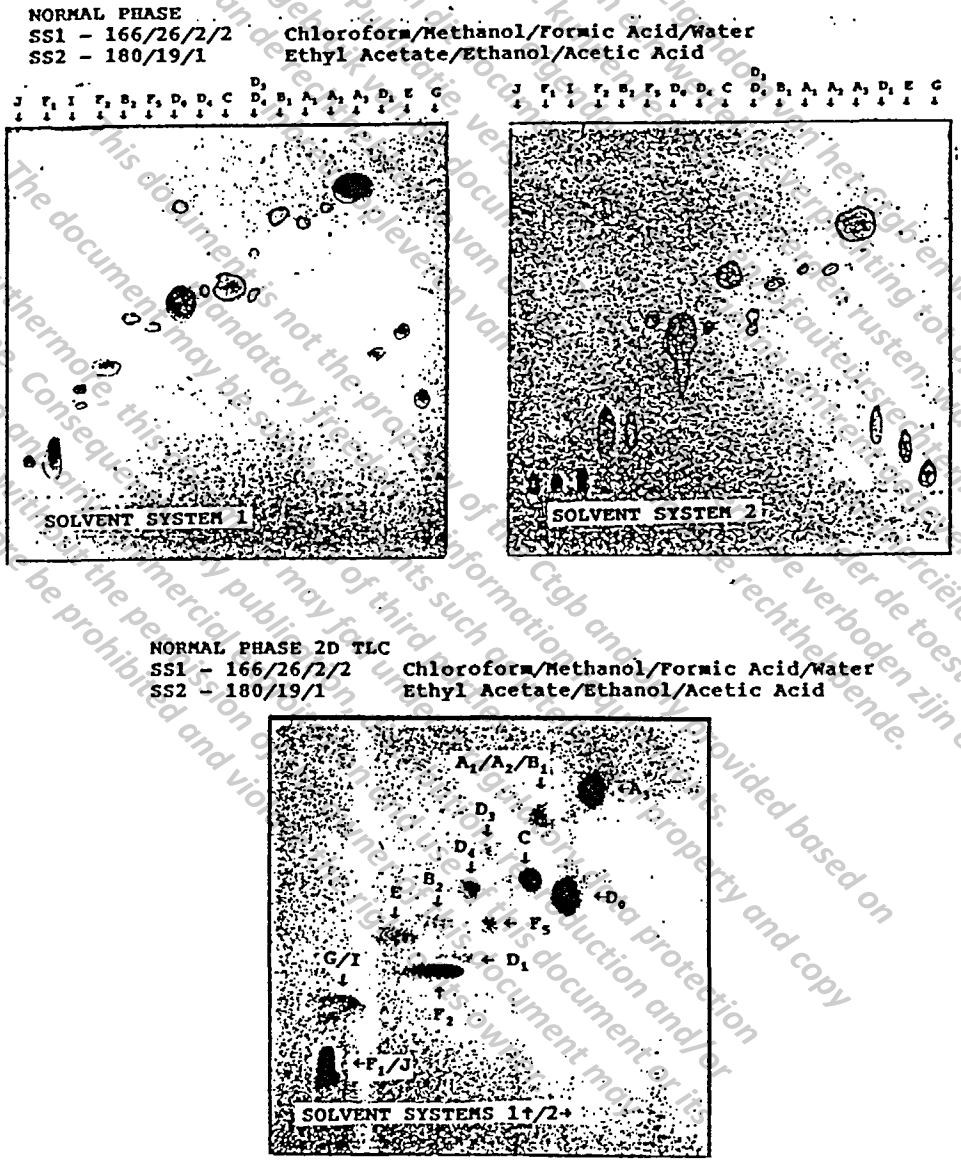
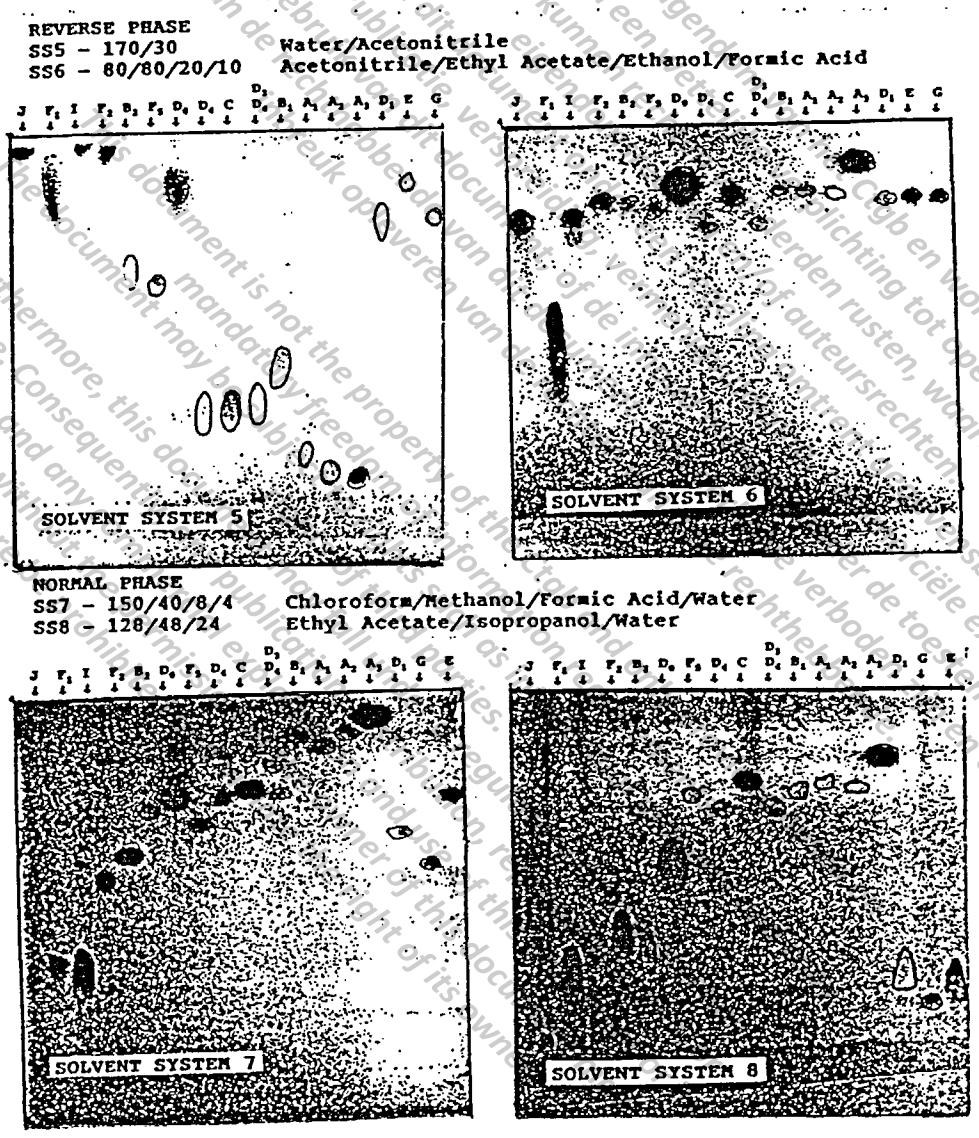


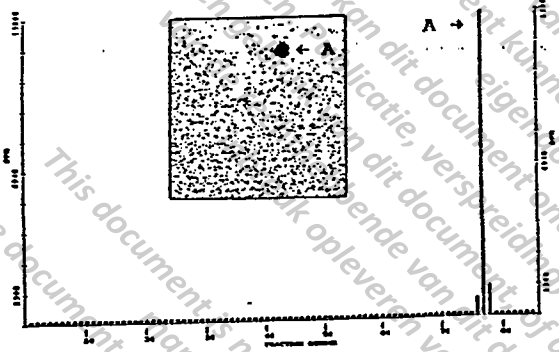
FIGURE 11. THIN LAYER CHROMATOGRAMS OF STANDARDS
(Continued)



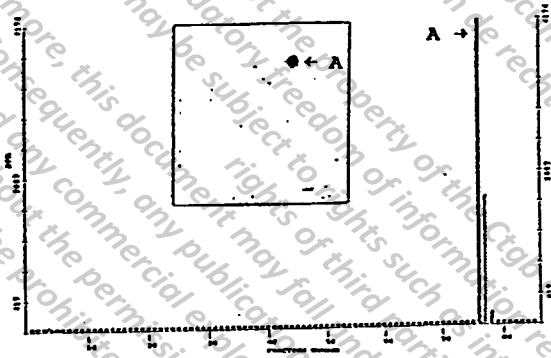
KEY

A_1 - CGA-67869	C - CGA-100255	D_4 - CGA-94689B	F_5 - CGA-37734
A_2 - CGA-48988	D_0 - CGA-67867	E - CGA-108905	G - CGA-79353
A_3 - CGA-67866	D_1 - CGA-107955	F_1 - CGA-68124	I - CGA-108906
B_1 - CGA-67868	D_3 - CGA-94689A	F_2 - CGA-119857	J - CGA-78532
B_2 - CGA-62826			

FIGURE 12. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE EXTRACTABLE RADIOACTIVITY IN SELECTED GREENHOUSE TOBACCO SOILS



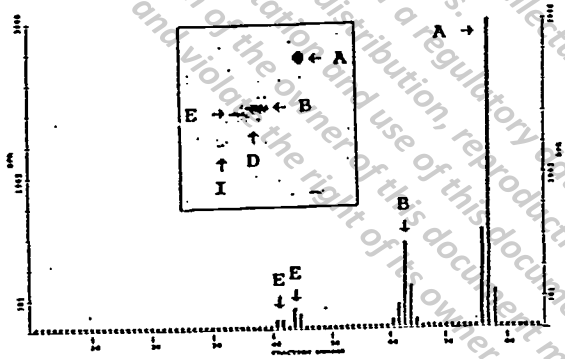
0-3" SOIL
TARGET TOBACCO PLANTING



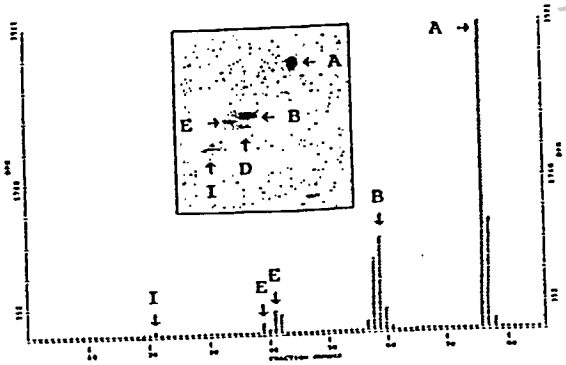
6-8" SOIL
TARGET TOBACCO PLANTING

KEY

A - CGA-48988
B - CGA-62826
D - CGA-107955
E - CGA-108905
I - CGA-108906

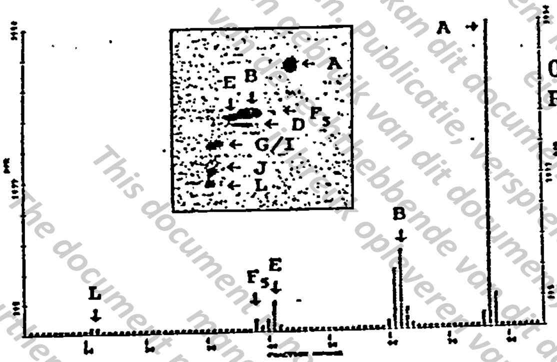


0-3" SOIL
MATURE TARGET
TOBACCO HARVEST

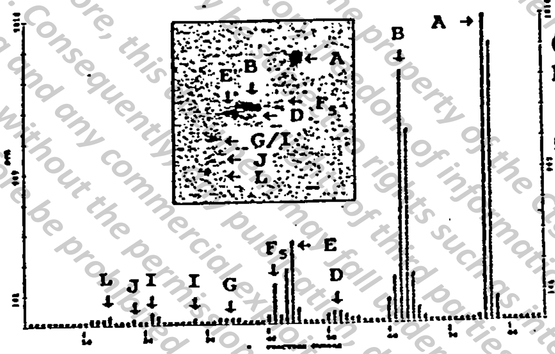


6-8" SOIL
MATURE TARGET
TOBACCO HARVEST

FIGURE 13. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE EXTRACTABLE RADIOACTIVITY IN SELECTED GREENHOUSE ROTATIONAL CROP SOILS



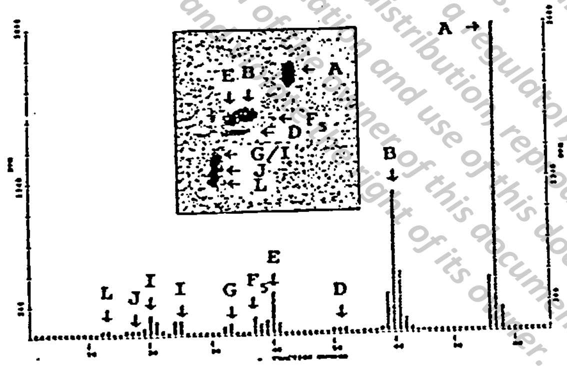
0-3" SOIL
ROTATIONAL CROP PLANTING



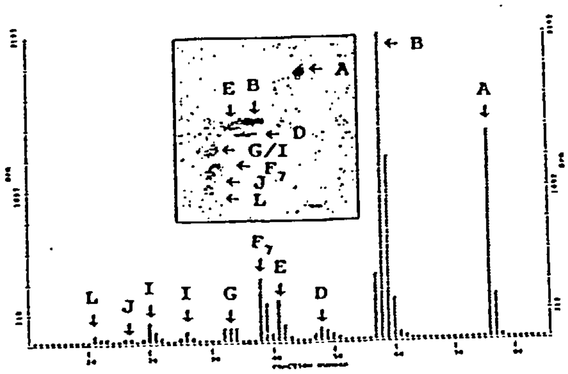
6-8" SOIL
ROTATIONAL CROP PLANTING

KEY

A - CGA-48988
B - CGA-62826
D - CGA-107955
E - CGA-108905
F_5 - CGA-37734
F_7 - N6
G - CGA-79353
I - CGA-108906
J - CGA-78532
L - S-1 (unknown)

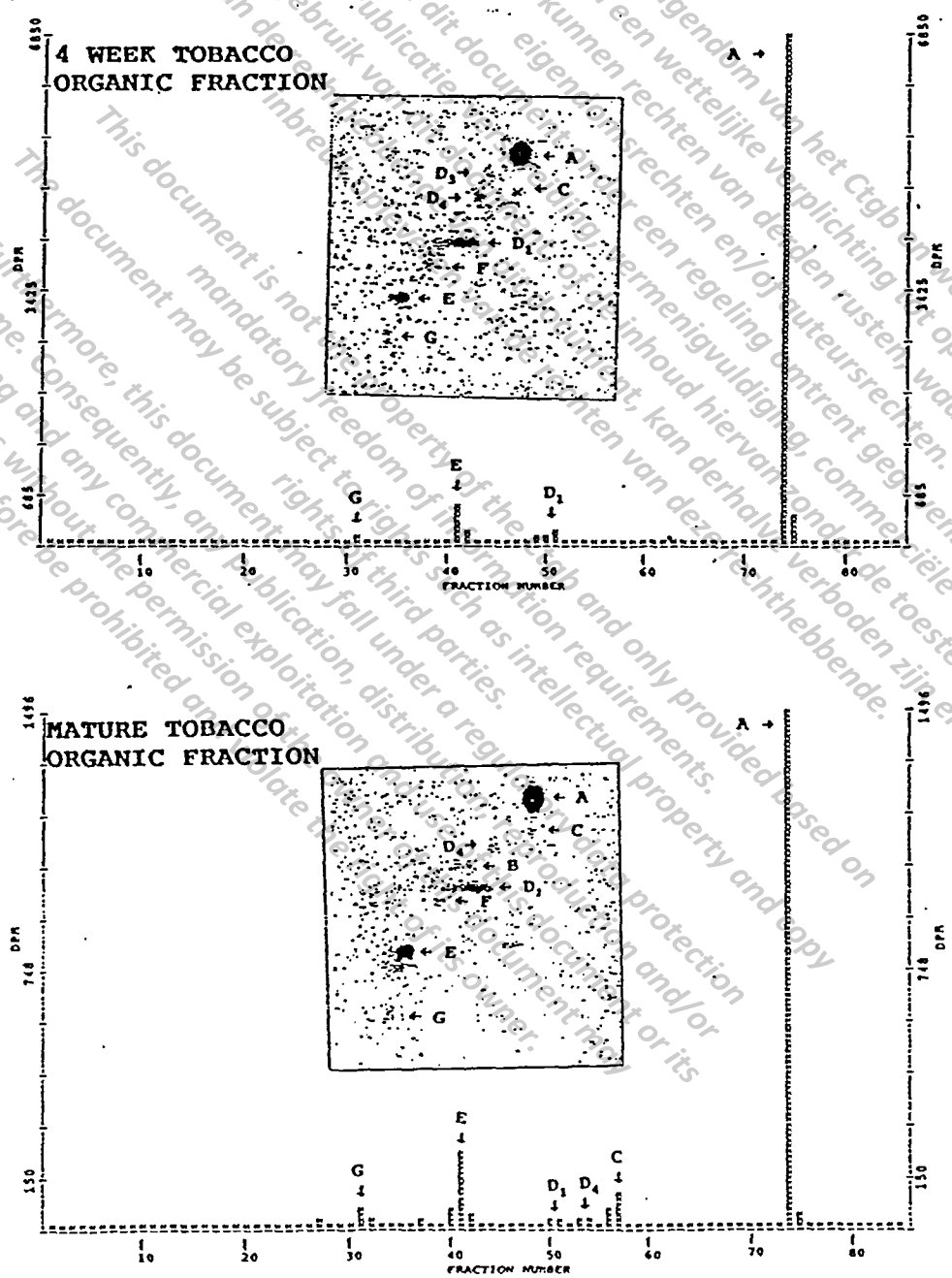


0-3" SOIL
MATURE ROTATIONAL
SOYBEAN HARVEST



6-8" SOIL
MATURE ROTATIONAL
SOYBEAN HARVEST

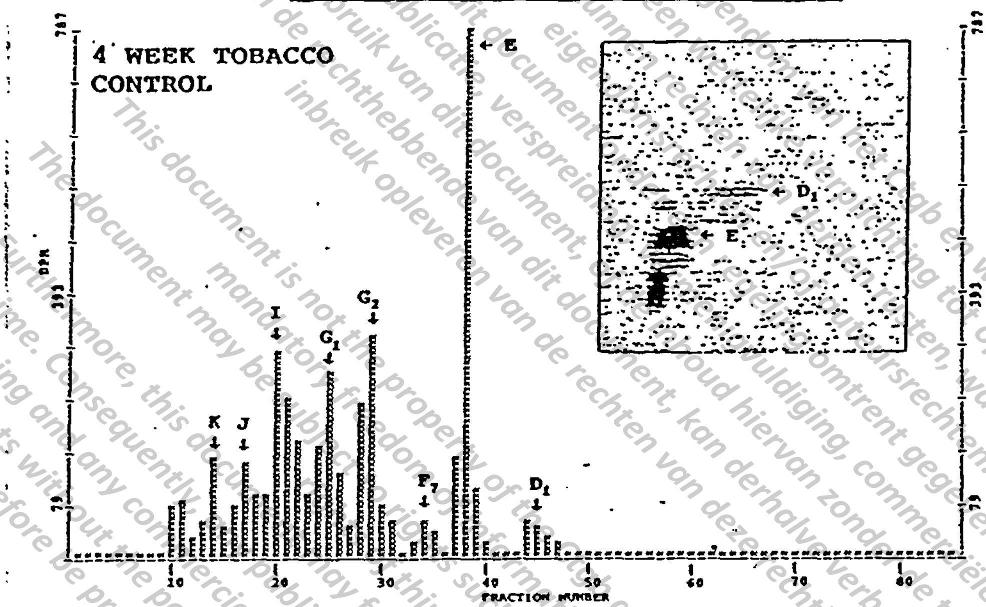
FIGURE 14. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE ORGANOSOLUBLE RADIOACTIVITY IN FOUR WEEK POST TREATMENT AND MATURE HARVEST TOBACCO LEAVES

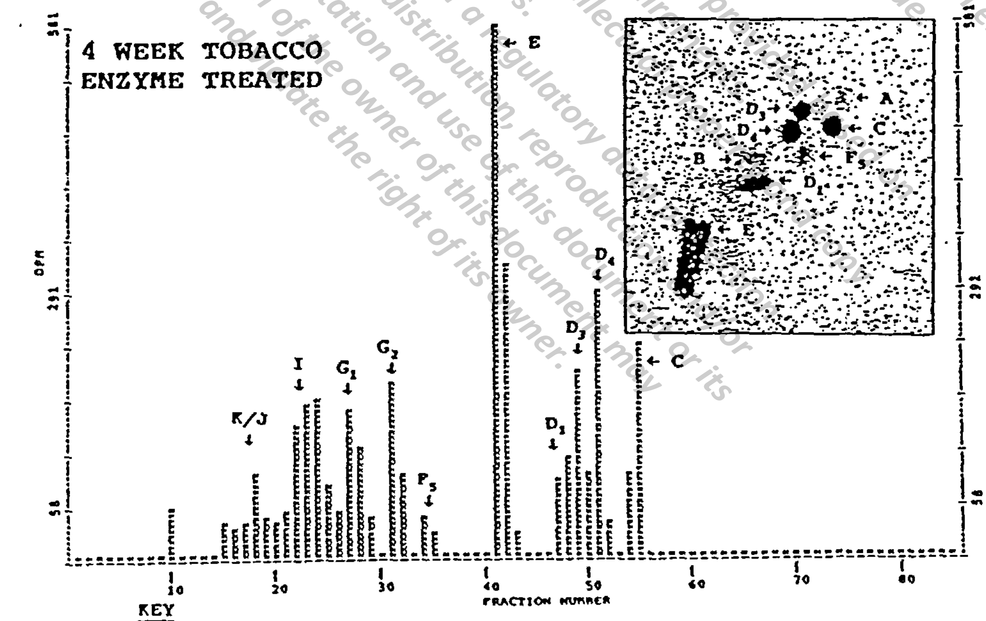


KEY

A - CGA-48988
B - CGA-62826/CGA-67868
C - CGA-100255
D_1 - CGA-107955
D_3 - CGA-94689A
D_4 - CGA-94689B
E - N7
F - CGA-119857
G - N4, N5

FIGURE 15. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF AQUEOUS SOLUBLE RADIOACTIVITY IN FOUR WEEK POST TREATMENT TOBACCO LEAVES

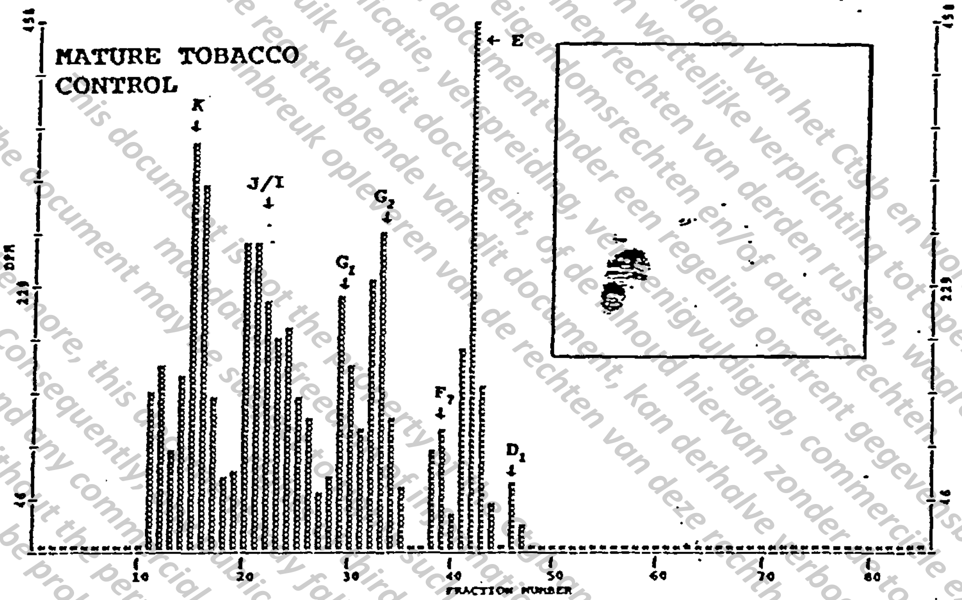


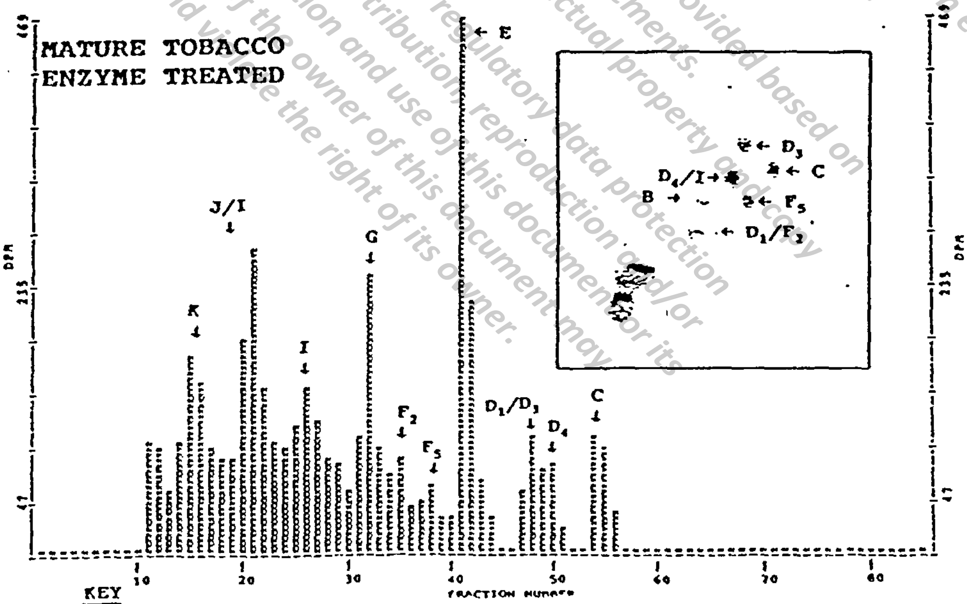


KEY

A - CGA-67869
B - CGA-62826
C - CGA-100255
D_1 - CGA-107955
D_3 - CGA-94689A
D_4 - CGA-94689B
E - N7
F_5 - CGA-37734
F_7 - N6
G_1 - N4, acidics
G_2 - N5, acidics
I - N3, N1a aglycone, acidics
J - N2
K - N1a

