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ABSTRACT

Soybean (Glycine max (L.) Merr. cv. Maple presto) seedlings, a green algae (Chlamydomonas segnis Ettl), a cyanobacterium (Anabaena spp.), and two nitrogen-fixing bacteria (Klebsiella pneumoniae strain M5A1 and Rhizobium japonicum strain 61A76) were separately exposed to concentrations of the s-triazine herbicide, prometryne, from 0.25 - 15.0 μg mL^{-1}. Growth parameters observed included fresh and dry weights (soybean), total protein content (soybean), cell number (C. segnis Ettl), heterocyst frequency (Anabaena spp.), and generation time (K. pneumoniae and R. japonicum).

Decreases in fresh and dry weights were apparent in soybean seedlings treated with the herbicide (2.5 - 15.0 μg mL^{-1} prometryne). Total protein content, as measured by the Hartree protein assay and the Kjeldahl nitrogen assay, was variable in all of the treated seedlings. Treated soybean seedlings exhibited poor leaf development and chlorosis.

A decrease in the population of C. segnis Ettl and heterocyst frequency in Anabaena spp. were noted after exposure to the herbicide (0.25 - 12.0 μg mL^{-1}). Full recovery was obtained in cleared cultures of C. segnis Ettl plated on pesticide-free nutrient agar. Similar recovery was not obtained in washed Anabaena spp. cells.

K. pneumoniae was relatively insensitive to the herbicide whereas the generation time of R. japonicum, when exposed to 6.8 μg mL^{-1} prometryne, was increased 3-fold as compared with untreated cultures (26.25 h vs. 9.5 h).

The relationship between nutritional nitrogen (nitrate, ammonium, urea) and changes in rhizosphere pH was established using axenic cultures of seedlings grown in nutrient agar. A nutrient-related rhizosphere pH was obtained which was modulated by the source of nitrogen in the medium. After 3 weeks of growth, a pH of 8.5 - 9.5 was generated by wheat seedlings fertilized with Hoagland's solution, No. 2. A comparable degree of alkalization was observed in nitrate-fertilized plants. A rhizosphere pH of less than 4.0 was obtained in plants fertilized with ammonium salts while no significant change was detected in wheat seedlings provided with urea as the sole source of nitrogen. These results are consistent with the concept of cation-anion uptake balance, maintenance of electroneutrality across the root surface, uptake by cotransporters, and chemiosmotic principles.

The remobilization of soil-bound ^{14}C-prometryne residues was affected by soil pH, fertilizer treatments (with or without plants), and the crop species, wheat (Triticum aestivum (L.) Merr. cv. Marquis) and soybean. Comparatively large changes in soil pH from pH 5.4 to pH 4.0 and pH 8.0 resulted in a 24 - 25% release of tightly bound radioactivity, respectively, while smaller changes from pH 5.4 to pH 4.9 released only 16% of the soil-bound residues. In addition, changes in pH resulted in differences in the extractability of prometryne and its hydroxy derivative, hydroxyprometryne, with more prometryne recovered following large changes in pH from pH 5.4.

Fertilizer treatments, at pH 5.4, also led to the remobilization of the soil-bound ^{14}C-prometryne. Use of ionic nitrogen sources (NO_3^- and NH_4^+) permitted the release of 4.0 - 16.7% of the bound residues. Negligible amounts (0.4%) of the extractable radioactivity was found following treatments with urea. These differences, however, were not apparent in the presence of rooting systems of wheat plants. Wheat plants grown in each of the fertilizer treatments resulted in approximately 15% remobilization of the total bound radioactivity.

Remobilization was plant specific, particularly in the rhizoplane. Soybean roots elicited a greater release of bound radioactivity (36.6%) in the rhizoplane than wheat plants (10.0%). However, the percent uptake of the remobilized derivatives was similar in both plant species (1% of the total bound radioactivity). Transport and metabolism of these residues were also plant specific.

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I. INTRODUCTION

A. REVIEW OF BACKGROUND LITERATURE

There is an increasing pressure on the production of food and other renewable resources to support the growth of the human population as it continues its upward spiral. Production losses to weeds and other pests may represent a significant proportion of a harvested crop (Table 1), ranging from 15 to 55%. Attempts have focused on minimizing such losses and increasing production. Thus, the use of fertilizers, pesticides, and other agrichemicals has become an integral part of western agriculture. These chemicals have allowed for increases in production of food and other resources on less land with lower labour and economic costs (Sanders 1981).

The use of xenobiotic pesticides to control pests has become very attractive to farmers. Pesticide usage has greatly increased within the past decade with herbicide use showing the most dramatic increase (Fig. 1). In the United States, in 1966, 227 million pounds (103×10^6 kg) of herbicidal active ingredients were applied and this increased to 625 million pounds (283×10^6 kg) in 1981 (Hileman 1982; Sanders 1981). Canadian sales of pesticides have increased from $70.9 million in 1978 to $212.6 million in 1981 (Anonymous 1983, 1980). Agricultural use of pesticides has increased from 72.3% of total sales in 1978 to 81.3% in 1981 (Anonymous 1983, 1980).

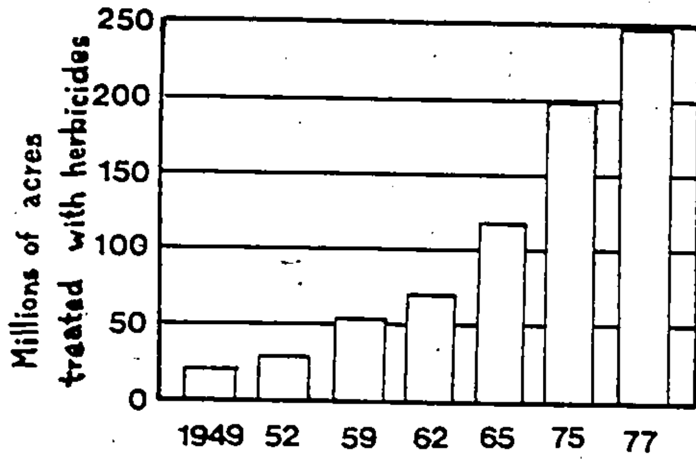
Table 1

Percentage of Losses in Major Agricultural Crops
on a World Basis Due to Insects, Diseases, and Weeds
(McEwen and Stephenson 1979)

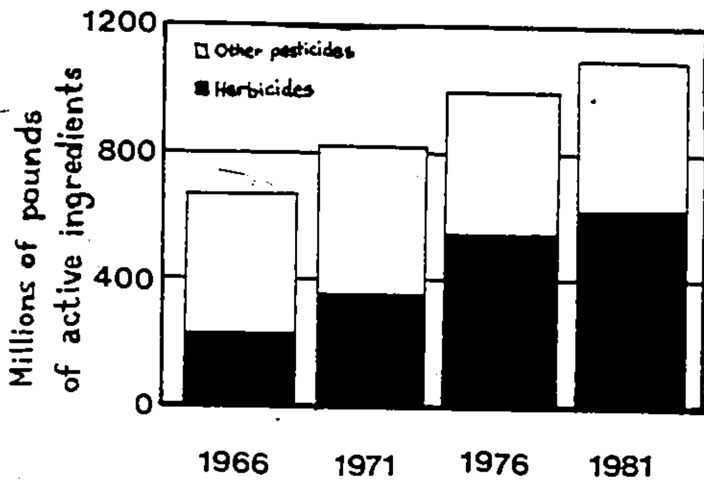
Crop	Insects	Diseases	Weeds	Total
Wheat	5	9	10	24
Oats	8	9	10	27
Barley	4	8	9	23
Rye	2	3	10	15
Rice	27	9	11	46
Millets and Sorghums	10	11	18	38
Maize (corn)	12	9	13	35
Potatoes	5	22	4	32
Sugar Cane	20	19	16	55
Citrus Fruit	8	9	4	22
Grapes	3	23	10	37
Oil Crops	11	10	11	32
Vegetables	9	10	9	28

Figure 1

Herbicide Usage in the U.S. (from Hileman 1982).



Millions of acres treated with herbicides
250
200
150
100
50
0
1949
52
59
62
65
75
77



Millions of pounds of active ingredients
1200
800
400
0
Other pesticides
Herbicides
1966
1971
1976
1981

Although the use of chemicals for controlling pests may be economically beneficial in agricultural systems and that they are easily accessible, serious ecological concerns must be addressed regarding the continual application of such xenobiotics in these systems. The application or entry of the pesticide into an ecological system exposes the compound to various biotic and abiotic interactions, particularly in the soil (Fig. 2).

Within the soil environment, pesticide and herbicide residues can be leached from the soil, sorbed by plants, consumed by animals, volatilized into the atmosphere, chemically or microbially degraded, or interact with the clay minerals and/or organic matter (Pramer and Bartha 1980). Thus, the soil plays an integral role in the cycling of pesticides and herbicides in the environment. These dynamic processes are also influenced by the chemical properties of the compound, ie. water/lipid solubility, polarity, and chemical reactivity (McEwen and Stephenson 1979).

From an ecological viewpoint, an important consideration is the persistence of the chemicals in the soil. Persistence is defined as "the residence time of a chemical species in a specifically defined compartment of the environment" (Greenhalgh 1980). In the past, pesticide analysis has focused on those residues which were easily extracted from the substrate by various solvents and then depletion curves were determined. When the fate of a pesticide was assessed, terms like "disappearance" and "volatilization" were used to describe unaccountable

