THE IDENTIFICATION, DISTRIBUTION AND PERSISTENCE OF OXAMYL AND ITS DEGRADATION PRODUCTS IN PLANTED CORN SEED, SEEDLING ROOT AND SOIL FROM OXAMYL-TREATED CORN SEEDS

by

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ABSTRACT

A simple method was developed for treating corn seeds with oxamyl. It involved soaking the seeds to ensure oxamyl uptake, centrifugation to draw off excess solution, and drying under a stream of air to prevent the formation of fungus. The seeds were found to have an even distribution of oxamyl. Seeds remained fungus-free even 12 months after treatment. The highest non-phytotoxic treatment level was obtained by using a 4.00 mg/mL oxamyl solution.

Extraction methods for the determination of oxamyl (methyl-N'N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thio-oxamimidate), its oxime (methyl-N',N'-dimethyl-N-hydroxy-1-thio-oxamimidate), and DMCF (N,N-dimethyl-1-cyanoformamide) in seed, root, and soil were developed. Seeds were processed by homogenizing, then shaking in methanol. Significantly more oxamyl was extracted from hydrated seeds as opposed to dry seeds. Soils were extracted by tumbling in methanol; recoveries ranged from 86 - 87% for oxamyl. Root was extracted to 93% efficiency for oxamyl by homogenizing the tissue in methanol. Nuchar-Attaclay column cleanup afforded suitable extracts for analysis by RP-HPLC on a C_{18} column and UV detection at 254 nm.

In the degradation study, oxamyl was found to dissipate from the seed down into the soil. It was also detected in the root. Oxime was detected in both the seed and soil, but not in the root. DMCF was detected in small amounts only in the seed.

ACKNOWLEDGEMENTS

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CHAPTER I - INTRODUCTION

A. Brief History of Carbamate Pesticides

The emergence of carbamates as viable pesticides occurred rather late when compared with other types of synthetic organic pesticides. For example, organochlorine compounds such as DDT (dichlorodiphenyltrichloroethane), 2,4-D (2,4-dichlorophenoxyacetic acid)[1,2] and organophosphorus compounds such as schradan and parathion[3] were already in commercial use as insecticides and/or herbicides by the Second World War. Because these organochlorine and organophorus compounds were effective as pesticides and economical to produce at that time, carbamate development was pushed back to the mid-to-late 1940's.

The first "true" carbamate insecticide was synthesized by Dr. Hans Gysin at the Geigy Chemical Company in Switzerland[4]. During his search for aromatic amide insect repellents, a series of cycloaliphatic carbamate esters was prepared. One of these, dimetan (Figure I - 1), performed poorly as a repellent, yet was found to be quite toxic to houseflies and aphids. This discovery led Geigy to shift away from insect repellents and toward development of carbamate insecticides. Derivatives of heterocyclic enols[5,6] especially Isolan (Figure I - 2) and dimetilan, together with dimetan reached commercial production in Europe during the 1950's. All of these insecticides were esters of dimethylcarbamic acid.

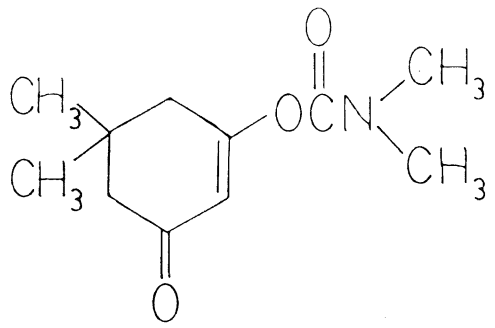

Figure I - 1: Structure of Dimetan

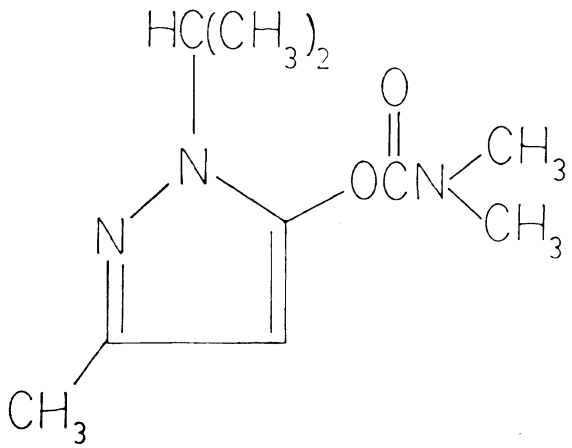

Figure I - 2: Structure of Isolan

In his search for effective herbicides, Dr. Joseph A. Lambrech of the Union Carbide Corporation in the United States synthesized the experimental compound UC 7744 in 1953[7,8]. The compound, carbaryl (Figure I - 3) with trade name Sevin, differed from the Geigy insecticides by substitution of an aryloxy group for the enol group and replacement of the dimethylcarbamyl moiety with the monomethyl counterpart. Because of its effectiveness on a large number of insects[9], as well as its low mammalian toxicity and rapid environmental degradability[10], production of this chemical in the United States grew from 1 million pounds in 1959[11] to more than 55 million pounds by 1971, making it one of the most commercially successful carbamates to date[12].

In the 1950's, it was discovered that the manner in which a large number of pesticides including carbamates acted on target pests was by inhibiting the activity of the neuroenzyme acetylcholinesterase. The hydrolysis of the impulse-conducting chemical acetylcholine was prevented, resulting in prolonged stimulation and eventual disorder of the nervous system, and ultimately to the death of the organism[13]. Because the structure of the impulse mediator acetylcholine was known (Figure I - 4), chemists at Union Carbide attempted to synthesize an N-methyl carbamate retaining some of the electronic characteristics of the earlier aryl compound, while having a closer spatial resemblance to acetylcholine. The result was the discovery of the oxime carbamates, particularly aldicarb (Figure I - 5) with the trade name Temik, which are not only effective as contact and

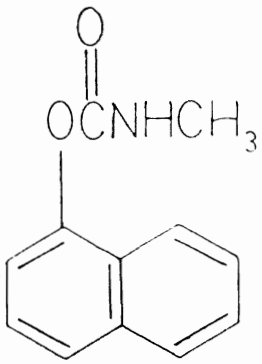

Figure I - 3: Structure of Carbaryl

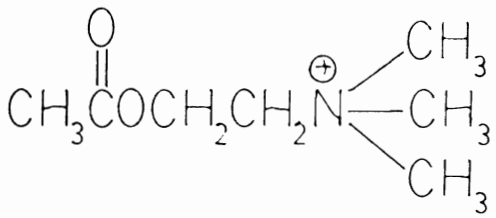

Figure I - 4: Structure of Acetylcholine

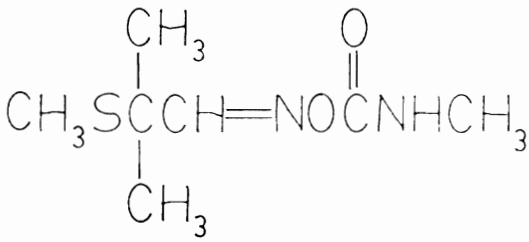

Figure I - 5: Structure of Aldicarb

systemic insecticides, but can be nematicidal and/or miticidal in activity as well[14,15].

In 1969, E.I. Du Pont de Nemours & Company (Inc.) of the United States introduced an experimental insecticide - nematicide designated as Du Pont 1410 (or DPX 1410)[16,17], presently known in the commercial formulation as Vydate™. The active ingredient in this pesticide is oxamyl (Figure I - 6), an N-methyl carbamate.

Depending upon the mode of application, oxamyl has been found to act widely on different organisms; when applied as a foliar spray, it works as a contact-type, moderately persistent insecticide and miticide; when applied to the soil, oxamyl functions as a systemic miticide/insecticide as well as a broad spectrum nematicide. The use of oxamyl has been allowed on a number of fruit, vegetable and field crops, but its main applications are in the production of ornamentals and turf. Oxamyl is the pesticide with which this thesis project is concerned.

B. Mode of Action of Oxamyl

Pesticides can generally be classified according to their mode of entry into the target pest as well as by their biological mode of action against the pest. Some types of pesticides are meant to be ingested and work as stomach poisons. Others enter the organism through the cuticle into the hemolymph by direct contact and subsequently spread throughout the interior of the tissue[18]. Still others are classified as systemic because of

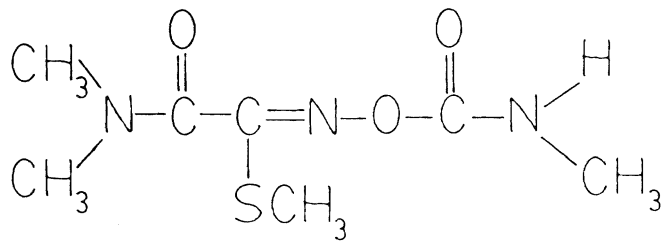

Figure I - 6: Structure of Oxamyl

their ability to translocate within the plant; after application, they move to parts remote from the site of application to combat the target pests[19]. Fumigants are gaseous chemicals which kill pests upon entering the respiratory system.

The term "biological mode of action" refers to the physiological mechanism by which death occurs once the pesticide has entered the pest. Carbamate pesticides (of which oxamyl is one) act according to a relatively well-understood mechanism, that of acetylcholinesterase inhibition. Acetylcholine is the chemical mediator of transmitted nerve impulses crossing cholinergic synapses within the nervous system of insects. Upon reaching the synapse, the impulse causes a release of this chemical transmitter acetylcholine which combines either with the "acetylcholine receptor" or with the enzyme acetylcholinesterase. This enzyme acts as a catalyst in the deactivation of the acetylcholine so that stimulation of the receptor stops and the synapse becomes available for a new transmission[20]. Acetylcholine decomposes to choline and acetic acid (Figure I - 7). It is generally thought that the enzyme contains 2 binding sites, a negative site and an ester-forming site. In a pictorial depicting the enzyme-substrate relationship (Figure I - 8), the enzyme initially combines with acetylcholine to form the intermediate complex (i), the acetylated enzyme is formed with the release of free choline (ii), with the acetylated enzyme finally hydrolyzed to regenerate the free enzyme and form acetic acid (iii).

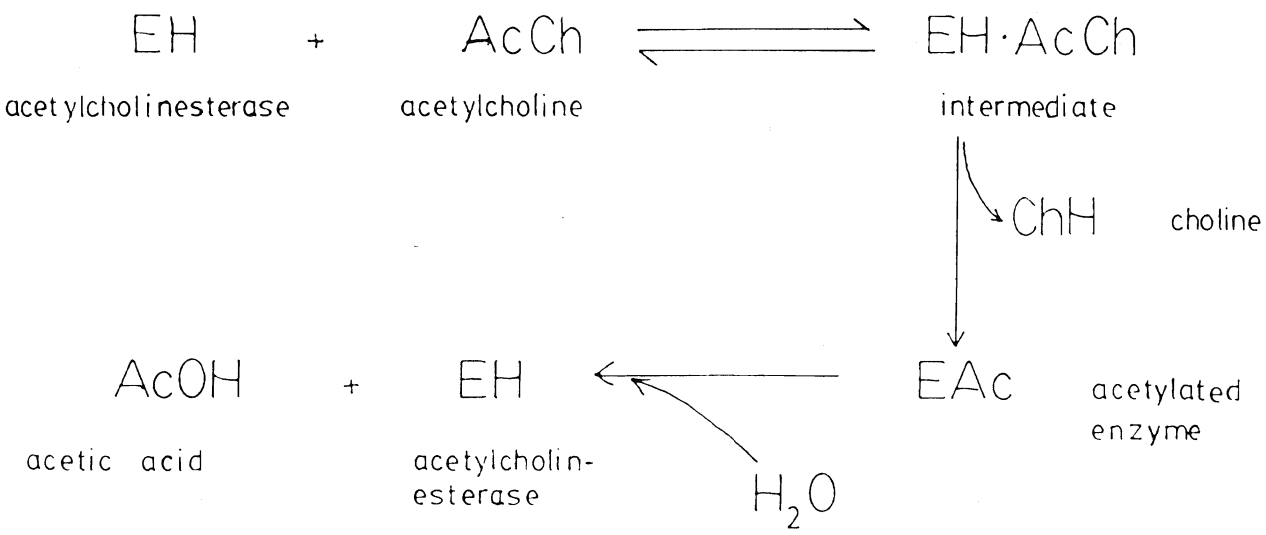

Figure I - 7: **Schematic of the Deactivation of Acetylcholine by Acetylcholinesterase**

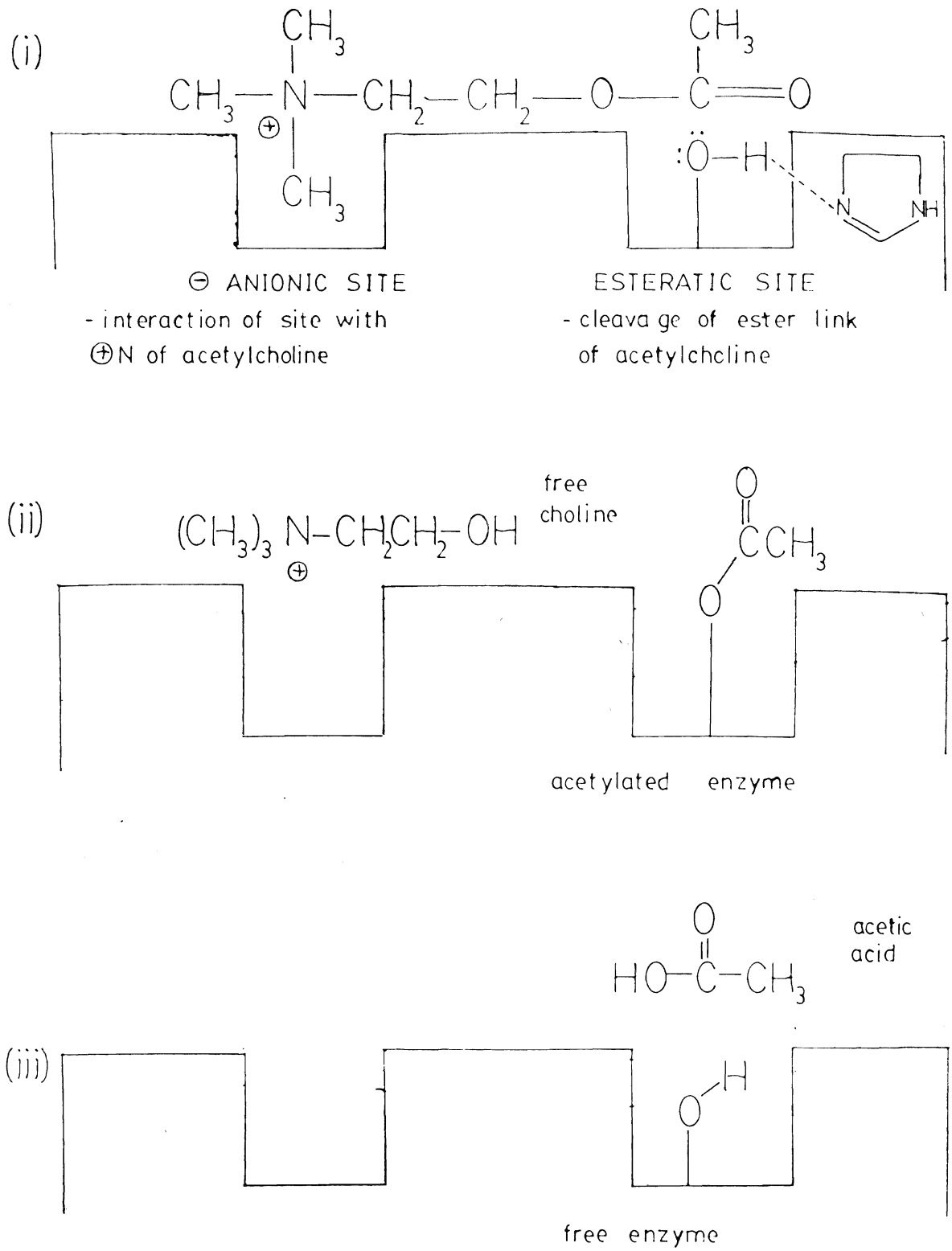

Figure I - 8: Pictorial of the Acetylcholinesterase Reaction

The essential feature of acetylcholinesterase inhibition produced by carbamate compounds is that they react with the enzyme in a manner exactly analogous to that of the acetylcholine, with the esteratic site on the enzyme becoming carbamylated. The reaction is analogous to that depicted in Figure I - 7. The inhibition is achieved because of the greater stability of the O-C bond of the carbamylated enzyme, making it more difficult to hydrolyze. The half-life of hydrolysis of the carbamate group and reactivation of the acetylcholinesterase has been calculated to be approximately 40 minutes[21], much longer than in normal cases. As a result, inhibition of the enzyme occurs.

The consequence of acetylcholinesterase inhibition is the accumulation of the transmitter acetylcholine in the synapse, causing prolonged postsynaptic response with eventual blockage in nervous transmission. Physiologically, poisoned insects show initial hyperactivity, followed by convulsive and uncoordinated movements, ending in paralysis and death. While the exact cause of death is not known, it is believed that it may be due to exhaustion following general failure in critical areas within the central nervous system[22].

C. The Chemistry of Oxamyl

Oxamyl is the common name given to the N-methyl carbamate compound methyl-N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thio-

oxamimidate, $C_7H_{13}N_3O_3S$ (Figure I- 6). It can be synthesized by the reaction of the corresponding oxime, methyl-N',N'-dimethyl-N-hydroxy-1-thio-oxamimidate, with methylisocyanate in a solvent such as acetone[23] (Figure I- 9). The oxime can be synthesized by reacting methoxycarbonylformhydroxamyl chloride with methylmercaptan in the presence of base, subsequently reacting the product with dimethylamine (Figure I -10). Oxamyl is a white, crystalline solid with a slight sulfurous odour. It melts at 100 - 102°C, changing to a different crystalline form which melts at 108 - 110°C. Its volatility is low as reflected by vapour pressures ranging from 2.3×10^{-4} mm Hg at 25°C to 7.6×10^{-3} mm Hg at 70°C.