FIGURE 16. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF AQUEOUS SOLUBLE RADIOACTIVITY IN MATURE HARVEST TOBACCO LEAVES



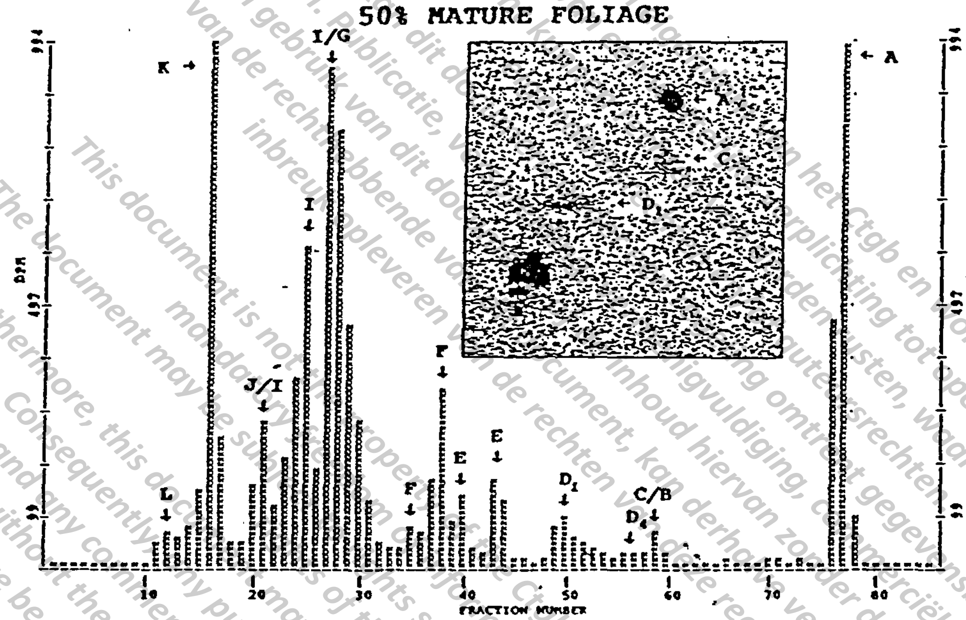


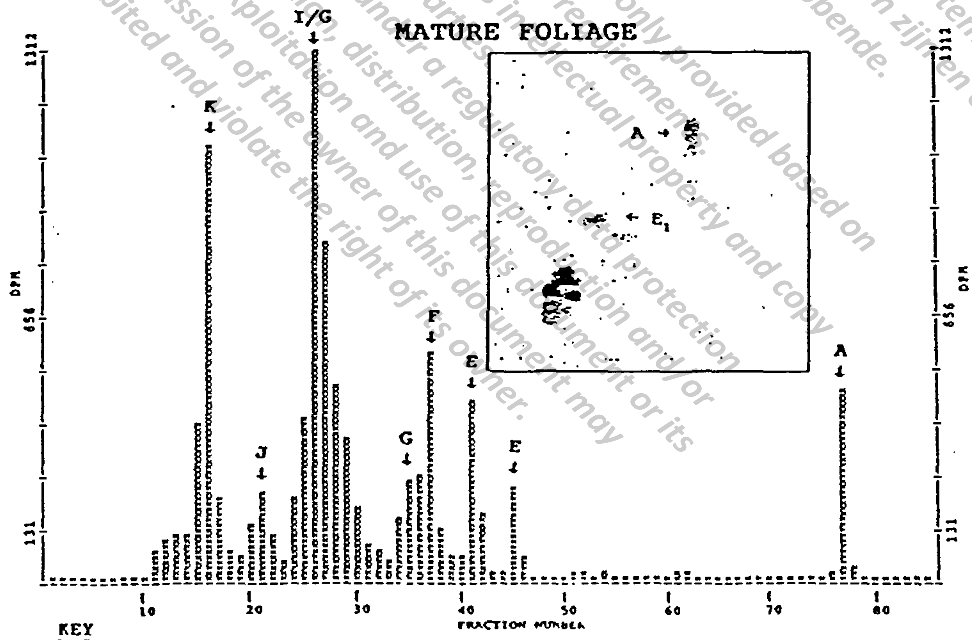
KEY

B – CGA-62826
C – CGA-100255
D_1 – CGA-107955
D_3 – CGA-94689A
D_4 – CGA-94689B
E – N7
F_2 – CGA-119857
F_5 – CGA-37734
F_7 – N6
G – N4, N5, acidics
G_1 – N4
G_2 – N5
I – N3, N1a aglycone, acidics
J – N2
K – N1a, acidics

FIGURE 17. <u>HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE EXTRACTABLE RADIOACTIVITY IN 50% MATURE AND MATURE LETTUCE FOLIAGE</u>

50% MATURE FOLIAGE

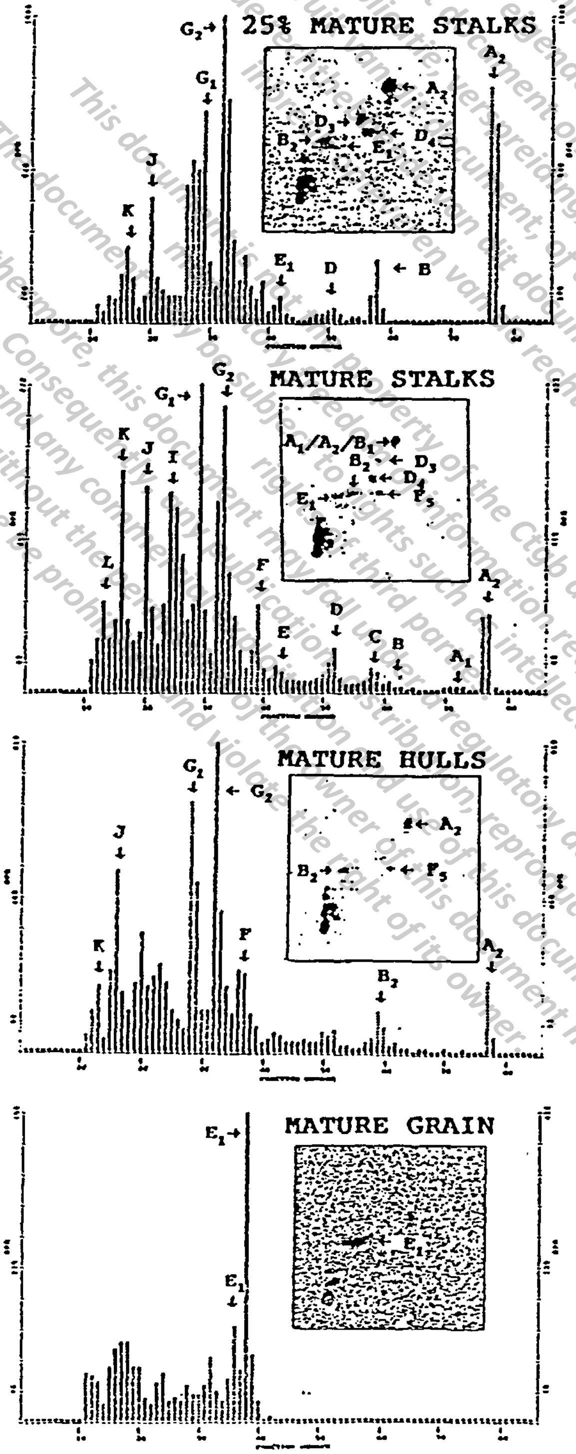




KEY

A – CGA-48988
B – CGA-67868/CGA-62826
C – CGA-100255
D_1 – CGA-107955
D_4 – CGA-94689B
E_1 – CGA-108905
E – N7, CGA-108905
F – N6, acidics, organic unknowns
G – N4, N5, acidics
I – N3, N1b aglycone, acidics
J – N2
K – N1b, acidics
L – acidics

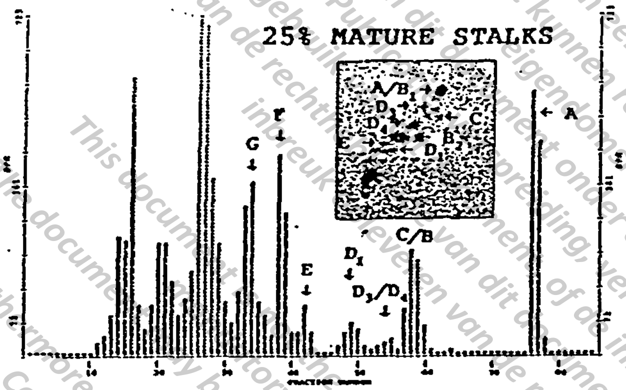
FIGURE 18. <u>HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE EXTRACTABLE RADIOACTIVITY IN 25% MATURE WHEAT STALKS, MATURE WHEAT STALKS, MATURE WHEAT HULLS AND MATURE WHEAT GRAIN</u>

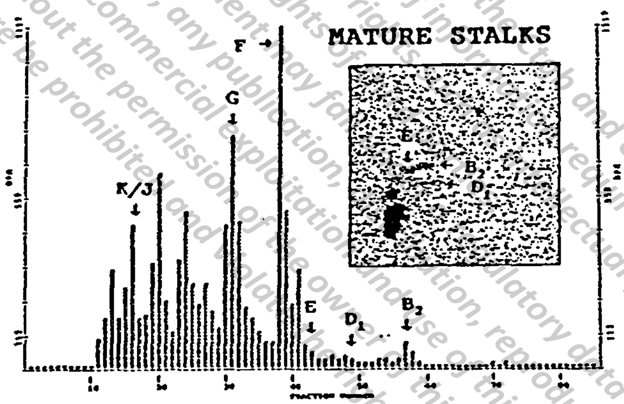


KEY

A_1 - CGA-67869
A_2 - CGA-48988
B - CGA-67868/CGA-62826
B_1 - CGA-67868
B_2 - CGA-62826
C - CGA-100255
D - CGA-107955, CGA-94689A/B, N8
D_1 - CGA-107955
D_3 - CGA-94689A
D_4 - CGA-94689B
E - N7, CGA-108905
E_1 - CGA-108905
F - N6, CGA-119857, CGA-37734
G_1 - N4, acidics
G_2 - N5, acidics
I - N3, N1a aglycone, B2, P0, acidics
J - N2
K - N1a, acidics
L - acidics

FIGURE 19. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE EXTRACTABLE RADIOACTIVITY IN 25% MATURE SOYBEAN STALKS, MATURE SOYBEAN STALKS AND MATURE SOYBEAN PODS





KEY

A – CGA-48988
B – CGA-67868/CGA-62826
B¹ – CGA-62868
B_2 – CGA-62826
C – CGA-100255
D_1 – CGA-107955
D_3 – CGA-94689A
D_4 – CGA-94689B
E – CGA-108905
F – N6
G – N4, N5, acidics
J – N2
K – N1a, acidics

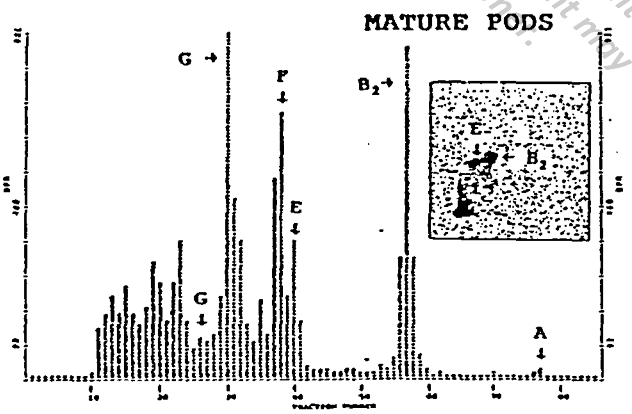


FIGURE 20. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE EXTRACTABLE RADIOACTIVITY IN 25% MATURE SUGAR BEET FOLIAGE, MATURE SUGAR BEET FOLIAGE AND MATURE SUGAR BEET ROOTS

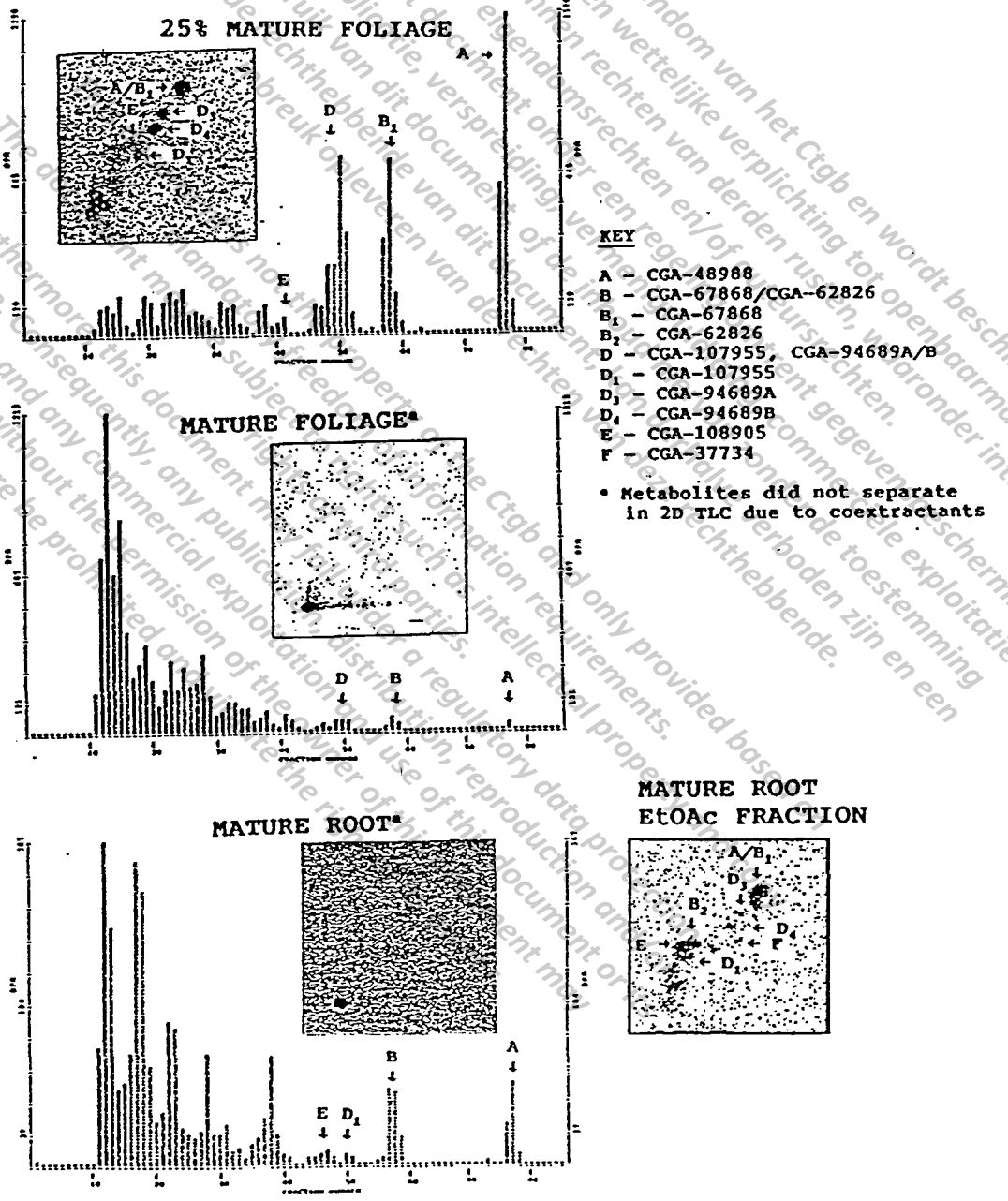
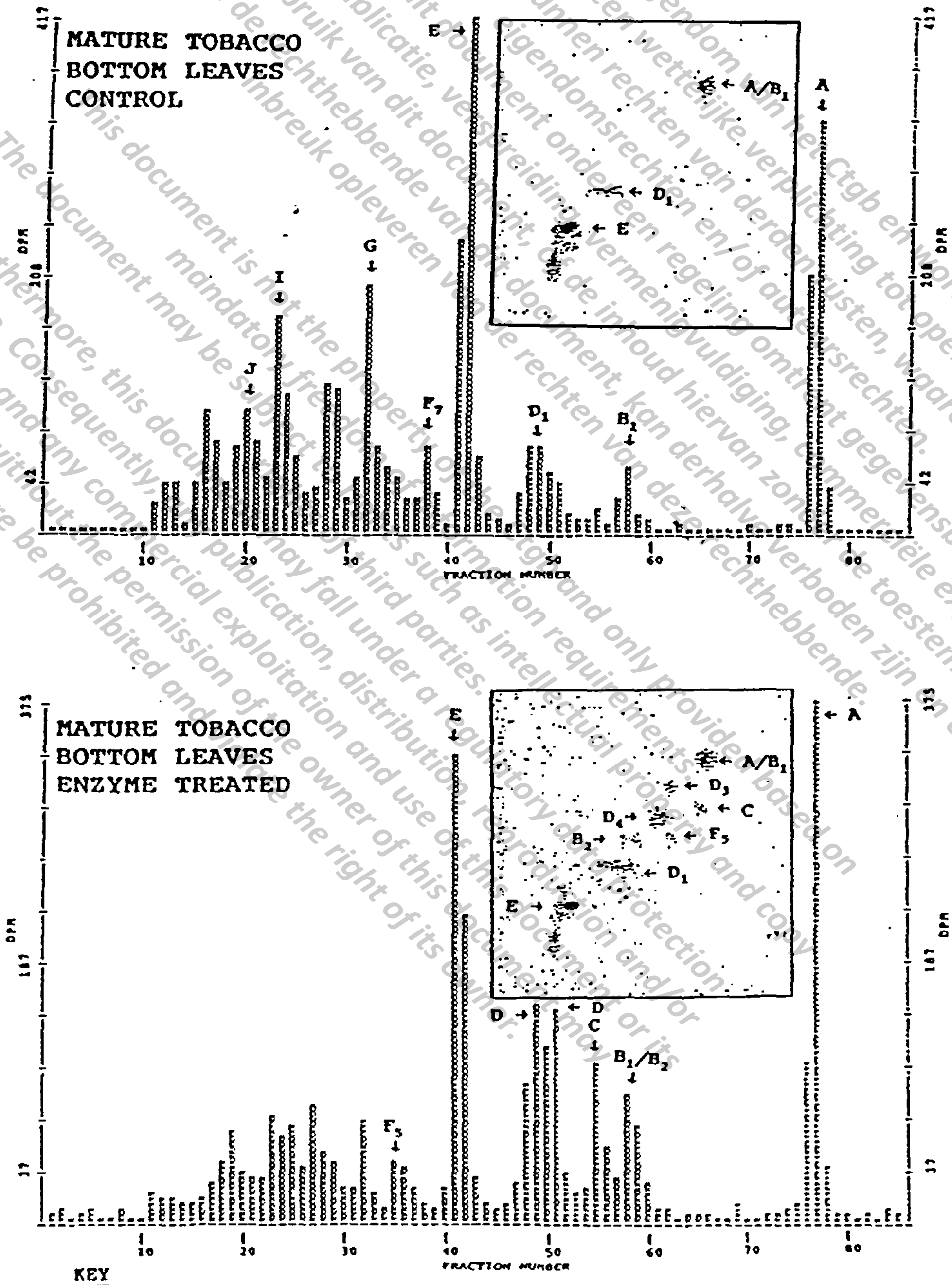


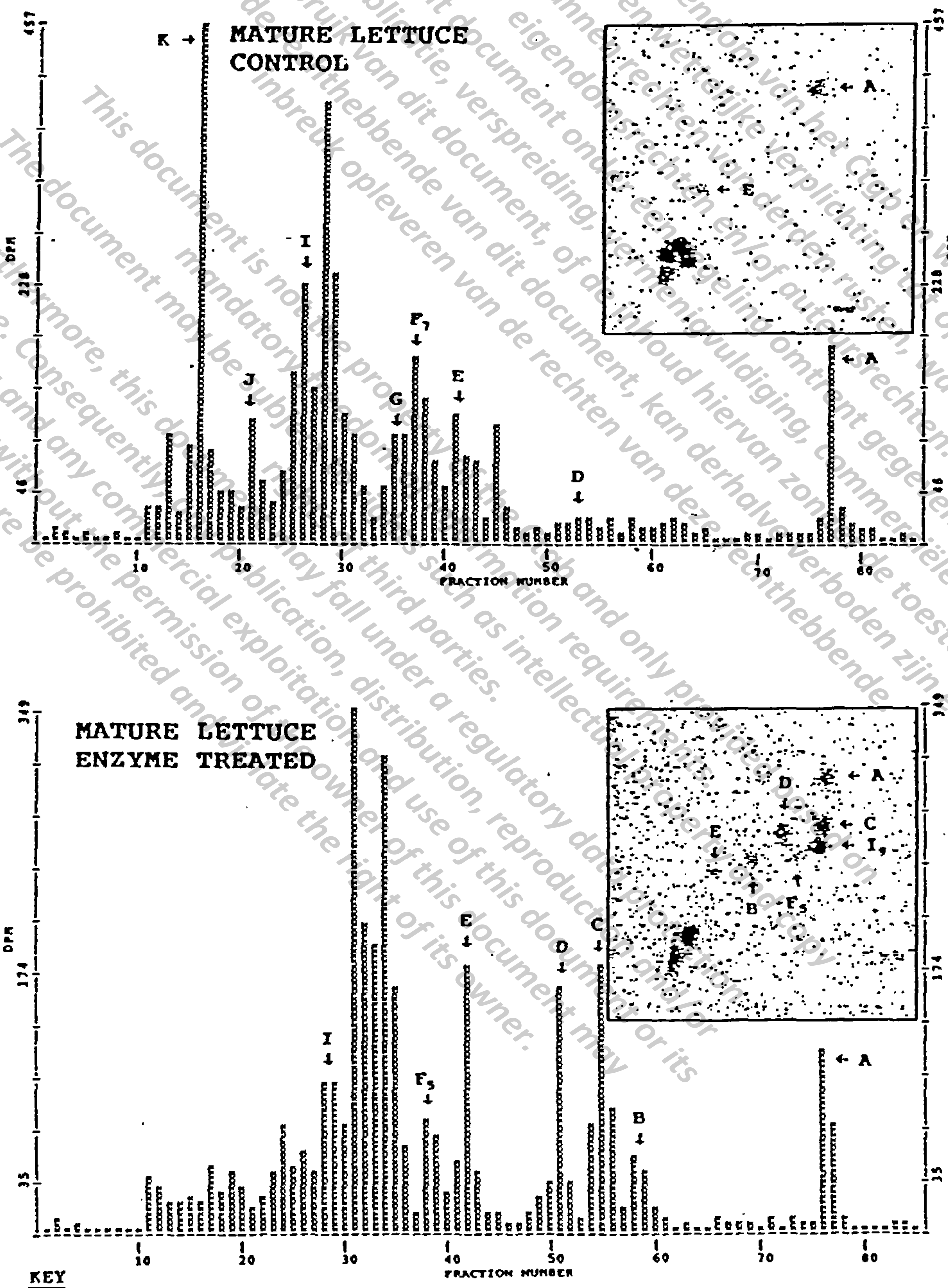
FIGURE 21. <u>HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF EXTRACTABLE RADIOACTIVITY IN MATURE TOBACCO BOTTOM LEAVES</u>



KEY

A – CGA-48988
B_1 – CGA-67868
B_2 – CGA-62826
C – CGA-100255
D – CGA-107955, CGA-94689A/B
D_1 – CGA-107955
D_3 – CGA-94689A
D_4 – CGA-94689B
E – N7
F_5 – CGA-37734
F_7 – N6
G – N4, N5
I – N3
J – N2

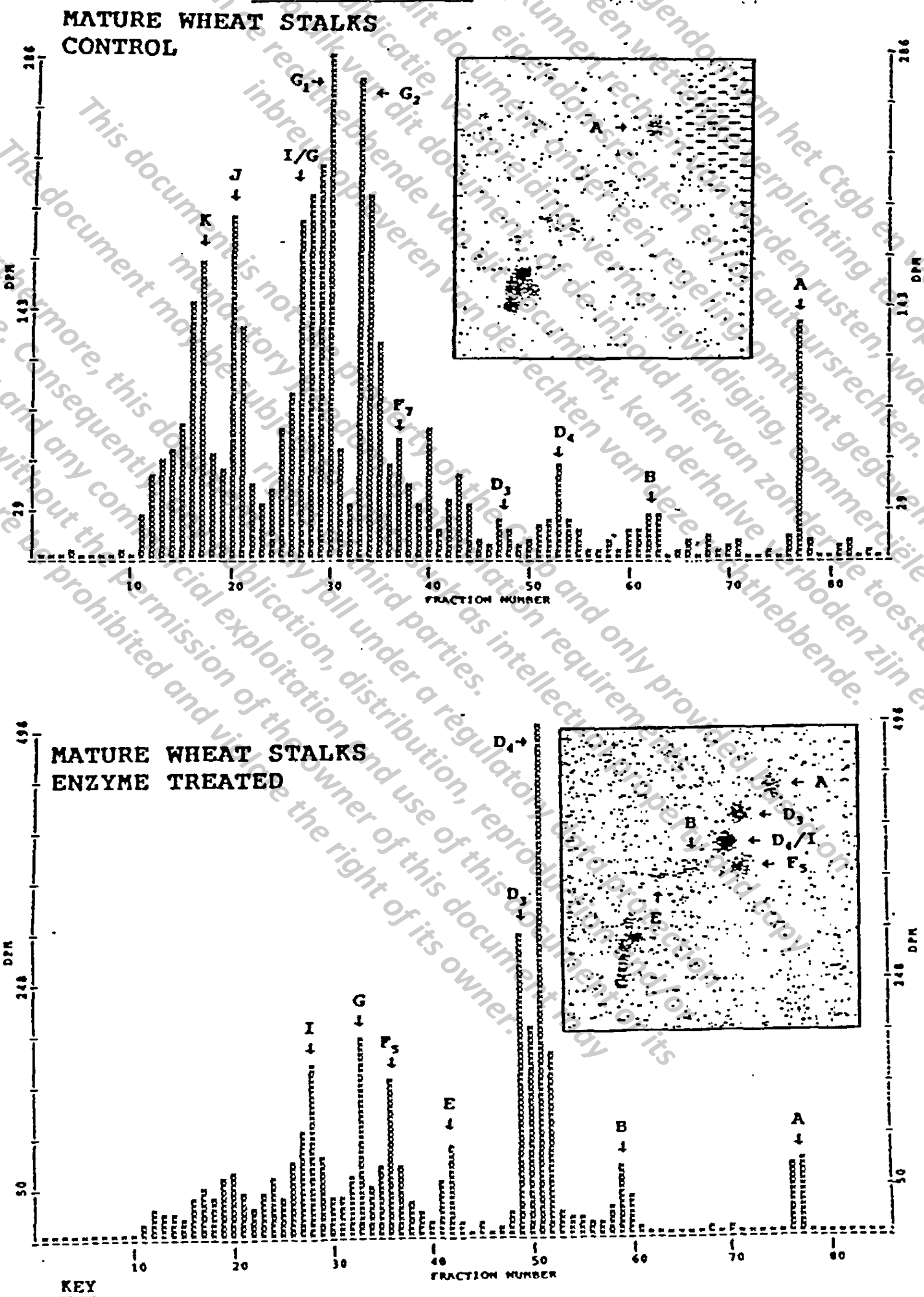
FIGURE 22. <u>HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF EXTRACTABLE RADIOACTIVITY IN MATURE LETTUCE FOLIAGE</u>