Figure 2

Factors Influencing the Behavior of Pesticides in Soils

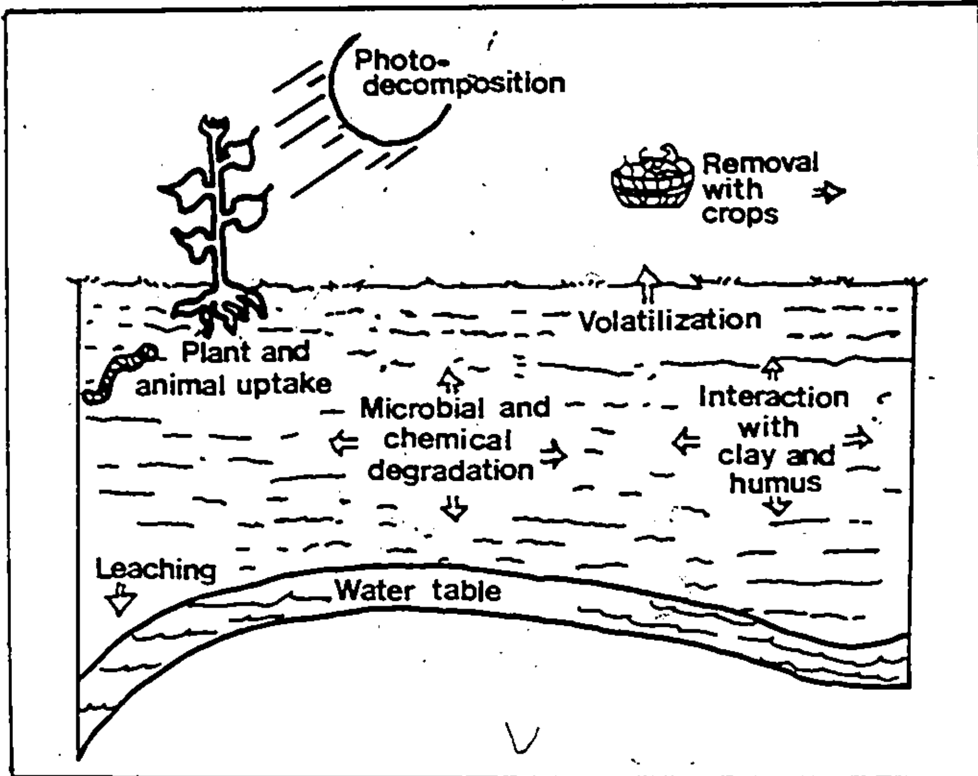


Photo-
decomposition
Removal
with
crops
Volatilization
Plant and
animal uptake
Microbial and
chemical
degradation
Interaction
with
clay and
humus
Leaching
Water table

residues. However, with the advent of the radiolabeled pesticides it has been shown that a significant proportion of these chemicals or their derivatives had in fact not "disappeared" or "volatilized", but rather remained unextractable in the substrate, even following exhaustive solvent extraction (Katan et al. 1976). These residues have been referred to as "bound" or "nonextractable" residues and have been quantified by combustion techniques followed by liquid scintillation counting (Khan and Hamilton 1980). Recently, Food and Agricultural Organization (FAO) and the International Atomic Energy Agency (IAEA), have defined nonextractable or bound residues as "chemical species originating from pesticide usage that cannot be extracted by methods commonly used in residue analysis and metabolism studies" (Anonymous 1982).

Concern for the environmental impact of these residues was the focus of a conference by the American Chemical Society (Kaufman et al. 1976). In addition, several workers have been able to demonstrate this binding phenomena for a variety of pesticides in soils. Khan (1982b) summarized most of the recent work on soil-bound pesticide residues and reported that the levels of binding of the tested compounds ranged from 7 to 90% of the initially applied radioactivity. In addition, DeAndrea et al. (1982) demonstrated binding levels of 40% for ^{14}C-parathion while 32.7 to 88.3% of the applied ^{14}C was bound in a variety of Egyptian soils incubated with ^{3}H-trifluralin (Mostafa et al. 1982). Pentachlorophenol was found to bind in flooded soils at levels of 25.6 to 28.6% (Weiss et al. 1982b). After 80 days of incubation, binding of mepronil in volcanic

ash soil and alluvial soil was 60.9 and 59.7% of the applied radioactivity, respectively (Yumita and Yamamoto 1980). The binding of naproanilide was found to be higher under oxidative than reductive conditions (Oyamada et al. 1980).

The formation of bound residues is not exclusive to the parent pesticide itself but have been found to occur for derivatives of the pesticide. Katan and Lichtenstein (1977) found that aminoparathion, a microbial metabolite of parathion, was significantly bound to sterile and nonsterile soils. In fact, they also observed that the amino derivatives of parathion, paraoxon and nitrophenol, were bound to a greater extent than the parent compound. Derivatives of chlornitrofen (Yamada 1983), monolinuron (Haque et al. 1982), and prometryne (Khan 1982a; Khan and Hamilton 1980) have also been reported to form bound residues in soils.

The extent of bound residues formation is also dependent on the characteristics of the soils. Fuhremann and Lichtenstein (1980) reported that the binding of six insecticides was less apparent in sandy soil than in loam soil. Bound paraquat levels have been found to be higher in peat than in other soil types (Riley et al. 1976). Stevenson (1972) demonstrated that atrazine adsorption increased as organic matter contents increased. Fuhr and Mittelstaedt (1980) postulated that the organic matter content of a soil is the determining factor for the binding of the pesticide in the soil indicating a direct relationship between pesticide binding and the soil's organic matter content. Khan

(1982a) found that soil-bound ^{14}C-prometryne residues were associated with the humic acid, fulvic acid, and humin fractions of a soil. Similar associations was found for ditalimfos (Meikle et al. 1976) and dinitroaniline herbicides (Helling and Krivonak 1978a).

The importance of such associations in the soil was expressed as concern for the long-term accumulation of these residues in soils and their possible environmental interactions (Anonymous 1982). Because these soil-bound residues may persist for long periods in the soil, they represent potential sinks which may be subject to biotic and abiotic interactions. For example, humic substances have been found to alter the hydrolysis of several organic pollutants (Perdue and Wolfe 1982), and thus affect degradation rates. Binding of pesticides in organic soils may be responsible for the alteration of degradation rates. In addition, humic substances are found in natural surface waters and in the interstitial waters of sediments and soils and binding of pesticides to these substances may provide another means by which pesticides may enter into aquatic systems. Humic complexation was demonstrated with polycyclic aromatic hydrocarbons and chlorinated hydrocarbons (Carlberg and Martinsen 1982). Studies have also shown that DDT is able to bind to dissolved humic materials and that this is dependent on the sources of the humic substances (ie. commercial preparation vs. soil extraction), pH, calcium concentration, ionic strength, and the concentration of the humic material (Carter and Suffet 1982).

The accumulation of soil-bound residues raises concern that these pesticide sinks are potentially available to plants and animals. Earthworms were able to extract 2.7% of the bound methyl ^{14}C-parathion residues (Fuhremann and Lichtenstein 1978) and 0.72% of bound ^{14}C-hydroxymonolinuron-β-D-glucoside residues (Haque et al. 1982). These residues are also subject to microbial activity. Khan and Ivarson (1981) showed that indigenous bacteria were able to release 27% of the total bound ^{14}C-prometryne residues and 23.5 to 27.1% by physiologically different bacterial groups (Khan and Ivarson 1982).

Plant uptake of these bound residues has been investigated by several workers. Oat plants were able to extract 5.1% of the soil-bound methyl ^{14}C-parathion residues after two weeks of growth with 46 to 62% of the radioactivity present in the shoots (Fuhremann and Lichtenstein (1978). When oat plants were grown for three weeks in soils containing bound prometryne, they were able to extract and absorb only 0.92% of the bound residues (Khan 1980). Soybeans grown for ten weeks with soil-bound dinitroaniline herbicides removed 0.46 to 1.06% (Helling and Krivonak 1978b). When grown in soils containing bound ^{3}H-trifluralin, soybeans had incorporated 0.20 to 0.87 ppm into the plant tissues after four weeks (Mostafa _et_ _al_. 1982). Fuhr and Mittelstaedt (1980) found that maize plants, after 29 days, had extracted 1.7% of the soil-bound methabenzthiazuron residues in a sandy soil. Roberts and Standen (1981) investigated the uptake of bound ^{14}C-cypermethrin by wheat plants. They found that after six weeks of growth, wheat plants were able to

extract and absorb 0.14 to 0.58% of the bound radioactivity whereas ryegrass extracted 0.23% of bound ^{14}C-hydroxymonolinuron-β-D-glucoside from soils after 21 days (Haque et al. 1982).

Although bound residues have been demonstrated to be bioavailable, very little is known of the means by which soil-bound residues become remobilized. Lichtenstein (1980) states that "combustion or strong hydrolysis of extracted soil can release these bound ^{14}C residues". Based on this observation and that alkali soil extractions are able to disrupt soil structures (Khan 1982a), we postulate that changes in soil pH may be responsible for the release of these soil-bound residues. Recently, it has been demonstrated that the plant roots are able elicit localized changes in pH depending on the type of nitrogen fertilizer available to the root (Weinberger and Yee 1984). Therefore, it was presently hypothesized that these localized pH changes mediated by plant roots may cause the release of the soil-bound pesticide residues.

Attendant on their release, soil-bound residues may be remobilized into biotic organisms, metabolized, and even become re-bound in biotic tissues, again. The metabolic pathways of many pesticides in biotic organisms have been extensively studied with emphasis on the free, conjugated, and bound species. Free metabolites are those derived from the parent pesticide which has not reacted with the natural components of the organism, while conjugated metabolites are derivatives which have reacted chemically with a natural component of the plant to form a new compound (Kaufman 1976; Khan 1980). For apolar pesticides, free

metabolites can be extracted from the organic substrate and will partition from water into organic solvents like chloroform or ether. Conjugation products, however, can be extracted with polar solvents but are unable to partition into the nonpolar solvents. Bound species are conjugation products which are unable to be removed from the substrates, even after thorough solvent extraction (Dorough 1976). Several workers have acknowledged the presence of the three metabolic species for a variety of pesticides and crop species. Pesticides found to be metabolized into free, conjugated, and bound species include ^{14}C-carbofuran and ^{14}C-phorate in potatoes, carrots, and radishes (Sonobe et al. 1983, 1982), ^{3}H-triforine in barley (Rouchaud et al. 1979, 1978), and ^{14}C-pentachlorophenol in rice plants (Weiss et al. 1982a).

The importance of pesticide residues in crop species is that the continual accumulation of these residues in tissues may result in their remobilization into other biotic components or trophic levels (McEwen and Stephenson 1979). In some experiments, it has been demonstrated that plant bound residues are not biologically available to animals since most of the radioactivity was excreted in the feces (Dorough 1976; Marshall and Dorough 1977; Paulson et al. 1975; Sutherland 1976). However, chicken liver homogenate was able to release significant quantities of the atrazine residues bound in corn plants, while bovine rumen liquor was unable to do so (Khan and Akhtar 1983). In addition, conjugated residues may possess some potential effects on the biota. Although amino acid conjugates of 2,4-D have been shown to have no mutagenic activity in Salmonella typhimurium strains (Rashid and Mumma

1983), they have been demonstrated to have some biological activity in Avena sativa coleoptiles (Feung et al. 1974). Soybean cotyledon tissues were rapidly able to conjugate 2,4-D to amino acids and these conjugates stimulated elongation in the coleoptiles. In some cases, growth stimulation of the conjugates exceeded that of 2,4-D, itself.

B. PURPOSE

The purpose of the research which formed the basis for this thesis was to investigate the release of bound residues from soil treated with prometryne and follow the subsequent uptake and metabolism of the released residues by two crop species, wheat and soybean. Primary focus was on these plants roots' abilities to alter the soil pH as a possible mechanism for the release of these soil-bound residues. In addition, with the release of these residues into the soil solution, concern was raised as to the potential effect they may have on soil microflora, particularly those which are associated with the roots. Consequently, these concerns were addressed.