The solubility of oxamyl in various solvents is presented in Table I - 1[24]. Note that oxamyl is soluble in polar organic solvents, virtually insoluble in non-polar organic solvents and only moderately soluble in water.

While oxamyl is stable in slightly acidic aqueous solutions, it hydrolyzes under alkaline conditions to the oxime and subsequently to methylamine and carbon dioxide (Figure I-11)[25]. Its stability in acidic, neutral and alkaline solutions is summarized in Table I - 2[24]. The results show that oxamyl is stable under slightly acidic conditions: even after 11 days in a pH 4.7 solution, no decomposition was observed. Slow hydrolysis to the oxime does occur in neutral solution . Under alkaline conditions, rapid hydrolysis takes place, with 30% conversion in the first 6 hours. (It should be noted that depending upon the

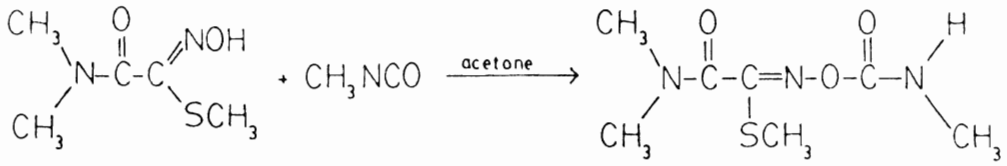

Figure I - 9: The Synthesis of Oxamyl

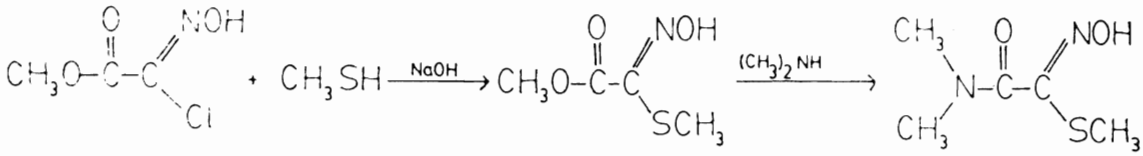

Figure I - 10: The Synthesis of Oxime

TABLE I-1: THE SOLUBILITY OF OXAMYL IN VARIOUS SOLVENTS AT 25°C

SOLVENT	g DISSOLVED PER 100 g SOLVENT
Methanol	144
DMF	108
Acetone	67
Ethanol	33
Cyclohexanone	29
Water	28
Isopropanol	11
Toluene	1

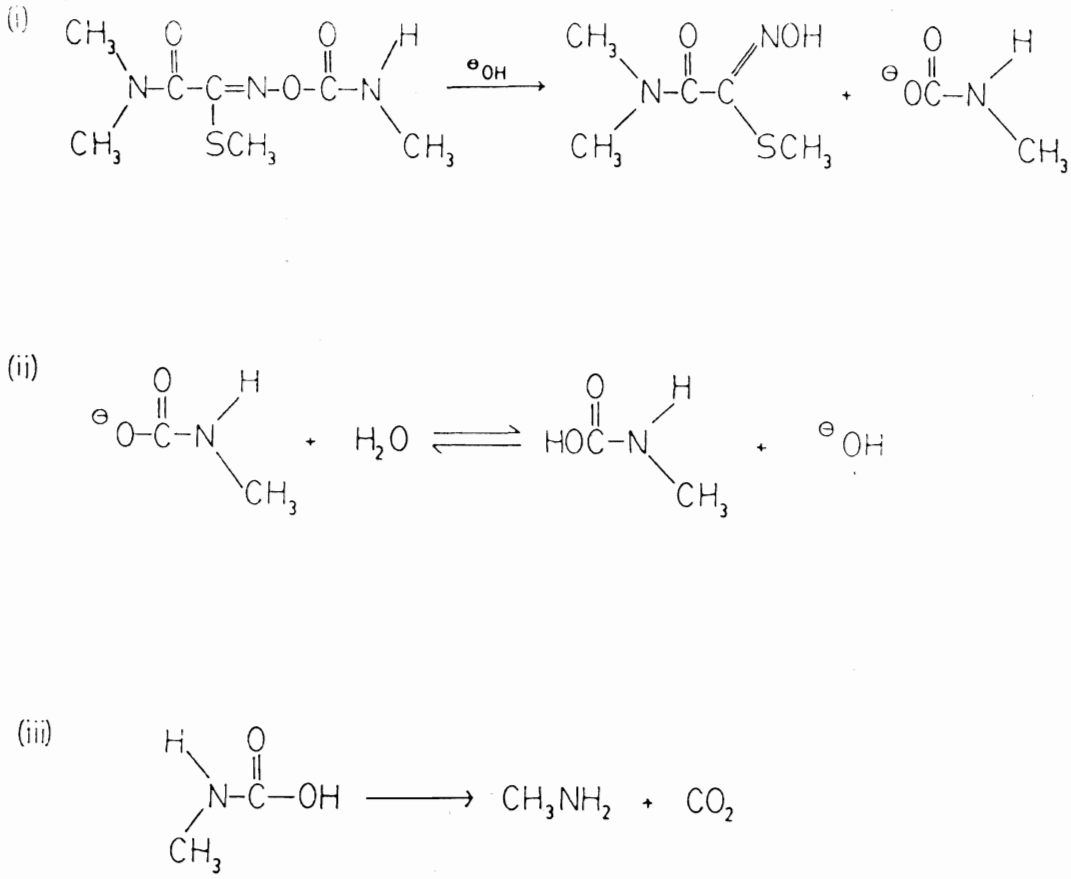

Figure I - 11: Alkaline Hydrolysis of Oxamyl

TABLE I-2: THE STABILITY OF OXAMYL IN AQUEOUS SOLUTIONS

TIME IN SOLUTION* (HOURS)	% OXAMYL LOSS (16 OZ./100 GALLONS) pH 4.7	pH 6.9	pH 9.1 **
0	0	0	0
6	0	0	30
24	0	3	45
48	0	9	47
72	0	16	60
96	0	26	66

* SOLUTIONS IN 0.01 M AQUEOUS BUFFERS; ROOM TEMPERATURE

** AFTER 6 HOURS, pH HAD DECREASED TO ABOUT pH 7

pH, this rate of decomposition will vary widely). During this period, the hydrolysis reaction resulted in the sodium bicarbonate buffer being neutralized, with the subsequent rate of decomposition approximating that seen in the neutral solution[24].

D. The Degradation of Oxamyl

Perhaps the most comprehensive study to date on the fate of oxamyl was presented in 2 papers by John Harvey, Jr. and his co-workers in 1978[26,27].

The initial phase of study involved tracing the metabolism of oxamyl in plants. Carbon-14-labelled oxamyl (Figure I - 12) was used to treat tobacco, alfalfa, peanuts, potatoes, apples, oranges and tomatoes. Its metabolic fate was followed using a combination of thin-layer chromatography (TLC), liquid scintillation counting (LSC), high-performance liquid chromatography (HPLC) with scintillation flow monitoring or ultra violet absorbance detection, and gas chromatography/mass spectrometry (GC/MS).

Fifteen days after the foliage of tobacco plants was treated by pipette with droplets of an aqueous solution of [^{14}C]oxamyl, it was found that 50% of the original radioactivity could be washed off the surface of the treated leaves. Analysis of this aqueous wash showed 96% oxamyl and 3% oxime.

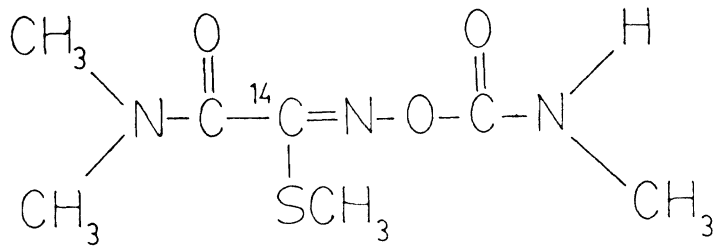

Figure I - 12: Oxamyl Labelled with ^{14}C

Radioactivity was distributed within the tissues of the plant with 37.6% in the treated leaves, 1.3% in new growth and 0.1% in the root. Analysis of the leaves indicated 56% to be [^{14}C]oxamyl, 5% to be the oxime and the remaining 39% to be a polar unknown fraction, of which 93% was shown to be a glucose conjugate of the oxime (Figure I - 13) and 7% identified as N,N-dimethyloxamic acid (Figure I - 14).

Seventeen days after the last of three spray treatments with solutions of [^{14}C]oxamyl, the analysis of alfalfa hay indicated that over 90% of the radioactivity was in the form of the glucose conjugate of oxime (Figure I - 13), with only traces of intact oxamyl (0.8%) and oxime (0.8%) detected.

Young peanut plants given a single foliar treatment of [^{14}C] oxamyl and sampled after four weeks were found to contain neither oxamyl nor oxime. Instead, over 99% of the radioactivity in the extract was highly polar and was found to contain not only the glucose conjugate of oxime, (Figure I - 13) but also that of methyl-N-hydroxy-N'-methyl-1-thio-oxamimidate (Figure I- 15). In mature hay which was harvested about seven weeks after a second [^{14}C] oxamyl treatment, or about 11 weeks after the first treatment, no oxamyl was present. However, 1% of the radioactivity was attributed to oxime. Most of the radioactivity was in the form of very polar conjugates (99%). Analyses indicated that neither of the earlier glucose conjugates (Figures I - 13 and 15) were present. Rather, it was concluded that the oxime moiety was attached to a polysaccharide-type compound

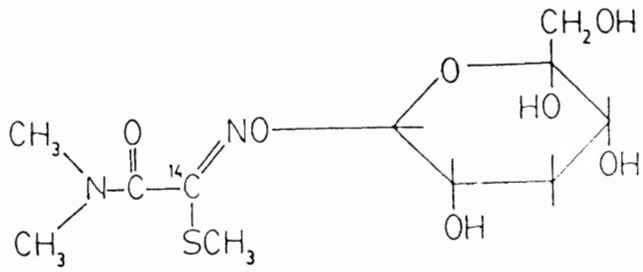

Figure I - 13: Glucose Conjugate of Oxime

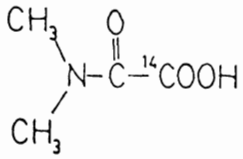

Figure I - 14: Structure of N,N-Dimethyloxamic Acid

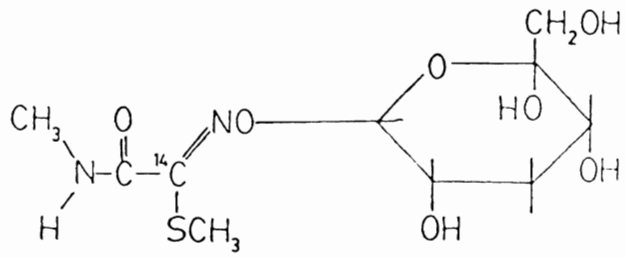

Figure I - 15: Glucose Conjugate of Methyl-N-hydroxy-N'-methyl-1-thio-oxamimidate

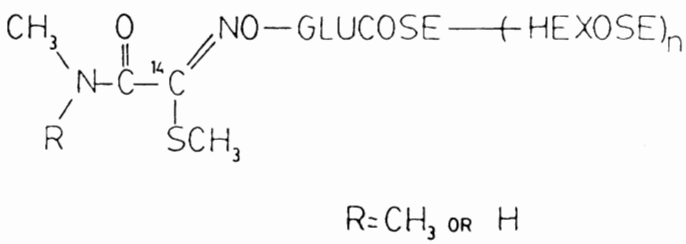

Figure I - 16: Polysaccharide Conjugates of Oxime

(Figure I - 16). Extraction of mature peanuts yielded no oxamyl or corresponding oxime.

Shortly after potato plants had emerged, five foliar treatments of [^{14}C] oxamyl were applied to the plants. Mature potato tubers contained a radioactive residue calculated to be 0.7 ppm oxamyl. Because the peels contained less than 10% of the radioactivity, only the residue of the interior portion of the tuber was characterized. Analysis indicated very little or no free oxamyl or oxime. However, it is probable that the majority of radioactivity was in the form of insoluble polysaccharide conjugates (Figure I - 16).

Apple fruits six weeks after direct application of [^{14}C] oxamyl contained ^{14}C residues evenly distributed throughout the fruit. The radioactive residues were found to contain 16% oxamyl, 42% oxime and 17% of a metabolite identified as N,N-dimethyl-1-cyanoformamide (Figure I - 17). Twenty-three percent appeared as polysaccharide-type conjugates (Figure I - 16).

Oranges harvested six weeks after direct application of a solution of [^{14}C] oxamyl contained a total ^{14}C residue equivalent to 2.5 ppm oxamyl. Characterization yielded the following results: of the total ^{14}C content, 9% was identified as oxamyl, 6% as the oxime, 20% as DMCF, 35% as the glucose conjugate of oxime, 22% as the glucose conjugate of methyl-N-hydroxy-N'-methyl-1-thio-oxamimidate, and 8% as unidentified polar metabolites.

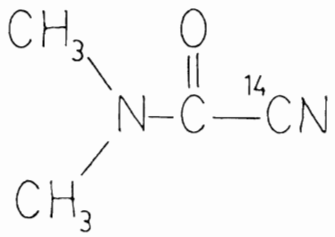

Figure I - 17: Structure of N,N-Dimethyl-1-cyanoformamide (DMCF)

Fourteen days after surface-spotting tomatoes with [^{14}C] oxamyl solution, the principal residue was found to be oxamyl itself (59%). The free oxime was determined to be 13%, with its glucose conjugate detected at 5%. Only 4% was observed as DMCF. The remainder of the residue was a mixture of polar metabolites or natural products (19%) that were not further characterized.

The results led the authors to conclude that oxamyl is initially hydrolyzed to the oxime, followed by conjugation with glucose. The resulting glucoside was gradually demethylated to yield the monomethyl species. Further reaction of these conjugates with hexose units led to the formation of polysaccharide conjugates. In several fruits, limited conversion of oxamyl to DMCF occurred.

The subsequent study involved investigating the decomposition of oxamyl in both water and soil under laboratory and field conditons. Again, liquid scintillation counting (LSC), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and mass spectrometry (MS) were used to trace the fate of radio-labelled oxamyl in the various experiments.

The decomposition of oxamyl when exposed to ultraviolet light in the laboratory was investigated in both distilled and river water samples. In both types of water, distilled at pH 6.2 and river at pH 6.5, an accelerated decomposition was observed. Within seven days, the oxamyl in the distilled water had decomposed to 28% of the original amount, with 43% converting to

oxime and 25% to the geometrical isomer of oxime. In the river water, the values were 22, 39 and 36% respectively.

The decomposition of oxamyl in river water exposed to sunlight outdoors for six weeks was even more extensive. Over the course of the experiment, the total radioactivity dropped by 17%, presumably lost by volatilization as carbon dioxide. Complete hydrolysis of the oxamyl to oxime was observed within one day. The oxime gradually converted to its isomer so that at the end of the study period, it accounted for 34% of the remaining radioactivity, with the oxime accounting for 46%. The remaining radioactivity manifested itself as a mixture of polar compounds; the major component, N,N-dimethyloxamic acid accounted for 14% of the radioactivity.

The decomposition of oxamyl in soils was studied using a soil-metabolism apparatus[28]. For 42 days, air was drawn over soil that had been treated with [^{14}C] oxamyl. Gas traps were used to "capture" $^{14}CO_2$. At the end of the study period, the soil was analyzed. The experiment was done under both aerobic and anaerobic conditions.

Under aerobic conditions, [^{14}C] oxamyl degraded to liberate 51% CO_2 in 42 days. Only 4% oxamyl was found at the end of the 42 day study period . A polar fraction accounted for 11%, with 26% remaining behind as unextracted residue. Under anaerobic conditions, oxamyl degraded almost as rapidly, with only 8% remaining after 42 days. The oxime accounted for 41%, while a polar fraction accounted for 42%. Unextracted residue

was determined to be 6%. The data indicated that anaerobic conditions did not interfere with hydrolysis, but did slow total decomposition to carbon dioxide.

A biodegradation study under field conditions indicated that radioactivity was rapidly lost from the soil by volatilization, presumably as CO_2. Oxamyl rapidly decomposed in the soil with less than 5% detected 1 month after treatment. While oxime was detected in the soil in the early days of the study, less than 5% was observed after one month. A detectable polar fraction appeared (8%), but could not be characterized. Analysis of the leachate after five months yielded insignificant amounts of oxamyl.

The field disappearance study confirmed earlier laboratory data on the degradation rate of oxamyl. It was concluded that under practical conditions, degradation of oxamyl to non-toxic forms occurs extremely rapidly. Its half-life under field conditions was determined to be approximately 8 days.

E. Treatment of Seed with Oxamyl

The potential for using nematicides on seeds to protect the seed and roots from nematode attack during the very beginning of root formation was recognized as early as 1955[29]. However, determining a suitable method of seed application was a problem. Initial attempts at dissolving the chemical in organic solvents, then soaking seeds in the resulting solution often ended with

failure: if the organic solvent was allowed to reach the embryo of the seed, the rate of germination was drastically reduced[30]. The alternative was to apply the chemical superficially to the dry seed. This approach does not result in poisoning, but makes the seed more susceptible to oxamyl loss during watering[31]. Furthermore, nematicides as seed treatments had not been widely used, because many nematicides, particularly the halogenated hydrocarbons, were too phytotoxic[32].

The value of oxamyl as a nematicide was realized in the 1970's. Rodriguez-Kabana and his colleagues evaluated a seed-treatment method using acetone as the solvent for delivering the nematicides oxamyl, carbofuran and phenamiphos to seeds of wheat and rye[32]. Seed treatment involved immersing the seeds for 30 seconds in solutions of various nematicide concentrations, then drying them quickly under a current of air. Germination studies revealed that phenamiphos tended to be the most phytotoxic of the three, suppressing the emergence of the plants. The three nematicides were all effective at controlling the plant-parasitic nematodes, with oxamyl capable of affording protection at lower levels in the short term. An extended experiment was not conducted to determine the length of time the nematicide, coated onto the seeds, would be effective against the target pests.