KEY

A - CGA-48988
B - CGA-62826
C - CGA-100255
D - CGA-94689B
E - CGA-108905
F_5 - CGA-37734
F_7 - N6
G - N5
I - N3, N1b aglycone, acidics
I_s - N1b aglycone
J - N2
K - N1b, glucose conjugate

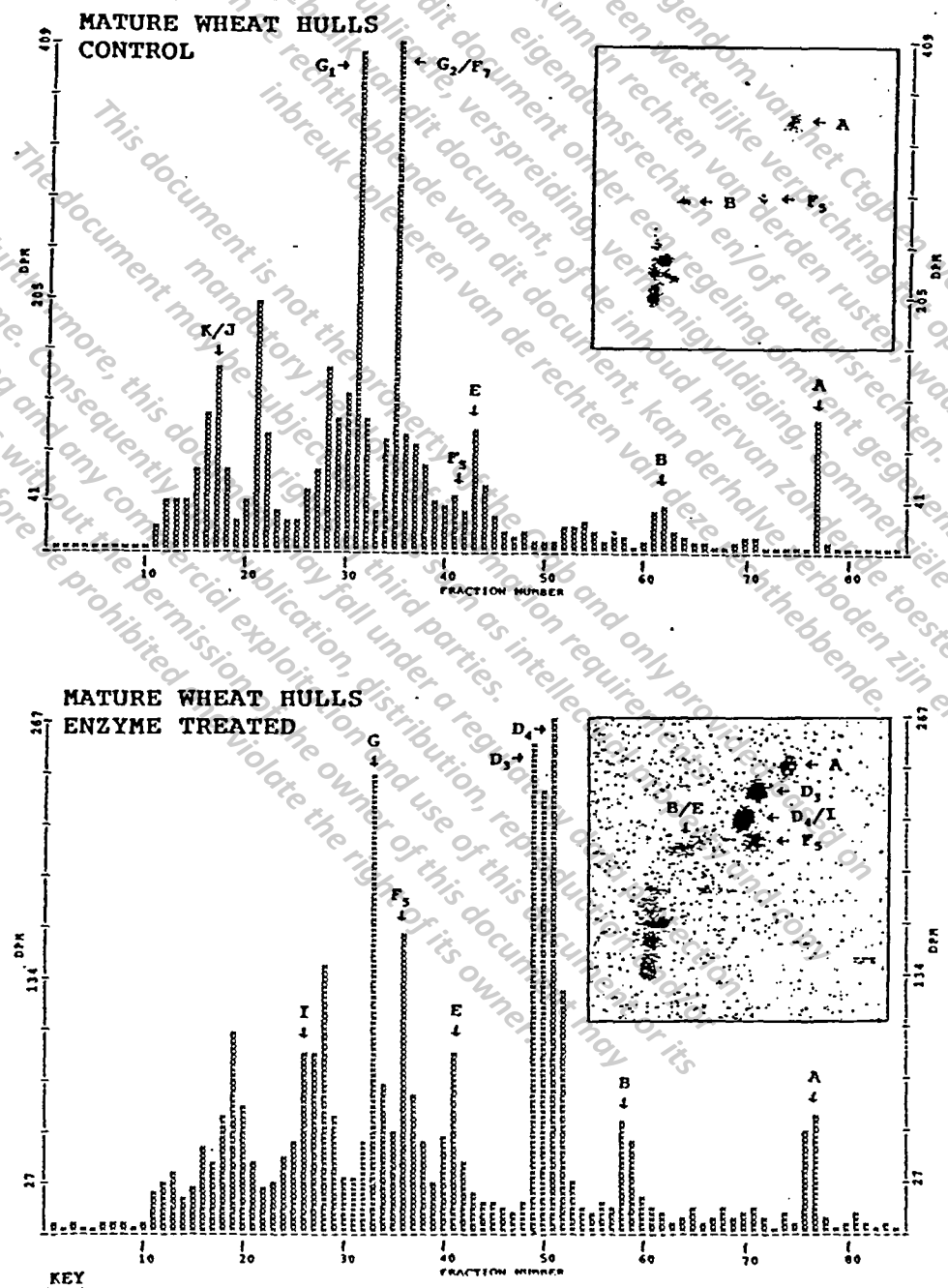
FIGURE 23. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF EXTRACTABLE RADIOACTIVITY IN MATURE WHEAT STALKS



KEY

A - CGA-48988
B - CGA-62826
D_3 - CGA-94689A
D_4 - CGA-94689B
E - CGA-108905
F_5 - CGA-37734
F_7 - N6
G - N4, N5, acidics
G_1 - N4, acidics
G_2 - N5, acidics
I - N3, N1a aglycone, acidics
J - N2
K - N1a, acidics

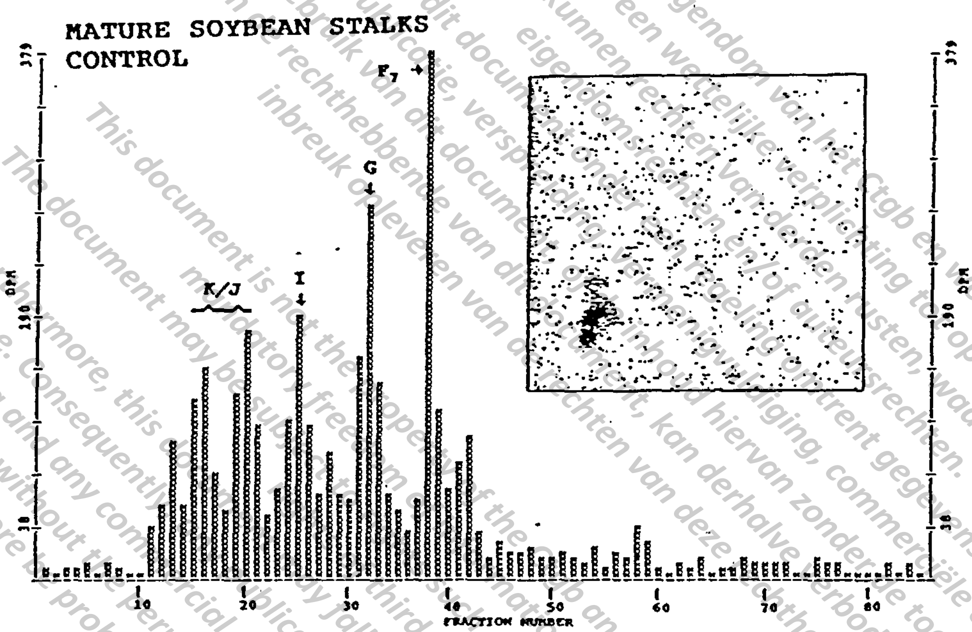
FIGURE 24. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF EXTRACTABLE RADIOACTIVITY IN MATURE WHEAT HULLS

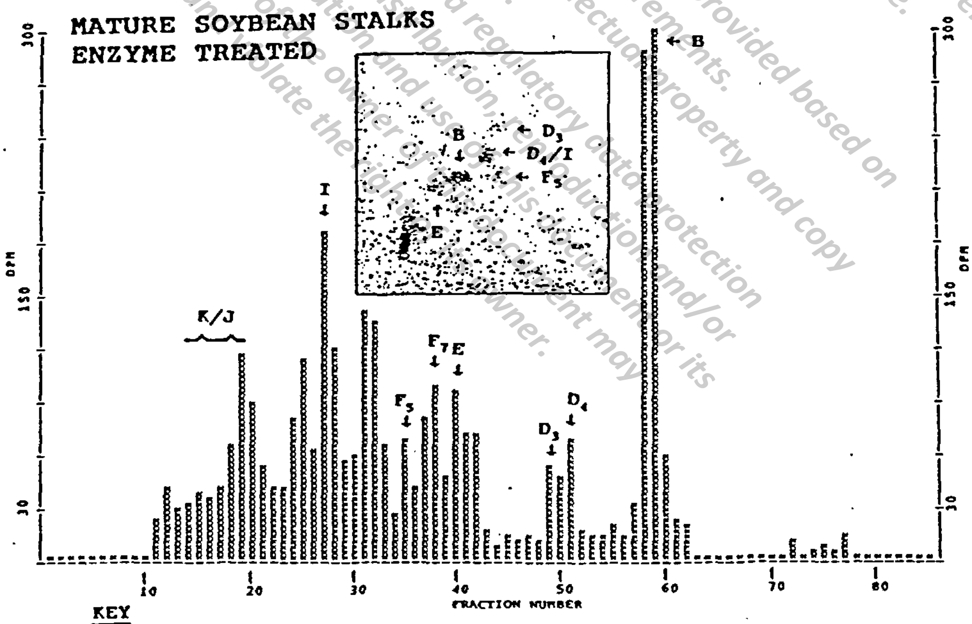


KEY

A – CGA-48988
B – CGA-62826
D_3 – CGA-94689A
D_4 – CGA-94689B
E – CGA-108905
F_5 – CGA-37734
F_7 – N6
G – N4, N5, acidics
G_1 – N4
G_2 – N5
I – N1a aglycone, acidics
J – N2
K – N1a, acidics

FIGURE 25. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF EXTRACTABLE RADIOACTIVITY IN MATURE SOYBEAN STALKS

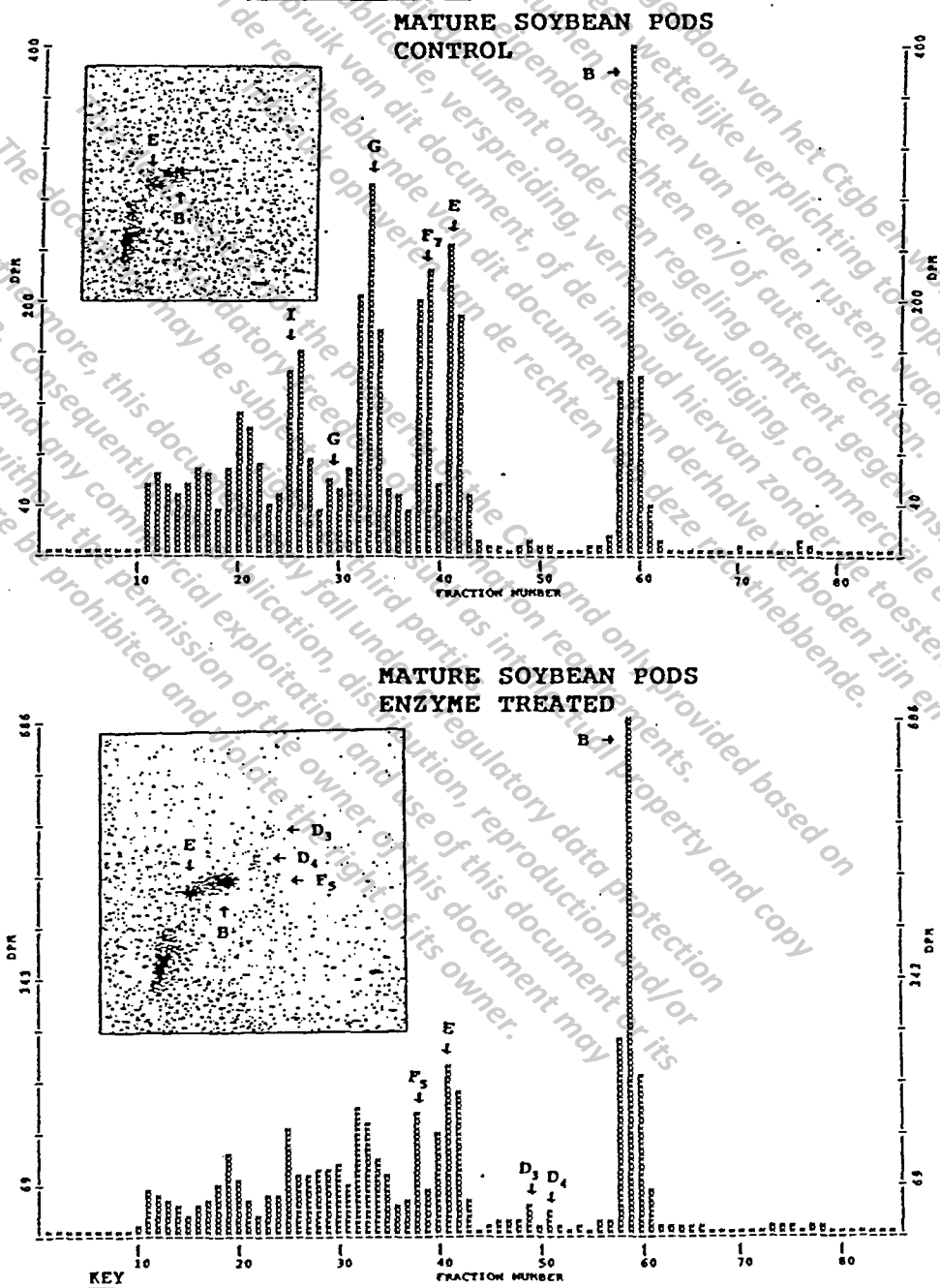




KEY

B – CGA-62826
D3 – CGA-94689A
D4 – CGA-94689B
E – CGA-108905
F5 – CGA-37734
F7 – N6
G – N4, N5, acidics
I – N1a aglycone, glucose conjugate, acidics
J – N2
K – N1a, acidics

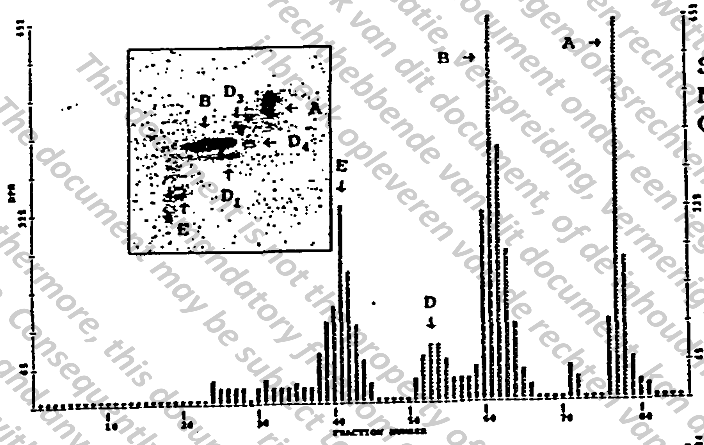
FIGURE 26. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF EXTRACTABLE RADIOACTIVITY IN MATURE SOYBEAN PODS



KEY

B – CGA-62826
D_3 – CGA-94689A
D_4 – CGA-94689B
E – CGA-108905
F_5 – CGA-37734
F_7 – N6
G – N4, N5, glucose conjugate, acidics
I – glucose conjugate, acidics

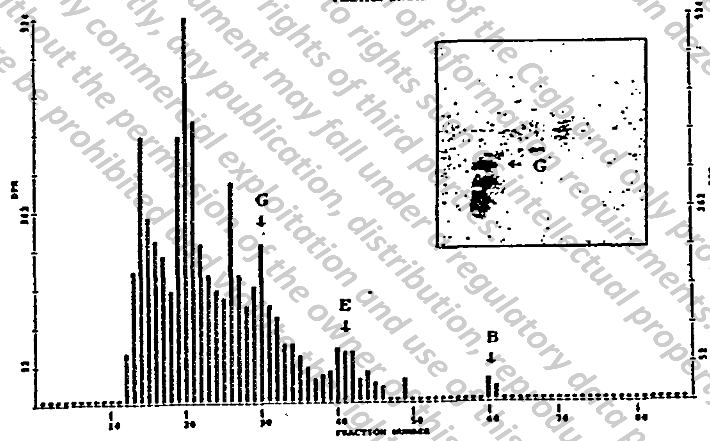
FIGURE 27. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE ORGANOSOLUBLE, AQUEOUS SOLUBLE, AND 6M HCL ACID HYDROLYZED AQUEOUS SOLUBLE RADIOACTIVITY IN MATURE SUGAR BEET ROOTS



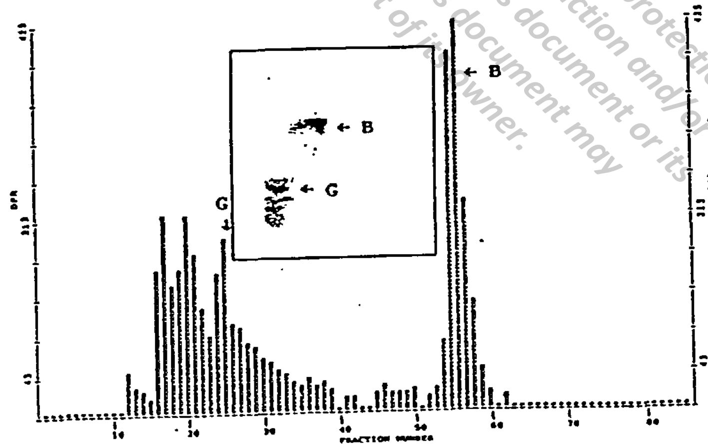
SUGAR BEET
MATURE ROOTS
ORGANIC FRACTION

KEY

A - CGA-48988
B - CGA-62826/CGA-67868
D - CGA-107955
CGA-94689A/B
D_1 - CGA-107955
D_3 - CGA-94689A
D_4 - CGA-94689B
E - CGA-108905, N7
G - CGA-79353

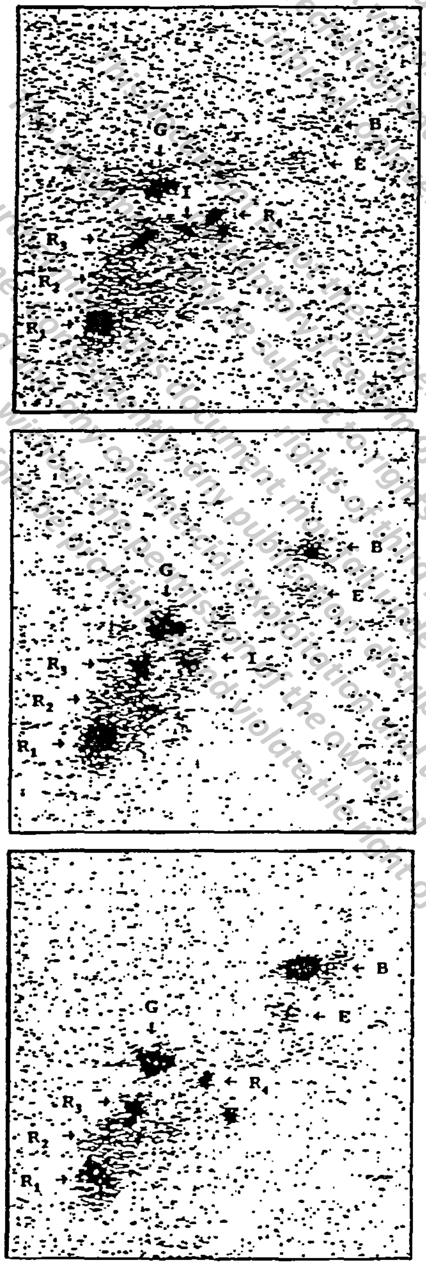


SUGAR BEET
MATURE ROOTS
AQUEOUS FRACTION



SUGAR BEET
MATURE ROOTS
AQUEOUS FRACTION
+ 6N HCl

FIGURE 28. NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A CELLULASE HYDROLYSIS AND A 6M HCL HYDROLYSIS OF AQUEOUS SOLUBLE RADIOACTIVITY IN MATURE SUGAR BEET ROOTS[1]



SUGAR BEET
MATURE ROOTS
CONTROL

KEY

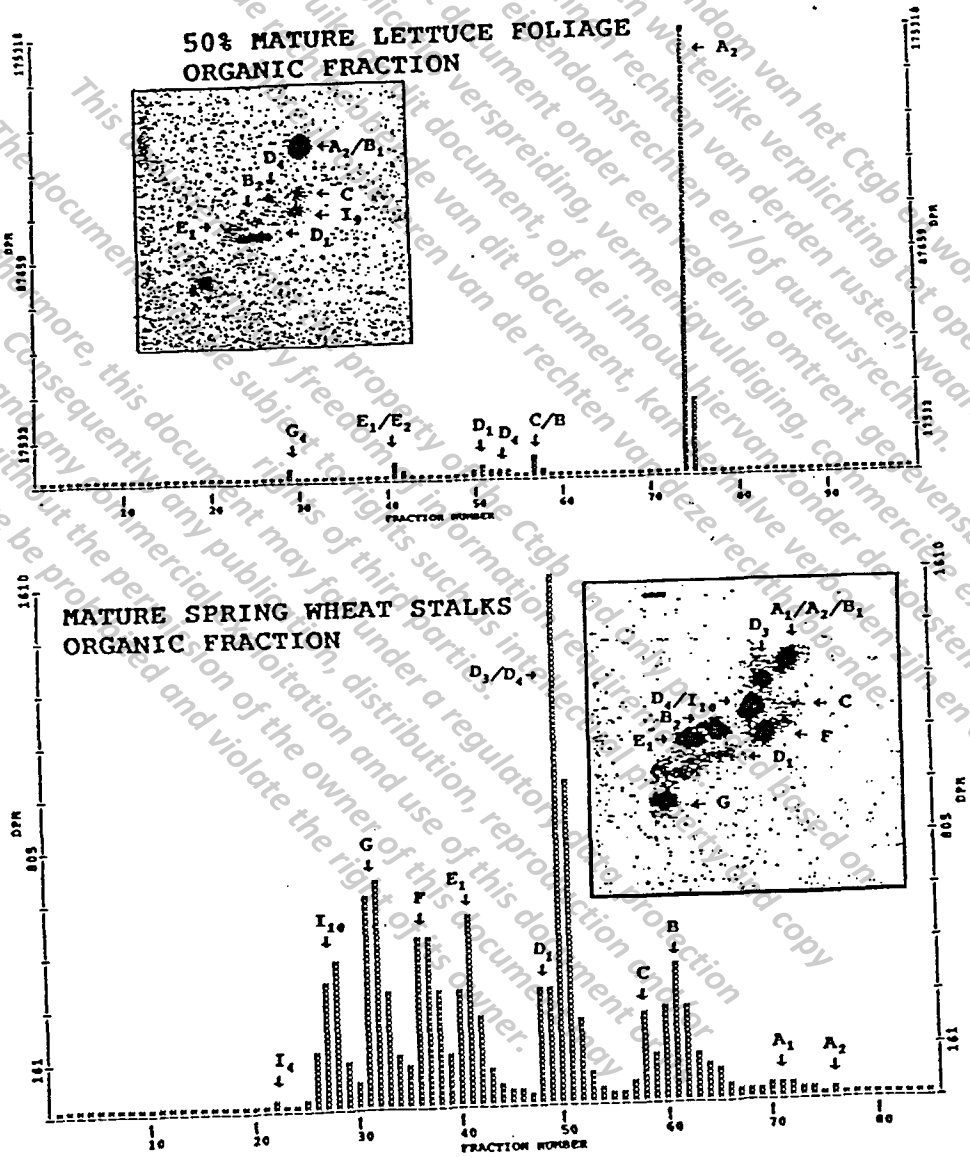
B – CGA-62826
E – CGA-108905
G – CGA-79353
I – CGA-108906
R_1 – REGION 1
R_2 – REGION 2
R_3 – REGION 3
R_4 – REGION 4

SUGAR BEET
MATURE ROOTS
CELLULASE HYDROLYSIS

SUGAR BEET
MATURE ROOTS
6N HCl HYDROLYSIS

[1]SS7 AND SS8

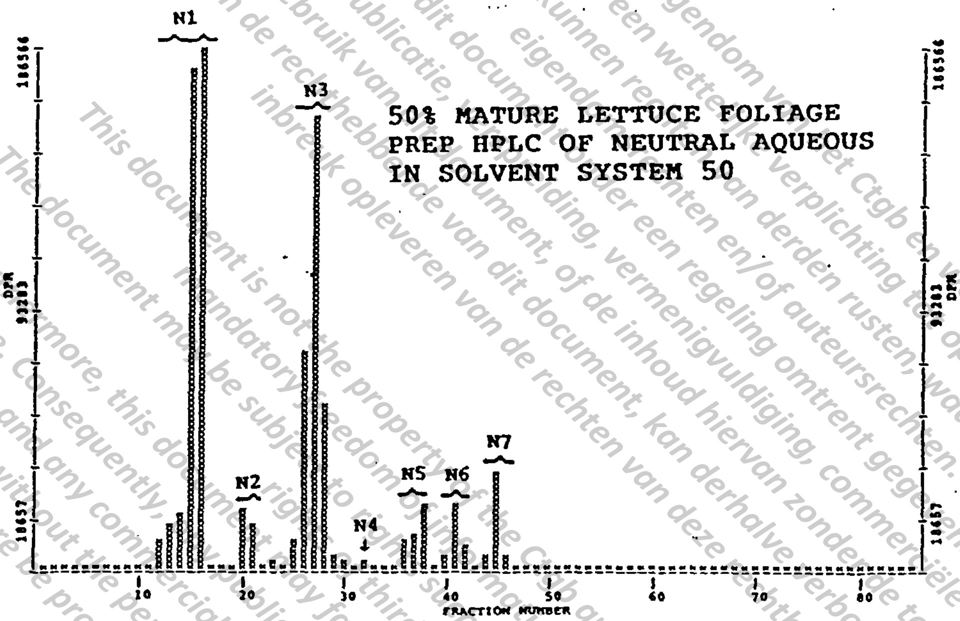
FIGURE 29. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE ORGANOSOLUBLE RADIOACTIVITY IN 50% MATURE LETTUCE FOLIAGE AND MATURE WHEAT STALKS



KEY

A_1 – CGA-67869
A_2 – CGA-48988
B – CGA-67868, CGA-62826
B_1 – CGA-67868
B_2 – CGA-62826
C – CGA-100255
D_1 – CGA-107955
D_3 – CGA-94689A
D_4 – CGA-94689B
E_1 – CGA-108905
E_2 – N7
F – CGA-37734
G – N4, N5
G_4 – LU2 (unknown)
I_4 – U0 (unknown)
I_9 – N1b aglycone
I_{10} – N1a aglycone