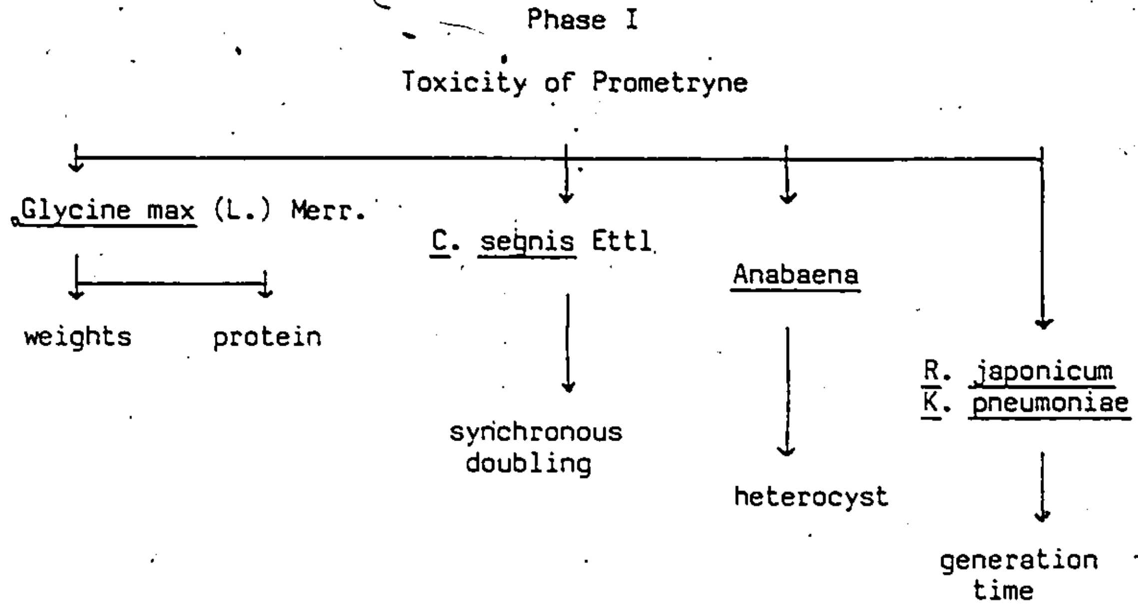
C. EXPERIMENTAL RATIONALE

The pesticide chosen for this study was the s-triazine herbicide, prometryne, primarily because experimental procedures for its analyses in soils and plant tissues is well documented. Also, prometryne has been shown to bind in significant levels in an organic soil (Khan and Hamilton 1980). Prometryne is widely used in eastern Canada for vegetable crops grown in organic soils (Khan, personal communication).

The presence of prometryne in the soil in a bound state raises serious questions about its possible release and its influence on various flora and microflora. Bioassays were required to determine the potential biocidal activity of prometryne, not only on susceptible crop species but also on indigenous soil microflora. To this end, soybeans were chosen as a test crop because of its potential role as a rotating crop (Robert and Schmidt 1983) while the algal and bacterial species were chosen for their role in the carbon and nitrogen cycles in the soil system (Haahtela et al. 1983; Kleeberger et al. 1983).

Chlamydomonas segnis Ettl was chosen as a representative green algae found in soils and important in carbon cycling. A nitrogen-fixing cyanophyte, Anabaena spp. was tested because of its relative abundance in soils and role in nitrogen cycling. Two agriculturally important root-associated nitrogen fixing bacteria namely, Rhizobium japonicum and Klebsiella pneumoniae, were used for assaying prometryne effects on growth.

There were two major approaches used in this study (Fig. 3). The first was to assay the effects of prometryne on the growth of a sensitive crop species, soybean, and of the soil and aquatic microphytes. This enabled an assessment to be made of the potential effects that prometryne may have and to develop a framework for future work to determine the biological activity of soil-bound prometryne residues.



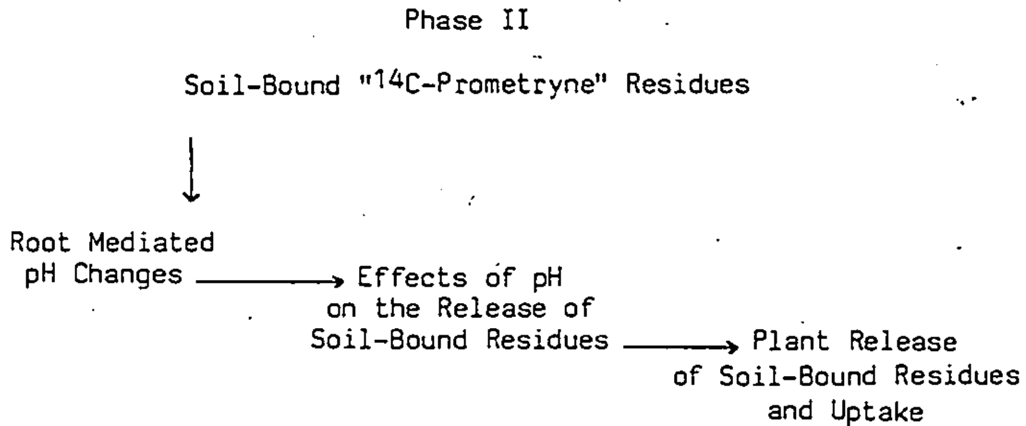


Fig. 3. Stages of Research

The second approach was to consider the role of root-mediated soil pH changes in the release of soil-bound ^{14}C-prometryne as a possible mechanism for the release of these residues. It was necessary to demonstrate that plant roots are able to elicit localized pH changes and that such changes are responsible for the release of the pesticide residues. Wheat and soybeans were chosen as the test crop species because of their economic importance in agricultural systems and because past research (Smiley 1974) had found that these two species are able to substantially modify the pH of the root environment.

The final steps were to show that the pH changes mediated by the plant roots could cause differential release of the soil-bound residues and to look at differences in uptake and metabolism of the residues.

II. MATERIALS AND METHODS

A. GENERAL METHODOLOGY

1. Chemicals

Technical grade (98.5%) prometryne (2-(methylthio)-4,6-bis(isopropropylamino)-s-triazine) and its derivaties ie. hydroxyprometryne (2-(hydroxy)-4,6-bis(isopropylamino)-s-triazine), deisopropylprometryne (2-(methylthio)-4-(amino)-6-(isopropylamino)-s-triazine), prometone (2-(methoxy)-4,6-bis(isopropylamino)-s-triazine), and the diamino derivative (2-(methylthio)-4,6-(diamino)-s-triazine) were gifts from Ciba-Geigy Ltd., Cambridge, Ont. Uniformly ring-labeled ^{14}C-prometryne (specific activity, 5.7 μCi mg^{-1}) was similarly obtained and tested by the Environmental Chemistry Section, Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, Ont. and found to be 96% pure. All

other chemicals for the growth media and analyses are listed in Table 2 along with their suppliers. These were reagent grade chemicals and used as received. Glass distilled, pesticide grade solvents (acetone, chloroform, ethanol, ether, hexane, and methanol), used for the extractions and cleanup procedures, were purchased from Caledon Laboratories Ltd., Georgetown, Ont. Deionized distilled water, used for the preparation of all solutions, was prepared by passing tap water through an Illinois Water Treatment Universal Ionxchanger model 1 (Sargent-Welch Scientific Co., Toronto, Ont.) before distilling in a glass enclosed system (O.H. Johns Scientific Ltd., Toronto, Ont.). The organic soil (Table 3) used in these experiments was the same as described by Khan and Hamilton (1980).

2. Equipment

The equipment used in the resolution of the project is listed in Table 4 along with either the manufacturer's or supplier's name. Three different gas-liquid chromatographs were used due to differences in availability and operational status of these machines at the various stages of this study. However, to maintain reproductibility, the same machine was used in each trial of the experiment.

Before use, all glassware was soaked overnight in a chromic acid bath followed by rinsing ten times with distilled water. The glassware was then rinsed with methanol or acetone and dried in an oven (90°C). Glassware that required sterilization was autoclaved for 15 min at 115°C and 1.4 kg cm^{-2}.

Table 2

List of Chemicals and Suppliers
(All reagent grade)

Chemical	Formula	Supplier
Acetic Acid	CH_3COOH	Canlab Ltd.[1]
Aluminium Oxide	Al_2O_3	Canlab Ltd.
Aluminum Potassium Sulphate	$AlK(SO_4)_2.12H_2O$	Canlab Ltd.
Amberlite XAD-2		BDH Chemicals[2]
Ammonium Chloride	NH_4Cl	Sigma Chemical Co.[3]
Ammonium Molybdate	$(NH_4)_6Mo_7O_{24}.4H_2O$	Sigma Chemical Co.
Ammonium Phosphate, Monobasic	$NH_4H_2PO_4$	Merck and Co., Inc.[4]
Ammonium Sulphate	$(NH_4)_2SO_4$	Canlab Ltd.
Arabinose	$C_5H_{10}O_5$	Sigma Chemical Co.
Bacto-agar		BDH Chemicals
Boric Acid	H_3BO_3	Sigma Chemical Co.
Bovine Serum Albumin (BSA)		Sigma Chemical Co.
Bromocresol Green		Fisher Scientific Ltd.[5]
Cadimum Nitrate	$Cd(NO_3)_2.4H_2O$	Canlab Ltd.
Calcium Chloride, Dihydrate	$CaCl_2.2H_2O$	Sigma Chemical Co.
Calcium Nitrate, Tetrahydrate	$Ca(NO_3)_2.4H_2O$	Merck and Co., Inc.
Carbo-sorb		United Technologies, Ltd.[6]
Chlorophenol Red		Fisher Scientific Ltd.
Citric Acid	$C_6H_8O_7.H_2O$	Mallinckrodt Chemicals Work, Ltd.[7]
Cobalt Chloride	$CoCl_2.6H_2O$	Fisher Scientific Ltd.
Cobalt Nitrate	$Co(NO_3)_2.6H_2O$	Canlab Ltd.
Cupric Sulphate	$CuSO_4.5H_2O$	Canlab Ltd.
Diazald	$CH_3C_6H_4SO_2N(CH_3)NO$	Aldrich Chemical Co.[8]
EDTA-Tetrasodium Salt	$C_{10}H_{12}N_2Na_4O_8$	Canlab Ltd.
Ferric Chloride, Anhydrous	$FeCl_3$	Sigma Chemical Co.
Ferric Citrate	$FeC_6H_5O_7.5H_2O$	Canlab Ltd.
Ferric Tartrate	$Fe_2(C_4H_4O_6)_3.H_2O$	Sargent-Welch Ltd.[9]
Ferrous Sulphate	$FeSO_4.7H_2O$	Fisher Scientific Ltd.
Florisil F-100		Fisher Scientific Ltd.
Folin-Ciocalteau Reagent		Fisher Scientific Ltd.
HEPES Buffer	$C_8H_{18}N_2O_4S$	Sigma Chemical Co.
Hydrochloric Acid	HCl	Canlab Ltd.
Magnesium Sulphate	$MgSO_4.7H_2O$	Canlab Ltd.
Manganese Chloride	$MnCl_2.4H_2O$	Fisher Scientific Ltd.
Manganese Sulphate	$MnSO_4.4H_2O$	Fisher Scientific Ltd.
Mercuric Oxide	HgO	Canlab Ltd.
Metacresol Purple		Fisher Scientific Ltd.
Methylene Blue		Fisher Scientific Ltd.
Methyl Red		Fisher Scientific Ltd.
Nickel Ammonium Sulphate	$NiSO_4(NH_4)_2.SO_46H_2O$	Canlab Ltd.
Oxisorb- $^{14}CO_2$		New England Nuclear[10]
Permafluor V		United Technologies Ltd.
Phenolphthalein	$C_{20}H_{14}O_4$	Fisher Scientific Ltd.
Potassium Biphthalate	$KHC_8H_4O_4$	Fisher Scientific Ltd.
Potassium Bromide	KBr	Canlab Ltd.