An alternative to coating seeds with pesticides dissolved in organic solvents was to introduce the chemical into the seeds in aqueous solutions. This had earlier been deemed impractical because the germination percentage for many seeds, in particular

certain legumes, was drastically reduced after prolonged soaking in water[33]. However, it had been shown that the ability of water-soaked seeds to germinate could often be restored by drying[34]. Truelove and his co-workers developed a technique for introducing chemicals in aqueous solutions into seeds, with the aim of conferring protection against nematodes on plants grown from the seeds[35]. The seeds used were bean, watermelon, soybean, cucumber and corn. Oxamyl was the nematicide of choice. Seeds were soaked in aqueous solutions of oxamyl for times ranging from 15 minutes for soybean to 6 hours for bean. After soaking, they were transferred to a sieve and thoroughly rinsed with running water to remove excess oxamyl solution. The seeds were then spread out on paper-towelling, placed in a circulating air incubator and dried. Treated seeds were then planted in nematode-infested soil to determine the efficacy of oxamyl in controlling the nematodes. For each crop-type, not only did the oxamyl repel the nematodes from the developing roots, but also reduced the nematode population in the soil. Phytotoxicity was not observed because preliminary experiments had determined the optimum seed treatment concentrations. However, because the growth-time lasted a maximum of 28 days, it was impossible to determine the long-term resistance to nematodes conferred by the seed treatment to the seeds, and subsequently to the seedlings.

Townshend and Chiba studied the control of root-lesion and root-knot nematodes, and the yield response of oxamyl-treated seeds[36]. Seeds were soaked for 20 minutes in water, acetone and

methanol solutions of oxamyl. The solutions were decanted after soaking and the seeds placed between 2 fine mesh sieves. The seeds were dried initially with a stream of air until they tumbled freely, and then dried further overnight on sheets of paper-towelling. They were planted in both root-lesion nematode-infested and root-knot nematode-infested soils. In both cases, not only was plant growth increased over the non-treated seeds, (62% and 32% respectively) but so was the nodulation in the roots (267% and 71% respectively). Accordingly, the densities of nematodes within the roots were significantly reduced (by 73% and 86% respectively). The authors concluded that oxamyl provided acceptable nematode control enabling alfalfa seedlings to become better established in the first 21 days of growth. Furthermore, plants from oxamyl-treated seed grew better as a result of early nematode control.

F. Analytical Methods for the Detection of Oxamyl

Over the years, a number of analytical approaches including titrimetric, spectrophotometric and chromatographic (GC and LC) methods have been used to determine oxamyl at various levels.

Singhal and his colleagues developed both titrimetric[37] and spectrophotometric[38] methods for the determination of oxamyl residues in both crops and soils. In both methods, oxamyl was refluxed with 10% potassium hydroxide solution, resulting in the

hydrolysis of the compound to methylamine and dimethylamine from the carbamate and amide ends of the molecule respectively. The solutions were then shaken with Cu^{2+}, CS_2, and ammonia solution, resulting in the formation of N-methyl and N,N-dimethyl dithiocarbamates. Using the titrimetric approach, the unreacted Cu^{2+} was titrated with a 0.001 M EDTA solution, with the amount of oxamyl calculated by difference. Spectrophotometrically, the copper complexes were determined by absorbance at 435 nm.

A gas chromatographic method reported by Holt and Pease[39] involved the alkaline hydrolysis of the extracted oxamyl to the more volatile, but stable, oxime, with final determination by GC with sulfur sensitive flame photometric detection. A detection limit of 20 ppb was attained. Bromilow[40] described a method whereby the oxamyl was reacted on-column with trimethyl-phenylammonium hydroxide to yield the methoxime derivative which was determined using a flame-photometric detector in the sulfur mode. A detection limit of 10 ppb was realized.

The problem with GC methods is that it is difficult to simultaneously determine oxamyl and its metabolites. Using ordinary gas chromatographic conditions, oxamyl degrades thermally to its oxime. Determination of oxamyl as its oxime assumes that no oxime was present in the sample initially. This is not acceptable because results obtained by this approach do not give any indication about the biological activity in the samples analysed. As a result, HPLC techniques were investigated.

Early work involved the use of reversed-phase HPLC with UV detection to determine oxamyl[41]. Thean and his co-workers found that in analyzing a variety of fruits and vegatables by RP-HPLC, extensive cleanup of the extracts was necessary[42]. Cleanup procedures were subsequently simplified, with a number of workers opting for the use of Sep-Pak™-type solid phase cartridges. McGarvey and his colleagues developed a method using LC determination of oxamyl residues in potato tubers[43]. Following florisil Sep-Pak cleanup, LC determination using a size-exclusion column and UV detection at 254 nm was performed. The method was successful in reducing the significant interferences produced by the sugars extracted from the samples. A detection limit of 10 ppb was obtained. Chiba and his colleagues published a method for the determination of oxamyl and its oxime in peach seeds, seedlings, soil and the clay pots in which the plants were grown. The method required an extensive cleanup, which included the use of C_{18} Sep-Pak cartridges and XAD-2 resin. The radial compression separation system by Waters was also used. The cleaned-up extracts were analysed by RP-HPLC using a C_{18} column and UV detection at 254 nm. Detection limits were 100 ppb for oxamyl and 50 ppb for the oxime. Prince published a method for the rapid determination of oxamyl and its oxime in water and soil by RP-HPLC[45]. The extract was cleaned up by silica Sep-Pak and chromatographed on a C_{18} column, with UV detection at 254 nm. Detection limits of 1 ppb for oxamyl and 5 ppb for oxime were obtained. This can be due to the fact that both soil and water

extracts tend to contain significantly less interferences than plant matrices.

G. Purpose of this Project

As a soil application, oxamyl has been shown to be effective against at least 18 varieties of nematodes[24]. Studies of oxamyl as a seed treatment have shown good potential in terms of protecting seedlings in the early stages of development from attack by root-lesion and root-knot nematodes[32,35]. The early studies were primarily nematological in nature, concentrating on the treatment levels of pesticide necessary to afford the plant protection against nematodes. The work done by Townshend and Chiba[36] on alfalfa was among the first to concurrently encompass both biological efficacy and chemical studies in which quantities of oxamyl on treated seeds were determined. It was thus decided that the behaviour of oxamyl treated onto corn seeds would be investigated. Although corn is a major crop, it had not been extensively studied. The project would include monitoring the distribution and persistence of oxamyl and its degradation products, specifically the oxime and DMCF, in planted corn seed, seedling root, and soil samples, using seeds previously treated with oxamyl for planting. Two types of soils, sterile and nematode-infested, would be used in this study. Nematode-infested soil would be extensively used to create an experimental soil 'climate' as close to that of problem field conditions (due

to nematode infestation) as possible. This is an important requirement, not only because concurrent biological studies to determine pesticide effectiveness would need conditions where nematode attack upon the early root systems could occur, but from a chemistry standpoint as well. The fate of oxamyl could be studied more realistically with soil containing a sizeable nematode population. Before this degradation study could commence, it would be necessary to develop several important methodologies. It would be necessary to develop a seed coating method which would give even distribution of the oxamyl onto the seeds. This would be important because the oxamyl coated onto the seeds would be the sole source of the nematicide. Its uniform distribution would be help achieve consistency of results. Furthermore, it would be necessary to determine the highest level of oxamyl applied to the seed which would not induce phytotoxicity in the seeding. Development of simple extraction and cleanup methods for the seed, root and soil samples would be necessary to ensure that analyses on samples generated through the degradation study could be fully completed within the time-frame of this project.

CHAPTER II - EXPERIMENTAL

A. Materials, Apparatus and Instrumentation

(1) Materials

(i) The Nematicide and its Related Compounds

Analytical grade oxamyl, (methyl-N',N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thio-oxamimidate) and its oxime (methyl-N',N'-dimethyl-N-hydroxy-1-thio-oxamimidate) were obtained from E.I. du Pont de Nemours and Co. Inc., Biochemicals Department, Wilmington, DE. DMCF (N,N-dimethyl-1-cyanoformamide) was synthesized in-house (see Appendix). Stock solutions of 1.00 mg/mL each were prepared by dissolving the above compounds in distilled water containing 0.5% phosphoric acid. A composite standard containing the three compounds at 100 µg/mL each was prepared, and standards of lower concentrations were prepared by further dilution with distilled water. Standard solutions were refrigerated at 4°C when not in use. The commerical product, Vydate L, a 24% formulation of oxamyl (w/v), was obtained from du Pont Canada, Toronto, Ontario.

(ii) Solvents

Methanol and acetonitrile, HPLC grade; acetone and diethyl ether, reagent grade were all from Caledon Laboratories Ltd., Georgetown, Ontario. The distilled water was prepared in-house at Agriculture Canada Research Station, Vineland Station, Ontario.

(iii) Chemicals

Dimethylcarbamoyl chloride was obtained from Aldrich Chemical Company Inc., Milwaukee, Wisconsin; Sodium cyanide - granular, sodium hydroxide - pellets, and sodium sulfate - granular were from Fisher Scientific Canada, Don Mills, Ontario; Nitric acid (16 M) was from Caledon Laboratories Ltd., Georgetown, Ontario.

(iv) Miscellaneous

Nuchar-Attaclay and silane-treated glass wool were from Supelco Inc., Bellefonte, PA. Disposable Pasteur pipettes (5-3/4" long) were from Allied/Fisher Scientific. Whatman #1 filter paper, 5 and 9 cm diameter sizes were from Whatman International Ltd., Maidstone, England. Acro LC-13 syringe-tip filters, 0.45 µm pore size, were from Gelman Sciences Inc., Montreal, Quebec.

(v) Soils

Nematode-free Delhi soil was sterilized by freezing outdoors over the course of a winter season at Agriculture Canada Research Station, Vineland Station, Ontario. Nematode-infested Delhi soil was prepared by tumbling soil, infested at 12 x 10^3 Pratylenchus root-lesion nematodes per kg of soil, with non-infested soil to obtain a resultant nematode population of 4 x 10^3 nematodes per kg of planting soil.

(vi) Seed

Stokes Hybrid Sweet Corn Seeds, designated Seneca Chief UT 125, were purchased from Stokes Seed Co., St. Catharines, Ontario.

(2) Apparatus

(i) GF-6 Centrifuge from MSE/VWR Scientific, England.

(ii) Polytron Model PT 10-35 Homogenizer with PT K generator containing knives from Brinkman Instruments Canada Ltd., Rexdale, Ontario.

(iii) Fisher-Kendall Mixer (Tumbler) Model 12-811V2 from Fisher Scientific Co., U.S.A.

(iv) Model 75 Wrist Action Shaker from Burrell Corp., Pittsburgh, PA.

(v) Model FG 2310 Vortex Genie from Scientific Industries, Springfield, MA.

(vi) Oster Model 202 Airjet Hair Dryer from John Oster Manufacturing Co., Milwaukee, WI.

(vii) Buchi Rotavapor <R> Model 18018 Rotary-evaporator, Brinkman Instruments of Canada Ltd., Rexdale, Ontario.

(viii) Chemtrix Type 45 R/R pH controller, Type 45R/ER pH meter from Chemtrix Inc., Hillboro, OR.

(3) LC Instrumentation

(i) Solvent Delivery System and Injector

(a) Minipump Model 396 from Milton Roy Industries, Rexdale, Ontario.

(b) Li-Chroma-damp II pulse dampener from Handy and Harmon Tube Co., Norristown, PA.

(c) Rheodyne Model 7037 pressure relief valve from Technical Marketing Associates Scientific Supply, Mississauga, Ontario.

(d) Autosampler equipped with a Rheodyne Model 7010 injector with a 100 µl sample loop from American Research Products Corp., Bethesda, MD.

(ii) LC Column

Spherisorb ODS-2, 5 µm packing, 25 cm x 4.6 mm reversed phase column was from Chromatography Sciences Company (CSC) Inc., Montreal, Quebec and was preceded by a Brownlee MPLC guard holder, equipped with a 3 cm RP-18 guard cartridge from Technical Marketing Associates Scientific Supply, Mississauga, Ontario.

(iii) Detector

Spectra Physics Model SP8200 fixed wavelength UV detector equipped with a mercury lamp and a 254 nm filter from Technical Marketing Associates Scientific Supply, Mississauga, Ontario.

(iv) Recorder

Schimadzu C-R3A Chromatopac from Tekscience, Oakville, Ontario.

B. Experiments

(1) Preliminary Greenhouse Study

(To determine the highest non-phytotoxic oxamyl concentration on the seeds).

Seeds treated in 0, 1.00, 2.00, 4.00, 8.00, and 16.00 mg/mL oxamyl solutions were used. Depending upon the treatment solution concentration and the type of soil the seeds were planted into, seven different types of samples were prepared. Seeds treated with the 0 mg/mL oxamyl solution (i.e. distilled water) were planted into both nematode-free and nematode-infested soils; these samples acted as the checks and controls respectively. The seeds treated with oxamyl were planted into nematode-infested soil. Triplicates of each sample type were prepared.

(i) Planting Procedure

(a) Soil (1.2 kg) was weighed into and lightly packed into a 6" diameter plastic azalea pot.

(b) One centimetre deep holes, 20 per pot, were made in the soil in rows of 3,4,6,4 and 3.

(c) With a pair of tweezers, seeds were placed one-by-one, narrow tip down, into the holes in the soil.

(d) The pot was lightly packed again so that the exposed seeds were just covered by the soil.

The growing period was 10 days in duration. Growth Room B39 at Agriculture Canada Research Station, Vineland Station, Ontario was held at 22°C, 50% humidity, and 1500 foot candles light intensity. Pots were distributed randomly beneath the light bed and misted daily with water. To prevent rapid evaporation of the moisture during the first 4 days (i.e. prior to seedling development), the misted pots were covered with clear plastic sheets.

Upon completion of the growth period, the samples were taken as follows:

(ii) Sampling Procedure

(a) The contents of the pot were turned out into a plastic pan.

(b) Seedlings (foliage, seeds, roots) were carefully worked free of the soil.

(c) The seedlings were subsampled into foliage, seed, and roots using scissors.

(d) Foliage was stored in 1 pint mason jars.

(e) Seeds were brushed free of adhering soil using a small paint brush and stored in 20 mL glass scintillation vials.

(f) Roots were carefully brushed free of as much of the adhering soil as possible and stored in a 125 mL glass jar.

(g) All samples were capped tightly and kept frozen at -15°C until required.

(2) Main Growth Experiment

(To study the fate of oxamyl with time).

In this study, the seeds used were those treated with the most concentrated oxamyl solution (4.00 mg/mL) giving no apparent phytotoxic symptoms to the seedling. Sampling was planned for specific time periods so that the fate of oxamyl and its metabolites, its oxime and DMCF, could be traced against time.

Seeds treated in 0 and 4.00 mg/mL oxamyl solutions were used. All the seeds were planted into nematode-infested soil. Seeds treated in the 0 mg/mL oxamyl solution (i.e. distilled water) acted as the controls. For each sampling day, four replicates each of the controls and oxamyl-treated seeds were planted. Samples were taken on days 0, 1, 3, 7, 14, 21, and 28.

The planting procedure was the same as that used in the preliminary greenhouse study.

The prepared pots were subjected to the growth conditions of Growth Room B26 (Wisconsin tank room;) at the Agriculture

Canada Research Station, Vineland Station, Ontario. The temperature was held at 22°C, the humidity at 70%, light intensity 3000 foot candles, and light duration, 16 hours/day. Pots were distributed randomly beneath the light bank and misted daily with water. To prevent rapid evaporation from the pots during germination and early growth, the pots were covered after misting with large sheets of brown waxed paper which was also wetted down. The papering was removed when seedlings appeared above the soil (after approximately 3 days).

The sampling procedure was the same as that used in the preliminary greenhouse experiment.

(3) LC Analysis

The LC system was operated isocratically with a mobile phase consisting of 15% acetonitrile/85% water for the analysis of oxamyl and DMCF, and with a mobile phase consisting of 4% acetonitrile/95% water when determining the oxime. Prior to use, the mobile phase solvents were degassed by stirring on a magnetic stirrer under a vacuum for several minutes. The flowrate in both cases was 1.3 ml/minute (approximately 35% pump-stroke). Determination of oxamyl, its oxime, and DMCF in the samples was by the external standard method, using standard concentrations similar to those in the samples. After each day of running samples, the mobile phase was changed to 100% acetonitrile and allowed to run overnight at the reduced pumpstroke of 10% to

flush the system and remove any strongly absorbed materials from the column.

C. Development of Methodologies

(1) Seed Coating Techniques

(i) "Classical" Technique

(a) Five hundred millilitres each of the oxamyl solutions at 0, 1.00, 2.00, 4.00, 8.00, and 16.00 mg/mL concentrations were prepared by diluting appropriate volumes of Vydate L commerical formulation (24% w/v active oxamyl according to the label specificiations but standardized to 25.30% by HPLC analysis).

(b) When seed treatment was to commence, the oxamyl solutions were transferred to 1 litre glass beakers and kept in a well ventilated area.

(c) Forty gram portions of dry corn seed were added to the respective solutions, stirring the mixtures with a glass rod to ensure total immersion of the seeds.

(d) The beakers were covered with aluminum foil and left for 18 hours.

(e) At the end of the treatment period, the soaking solutions were decanted from the seeds into a plastic pail. (The remaining oxamyl in the solution was treated with approximately 2 g of sodium hydroxide pellets to hydrolyze it to the oxime; after 24 hours, the solution was flushed down the drain with copious amounts of water.)

(f) The seeds were spread onto paper towels on a mesh screenbox and allowed to air-dry for 8 hours.

(g) The seeds were transferred to and stored in capped paper cups.

(ii) "Improved" Technique

Steps (a) through (e) of the "Classical" Technique were followed as the initial steps in this treatment, with the procedure completed using the following steps:

(a) Heavy-duty glass centrifuge bottles (250 mL) were prepared by packing them 1/3 full with dry sand, and covering the sand layer with a 5 cm diameter Whatman #1 filter paper.