FIGURE 30. REPRESENTATIVE PREPARATIVE HPLC HISTOGRAMS OF THE NEUTRAL AQUEOUS RADIOACTIVITY FROM 50% MATURE LETTUCE FOLIAGE AND MATURE WHEAT STALKS



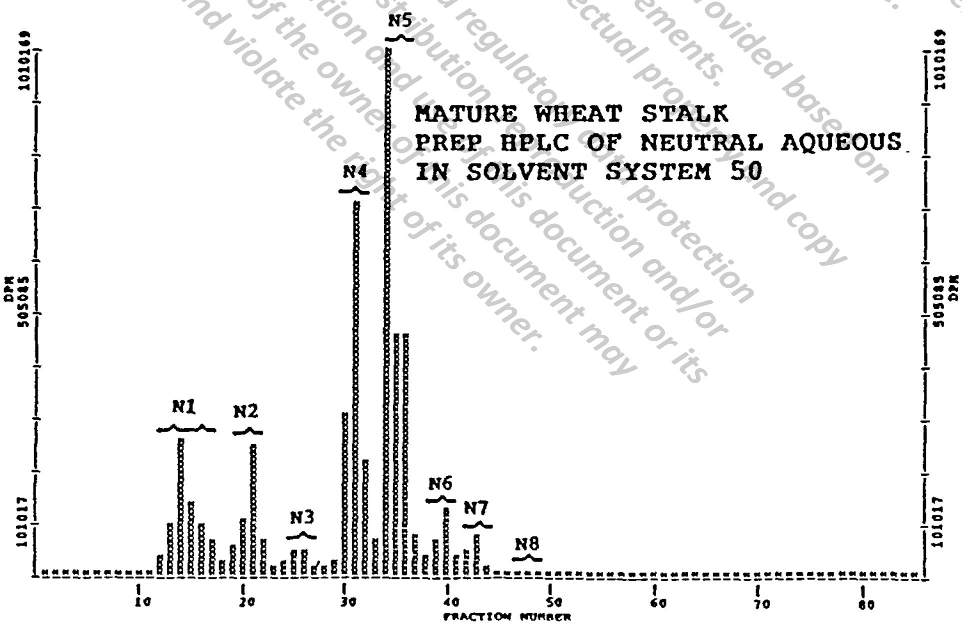
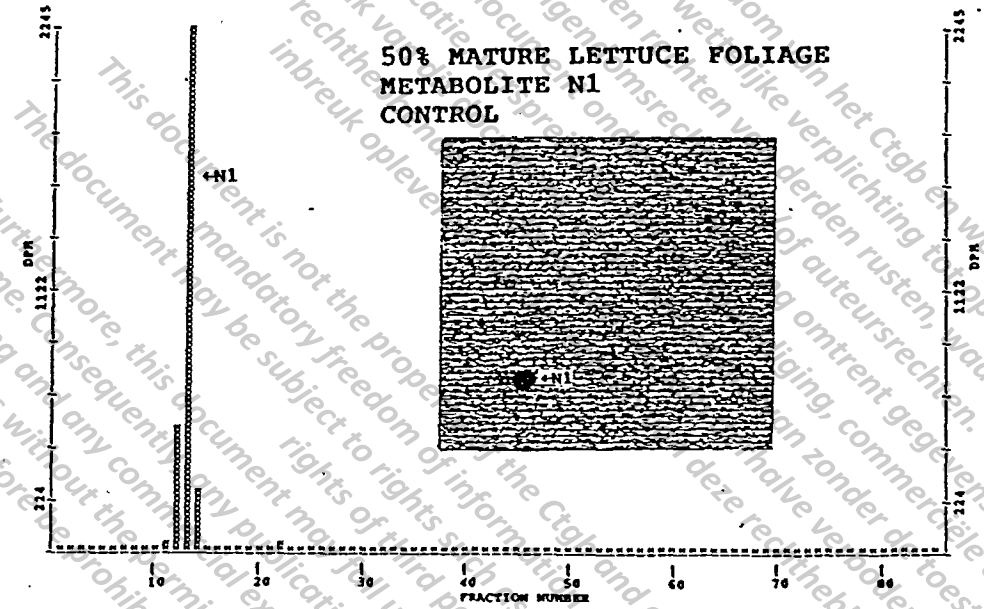


FIGURE 31. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N1 FROM 50% MATURE LETTUCE FOLIAGE



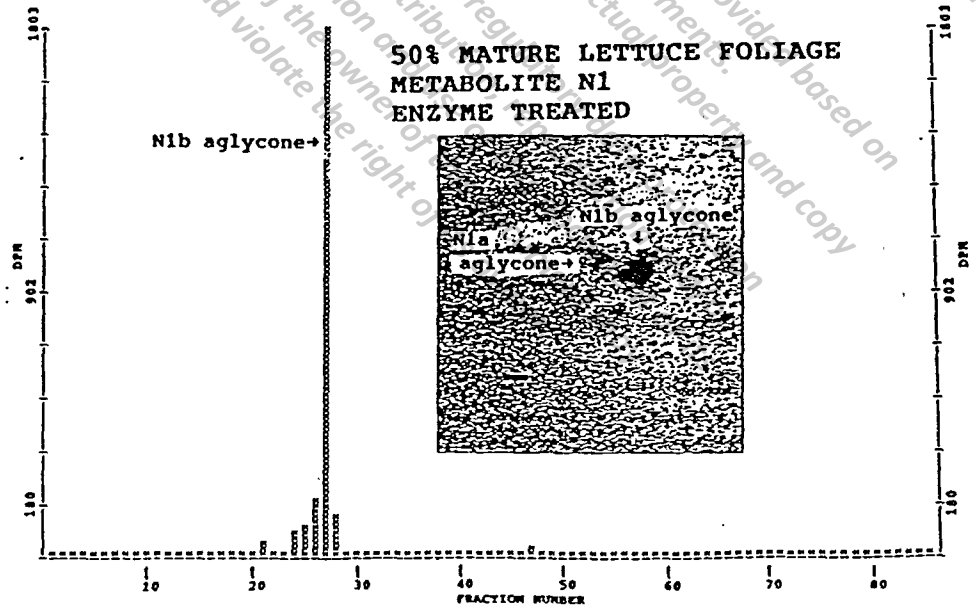
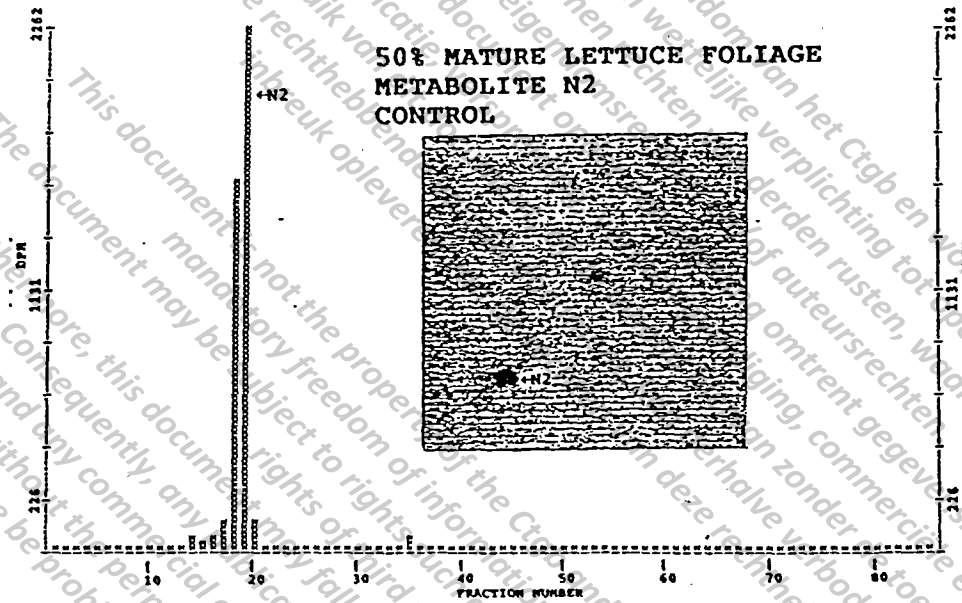


FIGURE 32. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N2 FROM 50% MATURE LETTUCE FOLIAGE



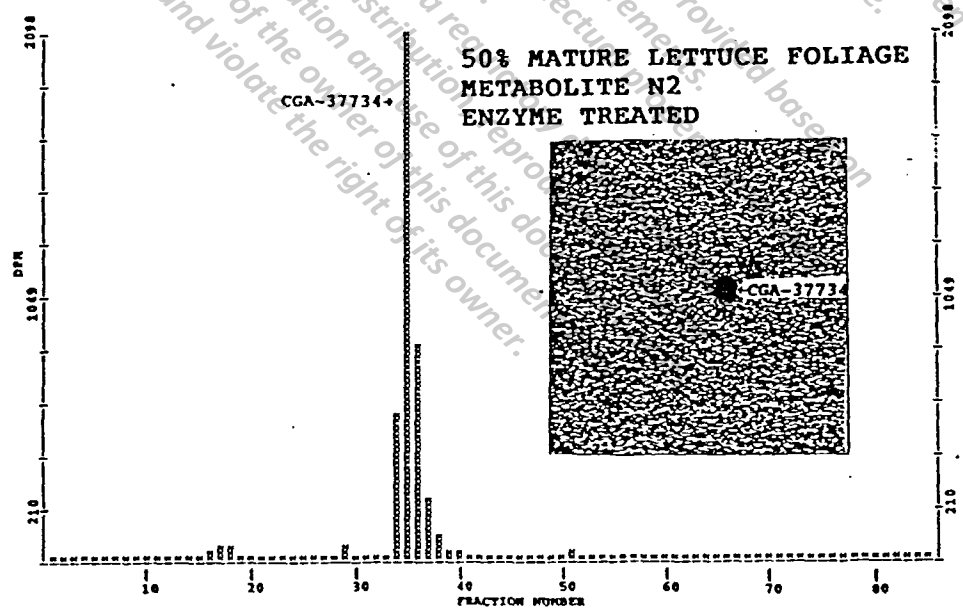
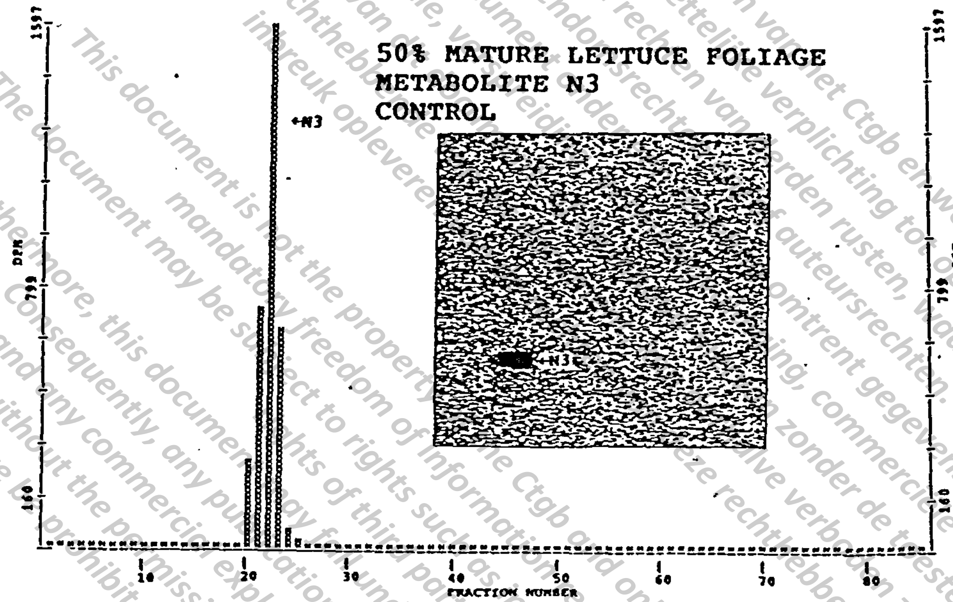


FIGURE 33. <u>HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N3 FROM 50% MATURE LETTUCE FOLIAGE</u>



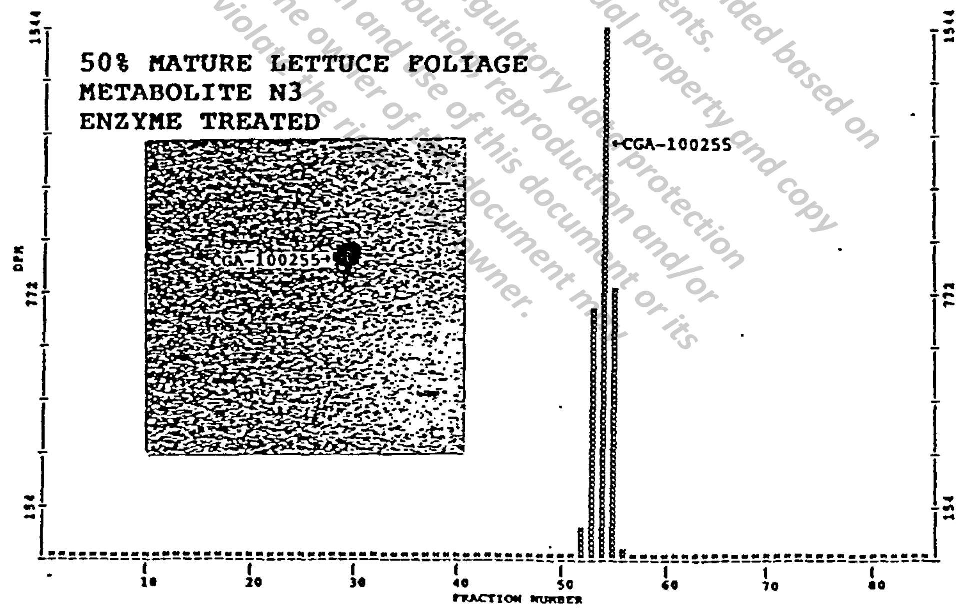
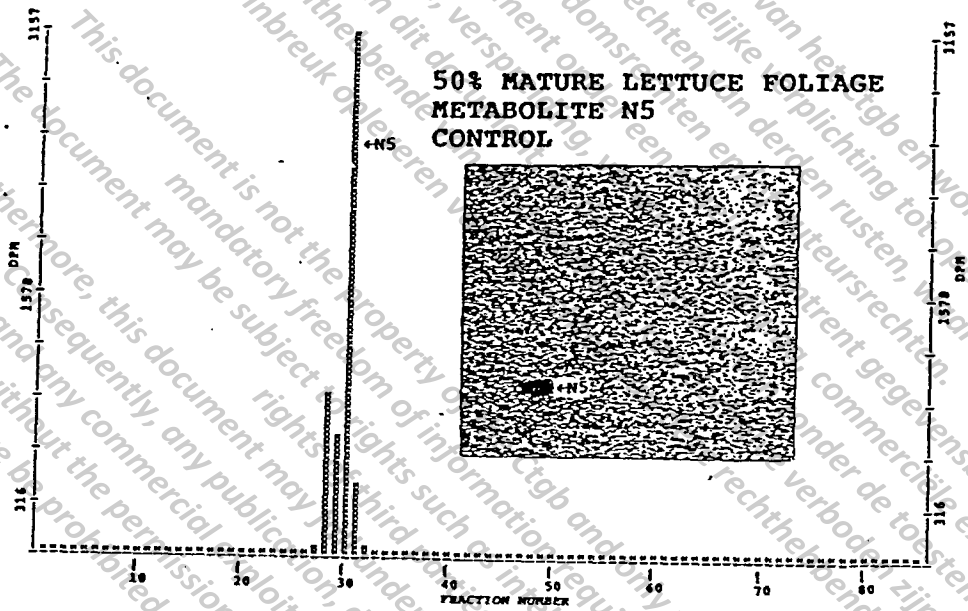


FIGURE 34. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N5 FROM 50% MATURE LETTUCE FOLIAGE



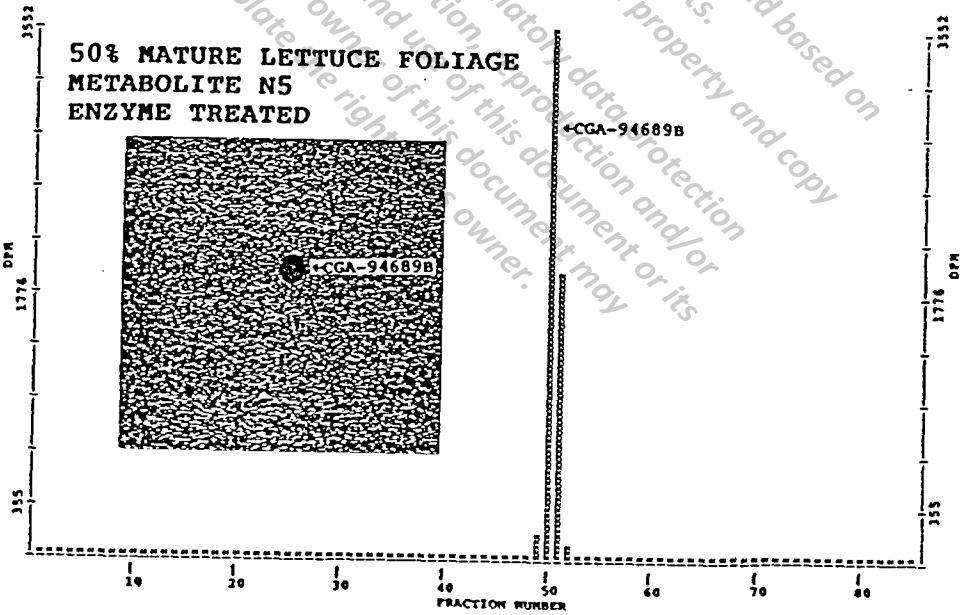
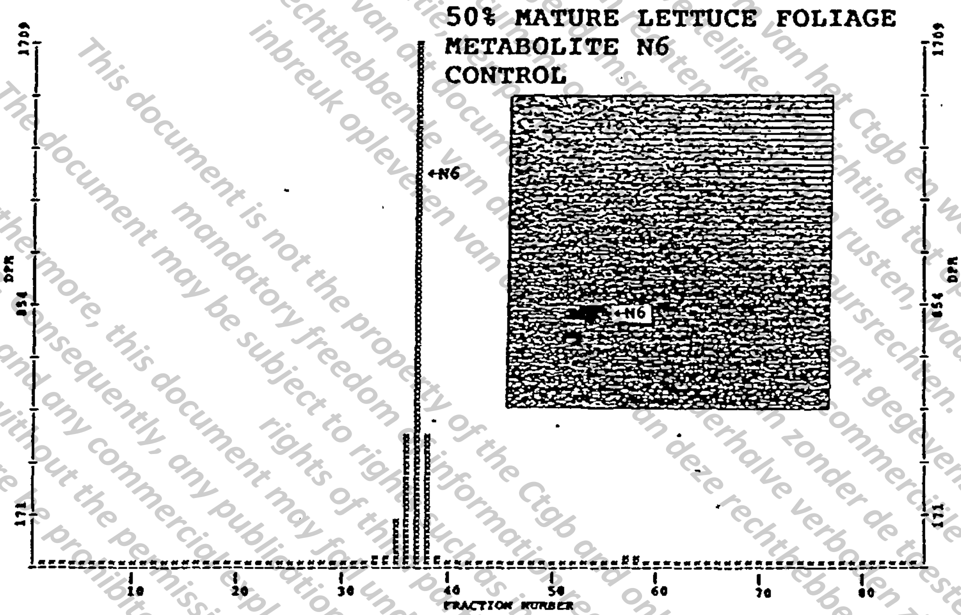


FIGURE 35. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N6 FROM 50% MATURE LETTUCE FOLIAGE



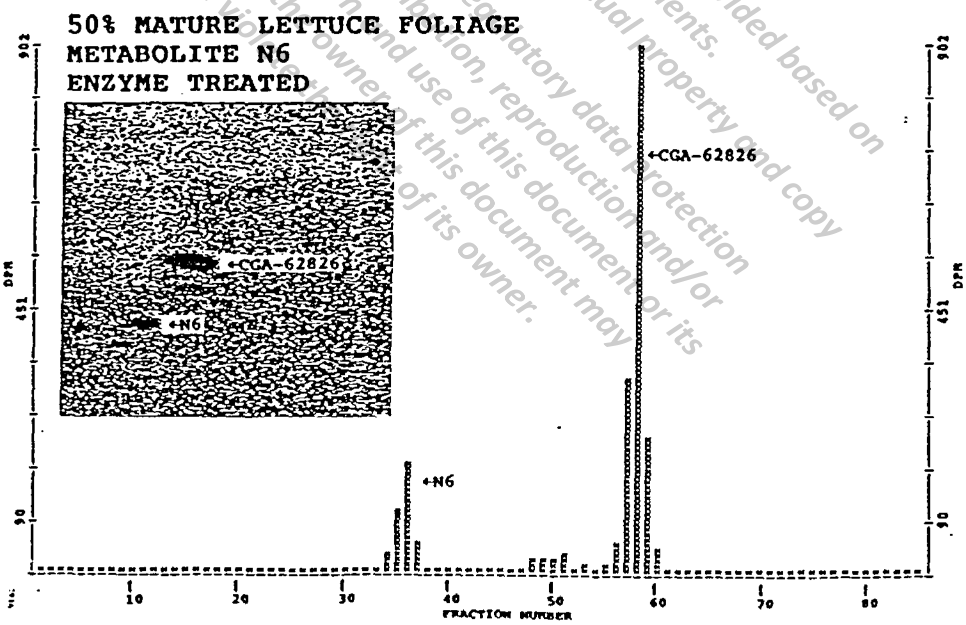
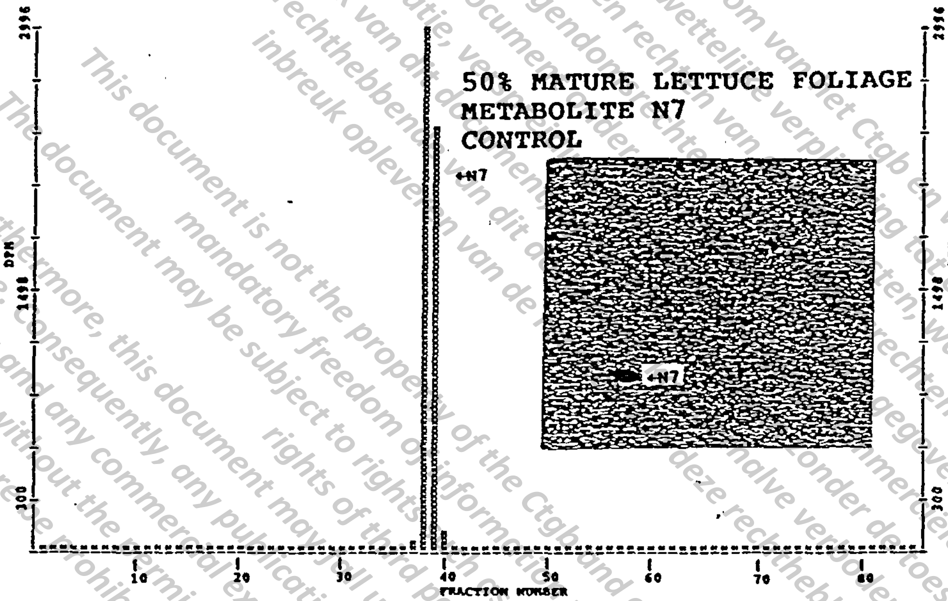


FIGURE 36. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N7 FROM 50% MATURE LETTUCE FOLIAGE



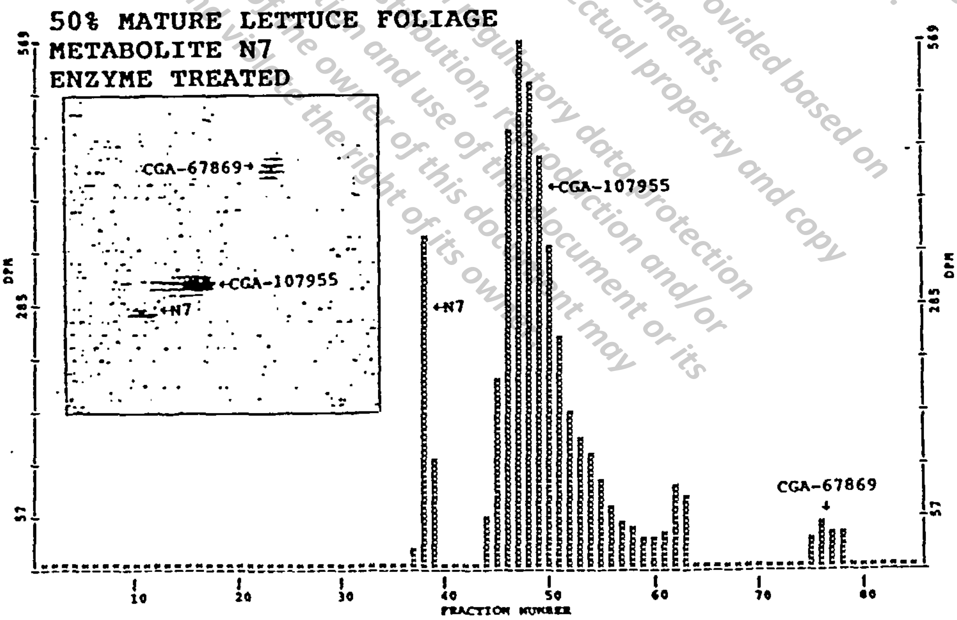


FIGURE 37. REVERSED PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM THE β-GLUCOSIDASE HYDROLYSES OF METABOLITES N1, N2, N3, N5, N6 AND N7 FROM 50% MATURE LETTUCE FOLIAGE