Table 2 (Cont')

Chemical	Formula	Supplier
Potassium Hydroxide	KOH	Canlab Ltd.
Potassium Iodide	KI	Fisher Scientific Ltd.
Potassium Nitrate	KNO_3	Canlab Ltd.
Potassium Phosphate, Dibasic	K_2HPO_4	Sigma Chemical Co.
Potassium Phosphate, Monobasic	KH_2PO_4	Sigma Chemical Co.
Potassium Sodium Tartrate	$KNaC_4H_4O_6 \cdot 4H_2O$	Fisher Scientific Ltd.
Potassium Sulphate	K_2SO_4	Canlab Ltd.
Scintiverse I		Fisher Scientific Ltd.
Sodium Acetate	$C_2H_3NaO_2.3H_2O$	Fisher Scientific Ltd.
Sodium Borate (Borax)	$Na_2B_4O_7 \cdot 10H_2O$	Fisher Scientific Ltd.
Sodium Carbonate	Na_2CO_3	Fisher Scientific Ltd.
Sodium Hydroxide	$NaOH$	Canlab Ltd.
Sodium Phosphate, Dibasic	$Na_2HPO_4.7H_2O$	Sigma Chemical Co.
Sodium Phosphate, Monobasic	NaH_2PO_4	Sigma Chemical Co.
Sodium Silicate	$Na_2SiO_3.9H_2O$	Canlab Ltd.
Sodium Sulphate, Anhydrous	Na_2SO_4	Canlab Ltd.
Sodium Thiosulphate	$Na_2S_2O_3.5H_2O$	Fisher Scientific Ltd.
Sodium Tungstate	$Na_2WO_4.2H_2O$	Fisher Scientific Ltd.
Tris(Hydroxymethyl)Aminomethane	$C_4H_{11}NO_3$	Sigma Chemical Co.
Universal pH Indicator Solution		Fisher Scientific Ltd.
Urea	H_2NCONH_2	Canlab Ltd.
Vanadyl Sulphate	$VOSO_4 \cdot 2H_2O$	Fisher Scientific Ltd.
Yeast Extract		Sigma Chemical Co.
Zinc Sulphate	$ZnSO_4 \cdot 7H_2O$	Merck and Co., Inc.

[1] Canlab Ltd., Toronto, Ontario. Canada.
[2] BDH Chemicals, Toronto, Ontario, Canada.
[3] Sigma Chemical Co., St. Louis, Missouri, U.S.A.
[4] Merck and Co., Inc., Toronto, Ontario, Canada.
[5] Fisher Scientific Ltd., Don Mills, Ontario, Canada.
[6] United Technologies, Ltd., Mississauga, Ontario, Canada.
[7] Mallinckrodt Chemicals Work, Ltd., St. Louis, Missouri, U.S.A.
[8] Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.
[9] Sargent-Welch Ltd., Weston, Ontario, Canada.
[10] New England Nuclear, Boston, Massachussets, U.S.A.

Table 3

Physical and Chemical Properties of Organic Soil

Classification	Humic Mesisol
% Carbon	45.4%
% Nitrogen	2.4%
% Mineral Matter	15.1%
Bulk Density	0.34
pH	5.4
Cation Exchange Capacity	163 meq/100g

Table 4

Equipment List and Suppliers/Manufacturers

Equipment	Model	Supplier/Manufacturer
Gas-Liquid Chromatograph	Hewlett-Packard 5830A	Hewlett-Packard(Canada)Ltd[1]
	Pye Unicam Series 104, Model 64	Pye Unicam Ltd.[2]
	Varian Vista Series 6000	Varian Canada Inc.[3]
Controlled Environment Chambers	Dubnoff Metabolic Shaking Incubator	Precision Scientific Co.[4]
	Hotpack Programmed Refrigerated Incubator, Model 352620	Hotpack(Canada) Ltd.[5]
	New Brunswick G24 Environment Incubator Shaker	New Brunswick Science Co., Inc.[6]
	Sherer Controlled Environment Lab Model CEL 255-6	Sherer-Gillett Co.[7]
Balances	Mettler H54AR Analytical Balance	Fisher Scientifc Ltd.[8]
	Mettler P163N	Fisher Scientific Ltd.
Centrifuge	International Clinical Centrifuge Model CL No. 10228H	International Equipment Co.[9]
	Sorvall Superspeed RC2-B Automated Refrigerated Centrifuge	Ivan Sorvall Inc.[10]
pH Probes and Meter	Orion Research Combination pH Probe, Model 91-04	Canlab Ltd.[11]
	Orion Research Digital Analyzer Model 501	Canlab Ltd.
	Sargent-Welch Miniature Combination pH Electrode	Sargent-Welch Ltd.[12]
Mixers	Corning PC-351 Hot Plate Stirrer	Canlab Ltd.
	Corning PC-353 Stirrer	Canlab Ltd.
	Polytron Type PT-10-20-3500	Brinkman Instruments Ltd[13]
	Scientific Products Deluxe Miser Model 58220	Scientific Products Int.[14]
Miscellaneous	Amsco General Purpose Autoclave	Amsco Inc.[15]
	Bausch and Lomb Spectronic 21 Spectrophotometer	Fisher Scientific Ltd.
	Beckman Liquid Scintillation Counter Model LS-3133P	Beckman Instruments, Inc.[16]
	Environmental Air Control Inc. Horizontal Laminar Flowhood, Model TT-3048	Fisher Scientific Ltd.
	Heidolph Rotary Evaporator, Type 51111	Canlab Ltd.
	Improved Neubauer Haemocytometer	Canlab Ltd.
	Labconco Kjeldahl Digestion Rack	Canlab Ltd.

Table 4 (Cont'd)

Equipment	Model	Supplier/Manufacturer
Miscellaneous	Lindberg Tube Furnace (Sola Basic S/B)	Canlab Ltd.
	Oven	Fisher Scientific Ltd.
	Packard Sample Oxidizer, Model 306	United Technologies Ltd.[17]
	SMI Micro/pettor Model 1075I	Scientific Manufacturing Industries[18]
	Sybron Thermolyne 2600 Hotplate	Canlab Ltd.
	Virtis Freeze Dryer, Model 10-100	Virtis Research Equipment, Inc.[19]
	Waterbath	Lab-Line Instruments Inc[20]
	Wild-Leitz M20 Microscope	Wild Leitz Canada Ltd.[21]

1 Hewlett-Packard (Canada) Ltd., Montreal, Quebec, Canada.
2 Pye Unicam Ltd., Toronto, Ontario, Canada.
3 Varian Canada Inc., Georgetown, Ontario.
4 Precision Scientific Co., Chicago, Illinois, U.S.A.
5 Hotpack (Canada) Ltd., Waterloo, Ontario, Canada.
6 New Brunswick Science Co., Inc., New Brunswick, New Jersey; U.S.A.
7 Sherer-Gillett Co., Marshall, Michigan, U.S.A.
8 Fisher-Scientific Ltd., Don Mills, Ontario, Canada.
9 International Equipment Co., Needham Heights, Massachussetts, U.S.A.
10 Ivan Sorvall, Inc., Newtown, Connecticutt, U.S.A.
11 Canlab Ltd., Toronto, Ontario, Canada.
12 Sargent-Welch Ltd., Weston, Ontario, Canada.
13 Brinkman Instruments Ltd., Rexdale, Ontario, U.S.A.
14 Scientific Products Inc., Evanston, Illinois, U.S.A.
15 Amsco, Inc., Erie, Pennsylvania, U.S.A.
16 Beckman Instruments, Inc., Fullerton, California, U.S.A.
17 United Technologies Ltd., Mississauga, Ontario, Canada.
18 Scientific Manufacturing Industries, Emeryville, California, U.S.A.
19 Virtis Research Equipment Inc., Gardiner, New York, U.S.A.
20 Lab-line Instruments, Inc., Melrose Park, Illinois, U.S.A.
21 Wild Leitz Canada Ltd., Ottawa, Ontario, Canada.

3. Biotic Organisms

a. Macrophytes

Wheat (*Triticum aestivum* (L.) Merr. cv. Marquis), lot no. PGR 5839 and soybean (*Glycine max* (L.) Merr. cv. Maple presto), lot no. PGR5358, seeds were gifts from Agriculture Canada. The seeds were surface-sterilized with 6% sodium hypochlorite and washed thoroughly with distilled water. Ten seeds were then placed in sterile glass petri dishes containing 15 mL (wheat) or 20 mL (soybean) of Hoagland's nutrient solution, No. 2 (Table 5). Germination of the seeds occurred in the dark in a controlled environment chamber at 27±2°C for three days. In the soybean-prometryne toxicity study, this period was extended to six days, as larger seedlings were required to prevent them from being totally immersed in the hydroponic media.

In all of the experiments involving either wheat or soybean, the seedlings were placed in a controlled environment chamber with the following conditions: 20/18°C day/night temperature, 5500 lux (fluorescent-incandescent) of lighting for 16 h day^{-1} (Sylvania cool white F48T12/CW/VHO - Sylvania 2500 hour Excel-line).

b. Microphytes

The green alga, *Chlamydomonas segnis* Ettl, was a gift from the culture collections of the National Research Council of Canada. Cultures of *C. segnis* Ettl, grown in Kuhl's media (Kuhl and Lorenzen 1964; Table

Table 5

Constituents of Nutrient Solutions and Media ($\mu g\ mL^{-1}$)[1]

Chemical	Hoagland's Nutrient Solution[2]	Kuhl's Media[3]	No.11 Media[4]	AIEHM[5]
$NH_4H_2PO_4$	115.04			
KNO_3	606.60	1011.1		
$Ca(NO_3)_2.4H_2O$	944.64			
$MgSO_4.7H_2O$	493.0	246.5	75.0	173.0
H_3BO_3	2.86	0.093	0.248	
$MnCl_2.4H_2O$	1.81			
$ZnSO_4.7H_2O$	0.22	0.100	0.023	
$CuSO_4.5H_2O$	0.08	0.00749		
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.121	0.006	0.007	
$Fe_2(C_4H_4O_6)_3.H_2O$				
$NaH_2PO_4.H_2O$		510.6		
$K_2HPO_4.2H_2O$		783.8	369.0	
$CaCl_2.2H_2O$		14.7	36.0	13.0
$FeSO_4.7H_2O$ (Fe-EDTA Complex)[6]		6.95		
$MnSO_4.4H_2O$		0.020	0.178	
$CoCl_2.6H_2O$		0.008		
Na_2CO_3			20.0	
$Na_2SiO_3.9H_2O$			58.0	
$FeC_6H_5O_7.5H_2O$			6.0	
$C_6H_8O_7.H_2O$			6.0	
EDTA			1.0	
$Co(NO_3)_2.4H_2O$			0.012	
$Na_2WO_4.2H_2O$			0.026	
KBr			0.010	
KI			0.007	
$Cd(NO_3)_2.4H_2O$			0.012	
$NiSO_4(NH_4)SO_4.6H_2O$			0.016	
$VOSO_4.2H_2O$			0.002	
$AlK(SO_4)_2.12H_2O$			0.038	
Yeast Extract				1000.0
HEPES Buffer				1100.0
Na_2HPO_4				125.0
Na_2SO_4				250.0
NH_4Cl				300.0
$FeCl_2$				4.87

1 All solutions autoclaved before use at 1.4 kg cm^{-2}, 115°C for 20 min.
2 From Hoagland and Arnon (1938)
3 From Kuhl and Lorenzen (1964)
4 From Hughes et al. (1958)
5 From Cole and Elkan (1973) and Kuykendall (1979)
6 For preparation of the Fe-EDTA complex. 0.69 g of $FeSO_4.7H_2O$ and 0.93 g of disodium salt of EDTA are dissolved in 80 mL of double distilled water by boilinf for a short time. The solution is then cooled to room temperature and made up to 100 mL. One mL of this solution contains $2.5X10^{-5}$ moles $FeSO_4.7H_2O$ in the form of Fe-EDTA complex (Kuhl and Lorenzen 1964).