(b) To the prepared centrifuge bottles, treated seeds were added, and the masses adjusted for centrifugation by the addition of appropriate amounts of glass beads.

(c) The packed centrifuge bottles were spun for 15 minutes at 5000 rpm.

(d) The seeds were removed from the bottles and spread onto paper towels in a fumehood.

(e) The fumehood door was lowered until a steady stream of ambient air flowed over the seeds; the seeds were air-dried in this manner for at least 48 hours or until the dried seeds were within approximately 0.5 g of the original weight of 40 g.

(f) Seeds were transferred to and stored in capped paper cups.

(2) Extraction Methods

(iA) Seed Extraction

(Initial version using a Polytron alone).

(a) To 20 dry corn seeds (approximately 5 g) in a 250 ml wide-neck Erlenmeyer flask, 100 mL of methanol was added.

(b) The seeds were homogenized for 1 minute using the Polytron unit set at speed 6.

(c) The sample mixture was vacuum-filtered on a 5 cm diameter Buchner funnel through a Whatman #1 filter paper. The flask was rinsed with 70 mL methanol and this was used to wash the residue on the filter.

(d) The combined methanol extracts were transferred to a 200 mL volumetric flask, diluted to mark with methanol and kept refrigerated at 4°C until required.

(iB) Seed Extraction

(Modification using Polytron and shaking).

(a) To 20 dry corn seeds (approximately 5 g) in a 250 mL wide-neck Erlenmeyer flask, 140 mL of methanol was added.

(b) The seeds were homogenized for 2 minutes using the Polytron unit set at speed 7.

(c) The Polytron probe was rinsed into the flask containing the sample with approximately 5 mL of methanol.

(d) The flask was stoppered tightly and the mixture shaken for 30 minutes at speed 4 on the wrist-action shaker.

(e) The sample mixture was vacuum-filtered on a 5 cm diameter Buchner funnel through a Whatman #1 filter paper. The flask was rinsed with 3 x 15 mL methanol and these portions were used to wash the residue on the filter.

(f) The combined extracts were transferred to a 200 mL volumetric flask, diluted to mark with methanol, and stored refrigerated at 4°C until further processing.

(iC) Seed Extraction

(Variation on (iB);Polytron and shaking of the wetted seeds).

(a) Moisture Determination in Corn Seed

The moisture uptake of dry, treated corn seeds was determined by planting 20 pre-weighed seeds in a 6" diameter azalea pot containing 1.2 Kg of dampened Delhi soil, misting the soil surface and allowing water absorption to occur over a 24-hour period. The seeds were then sampled, dusted off and reweighed, with the increase taken as water uptake. The % water absorbed was then calculated.

(b) Preparation of Water - Plumped Corn Seed for the Wetted Extraction

Twenty dry corn seeds (approximately 5 g) were weighed into a 1 pint Mason jar, and distilled water equivalent to that found in (iC)(a) was added. The jar was sealed and left for 24 hours refrigerated at 4°C to allow for complete water adsorption. The 'wetted' seeds were extracted as per the Polytron and shaking method (iB).

(iiA) Soil Extraction

(Tumbling in Methanol).

(a) Determination of Moisture in the Soil

Fifty grams of soil were weighed onto a watchglass and dried for 24 hours in a static air oven set at 105°C. The sample was then cooled to room temperature and reweighed. The % moisture in the soil was calculated by taking the difference in the damp and dried soil weights.

(b) Extraction of the Soil

1. To 100 g soil in a 32 oz. mason jar, 120 mL of methanol was added, and the jar sealed with an aluminum-foil-lined cap.

2. The sample was tumbled on a mechanical tumbler for 1 hour.

3. The mixture was vacuum-filtered on a 9 cm diameter Buchner funnel through a Whatman #1 filter paper, and the flask and soil rinsed with approximately 50 mL methanol.

4. The combined extracts were transferred to a 200 ml volumetric flask, diluted to mark with methanol, stoppered and refrigerated at 4°C until further processing.

(iiB) Soil Extraction

(Tumbling in Acid).

(a) Determination of Moisture in Soil

The moisture determination was performed as per (iiA)(a).

(b) Extraction of the Soil

1. To 100 g soil in a 32 oz. Mason jar, 120 mL of 0.2 M nitric acid was added, and the jar sealed with an aluminum-foil-lined cap.

2. The sample was tumbled on a mechanical tumbler for 1 hour.

3. The mixture was vacuum-filtered on a 9 cm diameter Buchner funnel through a Whatman #1 filter paper, and the flask and soil rinsed with approximately 50 mL of distilled water.

4. The extract was transferred to a 600 mL beaker and neutralized to pH 6.8 - 7.0 using 1 M sodium hydroxide.

5. The extract was then transferred to a 200 mL volumetric flask, diluted to mark with distilled water, stoppered and refrigerated at 4°C until further processing.

(iiC) Soil Extraction

(Modification of (iiA)(b)).

(a) Determination of Moisture in Soil

The moisture determination was performed as per (iiA)(a).

(b) Extraction of the Soil

1. To 100 g soil in a 32 oz. Mason jar, 100 mL of methanol was added, and the jar sealed with an aluminum-foil-lined cap.

2. The sample was tumbled on a mechanical tumbler for 1 hour.

3. The mixture was vacuum-filtered on a 9 cm diameter Buchner funnel through a Whatman #1 filter paper, and the flask and soil rinsed with 3 x 40 mL portions of methanol.

4. The combined extracts were transferred to a 250 mL volumetric flask, diluted to mark with methanol, stoppered and refrigerated at 4°C until further processing.

(iiiA) Root Extraction

(Using a Polytron).

(a) To 5 g of root in a 250 mL wide-neck Erlenmeyer flask, 140 ml of methanol was added.

(b) The sample was homogenized using a Polytron for 45 seconds at speed 7.

(c) The mixture was vacuum-filtered on a 5 cm diameter Buchner funnel through a Whatman #1 filter paper, and the flask and root rinsed with 3 x 35 mL portions of methanol.

(d) The combined extracts were then transferred to a 250 mL volumetric flask, diluted to mark with methanol, stoppered and refrigerated at 4°C until further processing.

(3) Cleanup Methods

(i) Syringe-tip Filtration of the Methanol Seed Extracts

(a) Two millilitres of methanol extract was pipetted into a calibrated 15 mL narrow-bottom centrifuge tube.

(b) Approximately 3 mL of distilled water was added to the tube.

(c) The tube was vortexed under vacuum and heated using a hair dryer until the solution level was approximately 1.5 mL.

(d) The aqueous solution was diluted to 2 mL with distilled water.

(e) The sample solution was transferred to a 5 mL glass syringe equipped with a Gelman syringe-tip filter and pushed through into a 2 ml polypropylene autosampler vial.

(f) The prepared extract was stored at 4°C until required for analysis by HPLC.

(ii) Centrifugation of the Acid-Extracted Soil Extracts

(a) Twenty millilitres of the neutralized acid extract was transferred to a 50 mL glass round-bottom centrifuge tube.

(b) The tube was centrifuged for 10 minutes at 2000 rpm.

(c) Approximately 5 mL of the clear top layer was pipetted into a 20 mL glass scintilllation vial, capped, and stored refrigerated at 4°C until analysis by HPLC.

(iii) Nuchar-Attaclay Cleanup of the Methanol Extracts

(a) A Nuchar-Attaclay 'column' was prepared by plugging the narrowed end of a 5-3/4" long disposable Pasteur pipette with silanized glass wool, filling it with 0.2 g Nuchar-Attaclay, and tapping it tip-down approximately 100 times to produce a tight bed of Nuchar-Attaclay.

(b) The prepared column was conditioned with 2 x 2 mL portions of methanol, air-forcing the solvent through the Nuchar-Attaclay. Special care was taken not to dry out the bed during this procedure.

(c) Two millilitres of the methanol extract was pipetted onto the column. The extract was air-forced through the column into a calibrated 15 mL narrow-tipped glass centrifuge tube. The column bed was washed with 2 mL methanol, which was added to the original methanol extract in the centrifuge tube.

(d) Approximately 3 mL of distilled water was added to the tube.

(e) The tube was vortexed under vacuum and heated with a hair dryer until the solution level was approximately 1.5 mL.

(f) The aqueous solution was diluted up to 2 mL with distilled water, transferred to a 2 mL polypropylene autosampler vial, and stored at 4°C until analysis by HPLC.

CHAPTER III - RESULTS AND DISCUSSION

A. Seed Coating Techniques

Within the scope of this study, it was necessary to develop a seed treatment method whereby the nematicide oxamyl could be imparted to the corn seed in an evenly distributed manner. This was important because the oxamyl absorbed by the seed was the sole source of the nematicide: if one seed received an insufficient amount, protection against nematodes would not be enough; if the dosage was too high, phytotoxicity (poisoning) would occur. Furthermore, in efficacy studies using the seeds, the biological effects would be assessed on each plant, bound in the assumption that each seed absorbed the same amount of oxamyl. In order to substantiate this assumption, one would have to be certain that the treatment method resulted in uniform coating of oxamyl onto the seeds. Non-uniformity in seed treatment could potentially result in variable responses by the nematodes to the nematicide. If this seed coating was to prove successful, it could offer a safer and more efficient application of the nematicide, as well as an economical alternative to both soil fumigation and foliage spraying. This was the goal in this part of the study.

The "classical" treatment involved soaking the seeds in the treatment solutions for a time period long enough to allow for maximum absorption into the seeds without causing them to germinate in the aqueous solutions. The damp seeds were then

spread out onto paper towels for a short time to dry. Table RD - 1 includes the weights of the corn seeds before and after treatment at various oxamyl concentrations.

Notice the obvious gains in weights for each seed group when treatment was finished and seeds were capped for storage. Initially, this was thought not to be much of a problem since there was no tangible change or deformation of the seed (such as germination). However, three days later, the growth of a blue-green fungus on the seeds was evident. Within a week, the spores had thickly covered each seed batch that was prepared. Not only did this occurrence bring into question the "storability" of the seeds even for the short term, but also created doubt as to how the fungus attack would ultimately affect their germination upon planting. Furthermore, the fungus-induced degradation of the oxamyl on or in the seeds was a possibility which, if these seeds were to be used for future plant growth/oxamyl degradation studies, could lead to false or inconclusive results. Finally, the variations in the seed weights after treatment could result not only in different amounts of moisture being present in the seed, but also in variations of the amounts of oxamyl absorbed by the seeds. Indeed, if one examines the surface of a dry corn seed, given the wrinkled contours it becomes obvious that pooling of the treatment solution in the crevices is a distinct possibility unless a more aggressive attempt at drawing off the excess solution is made. Because very high concentrations of

TABLE RD-1: CORN SEED WEIGHTS BEFORE AND AFTER "CLASSICAL" TREATMENT (i) VERSUS OXAMYL CONCENTRATIONS

CONCENTRATION OF OXAMYL IN THE VYDATE L TREATMENT SOLUTION (mg/mL)	WEIGHT OF THE SEEDS (g)		WEIGHT GAIN	
	PRE-SOAKING	POST-TREATMENT	DIRECT WT. GAIN (g)	RELATIVE WT. GAIN (%)
0	38.1	45.2	7.1	18.6
1.00	38.4	48.6	10.2	26.6
2.00	37.8	49.1	11.3	29.9
4.00	37.9	47.6	9.7	25.6
8.00	37.2	45.5	8.3	22.3
16.00	37.8	46.1	8.3	22.0

solutions were used, even one or two extra drops retained on the seed surface could lead to significant differences in the amount of nematicide taken up by one seed over another. With such questions having raised doubts in this initial seed coating technique, it was decided that another attempt would be made at seed treatment. In this case, the emphasis would be put on maximum removal of the excess oxamyl solution after treatment so as to prevent uneven distribution of the nematicide on the seed. Furthermore, to prevent fungus attack, a longer period of drying would be tried.

The "improved" technique included a centrifugation step. A heavy-duty glass centrifuge bottle was packed with sand to 1/3-full, with the damp coated seeds loaded on top. As a result of centrifugation, the excess coating solution adhering to the seed surface was drawn down into the sand. The drying scheme for the seeds was also altered; the centrifuged seeds were spread out onto paper-towelling in a fumehood and air was drawn over them for 48 hours. This period of time was usually sufficient to bring the seed weights back close to the pre-coated weights. Results are shown in Table RD - 2.

With this modified seed treatment, it was obvious that the weight gain compared with the classical treatment is substantially lower and more consistent. From a qualitative standpoint, the centrifugation served the purpose of removing excess surface solution. The additional drying clearly eliminated the fungus problem since even 12 months after

TABLE RD-2: CORN SEED WEIGHTS BEFORE AND AFTER "IMPROVED" TREATMENT (ii) VERSUS OXAMYL CONCENTRATIONS

CONCENTRATION OF OXAMYL IN THE VYDATE L TREATMENT SOLUTION (mg/mL)	WEIGHT OF THE SEEDS (g)		WEIGHT GAIN	
	PRE-SOAKING	POST-TREATMENT	DIRECT WT. GAIN (g)	RELATIVE WT. GAIN (%)
0	43.5	43.5	0	0
1.00	43.9	44.2	0.3	0.7
2.00	44.2	44.7	0.5	1.1
4.00	54.9	55.3	0.4	0.7
8.00	45.0	45.5	0.5	1.1

treatment, coated seeds that were kept stored in paper cups at room temperature remained fungus-free. Subsequent batches of coated seeds were prepared similarly.

B. Extraction Methods

(1) Extraction of Oxamyl, Oxime and DMCF from the Corn Seeds

It should be noted that when the first version of the extraction method (using only the Polytron) was studied, the seed sample was extracted twice; following the initial extraction, the seed residue was taken from the filter and re-extracted using the same method to determine how effective the first extraction was. Results are shown in Table RD - 3.

From these data, it is clear that a substantial portion of oxamyl was left behind in the seed following the initial extraction. Keeping in mind that the goal was to develop a method of extraction which was simple yet effective, the data from this first attempt indicated that further work was necessary. The wide variation in the total amounts of oxamyl extracted from replicate samples indicated rather inconsistent distributions of oxamyl on the seeds, because the extraction itself was done consistently from one replicate to another. At the same time, it was obvious from these results that one extraction was not sufficient to extract oxamyl from the seed quantitatively. As a result of these findings, the seed extraction method was altered in the first step with the aim of attaining improved extraction efficiency with a single

TABLE RD-3: AMOUNTS OF OXAMYL EXTRACTED FROM THE TREATED CORN SEEDS USING THE POLYTRON METHOD (iA).

CONCENTRATION OF OXAMYL IN THE VYDATE L TREATMENT SOLUTION (mg/mL)	AMOUNT OF OXAMYL EXTRACTED FROM THE SEEDS (ug/g)								
	REPLICATE 1			REPLICATE 2			REPLICATE 3		
	1st EXTR.	2nd EXTR.	TOTAL EXT'D.	1st EXTR.	2nd. EXTR.	TOTAL EXT'D.	1st EXTR.	2nd EXTR.	TOTAL EXT'D.
0	0	0	0	0	0	0	0	0	0
1.00	188	34	222	90	88	178	125	67	192
2.00	332	58	390	229	195	424	275	55	330
4.00	718	132	850	453	548	1001	700	111	811
8.00	1716	332	2048	986	916	1902	1236	416	1652
16.00	3561	527	4088	3307	1915	5222	802	2905	3707

* EXTR. = EXTRACTION

extraction. As such, the homogenizing was attempted for a longer time period at a higher speed setting in a greater volume of solvent. Furthermore, in order to increase the contact time between solvent and sample, the homogenized seeds, after the Polytron treatment, were shaken on a mechanical shaker for 30 minutes. The results generated from the modified extraction procedure (iB) are shown in Table RD - 4.

It should be stated at this time that the seeds used to conduct this modified extraction study were treated using the "improved" technique (i.e. centrifugation and longer drying time). As such, it was not necessary to contend with the fungus problem. The data in Table RD 4 clearly show much more consistency than those shown in Table RD - 3. This improvement was attributed to a more even distribution of oxamyl on the seeds. Furthermore, the amounts extracted at each coating concentration were substantially greater than those extracted using only the Polytron method. This is an obvious improvement. Although it is a common practice to carry out a recovery test, it was impossible to do so in this case because there was no practical way of absorbing a known quantity of oxamyl into the seeds. An alternative method of achieving this is by Soxhlet extraction, by which the analyte will be exhaustively extracted. However this was found to be unacceptable. A high proportion of oxamyl was degraded during the extraction process because the seeds were in contact with the warm methanol which was cyclically condensing. The majority of this oxamyl converted to its oxime.

TABLE RD-4: AMOUNTS OF OXAMYL EXTRACTED FROM THE TREATED CORN SEEDS USING THE POLYTRON AND SHAKING METHOD (iB)

CONCENTRATION OF OXAMYL IN THE VYDATE L TREATMENT SOLUTION (mg/mL)	AMOUNT OF OXAMYL EXTRACTED FROM THE SEEDS (ug/g) REPLICATE 1	REPLICATE 2	REPLICATE 3	MEAN	RELATIVE STANDARD DEVIATION* (%)
0	0	0	0	0	-
1.00	258	240	248	249	3.6
2.00	547	572	484	534	8.5
4.00	1013	1147	1081	1080	6.2
8.00	2196	2086	2119	2134	2.6

* OR COEFFICIENT OF VARIATION

If other degradation products such as methyl isocyanate and methyl isothiocyanate formed during Soxlet extraction, they would have been trapped in the methanol. However, they may not have been detected as the instrumentation was optimized for oxamyl, oxime and DMCF. While the use of the Polytron is presently accepted as the method of extraction for seeds, it was clearly not the best method here. Consequently, the combined method using the Polytron with subsequent shaking was used as the standard method of extraction for the remaining experiments.