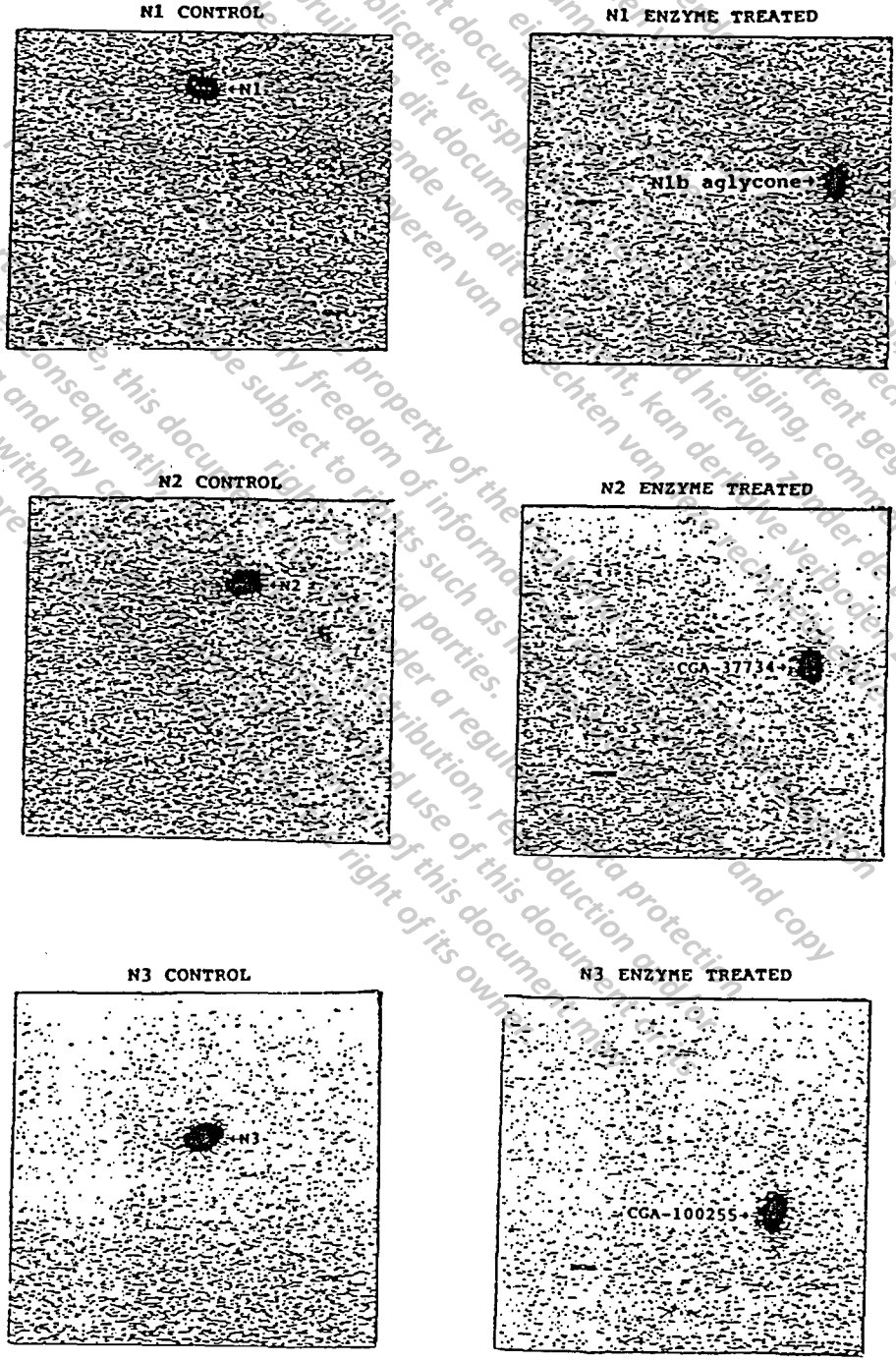


FIGURE 37. REVERSED PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM THE β-GLUCOSIDASE HYDROLYSES OF METABOLITES N1, N2, N3, N5, N6 AND N7 FROM 50% MATURE LETTUCE FOLIAGE (Continued)

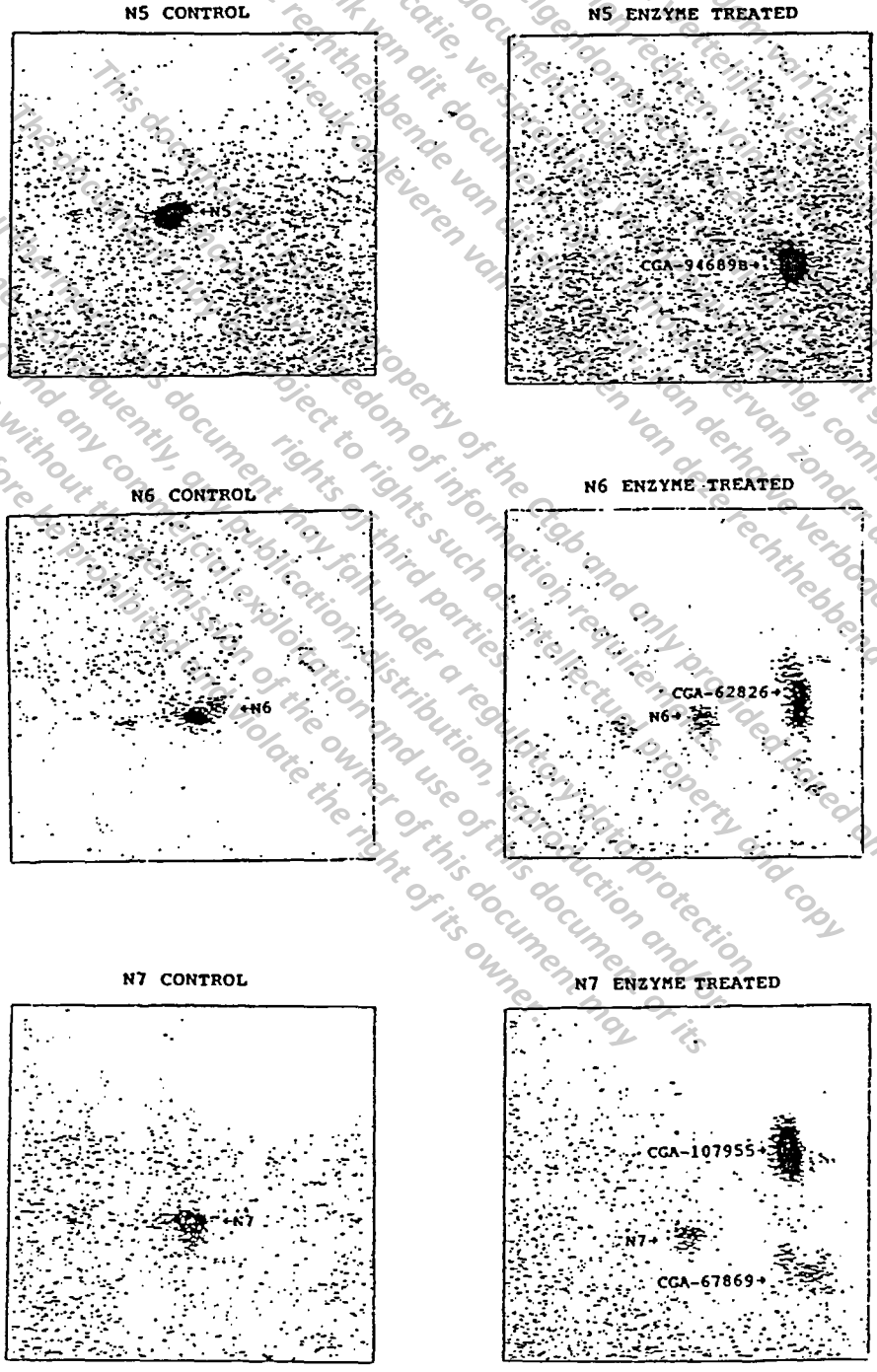


FIGURE 38. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N1 FROM MATURE WHEAT STALKS

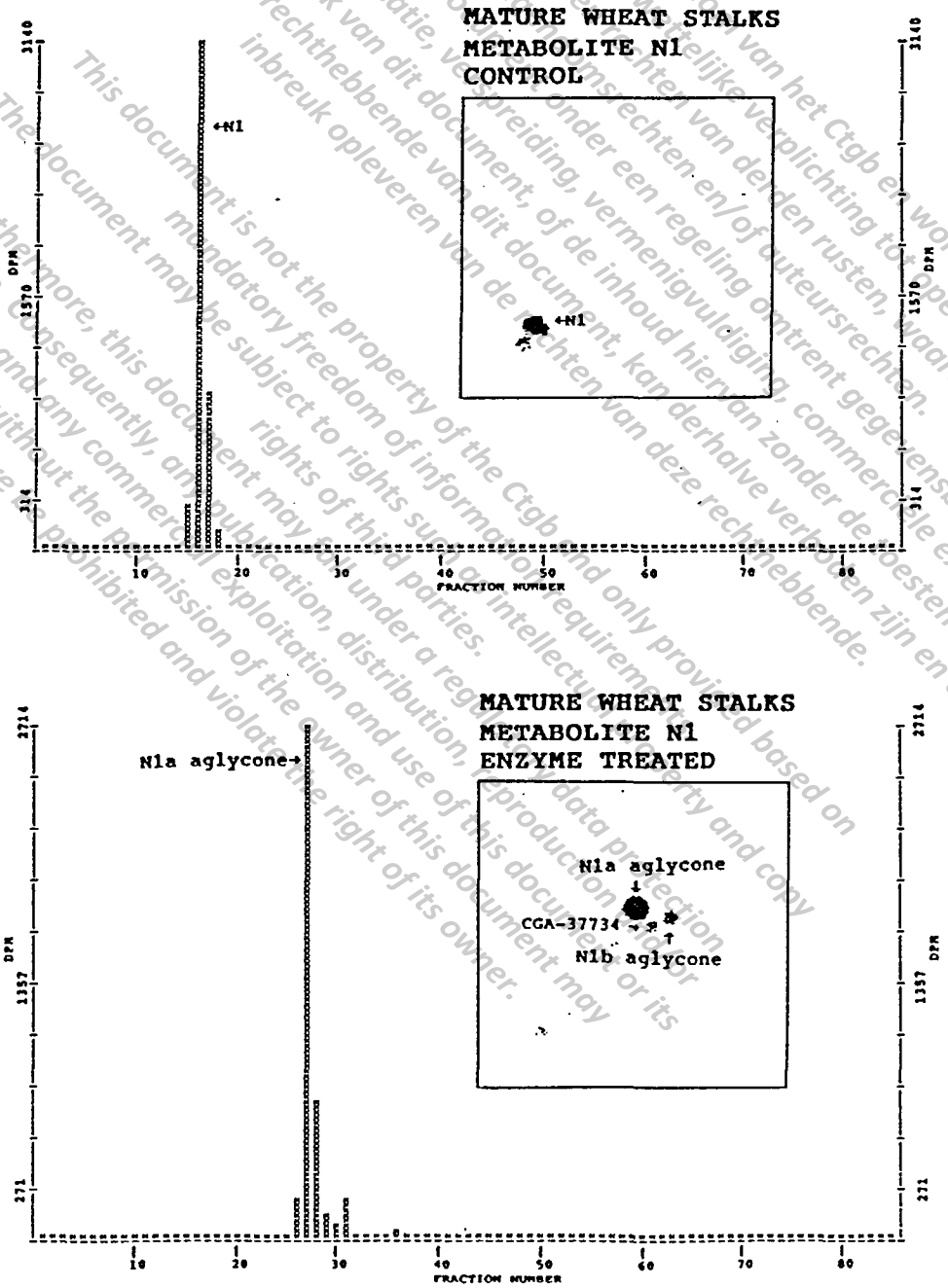
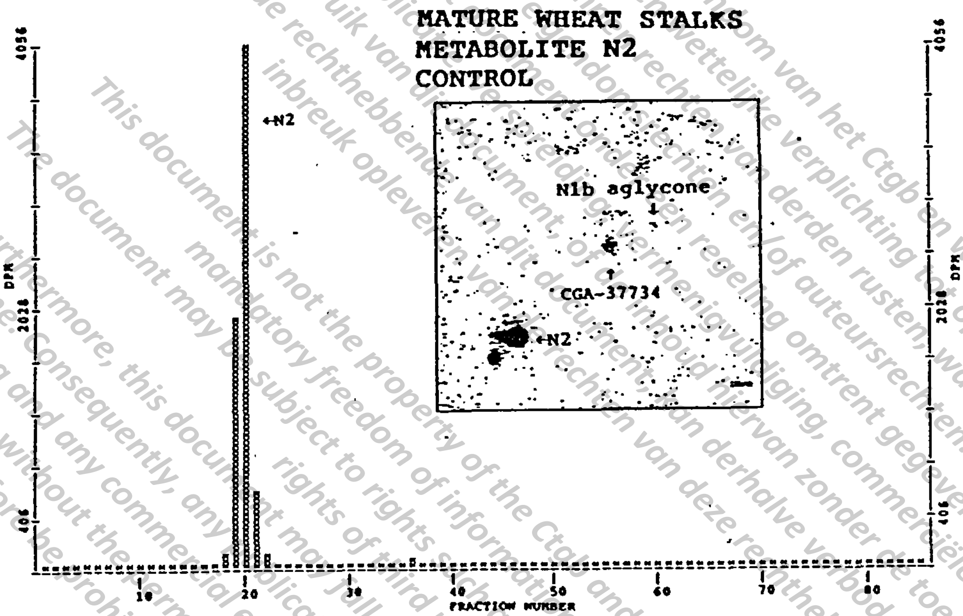


FIGURE 39. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N2 FROM MATURE WHEAT STALKS



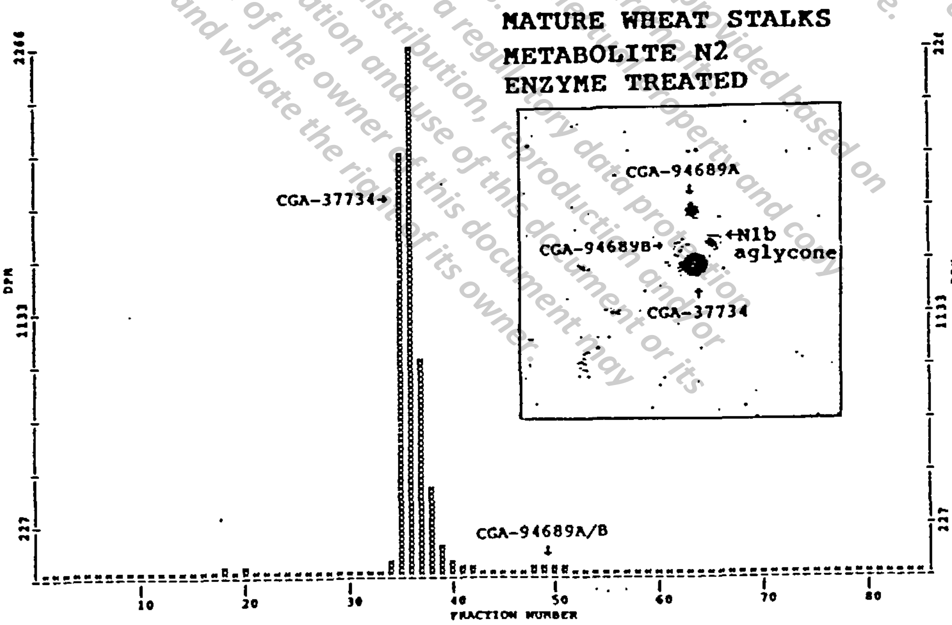
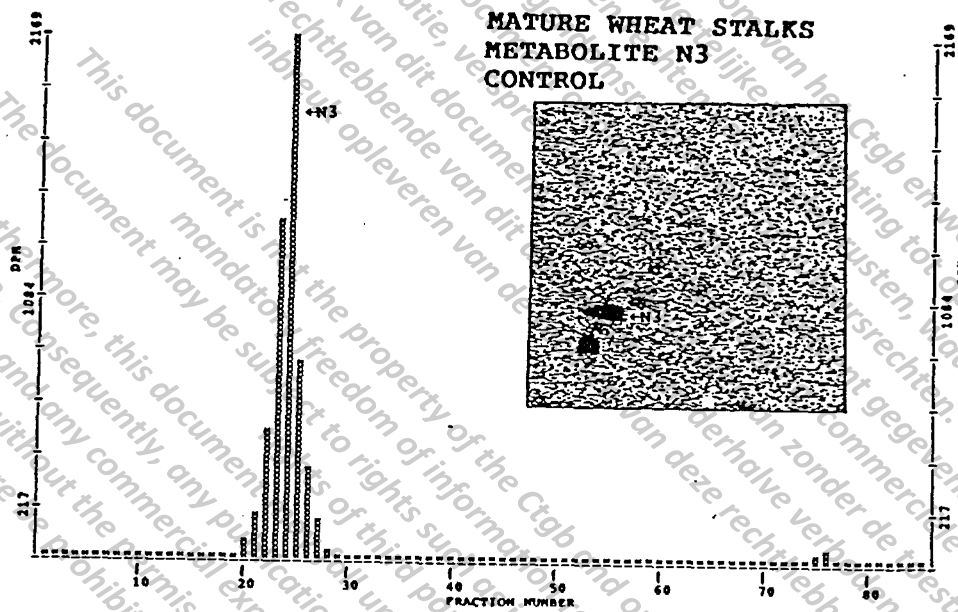


FIGURE 40. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N3 FROM MATURE WHEAT STALKS



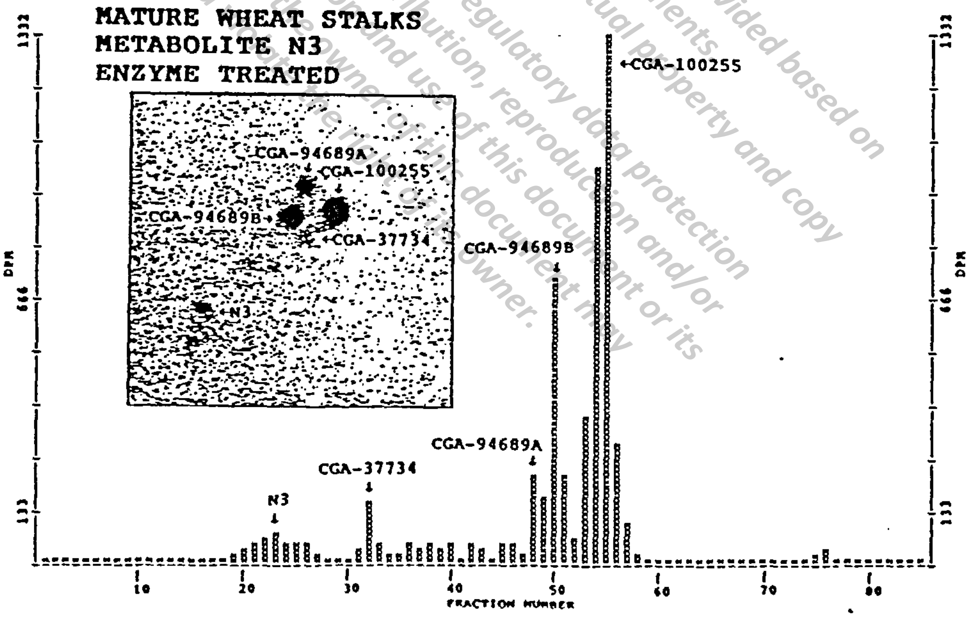


FIGURE 41. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N4 FROM MATURE WHEAT STALKS

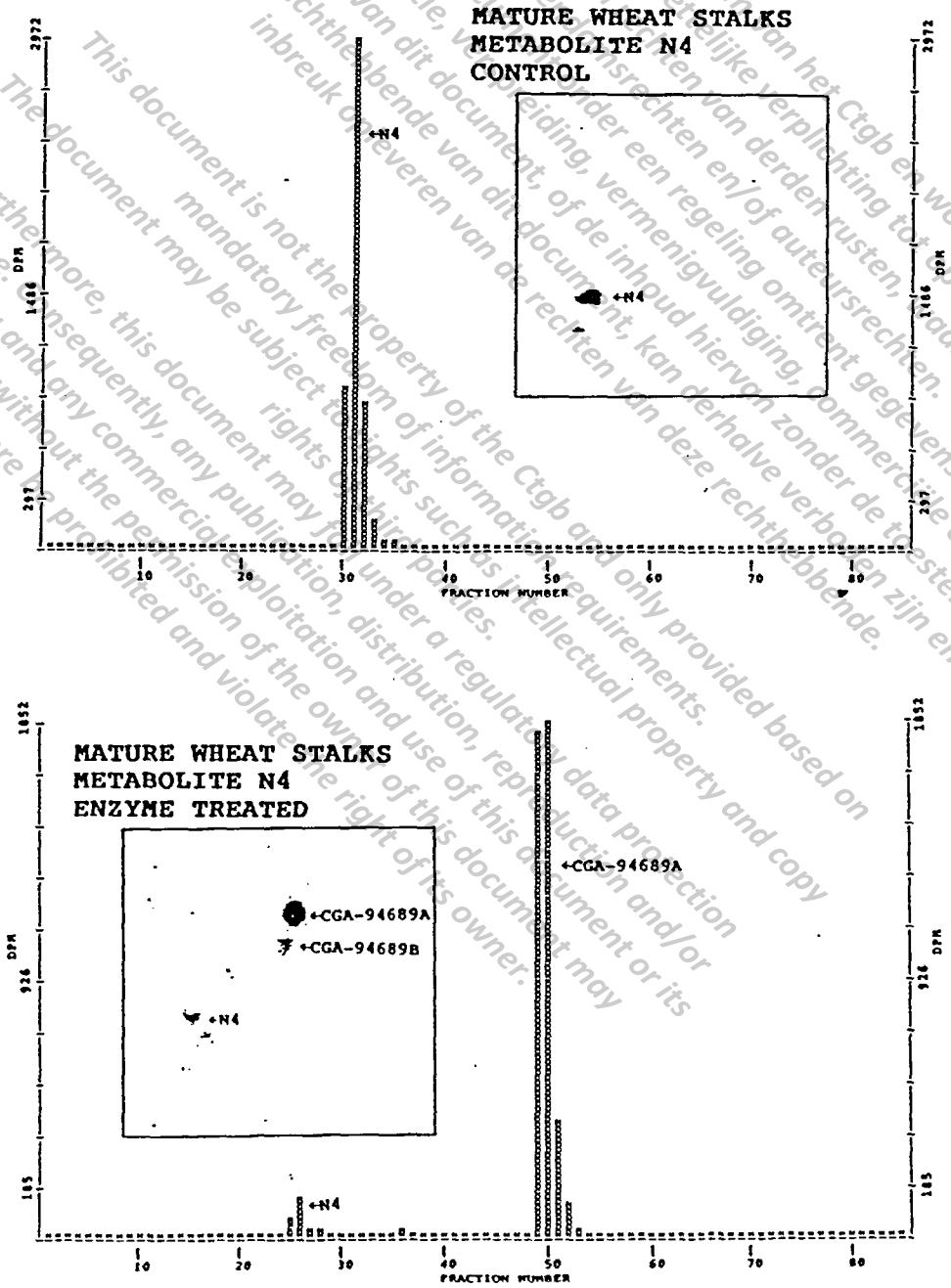


FIGURE 42. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N5 FROM MATURE WHEAT STALKS

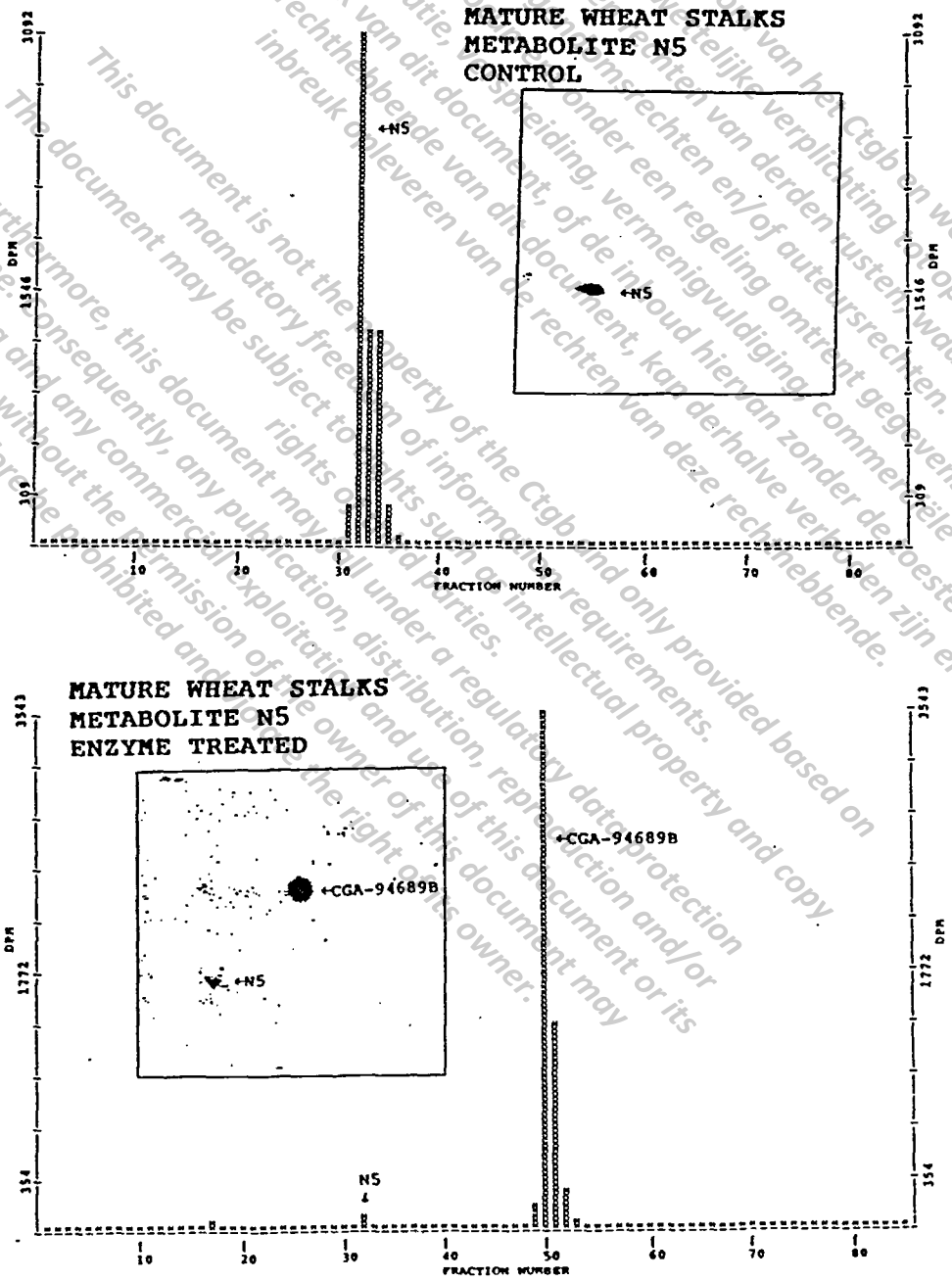
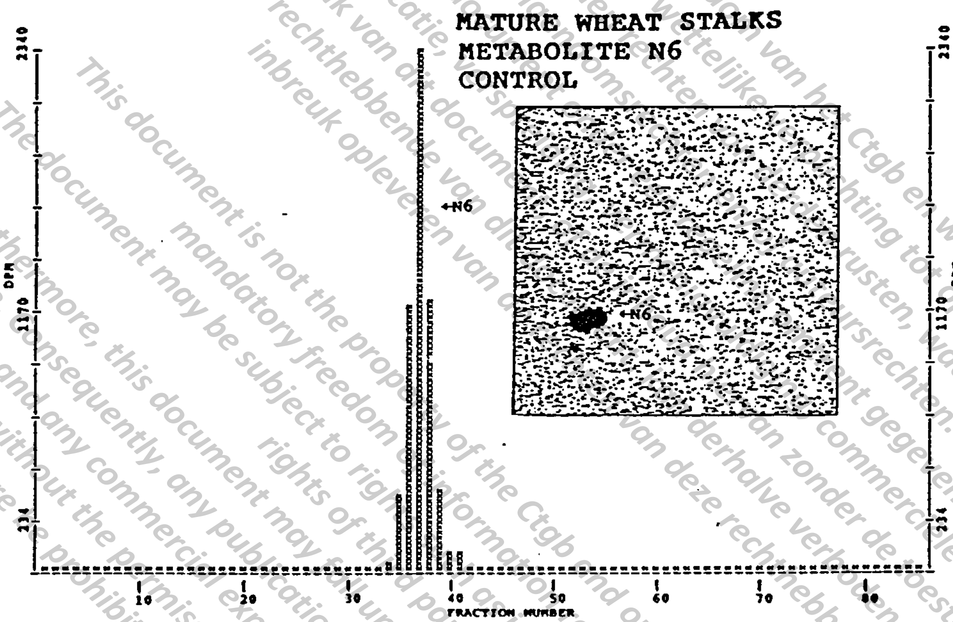