5) were synchronized according to the method of Badour (1981). Essentially, the cells were synchronized in Hotpack incubators at 25±2°C with a light/dark regime of 12/12 h and 2700 lux light (Sylvania cool white fluorescent F20T12-CW). Cultures were stirred magnetically and aerated (0.03% CO_2 v/v). The cultures were diluted at the beginning of each light period (S-phase of the cell cycle) with fresh media to a concentration of 2.0 x 10^5 cells mL^{-1} as determined by counts with an improved Neubauer haemocytometer.

Anabaena spp., the cyanobacteria, were purchased from Boreal Laboratories Ltd., Mississauga, Ont. The blue-green algae were established in culture tubes containing No. 11 media excluding $NaNO_3$ (Hughes *et al*. 1958; Table 5). Temperature and light regimes for *Anabaena* spp. were the same as required for *C. segnis* Ettl described above, except that the light intensity was 1600 lux. Heterocyst frequency was determined with the use of an improved Neubauer haemocytometer after vigorous shaking to disperse the filamentous growth and obtain a homogenous mixture.

Rhizobium japonicum strain 61A76 was purchased from Nitragin Co., Milwaukee, WI and *Klebsiella pneumoniae* strain M5A1 was a gift from the Department of Biology, Carleton University, Ottawa, Ont. The bacterial cultures were grown in A1EHM media (Cole and Elkan 1973; Kuykendall 1979; Table 5) in a New Brunswick G24 Environment Incubator Shaker at 30°C and shaken at 250 rpm.

4. Preparation of Soil-Bound ^{14}C Residues

Soil, containing bound ^{14}C residues, was prepared as detailed in Fig. 4 (Khan and Hamilton 1980). In 500 mL flasks, 20 g (oven-dry basis) of the organic soil was inoculated with 2.0 mL of acetone containing 164 µg of uniformly ring-labeled ^{14}C-prometryne (0.94 µCi) and 84 µg of unlabeled prometryne. This resulted in a final prometryne concentration of 12.4 mg kg^{-1}. The acetone was evaporated by a gentle stream of air and the soil was thoroughly mixed. The flasks were then stoppered with cotton wool and incubated in the dark at room temperature (23±2°C).

Weekly, distilled water was added as required to maintain the initial moisture content. When this soil was required, flasks were removed and extracted with methanol for 2 h in a mechanical shaker (Cotterill 1980; Khan and Hamilton 1980). The suspensions were then suction filtered and the extract concentrated to a small volume using a rotary evaporator. An aliquot (100 µL) was then removed for liquid scintillation counting using 10 mL Scintiverse-I as the scintillation cocktail. Methanol extractions were repeated until the radioactive counts were coincident with the background counts of a sample of control soil similarly extracted.

The soil was air-dried, wetted with distilled water, and incubated overnight at room temperature in the dark. The following day, the soil was re-extracted with methanol as described. The extracted soil was then air-dried and portions were removed for analysis. One portion was used

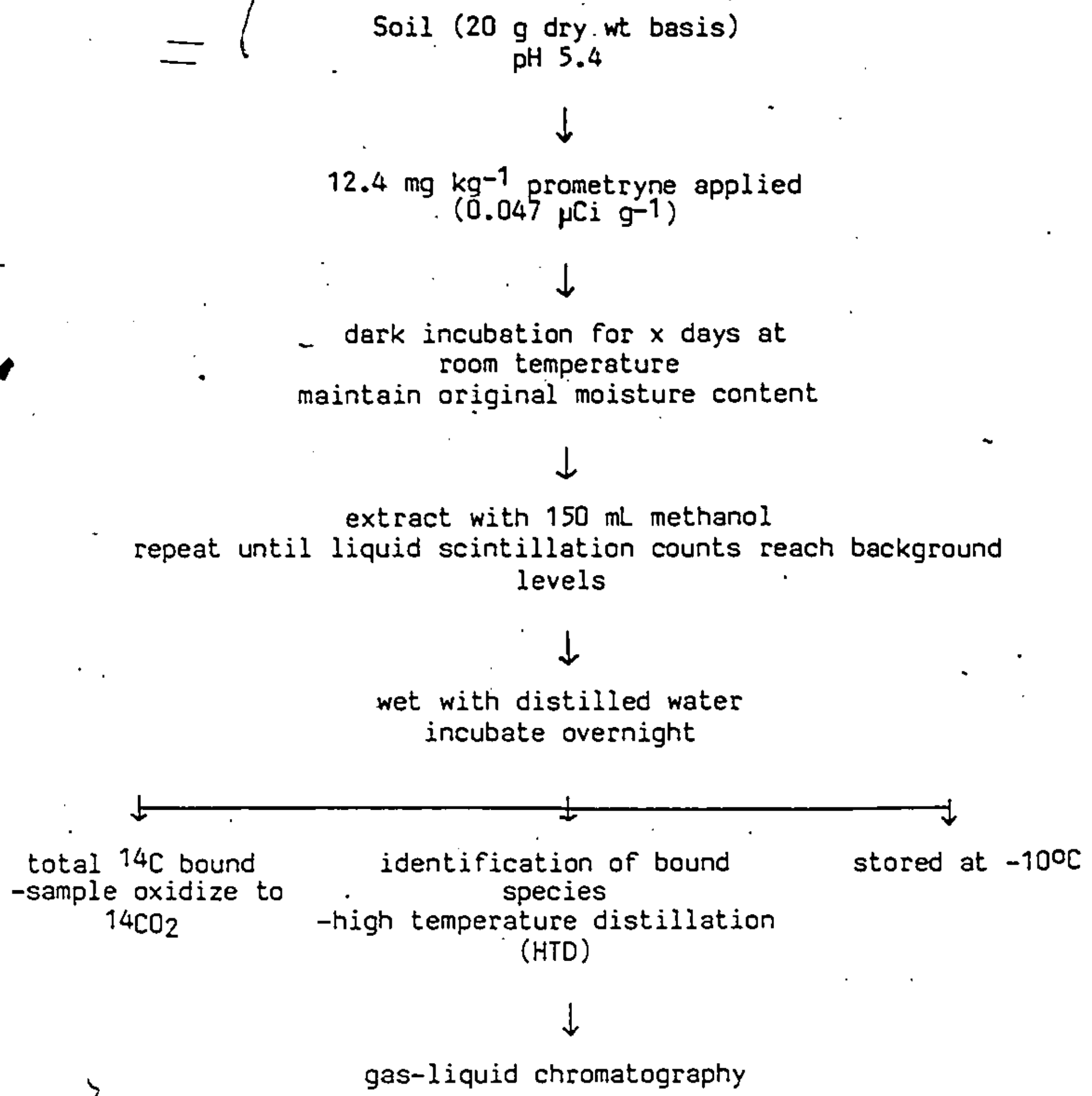


Fig. 4. Flow Diagram for the Preparation of Soil Containing Bound ^{14}C-Prometryne Residues

for sample oxidation to quantify the total bound radioactivity. The remainder of the bound soil was stored at -10°C until required. In order to identify the bound species present, a high temperature distillation (HTD) was carried out (section II.A.5; Khan and Hamilton 1980).

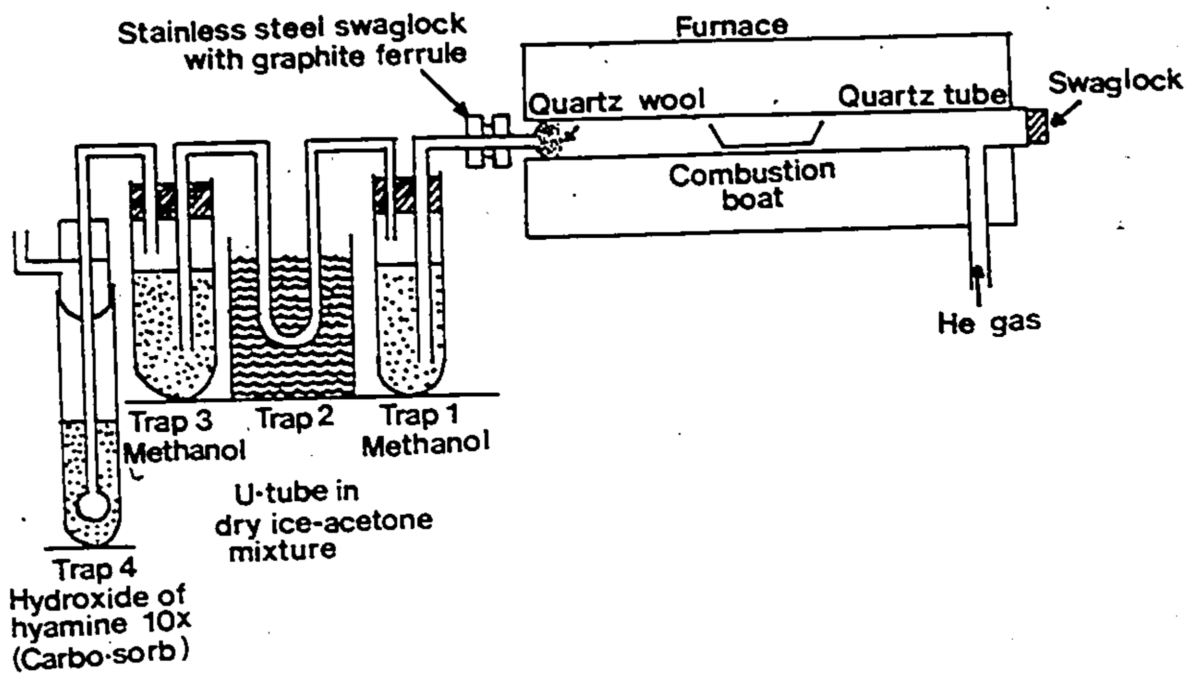
5. Identification of Soil-Bound Residues

In porcelain boats (0.2 cm wide, 0.5 cm deep, 4 cm long), 500 mg of soil containing bound ^{14}C residues were inserted into a quartz tube. The tube was inserted into a Lindberg Tube Furnace (Sola Basic S/B) and one end was attached to a helium gas line while the other end was connected to a series of methanol traps (Fig. 5). The methanol traps were required to absorb the volatilized residues as the temperature in the tube increased. Helium gas was used as a sweep gas so that minimal oxidation would occur as the bound residues were released and the final trap contained Carbo-sorb to absorb any $^{14}CO_2$ formed by oxidative reactions between the residues and humic substances.