During the large-scale growth experiment (to be discussed later), it was observed that the seeds, planted and sampled after only 1 day, gave significantly higher results for extracted oxamyl than those that were not even planted but analyzed immediately as zero-growth time controls. This was indeed odd, but upon further examination it was confirmed that a difference existed between the two seed samples. The unplanted seeds were as dry as they were on the day they were treated. The seeds that were planted and watered for only one day had already "plumped". Physically, the tissue of the "plumped" seeds was softer than that of the dry, harder seeds and thus, easily homogenized. To see if the extra moisture content actually made a difference in extraction efficiencies, an additional experiment was set up. Using seeds that had been coated in the 4.00 mg/mL oxamyl treatment solution, four different procedures were arranged to determine the effect of moisture content on the extraction efficiency; each procedure was assessed with three replicate

samples, 20 seeds per replicate.

The object of the experiment outlined in Table RD - 5 was to assess the improvement of the extractability of oxamyl from the softer hydrated seeds or seed residues. The four sample conditions essentially covered all the possible dry/wet combinations of seeds available for the first and second extractions. The results from this experiment are shown in Table RD - 6.

It is obvious that the physical difference in seed tissue does indeed affect the degree to which oxamyl can be extracted from the seed. For both the dry seed cases, at least 20% of the oxamyl was extracted in the second extraction. Hydrating the ground residue prior to re-extraction increased the efficiency of extraction. As indicated in Table RD - 6, almost twice as much oxamyl was extracted from the wet residue compared with the dry residue (Procedure 2 vs 1).

The results show a substantial increase in oxamyl extraction efficiency when the seeds were moistened prior to extraction. Because all these seeds were taken from the same 4.00 mg/mL soaking lot and because it was understood that the oxamyl was loaded onto the seeds quite uniformly, it is apparent that the nematicide is extracted much more easily from the wetted seeds. Approximately 75% more oxamyl was initially extracted from the wetted sample, compared with the dry sample. It is further apparent that of the total amount of extractable oxamyl, over 98% was retrieved in the first extraction from the

TABLE RD-5: COMPARISON OF OXAMYL EXTRACTABILITY IN WETTED VERSUS DRY SEEDS USING POLYTRON AND SHAKING METHOD (iB)

PROCEDURE	CONDITION OF SAMPLE	PROCESSING PROCEDURE
1	DRY SEED/ DRY RESIDUE	The dry seeds were initially extracted as per (iB), with the seed residue collected off the filter paper and re-extracted using the same method.
2	DRY SEED/ WET RESIDUE	The dry seeds were initially extracted as per (iB), with the seed residue collected off the filter paper, hydrated by the addition of water and re-extracted using the same method.
3	WET SEED/ DRY RESIDUE	The hydrated seeds were initially extracted as per (iB), with the seed residue collected off the filter paper, and re-extracted using the same method.
4	WET SEED/ WET RESIDUE	The hydrated seeds were initially extracted as per (iB), with the seed residue collected off the filter paper, re-hydrated by the addition of water, and re-extracted using the same method.

TABLE RD-6: AMOUNTS OF OXAMYL EXTRACTED FROM CORN SEEDS TREATED WITH A 4.0 mg/mL OXAMYL SOLUTION IN THE WET VS DRY SEED EXPERIMENT

PROCEDURE	CONDITION OF SAMPLE	REPLICATE	ug/g OF OXAMYL EXTRACTED DURING 1st EXTRACTION	MEAN	RELATIVE STANDARD DEVIATION (%)	ug/g OF OXAMYL EXTRACTED DURING 2nd EXTRACTION	MEAN	RELATIVE STANDARD DEVIATION (%)*
1	DRY SEED/ DRY RESIDUE	1	1109	1112	1.24	310	277	11.2
		2	1127			248		
		3	1100			274		
2	DRY SEED/ WET RESIDUE	1	1090	1077	3.16	443	539	17.4
		2	1038			542		
		3	1102			631		
3	WET SEED/ DRY RESIDUE	1	1844	1867	4.62	23	23	-
		2	1962			23		
		3	1794			**S.L.		
4	WET SEED/ WET RESIDUE	1	1936	2108	7.15	23	30	21.7
		2	2173			30		
		3	2216			36		

* OR COEFFICIENT OF VARIATION

**S.L. = SAMPLE LOST DUE TO SPILLAGE DURING EXTRACTION

wetted/plumped seed sample. While these results may put into question the usefulness of Polytron extraction of dry seeds, it appears that it is effective in processing wet, softened seeds. Fortunately, since all of the seed samples collected during the large-scale growth experiment contained a substantial amount of moisture, it would appear that the data generated from the growth experiment are acceptable.

Having established the suitability of the Polytron and shaking procedure for oxamyl in seeds, its applicability to the oxime and DMCF was also studied. Using the "improved" coating method, 2 sets of seeds were treated, one with 0, 100, 200, 400 and 800 ug/mL solutions of the oxime, the other with 0, 100, 200, 400 and 800 ug/mL solutions of the DMCF. These lower concentrations were chosen because it was expected that the levels of oxime and DMCF relative to oxamyl in actual samples would be significantly lower. The treated seeds were then extracted using the previously described Polytron and shaking method. Results are shown in Tables RD - 7 and RD - 8.

As with the case of oxamyl extraction from the corn seeds, there was no accurate way of determining the amounts of either oxime or DMCF loaded onto the seeds. However, based on the relatively good reproducibilities in the amounts extracted, as well as the proportions of the amounts of oxime and DMCF extracted relative to coating solution concentrations, it would seem to be reasonable to assume that both the oxime and DMCF

TABLE RD-7: AMOUNT OF OXIME EXTRACTED FROM CORN SEEDS TREATED WITH OXIME USING THE POLYTRON AND SHAKING METHOD (iB)

CONCENTRATION OF OXIME IN THE SEED TREATMENT SOLUTION (ug/mL)	AMOUNT OF OXIME EXTRACTED FROM CORN SEEDS (ug/g) REPLICATE 1	REPLICATE 2	REPLICATE 3	MEAN	RELATIVE STANDARD DEVIATION (%)*
0	0	0	0	0	-
100	26	28	33	29	12.4
200	49	'66	68	61	17.1
400	124	**S.L.	139	131	-
800	243	268	275	262	6.4

* OR COEFFICIENT OF VARIATION

**S.L. = SAMPLE LOST DUE TO SPILLAGE DURING EXTRACTION

TABLE RD-8: AMOUNT OF DMCF EXTRACTED FROM CORN SEEDS TREATED WITH DMCF USING THE POLYTRON AND SHAKING METHOD (iB)

CONCENTRATION OF DMCF IN THE SEED TREATMENT SOLUTION (ug/mL)	AMOUNT OF DMCF EXTRACTED FROM CORN SEEDS (ug/g) REPLICATE 1	REPLICATE 2	REPLICATE 3	MEAN	RELATIVE STANDARD DEVIATION (%)*
0	0	0	0	0	-
100	19	22	21	21	7.27
200	43	44	45	44	2.27
400	92	95	94	94	1.62
800	218	227	255	233	8.28

* OR COEFFICIENT OF VARIATION

could be just as successfully extracted from the corn seeds as oxamyl.

(2) Extraction of Oxamyl, Oxime and DMCF From Soil

When extracting chemical components from soil, tumbling of the sample in a pre-selected solvent is generally the method of choice[46]. As such, this was the direction taken with the aim of developing a simple procedure for extracting oxamyl, its oxime and DMCF in soil.

The initial attempt involved tumbling the soil sample in methanol, followed by filtration. Both nematode-infested and nematode-free soils were used to see if soil type made any difference in retrieving the spiked oxamyl. The spiking level for the soils was 2 ug oxamyl per gram soil. Following the addition of the spiking solution, the soil samples were tumbled for 1 hour, then left to stand for 18 hours to allow for oxamyl absorption/adsorption into/onto the soil. For each soil type, one blank and triplicate spiked samples were analysed. The soils were extracted twice. The filtered residue from the first extraction was re-extracted to determine the efficiency of the first extraction. Recovery data are shown in Table RD - 9.

While the recovery results are quite consistent for both the soil types, the recoveries themselves seem to be rather low, especially for the nematode-free soil. This may be due in part to the fact that it was a much drier sample, having been determined to have only 0.68% moisture content while the

TABLE RD-9: RECOVERIES OF OXAMYL FROM SOIL USING THE INITIAL TUMBLING METHOD (iiA)

SOIL TYPE	RECOVERED OXAMYL (%) 1ST EXTRACTION	2ND EXTRACTION
NEMATODE-FREE BLANK	-	-
NEMATODE-FREE SPIKE 1	69	0
NEMATODE-FREE SPIKE 2	70	0
NEMATODE-FREE SPIKE 3	63	0
	*X: 67 **R.S.D. = 5.65%	
NEMATODE-INFESTED BLANK	-	-
NEMATODE-INFESTED SPIKE 1	78	0
NEMATODE-INFESTED SPIKE 2	77	0
NEMATODE-INFESTED SPIKE 3	71	0
	*X: 75 **R.S.D. = 5.05%	

*X = MEAN

**R.S.D. = RELATIVE STANDARD DEVIATION OR COEFFICIENT OF VARIATION

nematode-infested soil was found to contain 5.39%. As such, the spiked oxamyl may have been absorbed/adsorbed to a greater degree by/to the dry nematode-free soil particles compared with the damp nematode-infested soil simply because of the lower moisture content. The biggest concern however, was that 25-33% of the spiked oxamyl could not be accounted for, even upon re-extraction. The question was raised as to whether the unaccounted portion now constituted "bound" residues (non-extractable) or whether the method itself was not very efficient at retrieving oxamyl. As a result, it was decided that 0.2 M nitric acid would be tried as the extracting solvent because it was thought the acid might release out the strongly absorbed oxamyl. Acids are generally used when attempting to extract "bound" residues. It was necessary to bring the acid extract back to pH 7 (avoiding pH levels above 7 because of the facile conversion of oxamyl to its oxime) before it could be analysed. For this trial, only 2 spiked replicates were used, with the initially extracted residue not subjected to re-extraction because it was felt that given an acidified solvent, significantly more oxamyl would be extracted with just a single extraction. Results are shown in Table RD - 10.

Needless to say, the results obtained from the acid extraction were indeed surprising, especially since it was expected that given the acidic extracting solvent, better recoveries would result. This expectation was found to be incorrect because it was found that a high proportion of the

TABLE RD-10: RECOVERIES OF OXAMYL FROM SOIL USING ACID EXTRACTION (iiB)

SOIL TYPE	RECOVERED OXAMYL (%) UPON SINGLE EXTRACTION
NEMATODE-FREE BLANK	-
NEMATODE-FREE SPIKE 1	35
NEMATODE-FREE SPIKE 2	36
NEMATODE-INFESTED BLANK	-
NEMATODE-INFESTED SPIKE 1	53
NEMATODE-INFESTED SPIKE 2	50

oxamyl was converted to oxime. Apparently this extraction method is not acceptable since it would make the accurate and separate quantitation of oxamyl and its oxime essentially impossible. It was thus decided to review the methanol tumbling method and attempt appropriate changes to improve recoveries.

Potentially, the most obvious mistake in the methanol tumbling method was an inefficient rinsing of the residues on the filter paper. Accordingly, a modification was made to the volume of methanol for the original extraction (from 200 to 250 mL). More importantly, rinsing was done 3 times with 40 mL of methanol each time. Previously, a single rinsing was made with 50 mL of methanol. Furthermore, after the first extraction, two successive extractions were made to see if all of the spiked oxamyl could be accounted for. Results from this latest spiking set are shown in Table RD - 11.

Examination of the data therein indicated that complete extraction of oxamyl was possible if three extractions were made. However, 86 and 87% recoveries with single extractions were deemed to be acceptable. As such, this method was used as the standard method of extraction for soil samples in the rest of the project.

(3) Extraction of Oxamyl, Oxime, and DMCF from Root

In view of the success of the Polytron method in the extraction of the corn seeds, especially when used on a dampened,

TABLE RD-11: RECOVERIES OF OXAMYL FROM SOIL USING THE MODIFIED TUMBLING METHOD (iiC)

SOIL TYPE	RECOVERED OXAMYL (%) 1ST EXTRACTION	2ND EXTRACTION	3RD EXTRACTION	TOTAL
NEMATODE-FREE BLANK	-	-	-	-
NEMATODE-FREE SPIKE 1	85	13	4	102
NEMATODE-FREE SPIKE 2	85	10	5	100
NEMATODE-FREE SPIKE 3	89	11	3	103
	*X: 86 **R.S.D. = 2.68%	*X: 11 **R.S.D. = 13.9%	*X: 4 **R.S.D. = 25.0%	
NEMATODE-INFESTED BLANK	-	-	-	-
NEMATODE-INFESTED SPIKE 1	88	9	3	100
NEMATODE-INFESTED SPIKE 2	85	10	3	98
NEMATODE-INFESTED SPIKE 3	87	8	***S.L.	-
	*X: 87 **R.S.D. = 1.76%	*X: 9 **R.S.D. = 11.1%	*X: 3	

*X = MEAN
**R.S.D. = RELATIVE STANDARD DEVIATION OR COEFFICIENT OF VARIATION
***S.L. = SAMPLE LOST DUE TO SPILLAGE DURING EXTRACTION

softer seed, it was decided that for the soft root samples, this type of extraction method would be attempted.

With the root sample spiked with oxamyl to a level of 1 µg/g, extractions were undertaken. Recovery results obtained from the extraction are shown in Table RD - 12.

As with the soils, the extraction efficiency of only oxamyl was determined. However, based on the good recoveries and reproducibilities, it would seem viable to assume that both the oxime and DMCF could be just as successfully extracted from the roots as the oxamyl. Therefore, this method was used as the standard method of extraction for root samples in the rest of the project.

C. Cleanup Methods

(1) Cleanup of Methanolic Extracts

The mobile phase used in the LC system throughout this project was a mixture of acetonitrile and water. Because the raw extracts for all three sample types studied here (seed, soil and root) were in methanol, (and not in either of the mobile phase component solvents), it was necessary to reduce the elution strength of the sample solvent substantially. This was achieved quite simply by performing a one-to-one phase transfer into water by taking a known volume of the methanol extract, adding distilled water to it, heating the mixture under vacuum and vortexing so that the methanol would evaporate, leaving the

TABLE RD-12: RECOVERIES OF OXAMYL FROM CORN ROOT USING THE POLYTRON METHOD

SAMPLE	RECOVERED OXAMYL (%)	MEAN	RELATIVE STANDARD DEVIATION (%)*
Root Blank	-	-	-
Root Spike 1	92	93	4.35
Root Spike 2	89		
Root Spike 3	97		

* OR COEFFICIENT OF VARIATION

residue dissolved in the water. The aqueous solution was then adjusted to that most suitable for HPLC analysis.

The necessity for some type of cleanup prior to injection became evident in the case of the corn seed extracts. While in methanol solvent, the extract was a clear, yellow gold colour. However, upon completing the transfer into water, the solution became dense, cloudy and white in colour. It was suspected at this time that some component of the seed (probably corn oil containing carotenoids), that was soluble in methanol but to a much lesser degree in water, had precipitated out in the water. The sample obviously could not be injected onto the LC in this state as damage to the system could have resulted.

The initial cleanup attempt involved forcing the final cloudy aqueous extract through a 0.45 μm syringe-tip filter, hopefully collecting a clear solution upon filtration. Unfortunately, the filter was ineffective at retaining what was most likely an emulsion of very small droplet size, as no difference could be detected between the filtered and unfiltered forms. This setback necessitated the use of a more rigorous cleanup. The use of Nuchar-Attaclay in the removal of dark pigments from methanolic extracts of chrysanthemum leaves had been shown to be quite effective[47]. It was therefore viewed as a viable cleanup method to apply to samples in this study. Visually, all of the extracts cleaned-up in this manner were completely devoid of colour; a yellow-gold from seed, a murky-brown from the soil, and a yellow-brown from root were completely

removed. Furthermore, the regions of the chromatograms in which oxamyl, its oxime and DMCF eluted were free of interferences (see Appendix II). To determine the recoveries of oxamyl, oxime and DMCF through the Nuchar-Attaclay treatment, 2 mL triplicate spike solutions in methanol containing 1 ug/mL each of oxamyl, oxime and DMCF (plus a methanol blank) were put through the Nuchar-Attaclay column treatment and compared with standards. The recovery results are shown in Table RD - 13.

The tabulated results indicate that losses of the target compounds are minimal for the oxamyl and its oxime, and slightly greater for the DMCF. This cleanup method was advantageous because it presented a cleaner extract for injection on the HPLC. The minor losses encountered using this cleanup were overshadowed by the "cleaner" chromatography observed during LC analysis. As a result, throughout this study, all the methanol extracts (seed, soil and root) were processed in this manner prior to instrumental analysis.

(2) Cleanup of the Acid-Extracted Soil Extracts

While pursuing the possibility of using the acid tumbling method for extracting soils, it became evident that after neutralizing the extract back to pH 6.8 - 7.0, a further treatment would be necessary. The neutralized extract was a murky, yellow-brown in colour, containing a fine suspension of a darker, dust-like solid, probably humic material that had passed through the filter. Since injecting samples of this nature

TABLE RD-13: RECOVERIES OF OXAMYL, OXIME, AND DMCF THROUGH THE NUCHAR-ATTACLAY COLUMN TREATMENT

SAMPLE	RECOVERED OXAMYL (%)	RECOVERED OXIME (%)	RECOVERED DMCF (%)
Blank	-	-	-
Spike 1	94	97	84
Spike 2	99	98	90
Spike 3	98	101	94
	*X: 97	*X: 99	*X: 89
	**R.S.D.: 2.72%	**R.S.D.: 2.10%	**R.S.D.: 5.66%

*X = MEAN

**R.S.D. = RELATIVE STANDARD DEVIATION OR COEFFICIENT OF VARIATION

directly onto the LC runs the risk of damage to the instrument, it was decided that the easiest way to remove the suspension from the extract would be to centrifuge the sample. This was found to be effective, with the upper portion of the extract clear after the treatment. It was from this layer that a portion was pipetted off for LC analysis. However, because poor recoveries for oxamyl were obtained (Table RD 10), and also because there was strong indication that oxamyl was degrading to its oxime, further work on this approach was discontinued.