FIGURE 43. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N6 FROM MATURE WHEAT STALKS



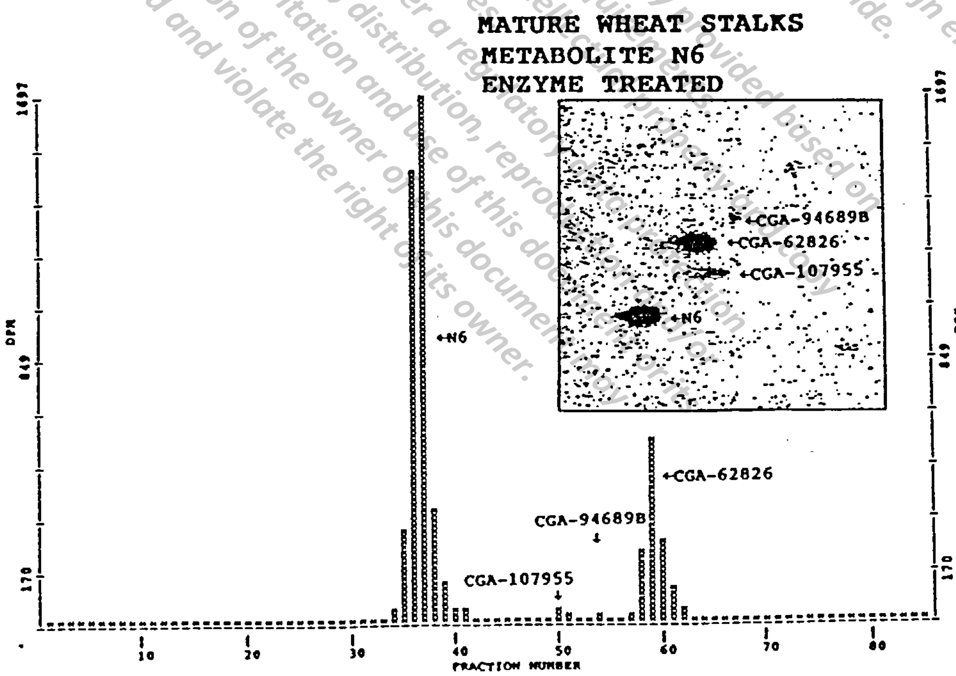
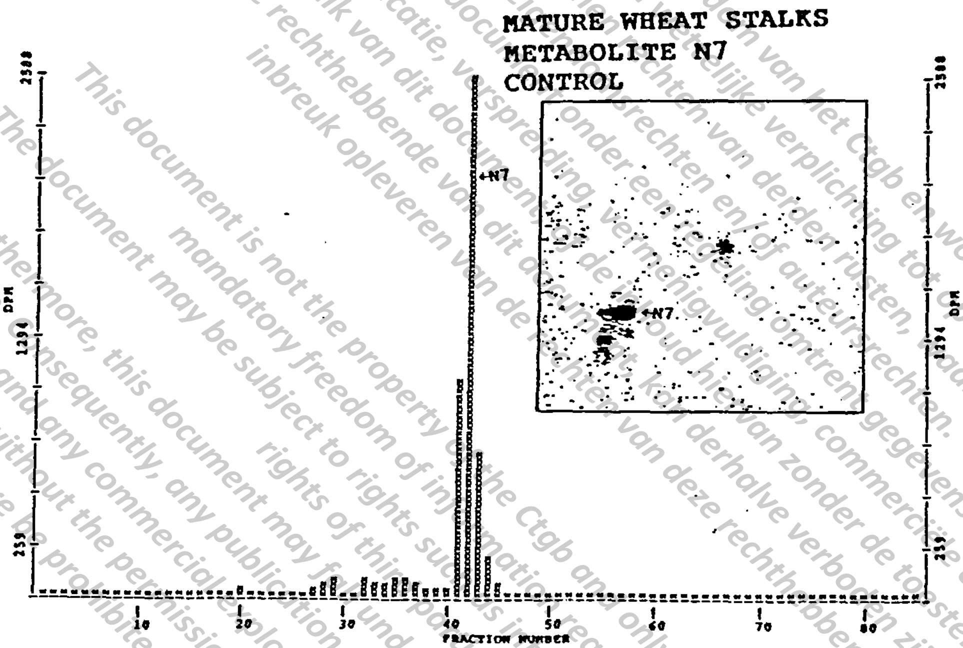


FIGURE 44. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM A β-GLUCOSIDASE HYDROLYSIS OF METABOLITE N7 FROM MATURE WHEAT STALKS



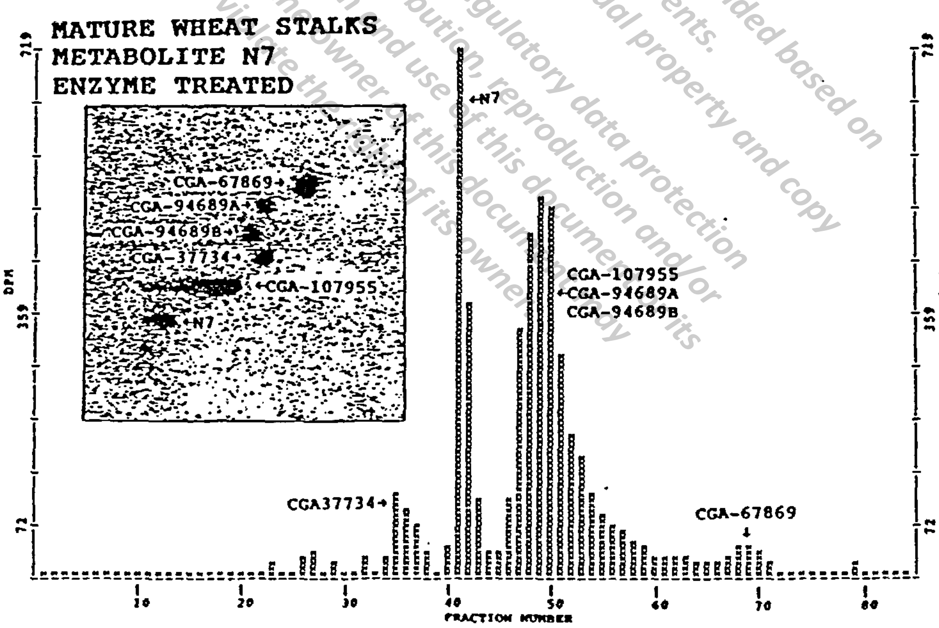


FIGURE 45. REVERSED PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM THE β-GLUCOSIDASE HYDROLYSES OF METABOLITES N1, N2, N3, N4, N5, N6 AND N7 FROM MATURE WHEAT STALKS

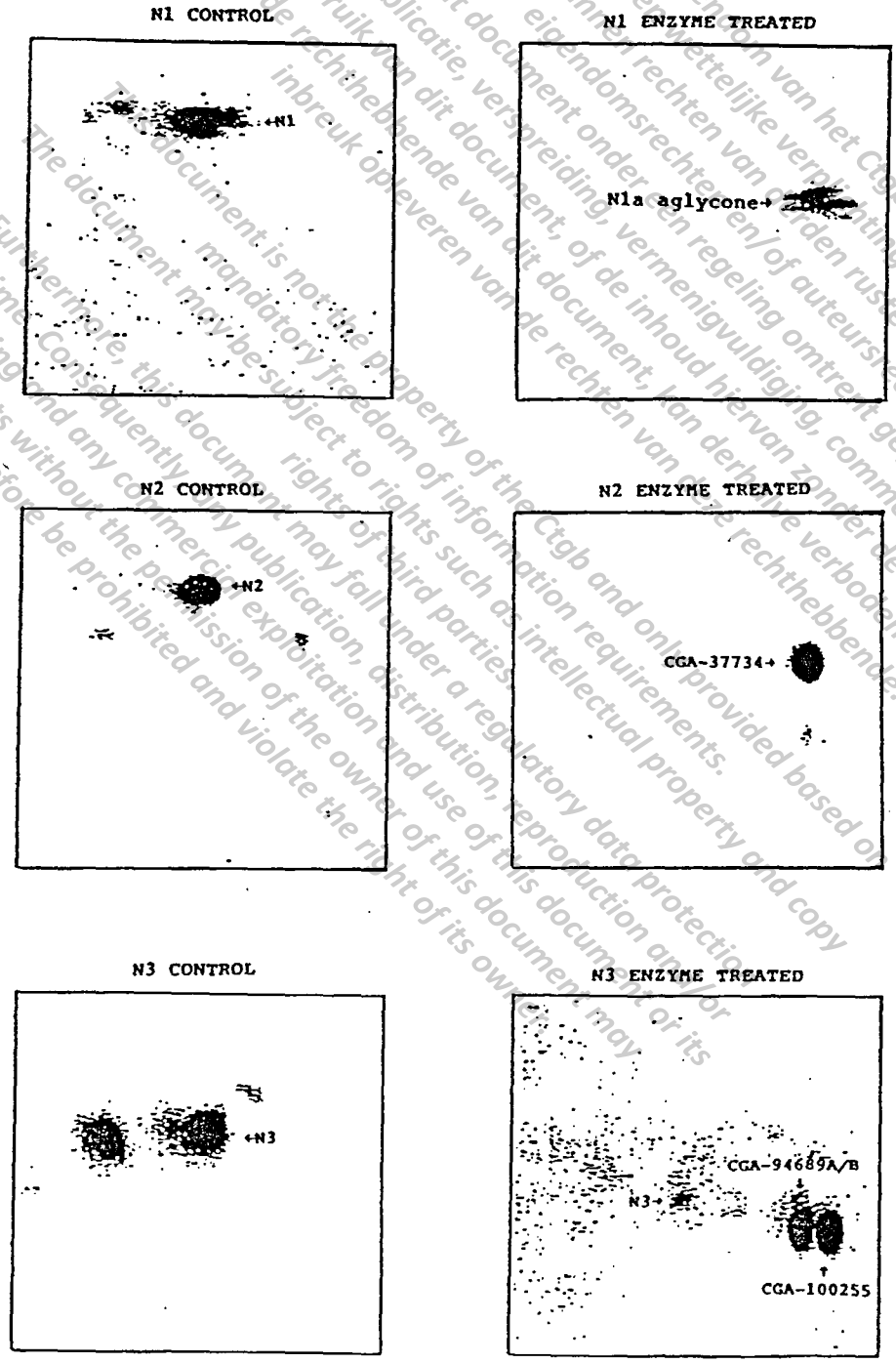


FIGURE 45. REVERSED PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE CONTROL AND TREATED REACTIONS FROM THE β-GLUCOSIDASE HYDROLYSES OF METABOLITES N1, N2, N3, N4, N5, N6 AND N7 FROM MATURE WHEAT STALKS (Continued)

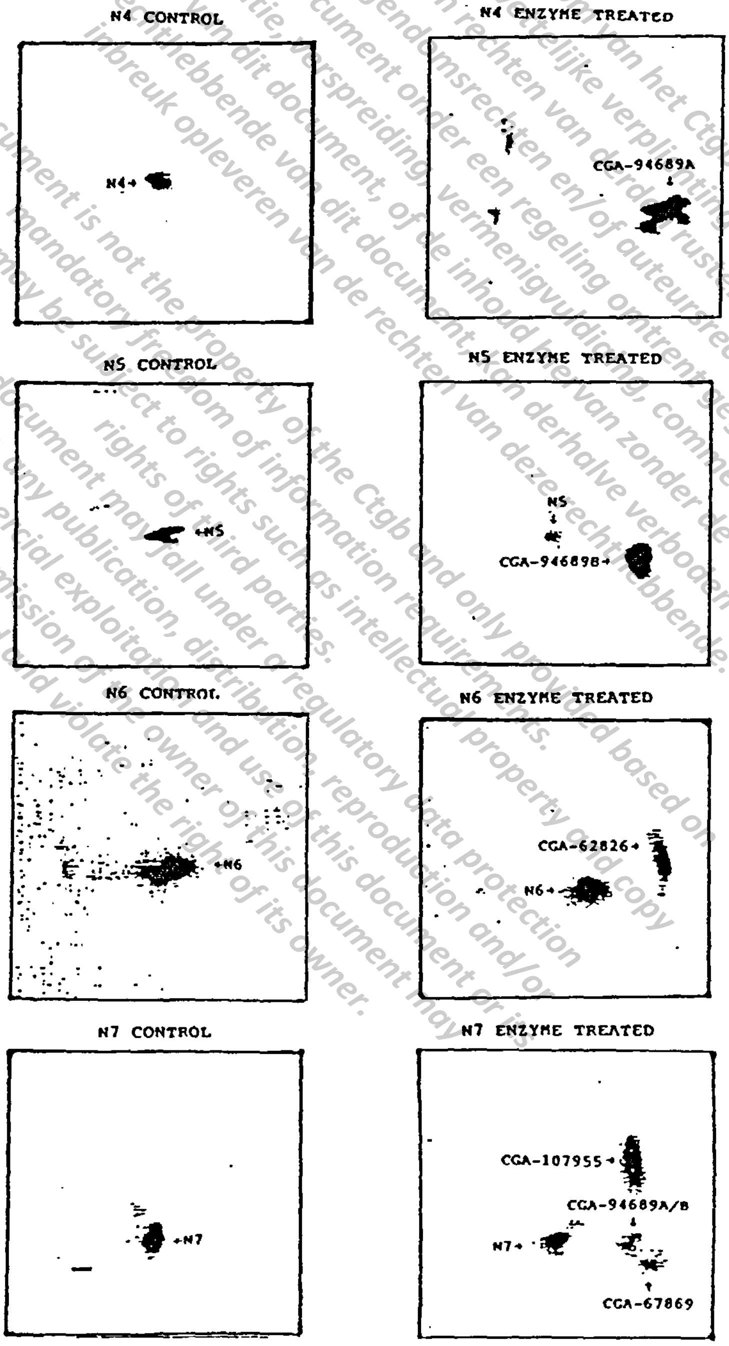


FIGURE 46. MASS SPECTRUM OF ACETYLATED METABOLITE N1a ISOLATED FROM MATURE WHEAT STALKS BY DCI AND + TSP ANALYSIS

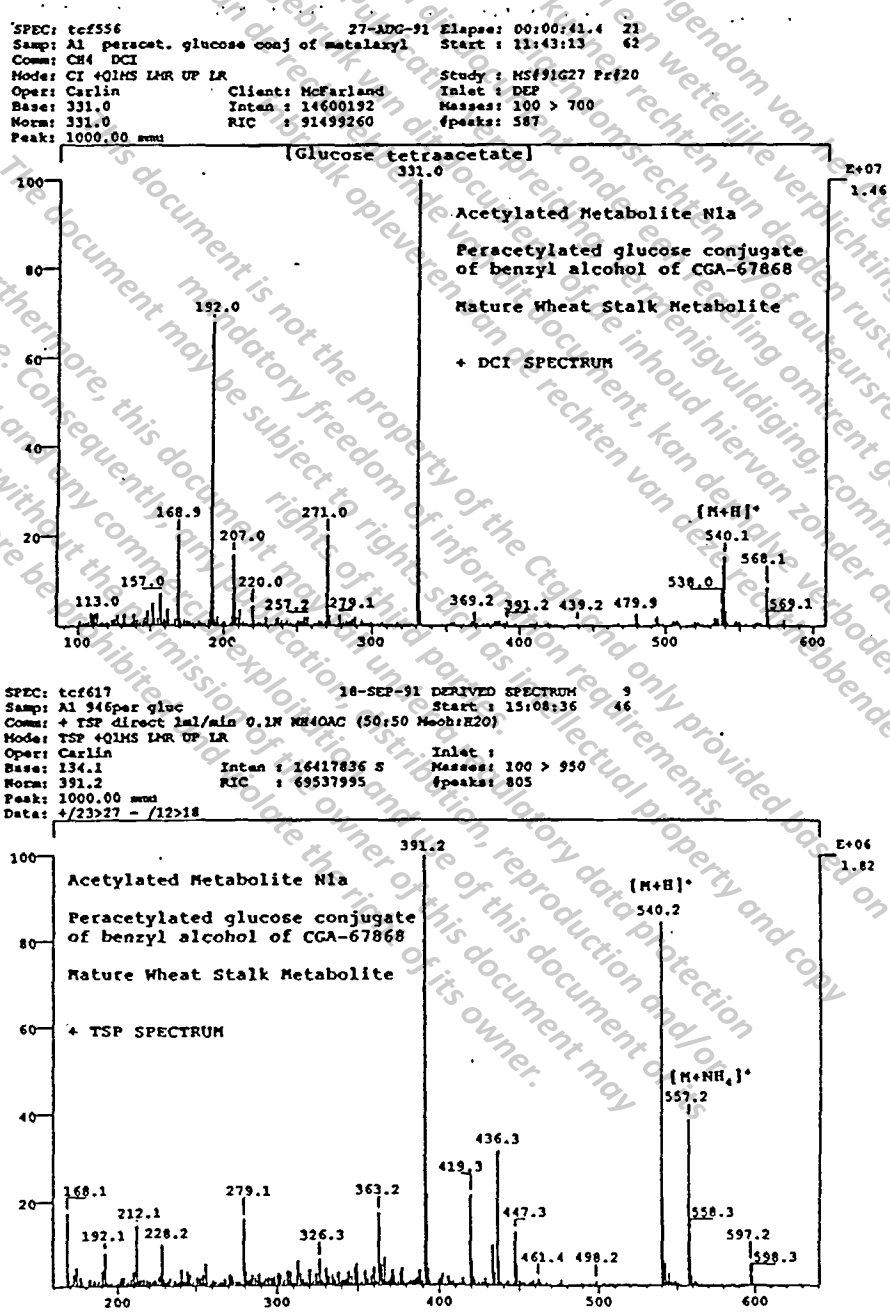


FIGURE 47. MASS SPECTRUM OF ACETYLATED METABOLITE N1b ISOLATED FROM 50% MATURE LETTUCE FOLIAGE BY + TSP ANALYSIS

SPEC: tcf679 09-JAN-92 Elapse: 00:00:45.6 23
Samp: N1-B Start : 13:57:46 30
Comm: +/- TSP direct 1 ml/min 0.1N NH4OAc (50:50 H2O/MeOH)
Mode: TSP +Q1MS LMR UP LR Study : MS91J5 Pr 203-89
Oper: Eberle Client: McFarland Inlet :
Base: 279.1 Inten : 1158724 Masses: 100 > 700
Norm: 279.0 RIC : 8703046 #peaks: 633
Peak: 1000.00 mmu Defect: 0 @ 1, 300 @ 1000