The furnace was heated from room temperature up to 800°C (ca. 15°C min^{-1}) with helium gas flowing through (45-50 mL min^{-1}). The temperature was maintained at 800°C for 15 minutes. After cooling, the quartz tube and connections were washed with methanol. The contents of the first three traps were combined and concentrated. An aliquot of the extract along with one from the $^{14}CO_2$ trap was then removed from scintillation counting. The extract was evaporated to dryness and redissolved in chloroform.

Figure 5

Apparatus from the High Temperature Distillation Technique (from Khan and Hamilton 1980).


Stainless steel swaglock
with graphite ferrule
Furnace
Quartz wool
Quartz tube
Swaglock
Combustion
boat
He gas
Trap 3
Methanol
Trap 2
Trap 1
Methanol
U-tube in
dry ice-acetone
mixture
Trap 4
Hydroxide of
hyamine 10x
(Carbo-sorb)

The extract was chromatographed on a 20.0 g acidic aluminum oxide column (24 x 70 mm, 10% water) topped with anhydrous sodium sulphate and prewashed with chloroform. The column was first eluted with 150 mL of chloroform to remove the nonpolar derivatives and then with 150 mL of methanol to remove the polar derivatives. The chloroform eluate was evaporated to dryness and redissolved in methanol. An aliquot was removed for injection into a gas-liquid chromatograph while another was removed for scintillation counting.

The methanol eluate was concentrated to 5.0 mL and an aliquot removed for scintillation counting. The remainder was methylated with diazomethane, evaporated, and redissolved in 2.0 mL 10% acetone in hexane. This mixture was then placed on a 5.0 g Florisil (60-100 mesh) column (oven-dried overnight) topped with anhydrous sodium sulphate and prewashed with hexane. The column was first eluted with 150 mL of hexane (the eluate was discarded) and then followed by 150 mL of 10% acetone in hexane and 150 mL of 50% acetone in hexane. The final two eluates were combined, evaporated to dryness, and redissolved in acetone. An aliquot was then analyzed by gas-liquid chromatography.

6. Gas-Liquid Chromatography

All gas-liquid chromatographs were equipped with a 1.8 m glass column, packed with 3% Carbowax 20M on 80/100 Supelcoport (Supelco Inc., Bellefonte, PA). The Pye Unicam Series 104 gas chromatograph, equipped with the Perkin-Elmer nitrogen-phosphorus detector (NPD), was used in

the study of pH release of soil-bound ^{14}C residues and for the high temperature distillation (HTD). The same instrument, except equipped with an alkali flame ionization detector (AFID), was used to quantify the amount of prometryne in the prometryne-saturated A1EHM media for the bacterial studies. A Hewlett-Packard model 5830A gas chromatograph fitted with a nitrogen-phosphorus detector (NPD) was used in the identification of extractable residues from the rhizosphere and rhizoplane soils while the Varian Vista series 6000 gas chromatograph with a thermionic specific detector (TSD) was used in the identification of the extractable residues from plant tissues.

In all cases, the flow of helium, hydrogen, and air was 30, 5, and 150 mL min^{-1}, respectively. The injection temperature was 200°C and the detector temperature was maintained at 300°C. Column temperatures were maintained at 220°C, except for the plant tissue analyses where temperature programming was required for resolution. In these cases, the initial column temperature was 210°C and this was held for 25 min. The column temperature was then increased at a rate of 5°C min^{-1} to a final temperature of 220°C which was held until the resolution of all peaks.

Except for the bacterial studies, gas-liquid chromatography was used to qualitatively identify the prometryne derivatives. Identification was based on the comparison of retention times of unknown compounds with known reference standards and on co-chromatography.

7. Determination of Radioactivity

Aliquots (100 µL) of all extracts were mixed with 10.0 mL of Scintiverse I solution and the radioactivity measured by liquid scintillation counting using an external standard and correcting the counts for quenching. Soil or tissue samples were combusted in a Packard sample oxidizer, model 306, to $^{14}CO_2$ which was absorbed and mixed with appropriate volumes of Carbo-sorb and Permafluor V. The $^{14}CO_2$ was then measured by liquid scintillation counting using an internal standard for quench correction.

8. Statistical Analysis

All of the data was analyzed by an analysis of variance (ANOVA) followed by a Student-Newman-Kuel's multiple range test. Procedures for calculation of the indices were obtained from the literature (Zar 1974).

B. PROMETRYNE TOXICITY TO MACROPHYTES AND MICROPHYTES

1. Soybean Susceptibility (Fig. 6)

a. Herbicide Application and Growth

In triplicate sets of 250 mL flasks, solid prometryne was mixed with 100 mL of Hoagland's nutrient solution, No. 2 Hoagland and Arnon 1938) to obtain herbicide concentrations of 15.0, 10.0, 7.5, 5.0, or 2.5 µg mL^{-1}. Controls containing no prometryne (0 µg mL^{-1}) were also initiated. The solutions were all adjusted to pH 5.4 with 0.01 N NaOH.

6-day old soybean seedlings

100 mL Hoagland's nutrient solution, No. 2 containing
0-15 μg mL^{-1} prometryne
(pH adjusted to 5.4)

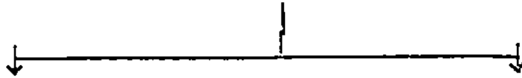
20:18°C day:night temperature
5500 lux light intensity
16 h light period

2 weeks growth

fresh weights

freeze dry

dry weights



Kjeldahl nitrogen assay

Hartree protein test

Fig. 6. Assay of Prometryne Toxicity to Soybean Seedling Growth

Into each flask, 6-day old soybean seedlings were transferred (3/flask). The flasks were then placed in a controlled environment chamber under the growth conditions specified for soybean (section II.A.3.a). The plants were grown for two weeks, with distilled water added as required to maintain a constant solution volume of 100 mL. Experimental sets were in triplicate.

b. Harvest

At the end of two weeks, the plants were removed from the flasks and the roots blotted dry with tissue paper. Fresh weights were then determined. The individual plants were placed into culture tubes and freeze-dried for 4 h at -50°C. Dry weights were determined and the plant tissues ground to form representative homogenous samples. The tissue samples were stored at -10°C for protein analysis.

c. Protein Analysis

i. Hartree Protein Analysis

Representative tissue samples from each treatment set were extracted according to Monahan (1974) and Varner (1964). Essentially, 100 mg of tissue was homogenized for 90 s with 2.0 mL of cold (2°C) 1.0 mM acetate buffer (pH 4.8) using the Polytron tissue homogenizer. The homogenate was transferred to a centrifuge tube with additional buffer (3 x 2.0 mL). This was combined and mixed for 30 s on a vortex mixer and then centrifuged at 30000 x g for 20 min at 2°C using the Sorval centrifuge. The supernatant was quantitatively transferred to a 25 mL volumetric flask and made up to volume with additional buffer.

This extract was then analyzed for total protein (Hartree 1972). In small test tubes, 0.9 mL of solution A (2.0 g $KNaC_4H_4O_6$-$4H_2O$ and 100 g Na_2CO_3 in 500 mL 1 N NaOH, diluted to 1 L) was added to 1.0 mL of the supernatent. The tubes were incubated in a water bath for 10 min at 50°C, then cooled to room temperature. To this, 0.1 mL of solution B (1.0 g $CuSO_4$-$5H_2O$ and 2.0 g $KNaC_4H_4O_6$-$4H_2O$ in 90 mL water and 10 mL 1 N NaOH) was added and left for 10 min. Following this, 3.0 mL of a freshly diluted Folin-Ciocalteau (1:15) reagent was rapidly forced into the solution using a 10 mL glass syringe to ensure complete mixing within 1 s. The tubes were returned to the 50°C water bath for 10 min and then cooled to room temperature. Absorbance of the mixture was determined in 1.0 cm cuvettes using a Bausch and Lomb spectrophotometer. The results at 650 nm were recorded relative to an acetate buffer blank. Protein quantification was determined from a calibration curve prepared from standard bovine serum albumin (BSA) solutions.

ii. Kjeldahl Nitrogen (Protein) Analysis

Kjeldahl digestion and steam distillation of the tissue was as described (Horwitz 1975) by adding 1.9 g K_2SO_4, 0.04 g HgO, and 5.0 mL H_2SO_4 to 100 mg of plant tissue in a Kjeldahl digestion flask. On the Kjeldahl digestion rack, the contents were brought to a quick boil avoiding excessive frothing by frequent turning of the flasks. The mixture was boiled for 1-1.5 h until a clear solution was obtained. The solutions were allowed to cool to room temperature and a minimal amount of deionized distilled water was slowly added to dissolve the precipitate. The solution was carefully transferred to the distillation

flask in a steam distillation unit. The digestion flask was rinsed five times with 2.0 mL of deionized distilled water and the washes were also transferred to the distillation flask.

In a 125 mL flask, the 25 mL volume was carefully marked and 5.0 mL of a saturated boric acid solution and 2 drops of 2:1 methyl red:methylene blue (v/v) indicator solution (0.2% alcoholic solutions) were added. The flask was placed below the condenser of the steam distillation unit with the tip below the surface of the boric acid solution. The digest was allowed to flow into the distillation tube and then 20 mL of sodium hydroxide/sodium thiosulphate reagent (40.0 g NaOH and 4.0 g $Na_2S_2O_3$-$5H_2O$ in 100 mL of water) was added to the digest. This mixture was steam distilled until 25.0 mL was collected in the receiving flask. The colour of the indicator changed from purple to green.

At the end of the distillation period, the condenser tip was rinsed into the receiving flask with deionized distilled water and the flask removed. The final volume of the distillate was made up to 50 mL and titrated with 0.02 N HCl to a grey endpoint. The per cent nitrogen was determined using the titre volume and this value was converted to per cent protein by multiplying by 6.25 (Horwitz 1975).

2. ALGAE - CHLAMYDOMONAS SEGNIS ETTL

a. Solvent System

Prometryne has a low water solubility (Weber 1970) and is generally dissolved in a minimum volume of an organic solvent to effect miscibility with water. Acetone was the solvent used in the present set of experiments and the volume proportion of acetone in solution for the various test media was held constant. In the algal experiments using C. segnis Ettl, the acetone concentration in the media was 0.13% (v/v) compared to 0.25% (v/v) for the trials containing the cyanobacteria, Anabaena spp. The differences in acetone and prometryne concentrations tested for the two species was due to differences in the volume of the media used in the growth studies.