D. Greenhouse Experiments

(1) Preliminary Growth Study

The early work in this project involved not only the development of an acceptable seed coating method, but also the determination of the highest level of oxamyl loading onto the seed without creating phytotoxicity. This preliminary growth study was designed to achieve this objective; seeds treated in oxamyl solutions at 0, 1.00, 2.00, 4.00, 8.00 and 16.00 mg/mL were planted and kept in a growth room for a period of 10 days after which time the seedlings were rated for development. The results are presented in Table RD - 14.

It is apparent from the tabulated observations that seeds treated in 8.00 and 16.00 mg/mL oxamyl solutions germinated in lower numbers and produced rather unhealthy looking seedlings. As a result, it was decided that the seeds treated in the 4.00 mg/mL oxamyl solution would be used for the subsequent main

TABLE RD-14: SEEDLING STATUS AT THE END OF THE 10-DAY GROWTH PERIOD

CONCENTRATION OF OXAMYL IN THE SEED TREATMENT SOLUTIONS (mg/mL)	REPLICATE	# OF OBSERVABLE SEEDLINGS PER POT*	SEEDLING CONDITION
0 (CHECK)	1 2 3	18 20 20	Strong, uniform growth with medium green-coloured foliage and a well-developed root system were observed.
0 (CONTROL)	1 2 3	18 20 20	Appearance similar to that of the check samples except that the seedlings were slightly shorter in height.
1.00	1 2 3	19 19 19	Same as the Control.
2.00	1 2 3	18 18 20	Same as the Control.
4.00	1 2 3	20 19 19	Same as the Control.
8.00	1 2 3	7 12 16	In the cases where germination did occur, marked stunting in growth was observed, although the green colouration in the foliage was retained.
16.00	1 2 3	7 11 4	A low rate of germination was observed, and in the cases where seedlings sprouted, extreme stunting with yellowing of the foliage was evident.

* EACH POT CONTAINED 20 PLANTED SEEDS.

growth experiment. Even at this coating level, the resulting seedlings showed strong growth with no apparent phytotoxic characteristics.

(2) Main Growth Experiment

Having determined a suitable oxamyl seed treatment level to ensure seemingly good seedling development, the next phase of study required carrying out a larger-scale growth experiment. It was expected that through this experiment, it would be possible to monitor the dissipation of oxamyl and the formation of its oxime and DMCF in seed, soil and root in relation to growth time. Both treated and untreated seeds were planted into nematode-infested soil (the untreated seeds acted as controls). Sampling was done at 0, 1, 3, 7, 14, 21 and 28 day intervals, with four replicates each of controls and treated seeds taken down at each sampling date. All samples (seeds, soil, roots) were then analysed to determine the amounts of oxamyl, its oxime and DMCF present at the time of sampling. Table RD - 15 summarizes the amounts of oxamyl, oxime and DMCF found in the corn seeds at the specified sampling date. Note that the amounts of chemicals detected in the samples were the total ug found in the 20 seeds per pot. These absolute amounts were used to eliminate the problems of accounting for different levels of moisture in the seed samples at the time of sampling.

Figures RD - 1 and 2 are plots of the amounts of oxamyl, oxime and DMCF extracted from the seeds versus elapsed growth

TABLE RD-15: AMOUNTS OF OXAMYL, OXIME, AND DMCF DETECTED IN THE CORN SEEDS (AS THE MEAN OF 4 REPLICATES) AT EACH SAMPLING DAY

SAMPLING DAY	TOTAL ug OF OXAMYL DETECTED IN THE 20 SEEDS	RELATIVE STANDARD DEVIATION (%)*	TOTAL ug OF OXIME DETECTED IN THE 20 SEEDS	RELATIVE STANDARD DEVIATION (%)*	TOTAL ug OF DMCF DETECTED IN THE 20 SEEDS	RELATIVE STANDARD DEVIATION (%)*
0	8776	5.67	583	21.4	38	9.70
1	6149	4.78	474	17.9	**N.D.	-
3	3389	26.3	268	31.0	54	54.0
7	1012	67.1	106	42.4	64	17.2
14	223	126	73	56.2	22	45.4
21	20	125	26	42.3	5	60.2
28	17	76.5	5	80.0	*N.D.	-

* OR COEFFICIENT OF VARIATION

**N.D. = NOT DETECTED

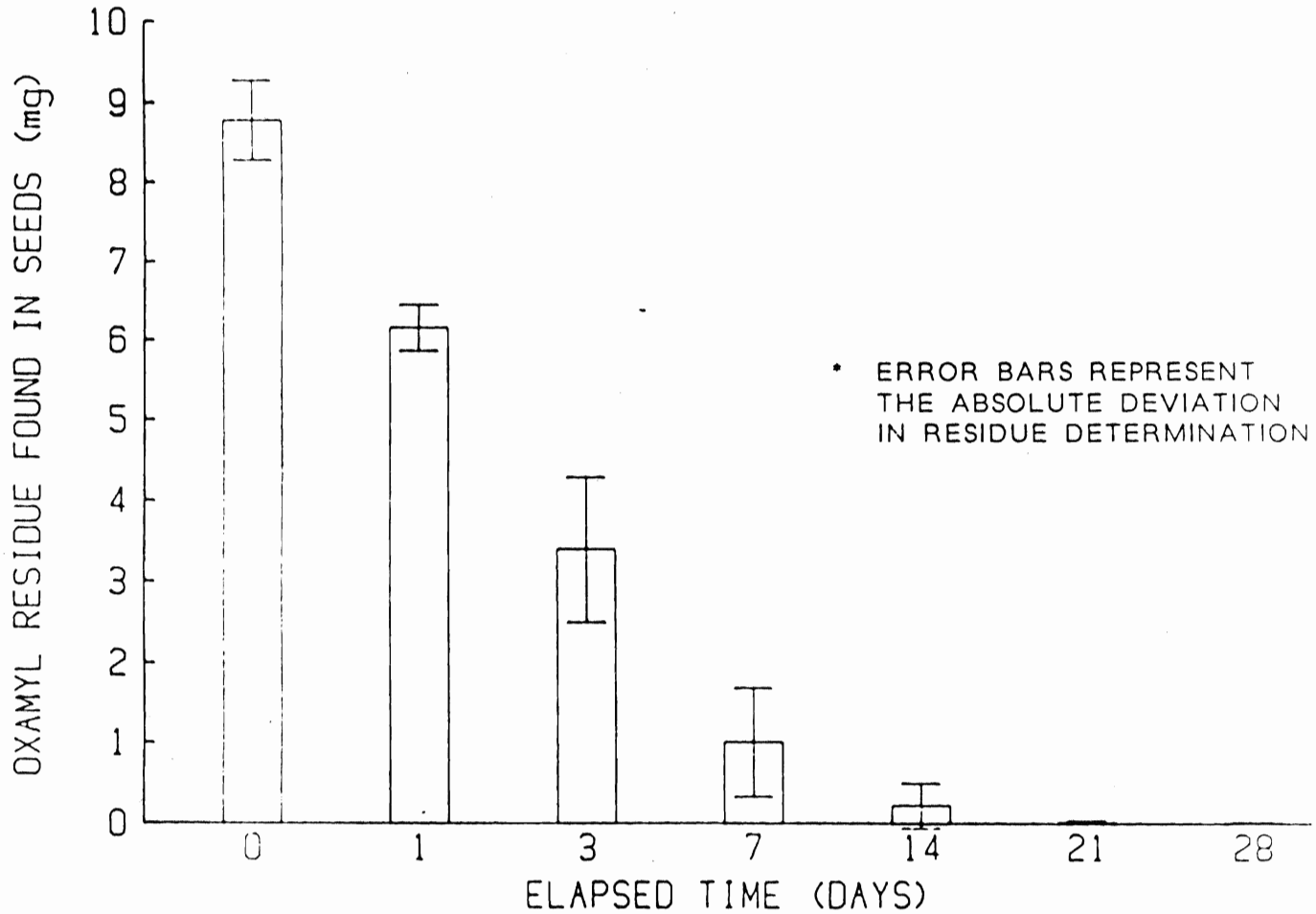

Figure RD - 1: Histogram Depiction of the Amount of Oxamyl Found in the Planted Corn Seeds Versus Time

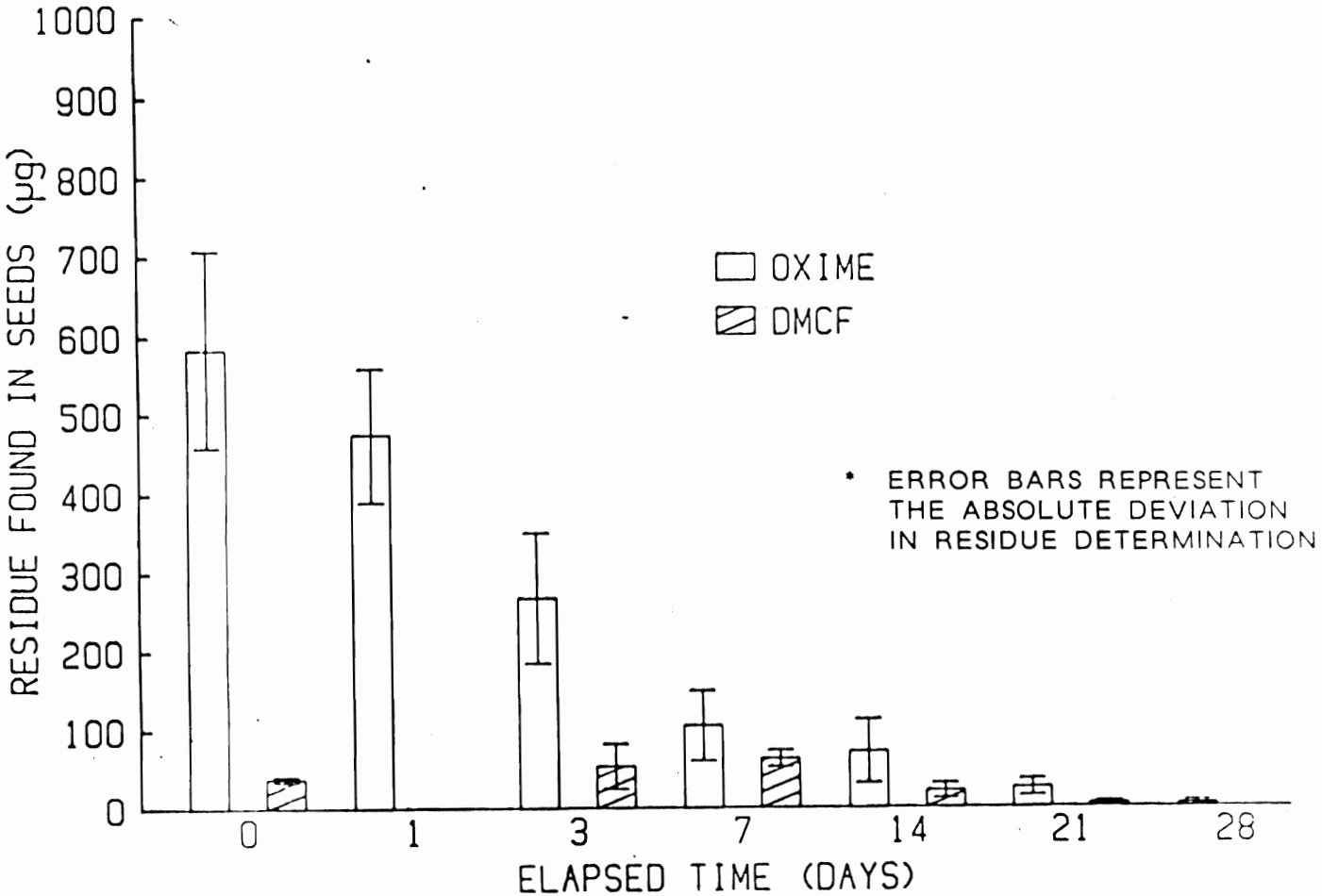

Figure RD - 2: **Histogram Depiction of the Amounts of Oxime and DMCF Found in the Planted Corn Seeds Versus Time**

time. It clearly shows a marked drop in oxamyl detected within a week of planting. The levels of oxime also decrease with time, although to a lesser degree than the oxamyl. (It is interesting to note that some oxime is present in the seeds before planting). The reductions in the amounts of both oxamyl and oxime with time appeared to be exponential in nature. To confirm this, a subsequent plot of the natural logs of the amounts of oxamyl and oxime detected versus elapsed time was made (Figure RD - 3) using the values listed in Table RD - 16. A linear regression analysis was also done on these two sets of points. Reasonably good correlation coefficients of 0.984 (for oxamyl) and 0.986 (for oxime) indicated that the dissipations of both these compounds were exponential.

While the data for oxamyl and its oxime in seeds indicated an obvious decrease with time, the DMCF data are somewhat inconclusive. From Table RD - 15, it appears that the amount initially increased, then decreased. Because DMCF is produced as a result of decomposition of oxamyl, the initial increase indicated that the rate of DMCF decomposition was slower than that of oxamyl. However, given the large deviations of the means, i.e. the low precision in generating each data point, it would be unwise to infer very much from these values.

Because it is well understood that the degradation of oxamyl is mainly to its corresponding oxime, it would be expected that when the level of oxamyl decreases, the level of the oxime would increase. This, however, was not observed here. Rather, it

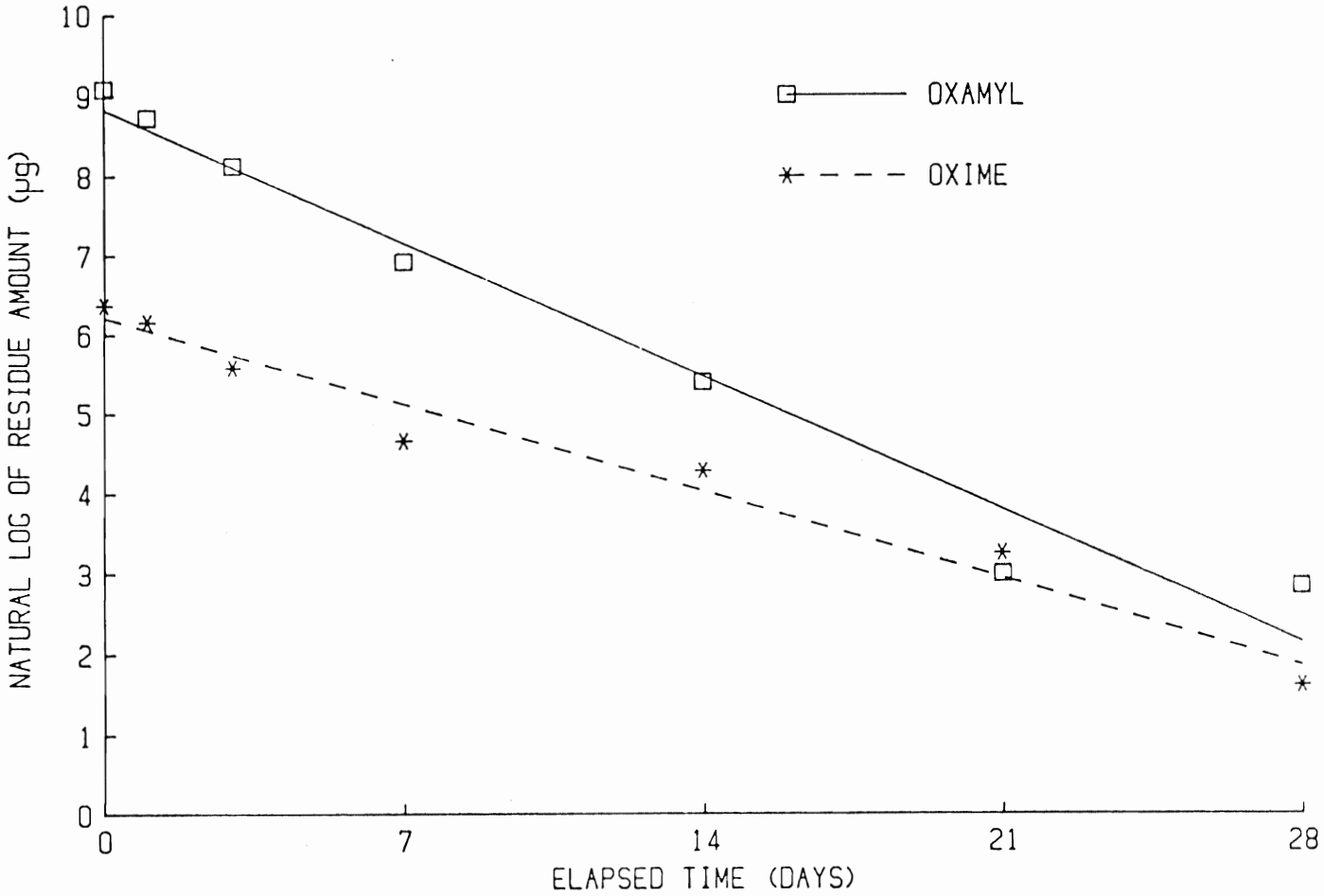

Figure RD - 3: Plot of the Natural Logs of the Amounts of Oxamyl and Oxime Found in the Planted Corn Seeds Versus Time

TABLE RD-16: NATURAL LOG OF THE AMOUNTS OF OXAMYL AND OXIME DETECTED IN THE SEEDS (AS A MEAN OF 4 REPLICATES) VERSUS EACH SAMPLING DAY

SAMPLING DAY	ln OF THE TOTAL ug OF OXAMYL DETECTED IN THE SEEDS	ln OF THE TOTAL ug OF OXIME DETECTED IN THE SEEDS
0	9.080	6.368
1	8.724	6.161
3	8.128	5.591
7	6.920	4.663
14	5.407	4.290
21	2.996	3.258
28	2.833	1.609
	*C.C.: 0.984	*C.C.: 0.986

*C.C. = CORRELATION COEFFICIENT

appears that both the oxamyl and its oxime tend to dissipate concurrently. Oxamyl may in fact degrade to oxime, but the rate of oxime degradation could be only slightly slower than that of oxamyl, resulting in little relative gain of the oxime. More probable, though, is that their decreases were due to uptake by the plant during seedling development or that both oxamyl and oxime were washed from the seed via watering. If watering was the cause, elevated levels of oxamyl and oxime would have been observed in the soil; if uptake by the plant occurs, then these chemicals would have been detected in either the roots or the foliage. Table RD - 17 summarizes the amounts of oxamyl and oxime found in the soil at the specified sampling date. DMCF was not detected in any of the soil samples. Note that in this table, the amounts of chemicals detected in the soils are expressed as ug per 100 g dry-weight sample. This was done because the moisture content in the soil samples ranged from 3 to 12%, with their appearances ranging from slightly dampened to mud-like. As a result, it was deemed necessary to normalize the data obtained for each sample so that a realistic comparison of all the data points could be made.