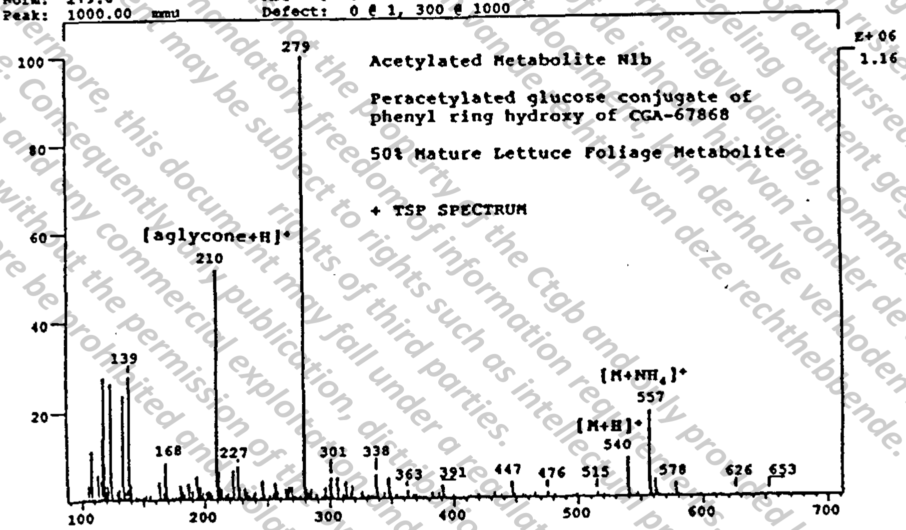


FIGURE 48. MASS SPECTRA OF ACETYLATED METABOLITE N2 ISOLATED FROM MATURE WHEAT STALKS BY +/- TSP ANALYSIS

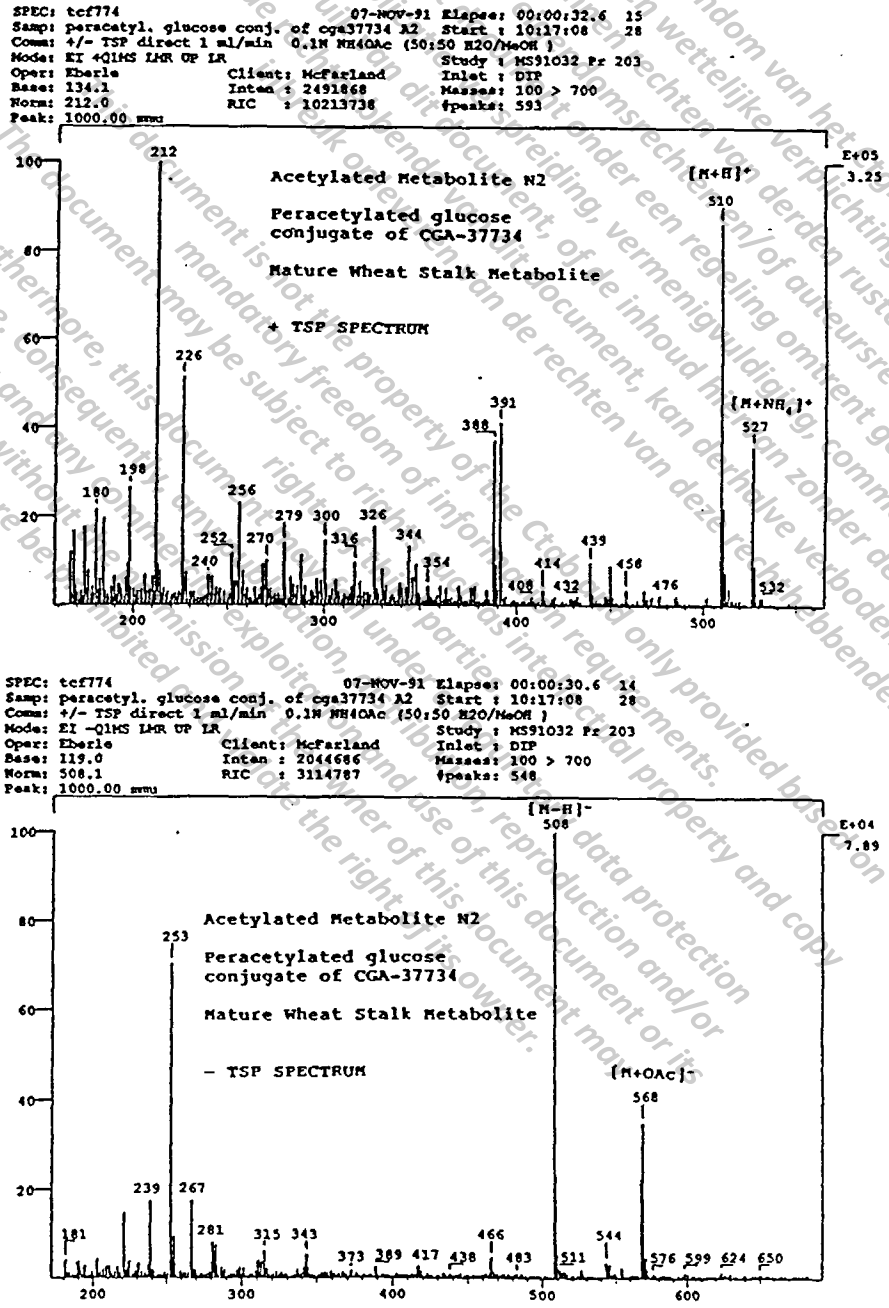


FIGURE 49. MASS SPECTRA OF ACETYLATED METABOLITE N4 ISOLATED FROM MATURE WHEAT STALKS BY +/- TSP ANALYSIS

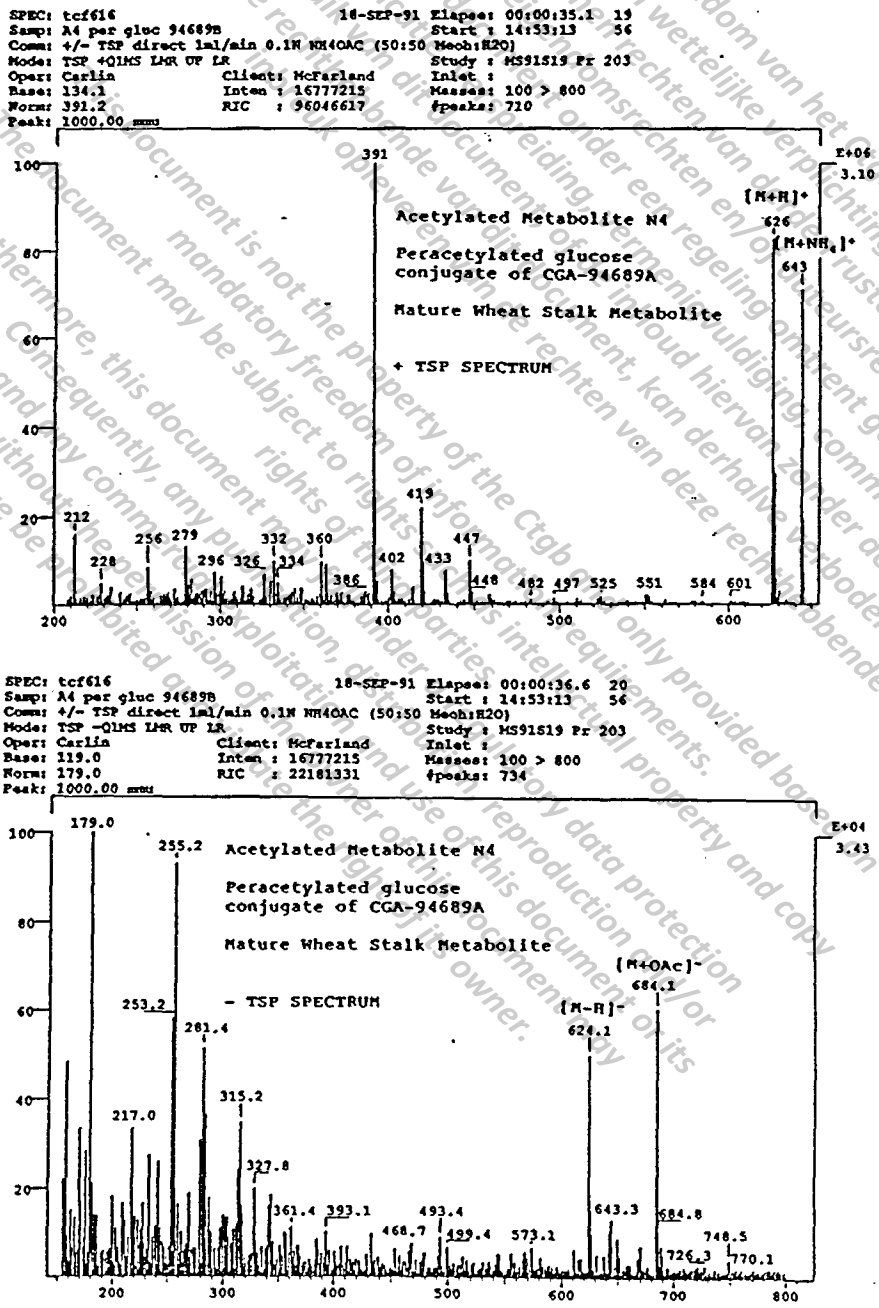


FIGURE 50. MASS SPECTRA OF ACETYLATED METABOLITE N5 ISOLATED FROM MATURE WHEAT STALKS BY +/- TSP ANALYSIS

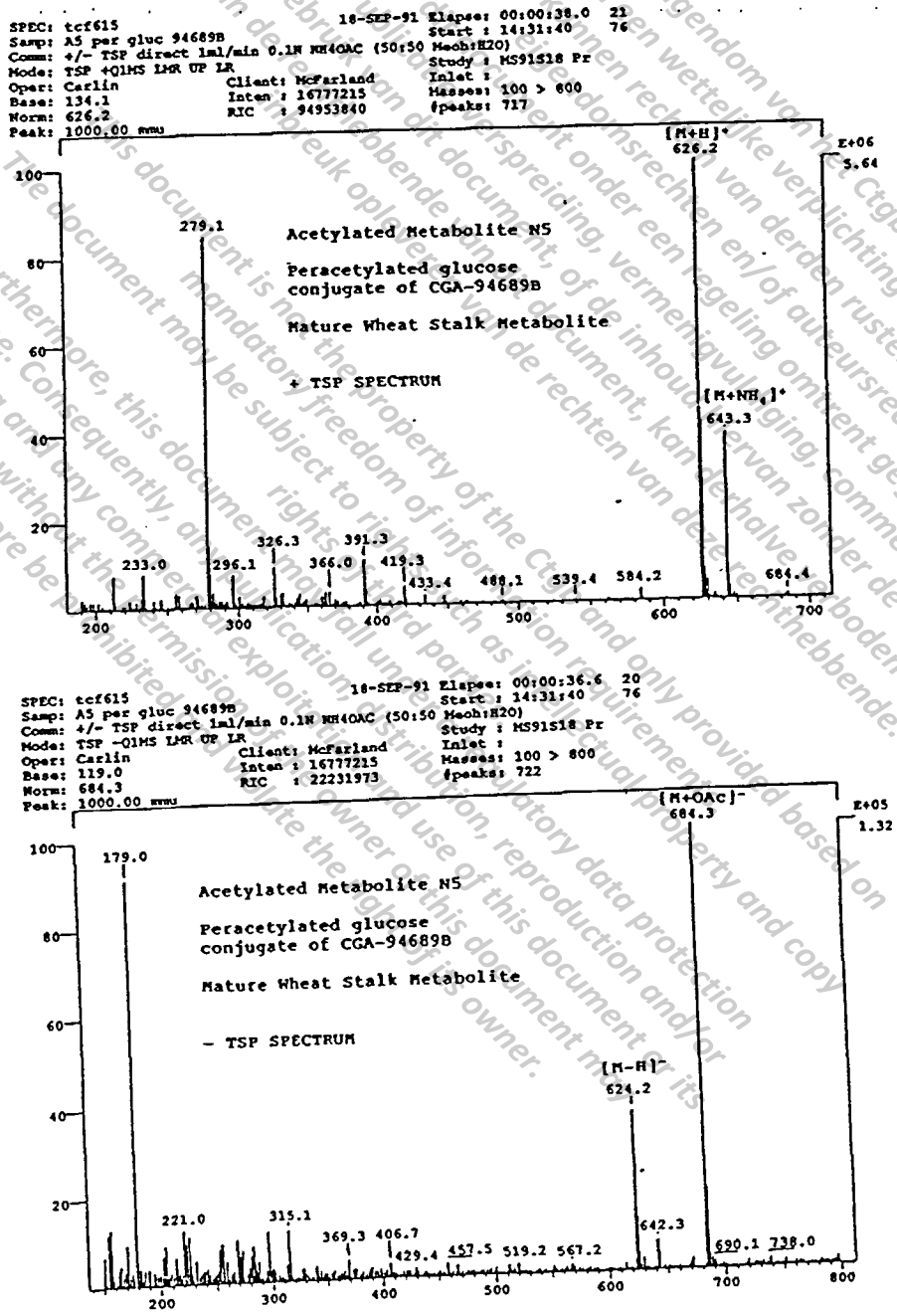


FIGURE 51. PROTON NUCLEAR MAGNETIC RESONANCE SPECTRUM OF ACETYLATED METABOLITE N5 ISOLATED FROM MATURE WHEAT STALKS

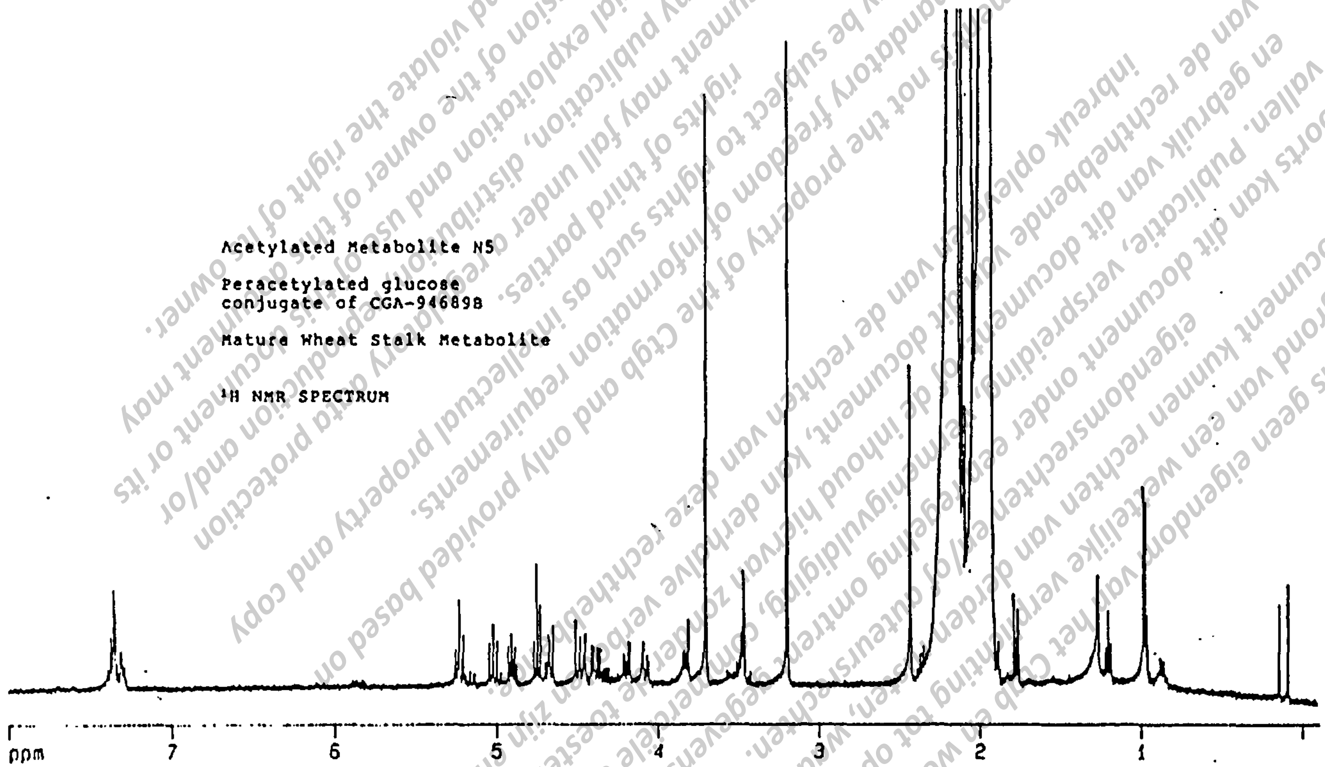
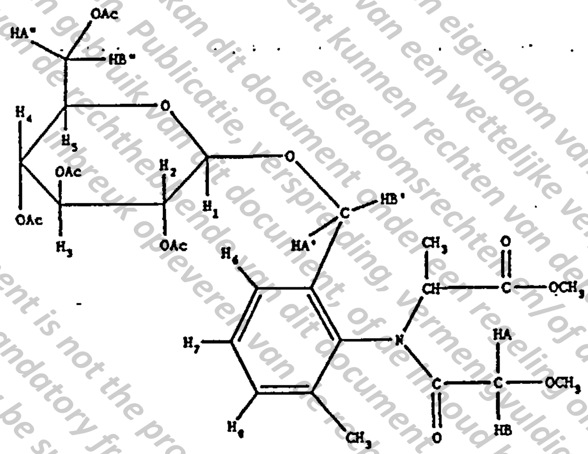


FIGURE 51. PROTON NUCLEAR MAGNETIC RESONANCE SPECTRUM OF ACETYLATED METABOLITE N5 ISOLATED FROM MATURE WHEAT STALKS (Continued)



Assignments for the major isomer are as follows[1]:

0.97 ppm	d - doublet	3 protons	CH-<u>CH3</u>
2.43 ppm	s - singlet	3 protons	Ar-<u>CH3</u>
3.19 ppm	s	3 protons	ether CH3
3.44 ppm	d	1 proton	H_A
3.48 ppm	d	1 proton $J(H_A-H_B)$ = 15.25 Hz	H_B
3.69 ppm	s	3 protons	ester CH3
3.70-3.85 ppm	complex	1 proton	H5
4.07 ppm	d of d	1 proton	$H_{A''}$
4.19 ppm	d of d	1 proton $J(H_{A''}-H_{B''})$ = 12.32 Hz $J(H_{A''}-H5)$ = 2.62 Hz $J(H_{B''}-H5)$ = 5.74 Hz	$H_{B''}$
4.36 ppm	q - quartet	1 proton	<u>CH</u>-CH3
4.49 ppm	d	1 proton	$H_{A'}$
4.66 ppm	d	1 proton $J(H_{A'}-H_{B'})$ = 11.91 Hz	$H_{B'}$
4.73 ppm	d	1 proton	H1
4.90 ppm	d of d	1 proton	H2
5.01 ppm	d of d	1 proton	H3
5.22 ppm	t - triplet	1 proton	H4
7.30-7.40 ppm	complex	3 protons	H6, H7, H8

[1]The methyl acetyl resonances are obscured by the CD_3CN "impurity" and DOH resonances.

FIGURE 52. MASS SPECTRA OF ACETYLATED METABOLITES N6a AND N6b ISOLATED FROM MATURE WHEAT STALKS BY + TSP AND TANDEM TSP ANALYSIS, RESPECTIVELY

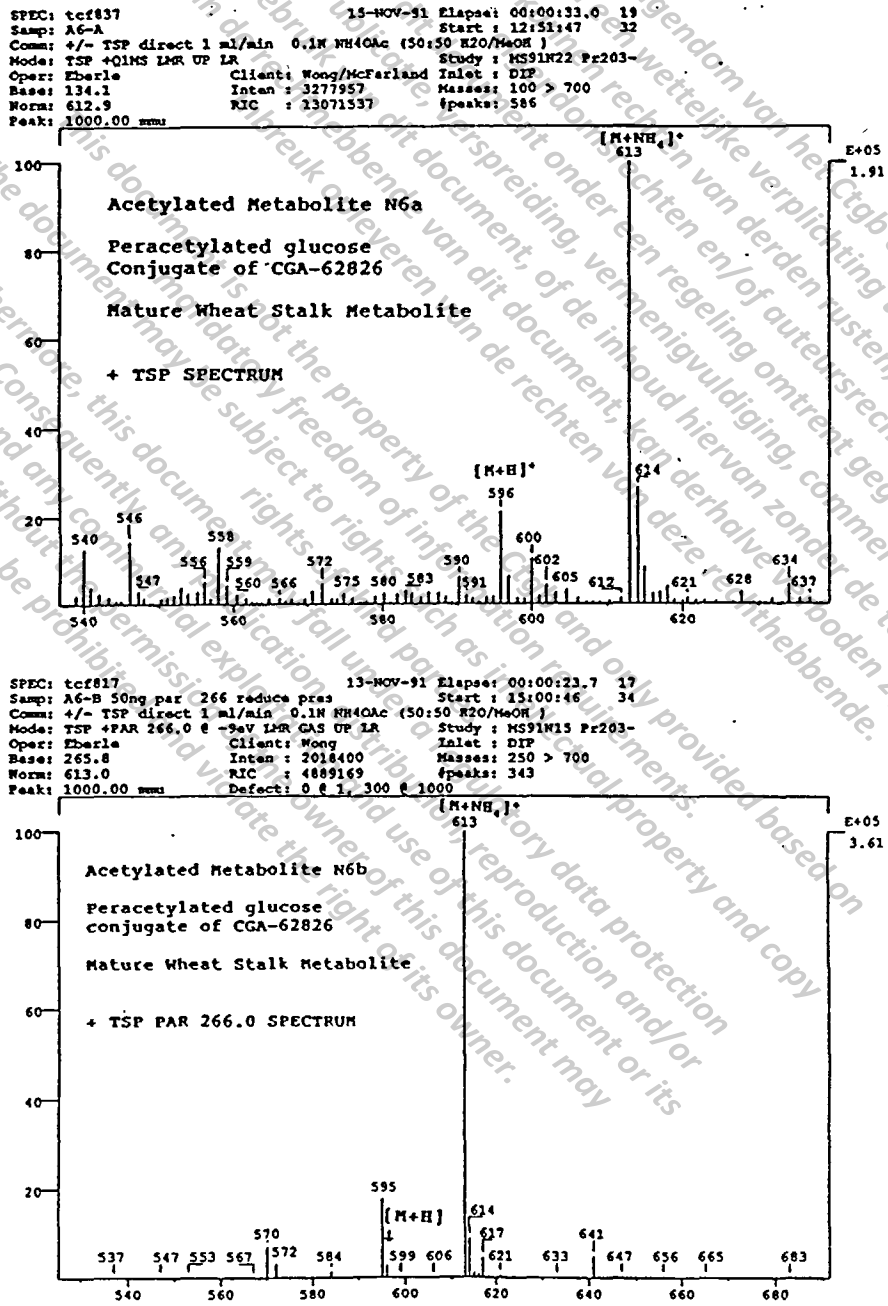
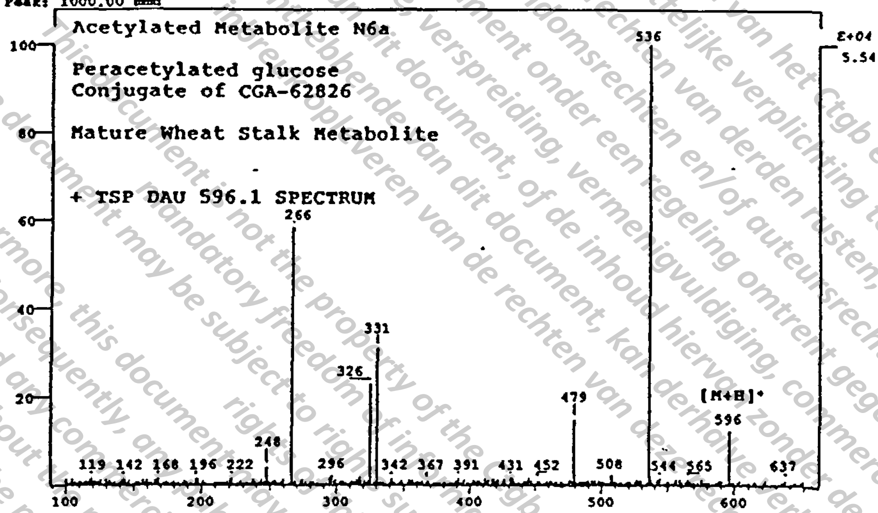


FIGURE 53. MASS SPECTRA OF ACETYLATED METABOLITES N6a AND N6b ISOLATED FROM MATURE WHEAT STALKS BY TANDEM TSP ANALYSIS

SPEC: tcf839 15-NOV-91 Elapse: 00:00:40.7 37
Samp: A6-A Start : 13:31:54 54
Comm: +/- TSP direct 1 ml/min 0.1N NH4OAc (50:50 H2O/MeOH)
Mode: TSP +DAU 596.1 @ -9eV LMR GAS UP LR Study : MS91N22 Pr203-
Oper: Eberle Client: Wong/McFarland Inlet : DIP
Base: 536.1 Inten : 55394 Masses: 100 > 650
Norm: 536.1 RIC : 417827 #peaks: 585
Peak: 1000.00 mmu



SPEC: tcf813 13-NOV-91 DERIVED SPECTRUM 9
Samp: A6-B 50ng Start : 09:34:20 54
Comm: +/- TSP direct 1 ml/min 0.1N NH4OAc (50:50 H2O/MeOH)
Mode: TSP +DAU 596.1 @ -9eV LMR GAS UP LR
Oper: Eberle Inlet : DIP
Base: 536.0 Inten : 53261 Masses: 100 > 610
Norm: 536.0 RIC : 175444 #peaks: 251
Peak: 1000.00 mmu Defect: 0 @ 1, 300 @ 1000
Data: +/35>40-/25>30

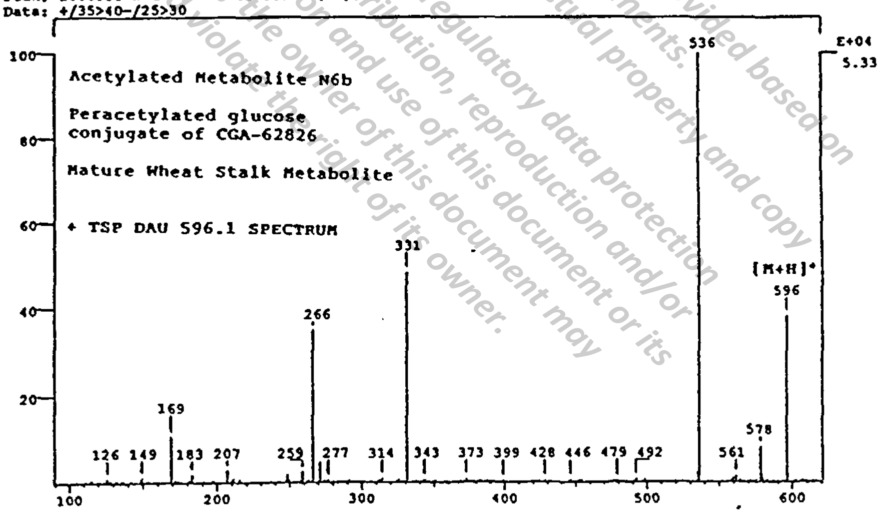


FIGURE 54. HPLC HISTOGRAM OF THE ACIDIC AQUEOUS SOLUBLE RADIOACTIVITY IN 50% MATURE LETTUCE FOLIAGE

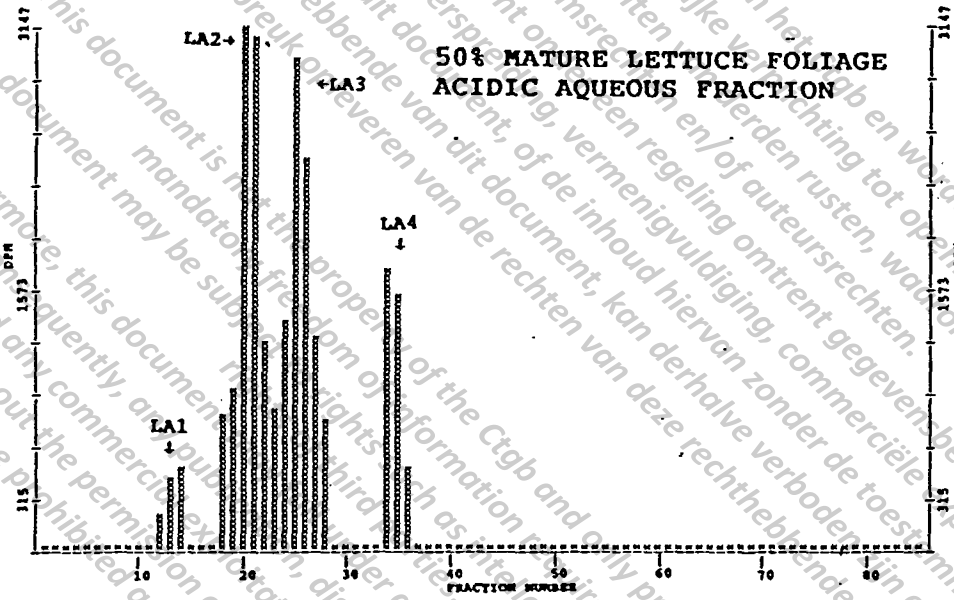
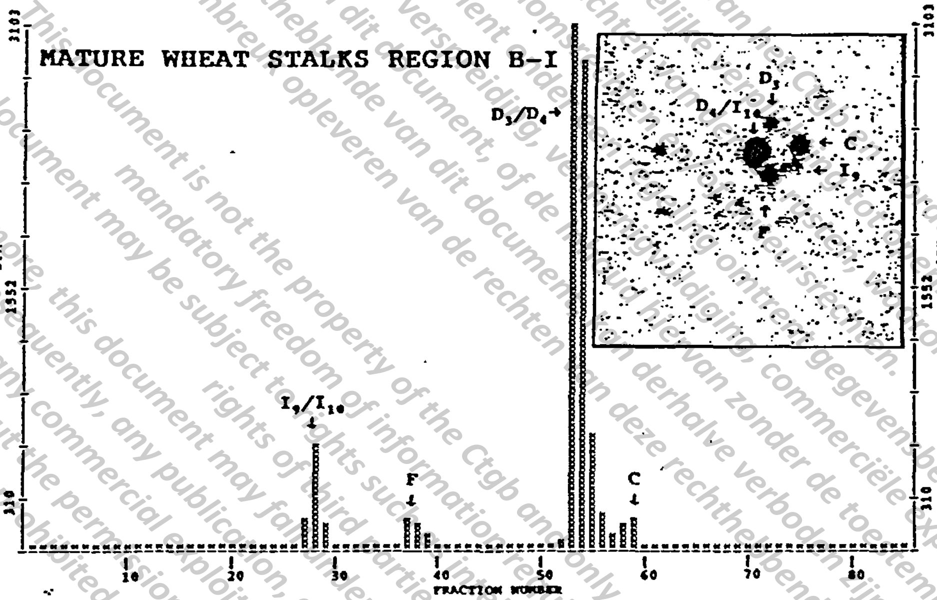


FIGURE 55. HPLC HISTOGRAM AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAM OF THE ACIDIC AQUEOUS SOLUBLE RADIOACTIVITY IN REGION B-I FROM MATURE WHEAT STALKS