By contrast, preliminary tests indicated that the bacteria, Rhizobium japonicum and Klebsiella pneumoniae, were sensitive to acetone and therefore, this solvent was not used to dissolve the herbicide into the media for the bacterial assays. Instead, serial dilutions of a prometryne-saturated medium was used. This was prepared by adding excess prometryne to 500 mL of A1EHM medium (Cole and Elkan 1973; Kuykendall 1979). After shaking and allowing the undissolved prometryne to settle, the suspension was filtered aseptically through a 0.20 µm sterile Nalgene membrane filter unit (Fisher Scientific Ltd., Canada) and the prometryne in solution was quantified by gas-liquid chromatography as follows.

A 10.0 mL aliquot of this medium was exhaustively extracted (4-times) with 50.0 mL of chloroform. The chloroform extracts were combined, evaporated to dryness, and redissolved in acetone. An aliquot was then injected into a gas-liquid chromatograph and the quantity of prometryne determined using reference standards. This medium, along with freshly prepared pesticide-free A1EHM medium used for serial dilutions, was then stored at 4°C until required.

b. Prometryne Effects on C. segnis Ettl (Fig. 7)

In seven parallel sets of experiments, synchronous cultures of C. segnis Ettl (2.0×10^5 cells mL^{-1}) were exposed to 12.0, 6.0, 3.0, 1.5, 0.75, or 0 μg mL^{-1} prometryne with 0.13% v/v acetone together with a control set of 0 μg mL^{-1} prometryne and 0% v/v acetone. The algal treatment occurred 2 h before the initiation of the dark phase corresponding to the sensitive S-phase of the cell cycle (Badour 1981). Cell counts were determined 14 h later at the beginning of the light phase which corresponds to G_1 of the cell cycle.

Aliquots (1.0 mL) were pipetted from each of the control, acetone treated, 12.0 μg mL^{-1}, 3.0 μg mL^{-1}, and 0.75 μg mL^{-1} prometryne treated sets into separate centrifuge tubes and then diluted to 10.0 mL with distilled water. Following this, the tubes were centrifuged in a clinical centrifuge at 3000 rpm for 10 min. In each case, the supernatant was removed and the algal pellet resuspended in distilled water, re-centrifuged and washed again. Finally, after discarding the supernatant, the pellet was resuspended in 10.0 mL distilled water,

150 mL of synchronous C. segnis cultures (2.0×10^5 cells/mL)
inoculated with 0, 0.75, 1.50, 3.0, 6.0, and 12.0 μg mL^{-1}
prometryne in acetone (0.13% v/v)

inoculated 2 h before dark
phase(s) of cell cycle

14 h (beginning of light or G_1 phase)

stirred and aerated (0.03% CO_2 v/v)

cell counts via haemocytometer

1.0 mL aliquots from the control, acetone treated, and
cultures treated with 0.75, 3.0, and 12.0 μg mL^{-1}
prometryne pipetted into separate centrifuge tubes

washed with distilled water

resuspend in 10.0 mL H_2O

0.1 mL plated on nutrient agar

Fig. 7. Assay of Procedure for Toxicity of Prometryne to *Chlamydomonas segnis*

vortexed, and 0.1 mL of the resultant suspension was plated on nutrient agar (1.5% v/v) fortified with Kuhl's media (Kuhl and Lorenzen 1964). The plates were incubated upside-down in a Hotpack incubator under the conditions noted above (section II.A.3.b). After 14 days, the number of colonies per plate were counted in all the treatment sets (three replicates per set).

3. Heterocyst Study - Anabaena spp. (Fig. 8)

Cultures of Anabaena spp. in No. 11 media (without $NaNO_3$) containing 1.0 x 10^5 heterocysts mL^{-1}, as determined by counting (improved Neubauer haemocytometer), were treated in parallel sets with 0.25% (v/v) acetone and a range of prometryne concentrations (10.0, 5.0, 1.0, 0.5, 0.1, or 0 µg mL^{-1}). In addition, a control set without added prometryne or acetone was also established. All the cultures were incubated under the conditions described above (section II.A.3.b). Following one week of growth, the culture tubes were vigorously shaken to break up the cyanobacterial filaments and a homogenous mixture for heterocyst counting was obtained. Furthermore, cells from the control, acetone treated, 10.0 µg mL^{-1}, 1.0 µg mL^{-1}, and 0.1 µg mL^{-1} prometryne treatment sets were washed as described for C. segnis Ettl (section II.F.2), and plated on nutrient agar (1.5% w/v) augmented with No. 11 media (Hughes et al. 1958). The plates were photographed after 14 days of growth in the incubator. Trials were completed in triplicate for a total of six per treatment set.

20 mL of <u>Anabaena</u> spp. ($1.0x10^5$ heterocysts mL^{-1})
treated with 10.0, 5.0, 1.0, 0.5, 0.1, and 0 µg mL^{-1}
prometryne in acetone (0.25% v/v)

one week subsequent growth
12 h light:12 h dark; 1600 lux
23±2°C

heterocyst counts via
haemocytometer

cultures washed in distilled water
(control, acetone, 10.0, 1.0, and 0.1 µg mL^{-1})

plated on nutrient agar
two weeks growth

photographed

Fig. 8. Procedure for Evaluating the Effect of Prometryne on the Growth of <u>Anabaena</u> spp.

4. Rhizobium japonicum and Klebsiella pneumoniae (Fig. 9)

In parallel experiments, populations of exponentially growing cells of R. japonicum and K. pneumoniae (optical density at 550 nm of 0.010) were grown in media augmented with 6.8, 2.3, 0.75, 0.25, and 0 µg mL^{-1} prometryne (as prepared by serial dilutions of the prometryne-saturated A1EHM media) and placed in an incubator under the same conditions as above, (section II.A.3.b). The growth of K. pneumoniae was monitored hourly by optical density (OD) readings taken at 550 nm in a Bausch and Lomb Spectronic 21 UV-VIS spectrophotometer (Miller 1972). As R. japonicum grew more slowly, OD_{550} readings were taken 17 h after inoculation and every 3 h thereafter. The OD_{550} readings obtained for both species were separately plotted on a semi-log scale and doubling times determined by regression analysis.

C. ROOT ALTERATION OF SUBSTRATE pH

1. Hydroponic pH Changes (Fig. 10)

In parallel sets of experiments, three wheat seedlings (3-day old) were transferred to 250 mL flasks containing 100 mL of one of the fertilizer solutions described in Table 6. The pH of all solutions were initially adjusted to pH 5.4. The plants were then transferred to a controlled environment chamber set for the conditions described above for wheat (section II.A.3.a). The plants were grown for 12 days with daily replenishment of the fertilizer solutions to the initial 100 mL using distilled water. The pH of the hydroponic solutions was monitored every two days. The experiments were conducted in triplicate sets.

Rhizobium japonicum and *Klebsiella pneumoniae* cultures inoculated into Klett flasks containing prometryne dissolved in AIEHM media (6.8, 2.3, 0.75, and 0.25 μg mL^{-1}) initial OD_{550} = 0.010

30°C, 250 rpm

OD_{550} taken hourly for *K. pneumoniae*;
OD_{550} 17 h later and every 3 h thereafter for *R. japonicum*

plot on semi-log scale

doubling times by regression analysis

Fig. 9. Schematic Diagram for Assaying the Toxicity of Prometryne to N_2-Fixing Bacteria, *R. japonicum* and *K. pneumoniae*

3-day old wheat seedlings

100 mL of fertilizer solutions (Table 6)
adjust to pH 5.2

20:18°C day:night temperature
5500 lux intensity
16 h light period

measure solution pH every 2 days
using a combination pH electrode

12 days growth

Fig. 10. Schematic Diagram for Measuring Hydroponic pH Alterations by Wheat Roots

Table 6

Fertilizer Solutions for Hydroponic Growth

Solution	Nitrogen Source	Concentration of nitrogen ($\mu g\ mL^{-1}$)
1. Distilled Water	-	-
2. Hoagland's Nutrient Solution, No. 2	NO_3^- NH_4^+	196 14
3. $Ca(NO_3)_2$	NO_3^-	25
4. $Ca(NO_3)_2$	NO_3^-	50
5. $Ca(NO_3)_2$	NO_3^-	1000
6. $(NH_4)_2SO_4$	NH_4^+	25
7. $(NH_4)_2SO_4$	NH_4^+	50
8. $NH_4H_2PO_4$	NH_4^+	25
9. Urea	H_2NCONH_2	25

2. pH Microelectrode Analysis

As well as observing overall changes in substrate pH, more localized changes were monitored. To this end, pH changes in solid media were also followed.

Into 40 mL centrifuge tubes (Pyrex No. 8260) containing 10.0 g of a pesticide-free organic soil-silica sand mixture (1:1.25 w/w) was planted four 3-day old wheat seedlings (Fig. 11). In triplicate sets, 1.0 µg of nitrogen g^{-1} of soil was added to each set as either $Ca(NO_3)_2$ or $(NH_4)_2SO_4$. A control set with no fertilizer was also established. The plants were then placed in an environment growth chamber under the conditions described above (section II.A.3.a). All sets were watered daily to field capacity. Using a Sargent-Welch miniature combination pH electrode, the probe was inserted into the rhizospheric regions and pH readings were taken in triplicate. The rhizosphere pH was monitored every two days.

3. Wheat Rhizosphere and Rhizoplane pH

To further define the detection of localized changes in pH into rhizosphere and rhizoplane, the soil surrounding the root was separated into two fractions. The experimental details are shown in Fig. 12.

Into small glass petri dish bottoms (60 x 15 mm), 5.0 g of the pesticide-free organic soil was weighed. Ten wheat seedlings (3-day old) were then planted in the soil. Each triplicate set was then fertilized

3-day old wheat seedlings
planted in 10.0 g soil:sand
mixture (1:1.25 w/w)

fertilize with 0 or 1.0 μg of nitrogen
g^{-1} soil as $Ca(NO_3)_2$ or $(NH_4)_2SO_4$

20:18°C day:night temperature
5500 lux light intensity
16 h light period

monitor rhizosphere pH by miniature
pH combination electrode every 2
days

2 days growth

Fig. 11. Flow Chart for Monitoring Rhizosphere pH With a Miniature Combination pH Electrode

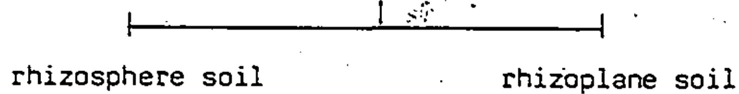
Ten 3-day old wheat seedlings planted in
5.0 g organic soil in 60X15 mm petri dish
bottoms

fertilized with 0 or 1.0 µg N g^{-1} soil
as $Ca(NO_3)_2$ or $(NH_4)_2SO_4$

20:18°C day:night temperature
5500 lux light intensity
16 h light period

3 weeks growth

separation of rhizosphere
and rhizoplane soils by shaking root-soil
mass



rhizosphere soil | rhizoplane soil

measure soil pH with 1:1 (v/v)
soil solution with 0.01 M $CaCl_2$
using pH electrode

Fig. 12. Flow Diagram of pH Measurements of Rhizosphere and Rhizoplane Soils

with one of the fertilizer solutions containing 1.0 µg of nitrogen g^{-1} of soil as either $Ca(NO_3)_2$ or $(NH_4)_2SO_4$. Control sets in triplicate without any additional nitrogen source were also initiated. The seedlings were then grown for three weeks under the conditions previously specified (section II.A.3.a).