Figure RD - 4 is a plot of the amounts of oxamyl and oxime extracted from the soil versus elapsed growth time. It is obvious that a significant portion of the oxamyl was washed from the seeds down onto the soil. This down-loading appears to have occurred quickly within the first three days of the experiment, after-which the oxamyl level decreased only slowly. However,

TABLE RD-17: AMOUNTS OF OXAMYL AND OXIME DETECTED IN SOIL (AS THE MEAN OF 4 REPLICATES) AT EACH SAMPLING DAY

SAMPLING DAY	ug of OXAMYL DETECTED PER 100 g DRY-WEIGHT SOIL	RELATIVE STANDARD DEVIATION (%)*	ug OF OXIME DETECTED PER 100 g DRY-WEIGHT SOIL	RELATIVE STANDARD DEVIATION (%)*
0	**N.D.	-	**N.D.	-
1	158	12.0	19	52.1
3	227	58.6	19	105
7	212	36.3	50	18.3
14	167	32.9	38	29.4
21	134	29.9	22	41.2
28	110	56.4	18	28.1

* OR COEFFICIENT OF VARIATION

**N.D. = NOT DETECTED

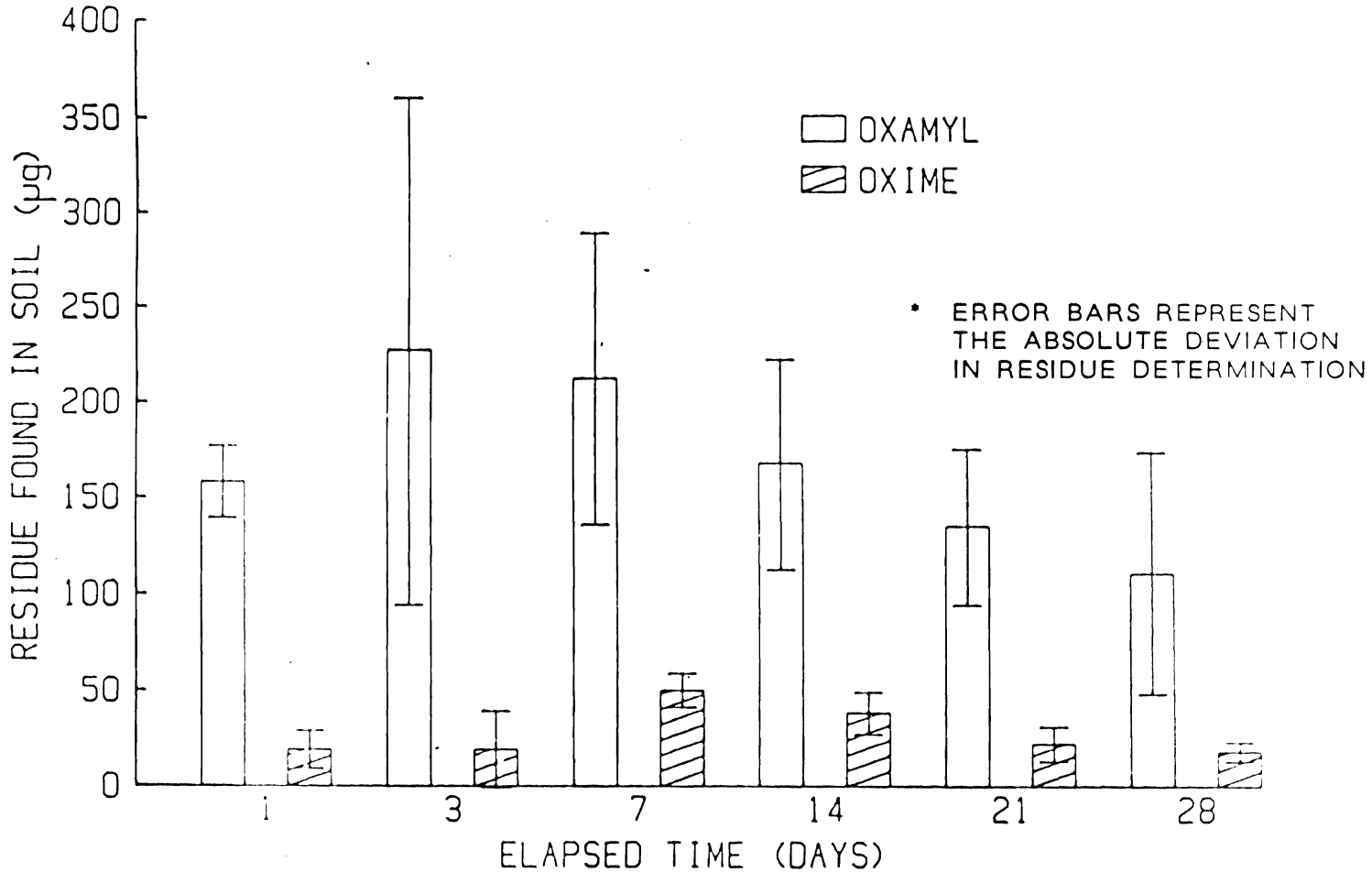

Figure RD - 4: Histogram Depiction of the Amounts of Oxamyl and Oxime Found in the Soil Versus Time

because of the large relative standard deviations in the mean values, it is difficult to make additional inferences from the data with confidence.

Although the DMCF data are inconclusive, the oxime results are interesting. While some of the oxime present in the soil may have been introduced from the seeds, the pattern in the decrease in its levels with time differed from that observed with the seeds, where wash-off tended to be the main reason for its disappearance. Here, an increase, albeit small, in the oxime level over the middle range of growth time was evident and may have been due to the degradation of oxamyl that had been rinsed onto the soil from the seeds.

Because of the potential for non-sterilized soil to contain micro-organisms, it is not inconceivable that oxamyl may degrade to compounds other than oxime and DMCF as a result of bacterial or microbial action.

Table RD - 18 shows the amount of oxamyl found in the roots at the specified sampling date. Neither oxime nor DMCF was detected in any of the root samples. In this case, all of the root collected from each pot was used as a single sample for analysis. As a result, the total amounts of chemical found were reported. Figure RD - 5 is a plot of the amount of oxamyl extracted from the roots versus elapsed growth time.

For the first week of study, there was insufficient root available to conduct an acceptable analysis. By Day 7, enough seedling growth had occurred to generate sufficient root for

TABLE RD-18: AMOUNT OF OXAMYL DETECTED IN THE ROOT (AS THE MEAN OF 4 REPLICATES) AT EACH SAMPLING DAY

SAMPLING DAY	TOTAL ug of OXAMYL DETECTED USING ALL OF THE ROOT FROM EACH POT	RELATIVE STANDARD DEVIATION (%)*
0	**S.N.A.	-
1	**S.N.A.	-
3	**S.N.A.	-
7	12	73.1
14	24	59.2
21	24	23.6
28	20	61.2

* OR COEFFICIENT OF VARIATION

**S.N.A. = SAMPLE WAS NOT AVAILABLE AS ENOUGH ROOT HAD NOT YET FORMED

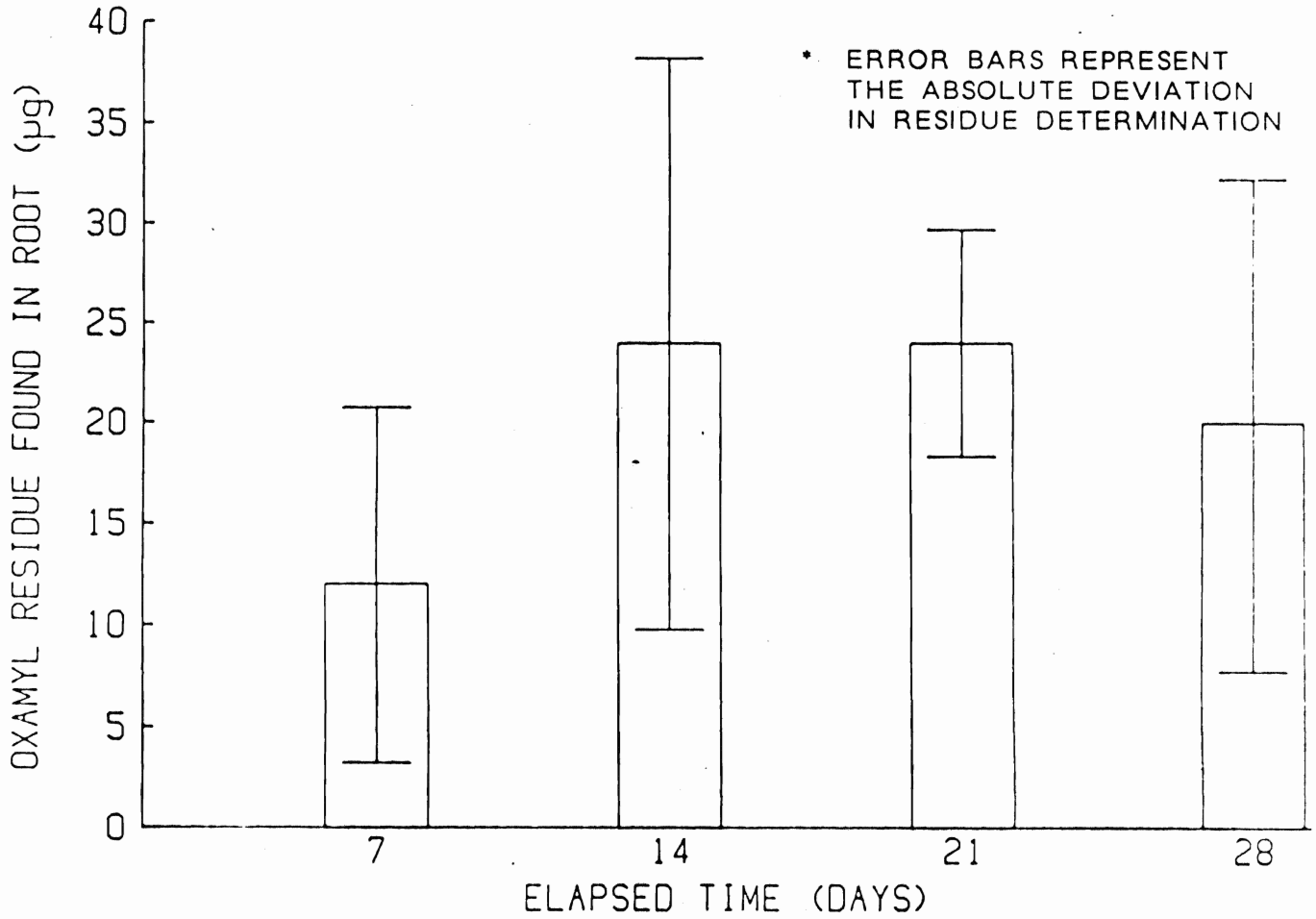

Figure RD - 5: Histogram Depiction of the Amount of Oxamyl Found in the Seedling Root Versus Time

analyses.

While neither oxime nor DMCF was detected in any of the root samples, this was not altogether unexpected because their proportions relative to oxamyl in previous analyses of seed and soil were quite small. Perhaps a more sensitive method of instrumental analysis would have yielded positive results at lower levels. Although the errors in the the mean oxamyl measurements are large, the results are important in confirming the presence of oxamyl in the root. This suggests the uptake of the chemical by the root either directly from the seed during growth, or through uptake from the surrounding soil.

CHAPTER IV - CONCLUSIONS

A simple method for coating oxamyl onto dry corn seeds from an aqueous oxamyl (Vydate L) solution was developed. The method entailed soaking the seeds in the treatment solution, then decanting off the excess liquid. Subsequent centrifugation of the damp seeds, followed by drying under a stream of air resulted in dry, treated seeds containing an even distribution of oxamyl. Initial fungus problems were overcome, with coated seeds remaining fungus-free even 12 months after treatment. However, phytotoxic effects upon germination and upon development of seedlings were observed when the oxamyl treatment solutions were 8.00 or 16.00 mg/mL.

Methods of extraction necessary for the determination of oxamyl, oxime, and DMCF in seed, seedling root, and soil were developed. While the Polytron and shaking method for the seeds provided consistent results, hydrating the seeds to soften the tissue and then extracting provided a dramatic increase in the extraction efficiency of oxamyl. Because of the nature of the treated corn seed samples, recoveries could not be determined. Tumbling the soils in methanol gave recoveries of 86 and 87% for oxamyl in nematode-free and nematode-infested soils respectively. Polytron extraction of the root yielded a mean recovery of 93% for oxamyl. Nuchar-Attaclay column cleanup of the methanol sample extracts afforded interference-free chromatography for

oxamyl, oxime, and DMCF. Excellent recoveries of 97, 99, and 89% for oxamyl, oxime, and DMCF respectively were obtained through this cleanup procedure.

In the degradation study, oxamyl was found to disappear from the planted corn seeds very rapidly, with only 11% of the original dose detected in the seeds after 7 days of growth. Interestingly, oxime was present initially on the seed; its disappearance was somewhat slower than that of oxamyl. Regression analysis of both data sets indicated exponential decline for both compounds. Oxamyl dissipated from the seeds into the soil. Its degradation with time was expected to give a concurrent rise in the oxime levels. While a slight increase was observed, it appeared that oxime, produced by the degradation of oxamyl in the soil, further degraded at a rate similar to that of oxamyl. Analysis of root samples confirmed the presence of oxamyl in the root during seedling growth. This would suggest uptake of the nematicide by the root either directly from the seed or from the soil during growth. Since root-lesion nematodes were the target of the oxamyl in this project, this finding may be important in establishing how the nematicide actually rebuffs nematode attack, whether by its presence in the root, or in the surrounding soil.

Work in extension of this project could take a number of forms. From a physical chemistry point of view, much more rigorous experimentation could be undertaken to quantitatively establish the kinetics of oxamyl degradation, not only to its

oxime and DMCF, but also to other postulated breakdown products such as methyl isocyanate and methyl isothiocyanate. In terms of practical applications of pesticide-treated seeds for commercial operations, it would be important to obtain biological efficacy data to compliment the chemical data produced here. If nematode studies were to show clearly protection of early root systems from nematode attack owing to the nematicide treated onto seeds, then this seed treatment approach would be a very viable alternative to present methods of pesticide application. It is a safer, less expensive and environmentally acceptable method of application, and the benefit to the agriculture industry is accepted to be substantial.

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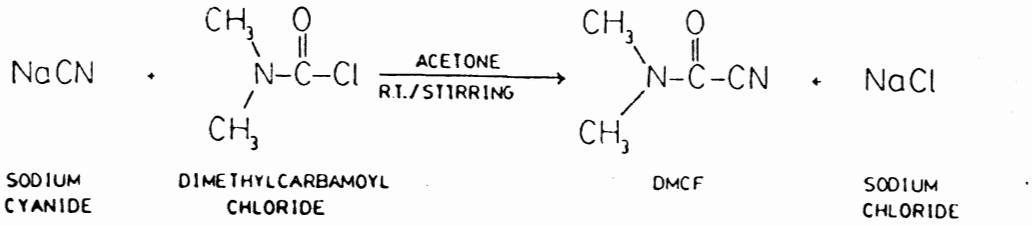
APPENDIX I

Synthesis of DMCF (N,N-dimethyl-1-cyanoformamide)[23]

DMCF was synthesized using the following reaction scheme:

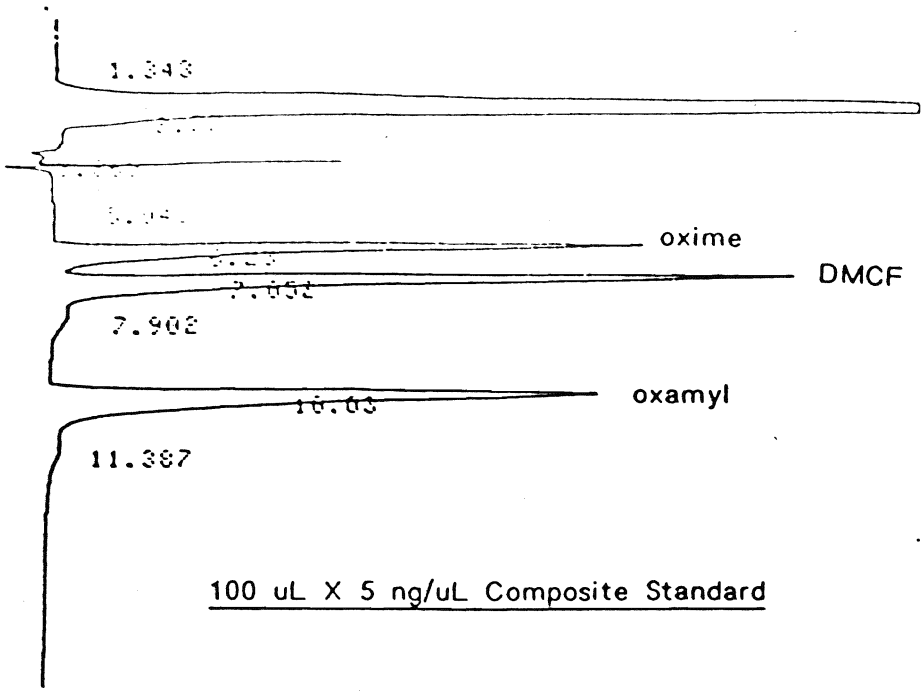


The steps in its preparation were as follows:

1. Approximately 250 mL acetone was distilled, with the middle 200 mL fraction collected, and stored tightly stoppered until further use.
2. Approximately 20 g of NaCN was ground to a fine powder using a mortar and pestal, then oven-dried at 103°C for 2 hours and finally cooled in a desiccator.
3. Dimethylcarbamoyl chloride (21.9 g, 0.2 mole) was added to 10 g of NaCN (0.2 mole) in 200 mL of acetone in a 500 mL flat-bottom and spherical glass flask; the mixture was tightly capped and stirred on a magnetic stirrer for 48 hours.
4. The mixture was vacuum-filtered through a Whatman #1 filter paper into a 500 mL round-bottom flask.