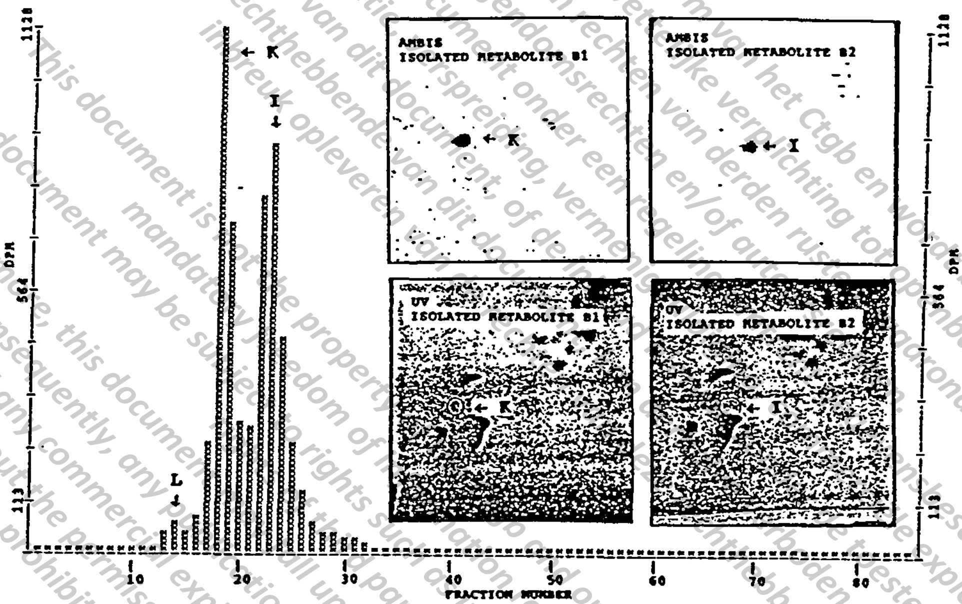


KEY

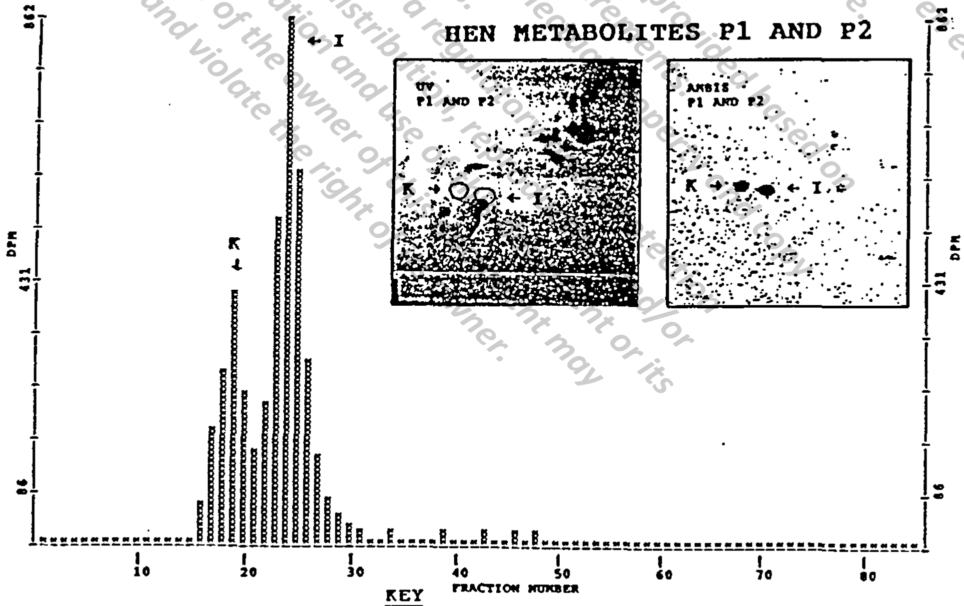
C - CGA-100255
D_3 - CGA-94689A
D_4 - CGA-94689B
F - CGA-37734
I_9 - N1b aglycone
I_{10} - N1a aglycone

FIGURE 56. HPLC HISTOGRAM AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE ACIDIC AQUEOUS SOLUBLE RADIOACTIVITY IN REGION B-II FROM MATURE WHEAT STALKS AND HPLC HISTOGRAM AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAM OF THE METALAXYL HEN METABOLITES P1 AND P2

MATURE WHEAT STALKS REGION B-II



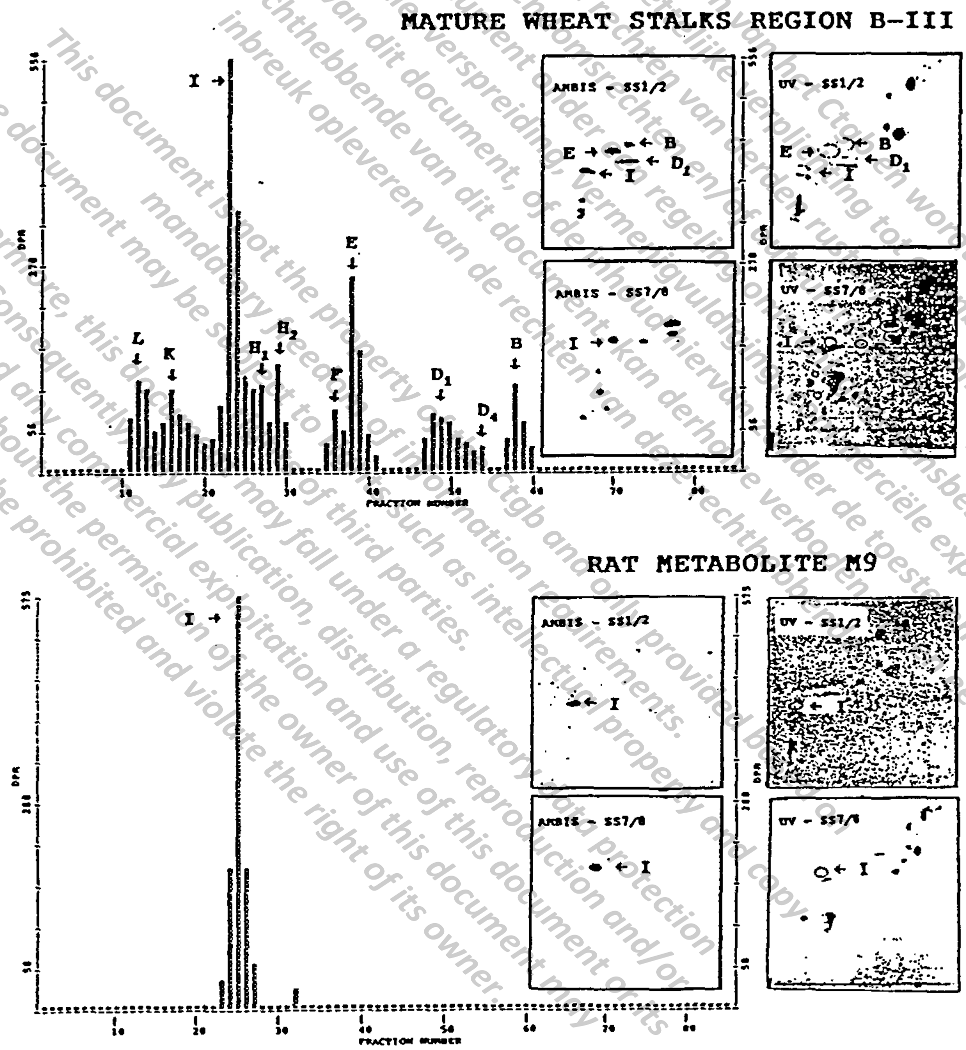
HEN METABOLITES P1 AND P2



KEY

I - B2-HEN P1
K - B1-HEN P2
L - U3 (unknown)

FIGURE 57. HPLC HISTOGRAM AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE ACIDIC AQUEOUS SOLUBLE RADIOACTIVITY IN REGION B-III FROM MATURE WHEAT STALKS AND HPLC HISTOGRAM AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF THE METALAXYL RAT METABOLITE M9

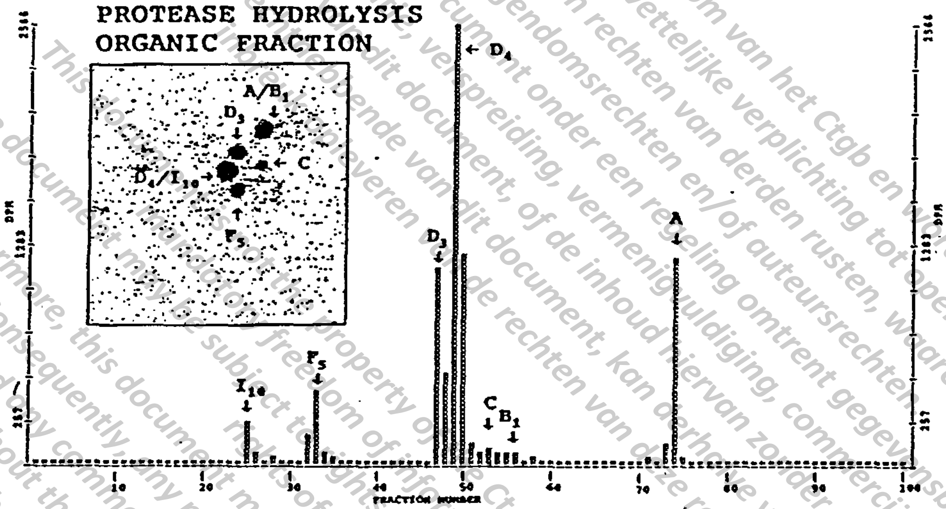


KEY

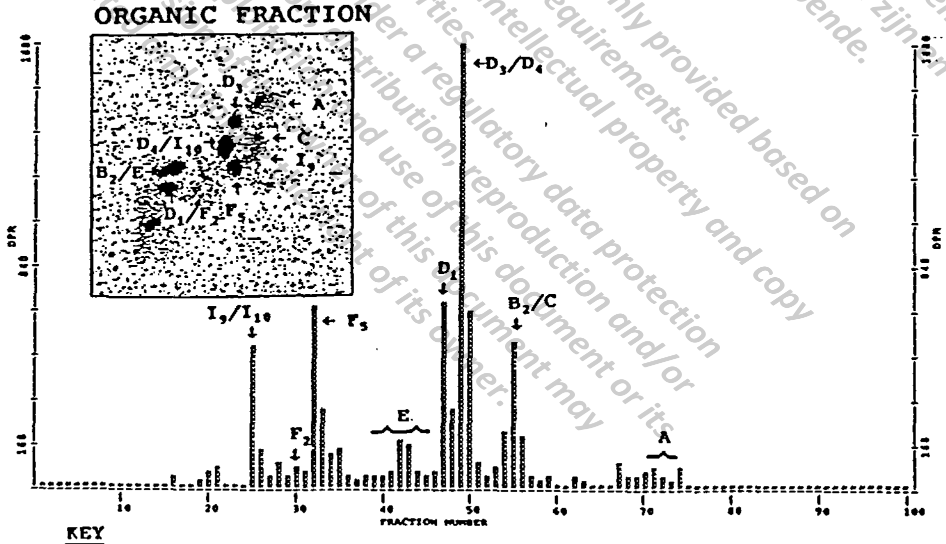
B - CGA-62826
D1 - CGA-107955
D4 - CGA-94689B
E - CGA-108905
F - CGA-119857
H1 - U6 (unknown)
H2 - U7 (unknown)
I - HEN PO-RAT M9
K - U5 (unknown)
L - U4 (unknown)

FIGURE 58. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF NON-EXTRACTABLE RADIOACTIVITY RELEASED FROM MATURE WHEAT STALKS BY PROTEASE AND β-GLUCOSIDASE HYDROLYSES

**MATURE WHEAT STALKS
PROTEASE HYDROLYSIS
ORGANIC FRACTION**



**MATURE WHEAT STALKS
PROTEASE/β-GLUCOSIDASE HYDROLYSES
ORGANIC FRACTION**



KEY

A – CGA-48988
B_1 – CGA-67868
B_2 – CGA-62826
C – CGA-100255
D_1 – CGA-107955
D_3 – CGA 94689A
D_4 – CGA 94689B
E – CGA-108905
F_2 – CGA-119857
F_5 – CGA-37734
I_9 – N1b aglycone
I_{10} – N1a aglycone

FIGURE 59. HPLC HISTOGRAM AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF NON-EXTRACTABLE RADIOACTIVITY RELEASED FROM MATURE WHEAT GRAIN BY CELLULASE HYDROLYSIS

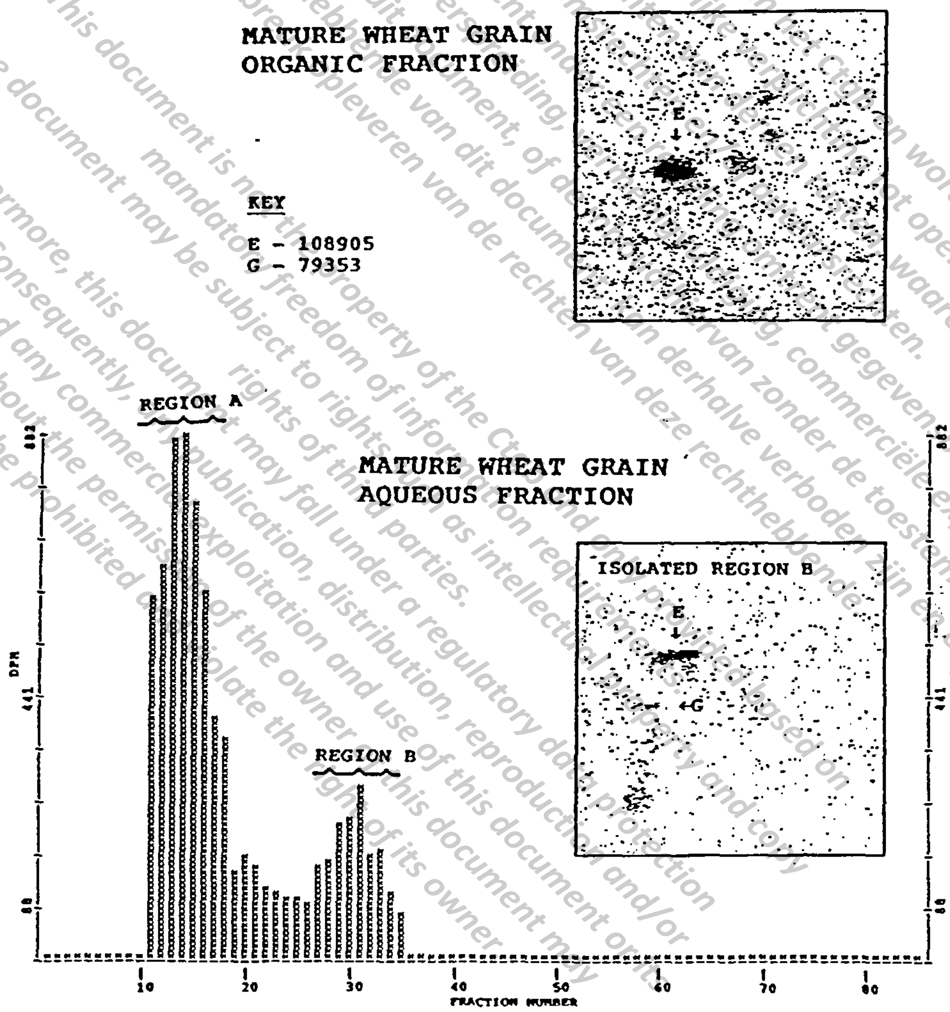
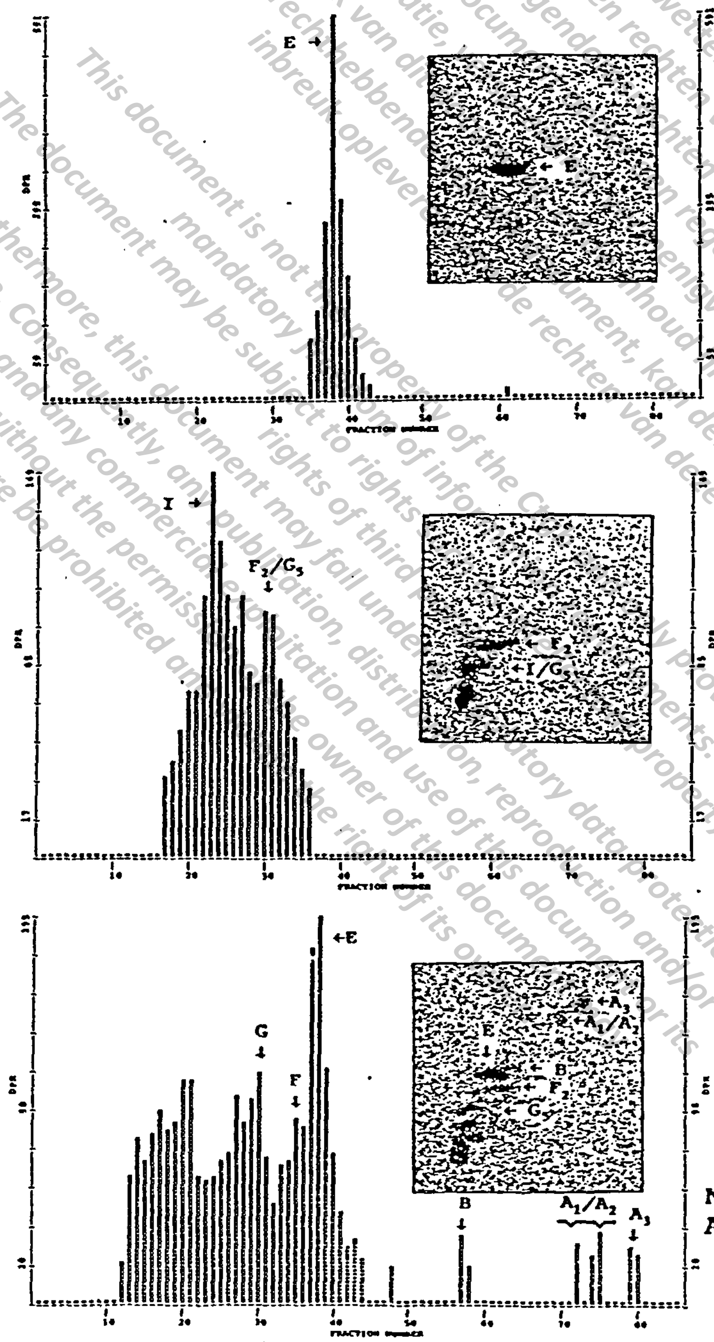


FIGURE 60. <u>HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAMS OF NON-EXTRACTABLE RADIOACTIVITY RELEASED FROM MATURE WHEAT GRAIN BY ACID HYDROLYSIS WITH 0.1M HCl</u>



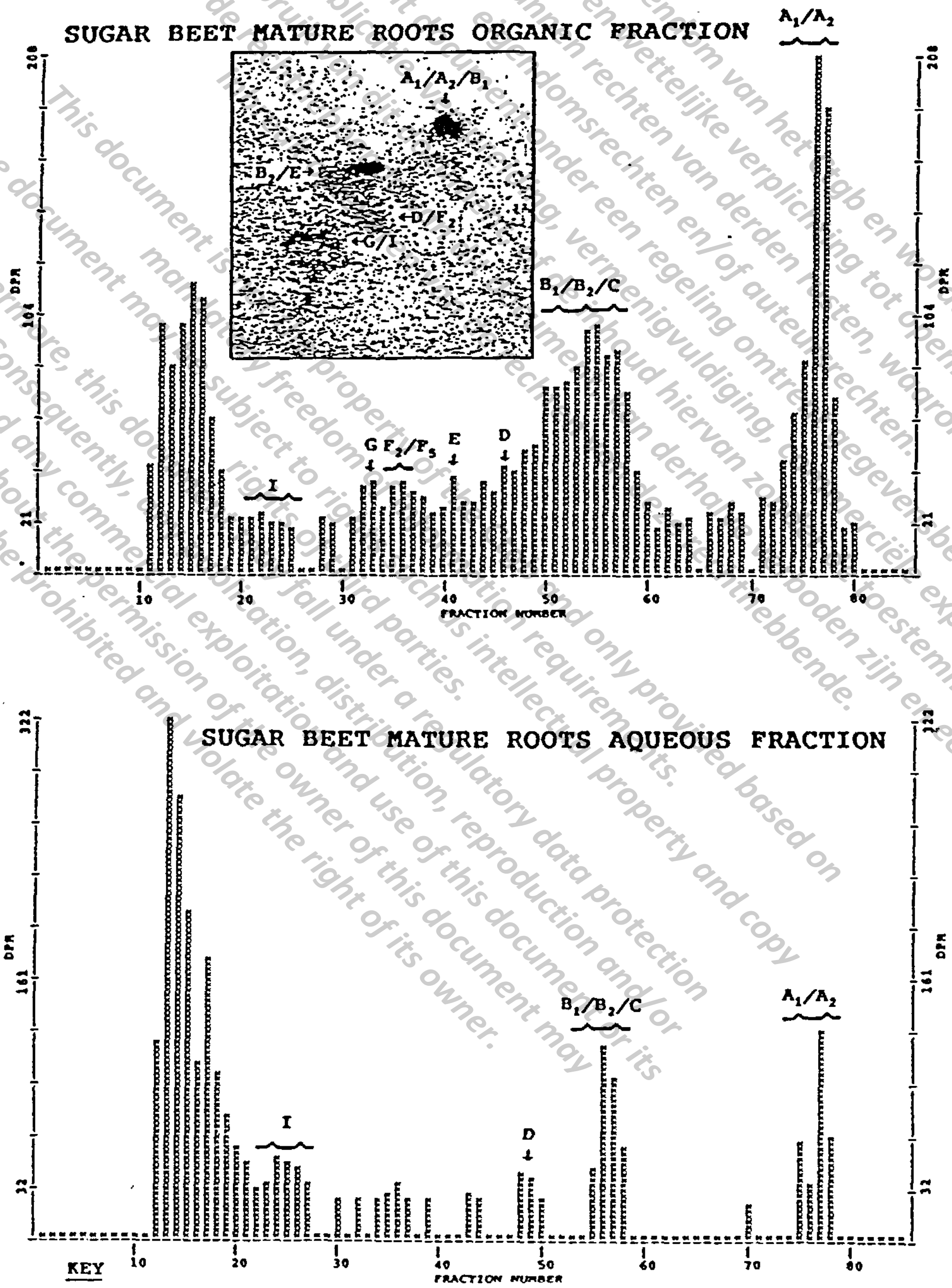
MATURE WHEAT GRAIN ORGANIC FRACTION REGION 2

MATURE WHEAT GRAIN ORGANIC FRACTION REGION 1

KEY
A_1 - CGA-67869
A_2 - CGA-48988
A_3 - CGA-67866
B - CGA-62826
E - CGA-108905
F - CGA-119857, acidics
F_2 - CGA-119857
G - CGA-79353, acidics
G_5 - CGA-79353
I - CGA-108906

MATURE WHEAT GRAIN AQUEOUS FRACTION

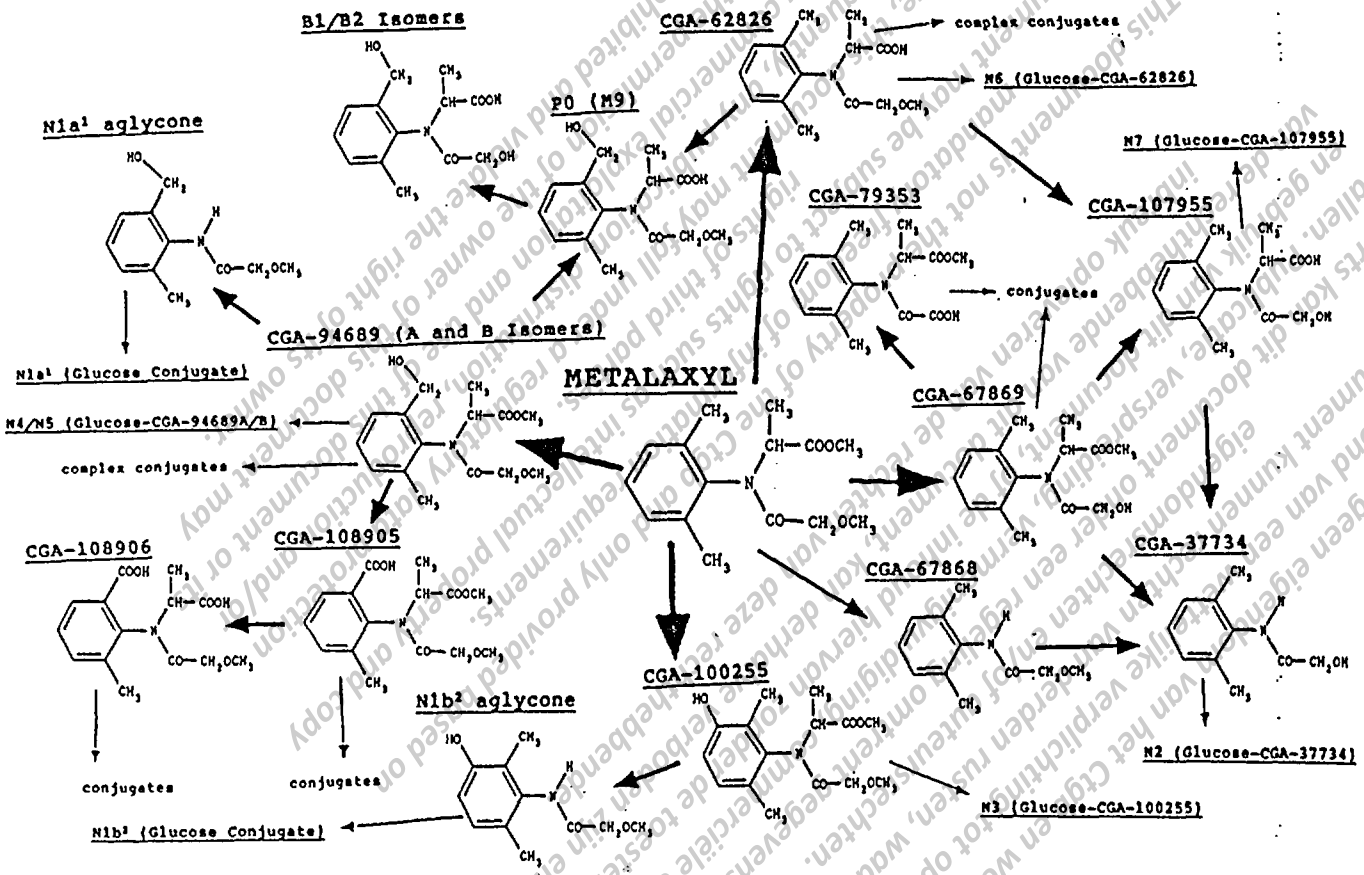
FIGURE 61. HPLC HISTOGRAMS AND NORMAL PHASE 2D-THIN LAYER CHROMATOGRAM OF NON-EXTRACTABLE RADIOACTIVITY RELEASED FROM MATURE SUGAR BEET ROOTS BY ACID HYDROLYSIS WITH 6M HCl



KEY

A_1 – CGA-67869	C – CGA-100255	F_2 – CGA-119857
A_2 – CGA-48988	D – CGA-107955	F_5 – CGA-37734
B_1 – CGA-67868	E – CGA-108905	G – CGA-79353
B_2 – CGA-62826		I – CGA-108906

FIGURE 62. METABOLIC PATHWAY OF METALAXYL IN ROTATIONAL CROPS



[1]Postulated structures based on Mass Spectra of N1a isolated from Mature Wheat Stalk.
[2]Postulated structures based on Mass Spectra of N1b isolated from 50% Mature Lettuce.

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2. CIBA-GEIGY Corporation, Ridomil® 2E Fungicide Label, 1992.

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6. 5.1.2.e Woo, "Identification of Four Sugar Conjugate Metabolites of ϕ-^{14}C-CGA-48988 in Tobacco," ABR-80035.

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22. [redacted]., "Uptake and Characterization of ϕ-^{14}C-CGA-48988 and Its Soil Metabolites in Field Rotation Corn," ABR-79004.

23. [redacted], "Uptake and Characterization of ϕ-^{14}C-CGA-48988 and Its Soil Metabolites in Field Rotation Spring Oats," ABR-79002.

24. [redacted], "Metabolism of [ϕ-^{14}C]-Metalaxyl in Goats," ABR-90078.