5. The filtrate was rotary-evaporated in a water-bath held at 60°C until no acetone remained.
6. One hundred millilitres of distilled water were added to the flask and the mixture stirred for 15 minutes to hydrolyze the unreacted dimethylcarbamoyl chloride.
7. The solution was gravity-filtered through Whatman #1 filter paper into a 250 mL glass beaker.
8. The dimethylcyanoformamide (DMCF) was extracted from the aqueous solution using 5 x 50 mL portions of anhydrous diethyl ether, each time stirring the mixture in an ice bath on a magnetic stirrer for 30 minutes, transferring the solution to a 250 mL glass separatory funnel, allowing the phases to separate, and collecting the upper ether layer in a 400 mL glass beaker.
9. Five grams of anhydrous sodium sulfate were added to the combined ether extracts to absorb any remaining water.
10. The ether extract was gravity-filtered through a Whatman #1 filter paper into a 250 mL glass round-bottom flask.
11. The filtrate was rotary-evaporated in a water-bath held at 50°C until the ether had evaporated, yielding DMCF as a clear, pale yellow liquid.
12. The DMCF was transferred to a 20 mL glass scintillation vial, capped tightly, and stored in a freezer at -4°C until required.

APPENDIX II



Mobile phase:15% acetonitrile/85% water

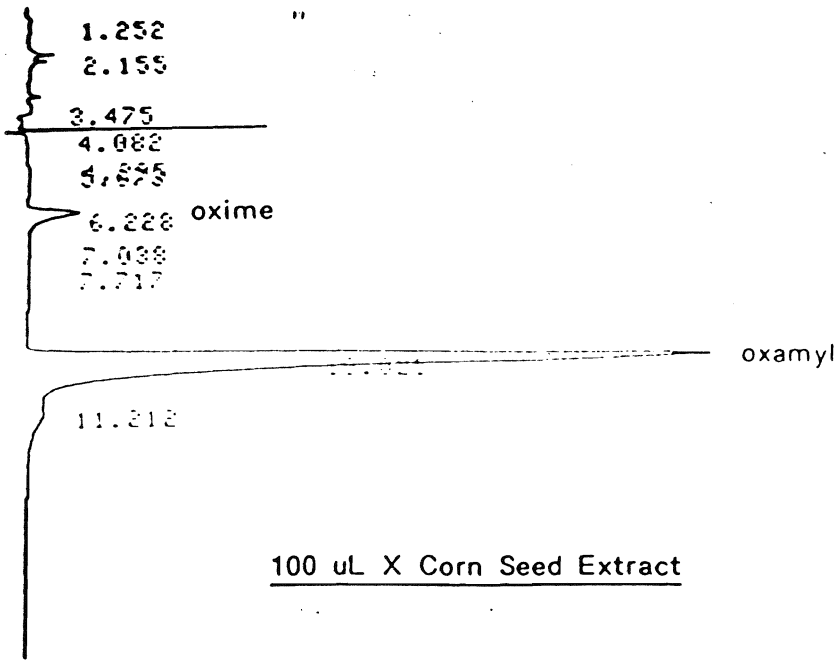
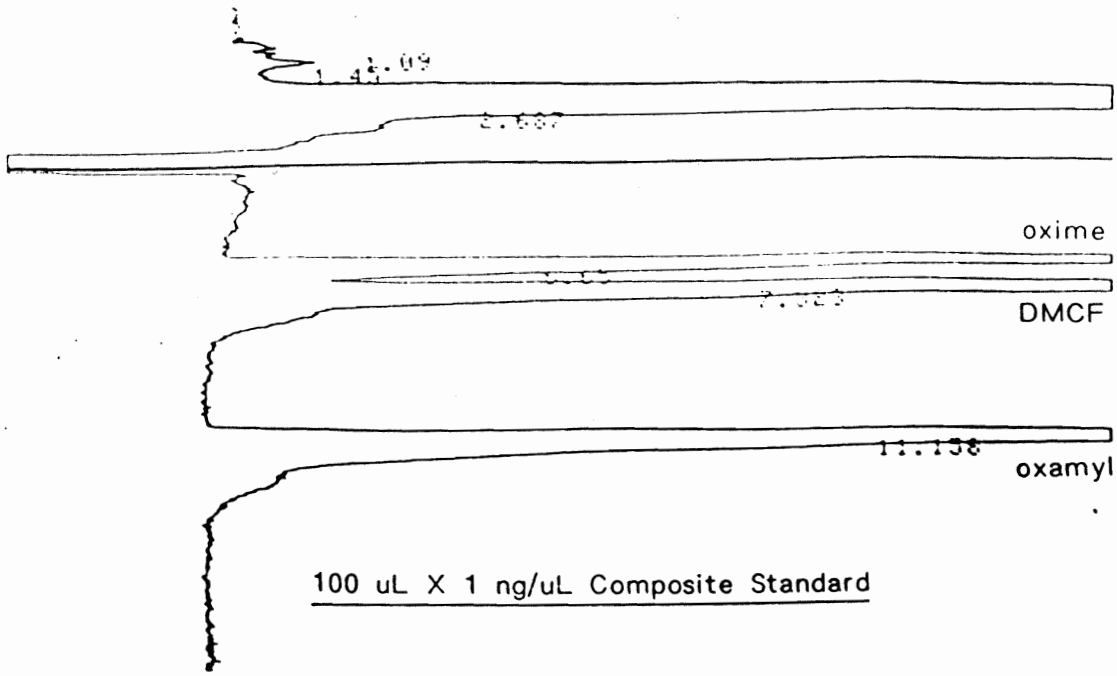


Figure AII-1: Chromatogram of a Typical Corn Seed Extract (accompanied by its corresponding standard chromatogram)



Mobile phase: 15% acetonitrile/85% water

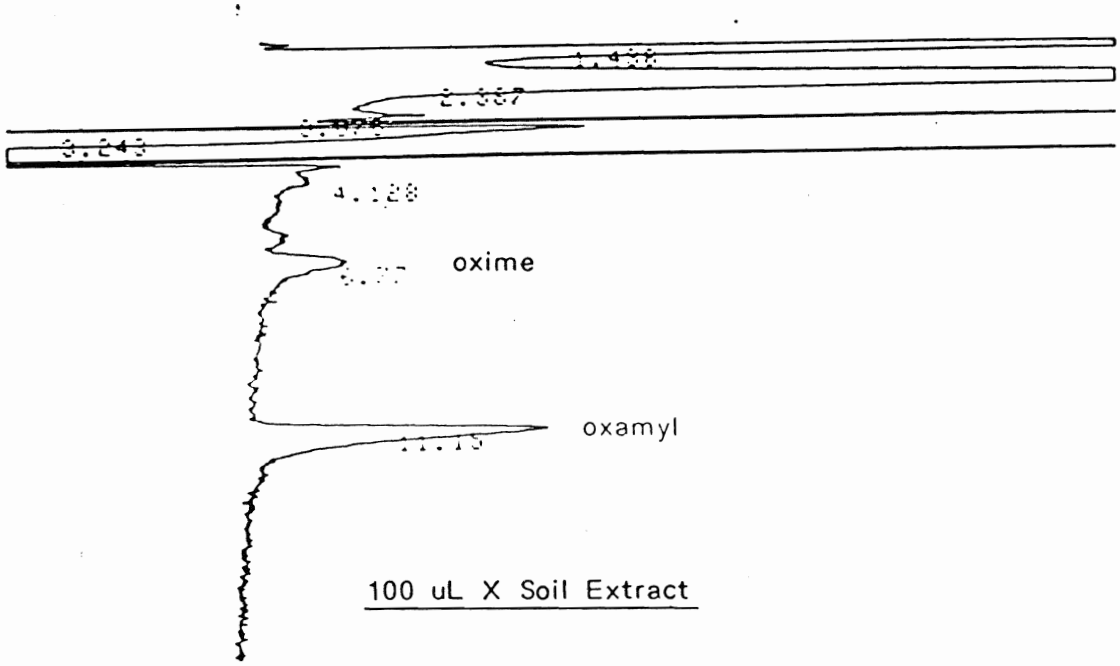


Figure AII-2: Chromatogram of a Typical Soil Extract (accompanied by its corresponding standard chromatogram)

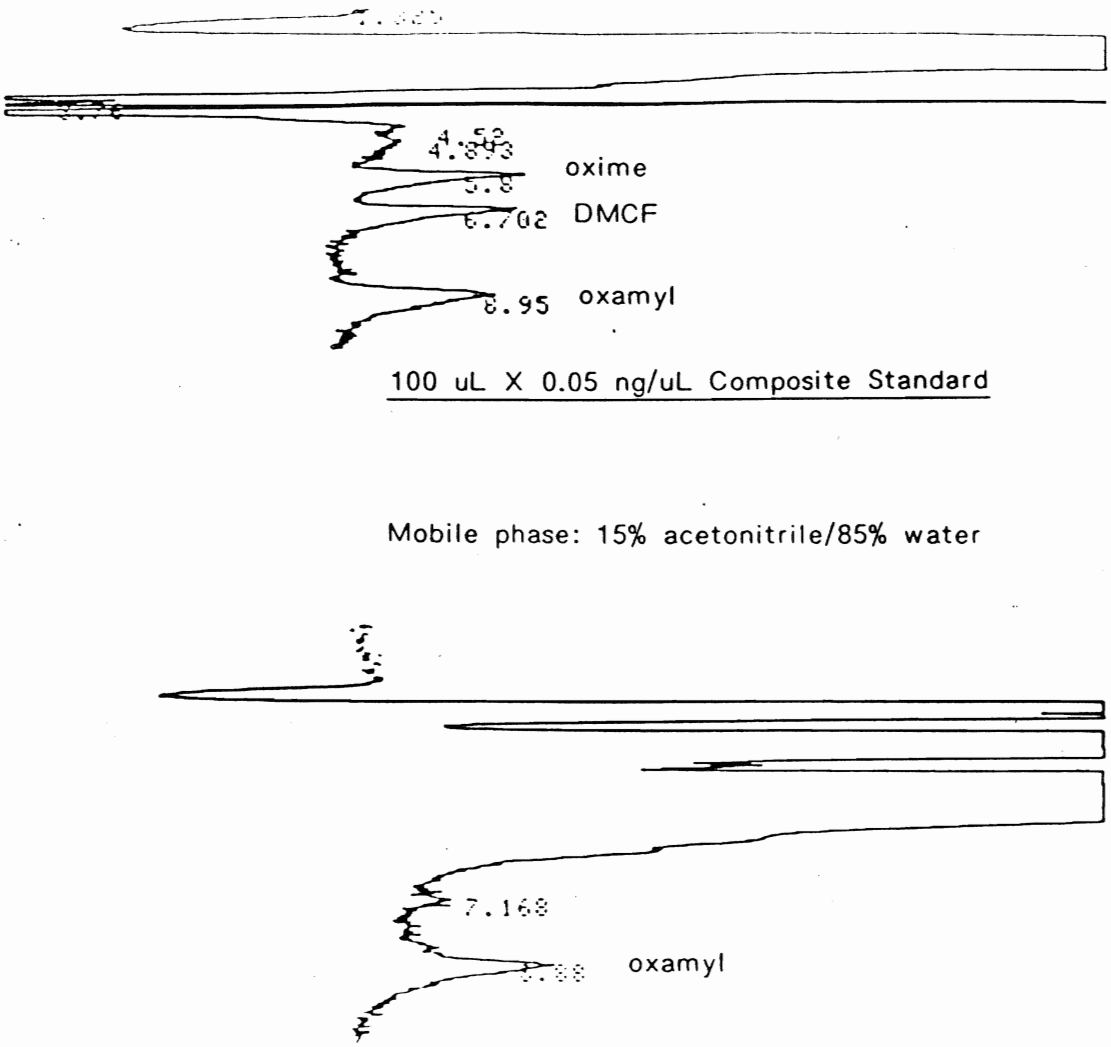
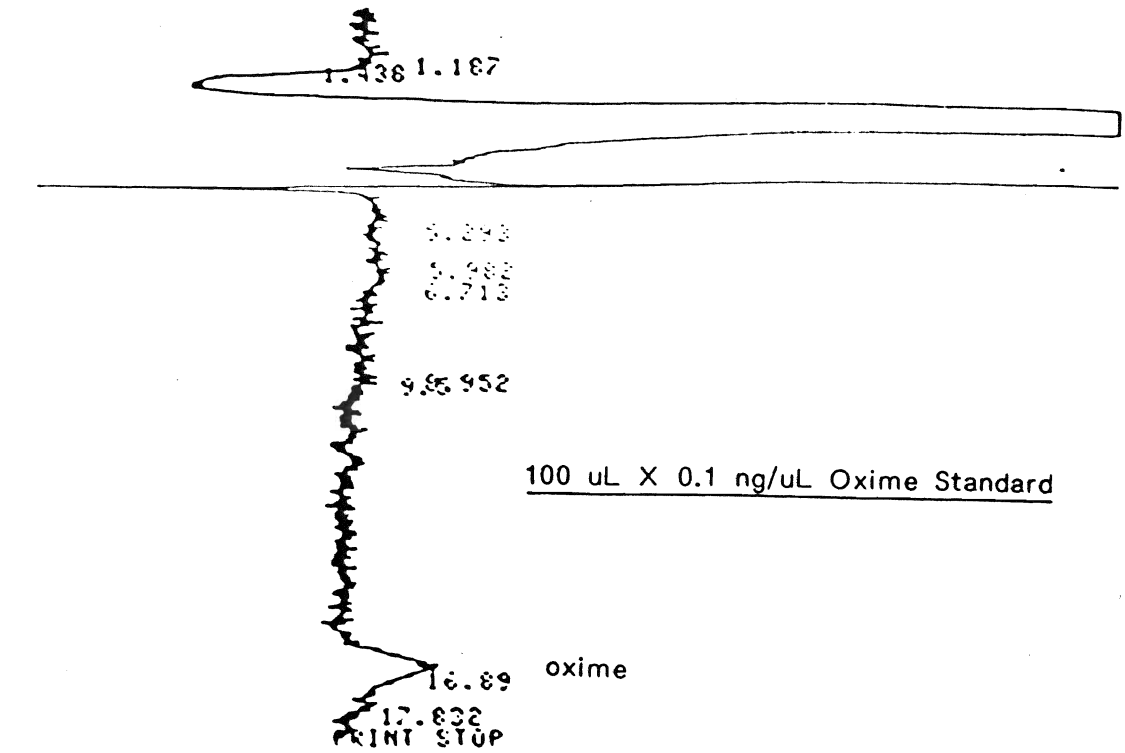


Figure AII-3: Chromatogram of a Typical Corn Root Extract
(accompanied by its corresponding standard chromatogram)



Mobile phase: 4% acetonitrile/96% water

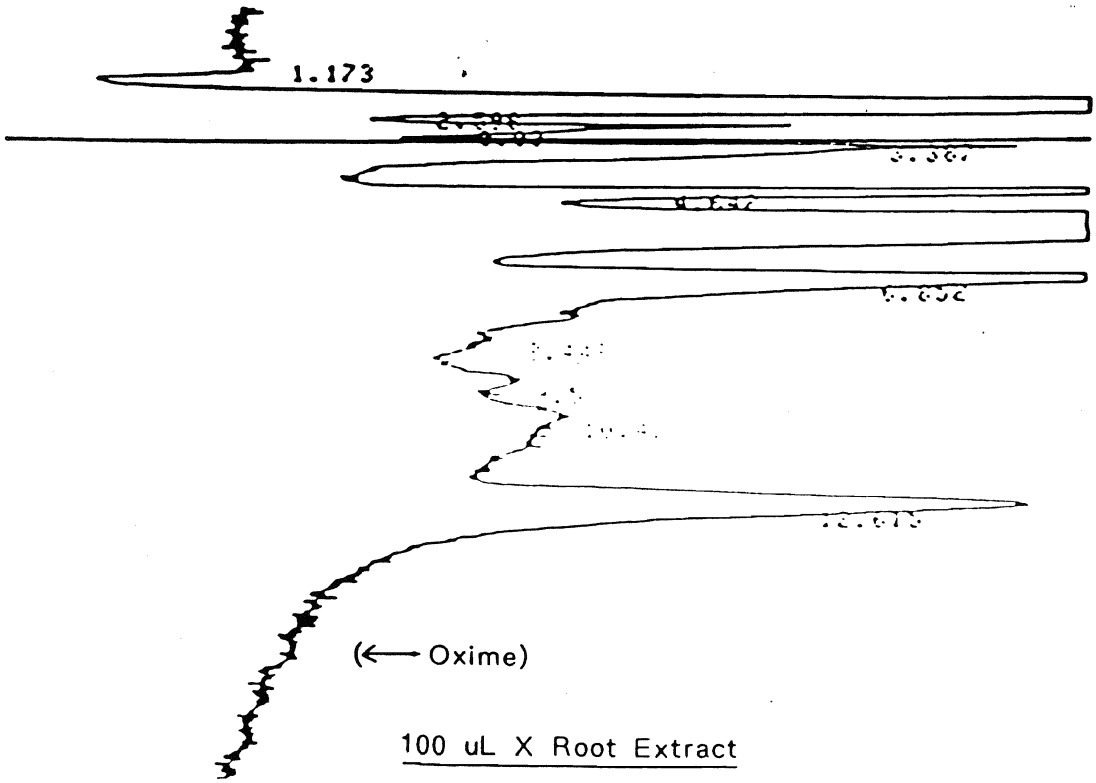


Figure AII-4: Chromatogram of a Typical Corn Root Extract (accompanied by its corresponding standard chromatogram)