The plants were watered daily to field capacity with deionized distilled water. At the end of the growth period, the plants were removed from the chamber and the soil fractions carefully separated by vigorously shaking the root-soil mass over a clean sheet of paper. The loosened soil was collected and termed "rhizosphere soil" since the root mass was extensive enough to warrant elimination of "bulk soil" as described by Clark (1949). Soil tightly adhering to the roots was then carefully removed and collected as the "rhizoplane soil" sample. After removing any visible severed root fragments from the soil, the pH of the two soil fractions was determined using a 1:1 (v/v) soil solution with 0.01 M $CaCl_2$ (Smiley 1974; van Lierop 1981). The $CaCl_2$ solution was used as the suspending media in order to minimize the variations in soil pH.

4. Fertilizer Influence on Soil pH

In triplicate sets, 5.0 g (oven-dry basis) of the pesticide-free organic soil was fertilized with 40 µg of nitrogen as either $Ca(NO_3)_2$, $(NH_4)_2SO_4$, or urea. This gave a final nitrogen concentration of 8.0 µg g^{-1}. The fertilizers were added as deionized distilled water solutions. A set fertilized with Hoagland's nutrient solution, No. 2,

and one with distilled water alone were also established. After 3 weeks, pH measurements were taken of 1.0 g soil samples randomly sampled from each treatment set. The soil was mixed with 0.01 M $CaCl_2$ (1:1 v/v). A combination pH electrode was used for the pH determination.

5. Root Mediated Changes in Agar pH

In order to observe the localized changes mediated by the plant's root system as a function of the nitrogen fertilizer source, plants were grown in agar (Fig. 13). To fertilizer solutions (pH 5.4) containing 40.0 µg of nitrogen mL^{-1} as $Ca(NO_3)_2$, $(NH_4)_2SO_4$, or urea, Bacto-agar was added to give a concentration of 1.5% (w/v). Additionally, a set with Hoagland's nutrient solution, No. 2 (pH 5.4), containing 210 µg of nitrogen mL^{-1} was also used as a nitrogen source. Each of the solutions were then autoclaved for 20 min at 1.4 kg cm^{-2}, cooled to 55°C, separately poured into sterile plastic petri dishes, and cooled to room temperature. Under aseptic conditions, one 3-day old wheat or soybean seedling was transferred to each agar plates. Each fertilizer treatment set consisted of twelve plates, of which nine contained seedlings. Control plates (plants excluded) individually augmented with a different nitrogen source were also initiated. The plates were then transferred to a controlled environment chamber under the conditions previously described for wheat and soybean (section II.A.3.a). Plates from all treatment sets were sampled weekly for 3 weeks. At each sampling period, 5.0 mL of one of the pH indicator

Agar plates (1.5% w/w) containing 25 μg N mL^{-1}
as either $Ca(NO_3)_2$, $(NH_4)_2SO_4$, or urea, or
Hoagland's nutrient solution, No. 2

aseptically transfer to each plate one 3-day
old wheat seedling. To some plates augmented
with Hoagland's nutrient solution, No. 2, one
3-day old soybean was aseptically added

20:18°C day:night temperature
5500 lux light intensity
16 h light period

subsample plates weekly for
3 weeks

add 5.0 mL of pH indicator
solutions

photographed

pH determined

Fig. 13. Procedure for Studying Root-mediated Changes of the pH in Agar

solutions were added to each plate (Table 7). After the indicator solutions had permeated into the agar, all the plates were photographed. The pH was then determined from the colours elicited in the agar.

D. EFFECT OF CHANGE IN pH ON THE RELEASE OF SOIL-BOUND ^{14}C-PROMETRYNE RESIDUES

1. Incubation

The procedure used to determine the release of soil-bound residues as a function of pH is detailed in Fig. 14. Buffer solutions (pH 4-8) were prepared as described earlier (Robinson and Stokes 1955). The pH of these solutions was then measured using a combination electrode. These buffer solutions were used to adjust the pH of the soil during incubation providing precise adjustments.

In 50 mL Pyrex culture tubes, triplicates of 1.0 g (oven-dry basis) of the air-dried organic soil containing bound ^{14}C-prometryne residues were mixed with 10.0 mL of one of the buffer solutions. The tubes were then loosely stoppered with cotton wool and placed into a controlled environment chamber. The soil samples were incubated in the dark at $20 \pm 2^{o}C$ for two weeks.

2. Extraction

At the end of the incubation period, the samples were exhaustively extracted with methanol (10.0 mL each time) and water until the radioactivity of the extracts was coincident with background levels.

Table 7

pH Indicator Solutions Used for Determining pH Changes in Agar

Indicator	Constituents	pH Range	Color Change
Universal pH Indicator Solution		4-10	red-purple
Bromocresol Green	0.1 g in 14.3 mL 0.01 N NaOH 235.7 mL water	3.8-5.4	yellow-blue
Chlorophenol Red	0.1 g in 23.6 mL 0.01 N NaOH and 236.4 mL water	5.3-6.8	yellow-red
Metacresol Purple	0.1 g in 26.2 mL 0.01 N NaOH and 223.8 mL water	1.2-2.8 7.4-9.0	red-yellow yellow-purple
Phenolphthalein	0.05 g in 50 mL EtOH and 50 mL water	8.2-10.0	colorless-pink

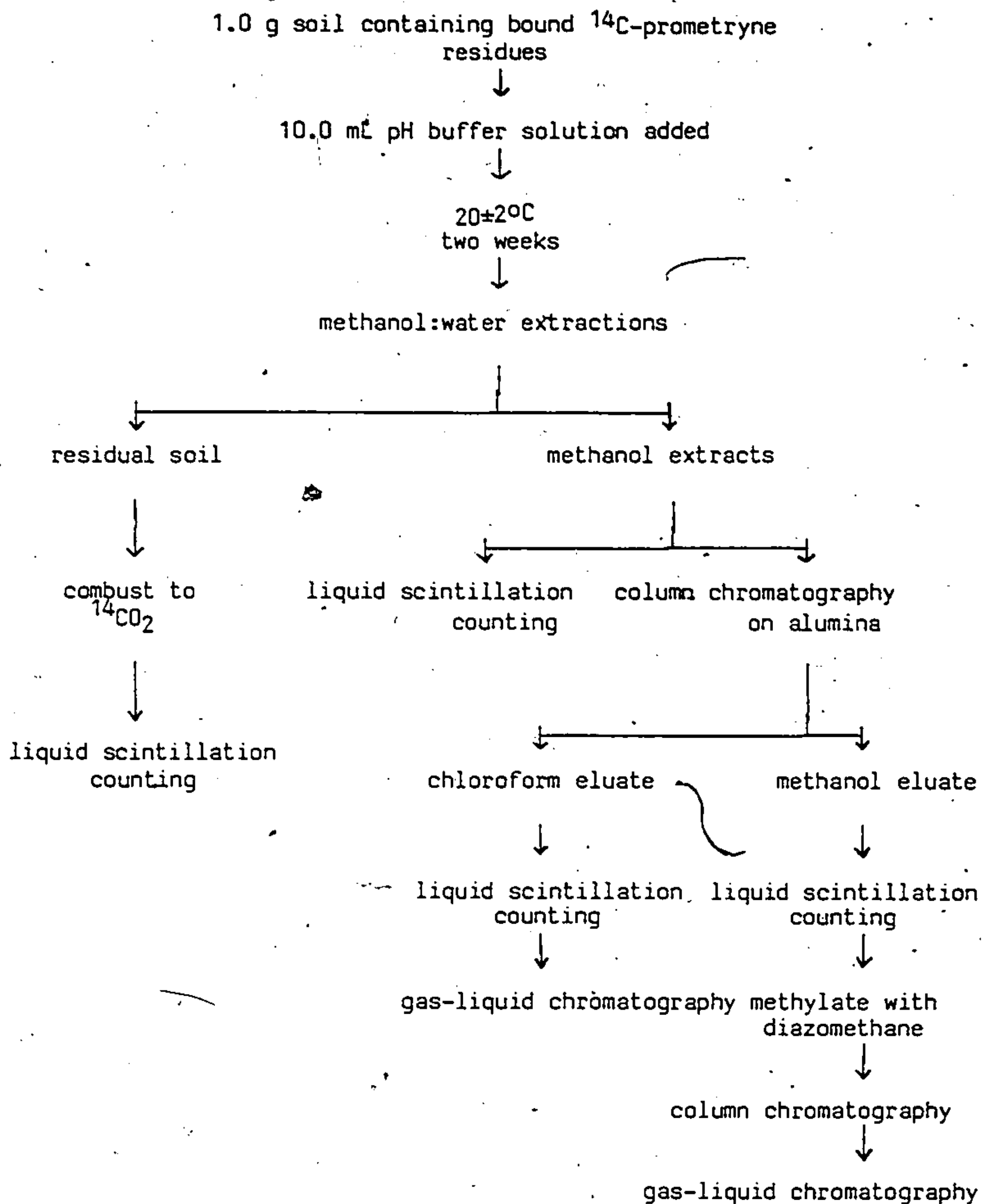


Fig 14. Procedure for Studying the Effects of pH on the Release of Soil-Bound ^{14}C-Prometryne

Extractions were carried out using a mechanical shaker and shaking the suspension for 2 h as described above (section II.A.4). The combined extracts were concentrated to a small volume (ca. 1 mL) on a rotary evaporator at room temperature. Aliquots of the extracts were then removed for liquid scintillation counting to give the total extractable radioactivity after the pH incubation. The remainder of the extracts were subjected to column clean-up on acidic aluminium oxide and eluted with chloroform (non-polar fraction) and methanol (polar fraction) as described earlier (seciton II.A.4). The methanol eluates were methylated with diazomethane. Aliquots were injected into the Pye gas-liquid chromatograph.

3. Residual Soil

The radioactivity of residues bound in the soil after pH incubation were determined by combustion in a sample oxidizer. The $^{14}CO_2$ produced was absorbed and mixed with appropriate volumes of Carbo-sorb and Permafluor V. The activity was measured by liquid scintillation counting using an internal standard for quench correction.

E. RELEASE OF SOIL-BOUND ^{14}C-PROMETRYNE BY PLANTS (FIG. 15)

1. Exposure and Growth

Five grams (oven-dry basis) of air-dried soil with bound ^{14}C residues was weighed into 60 x 15 mm sterile glass petri dish bottoms. In triplicate sets, ten 3-day old wheat seedlings or four comparably aged,

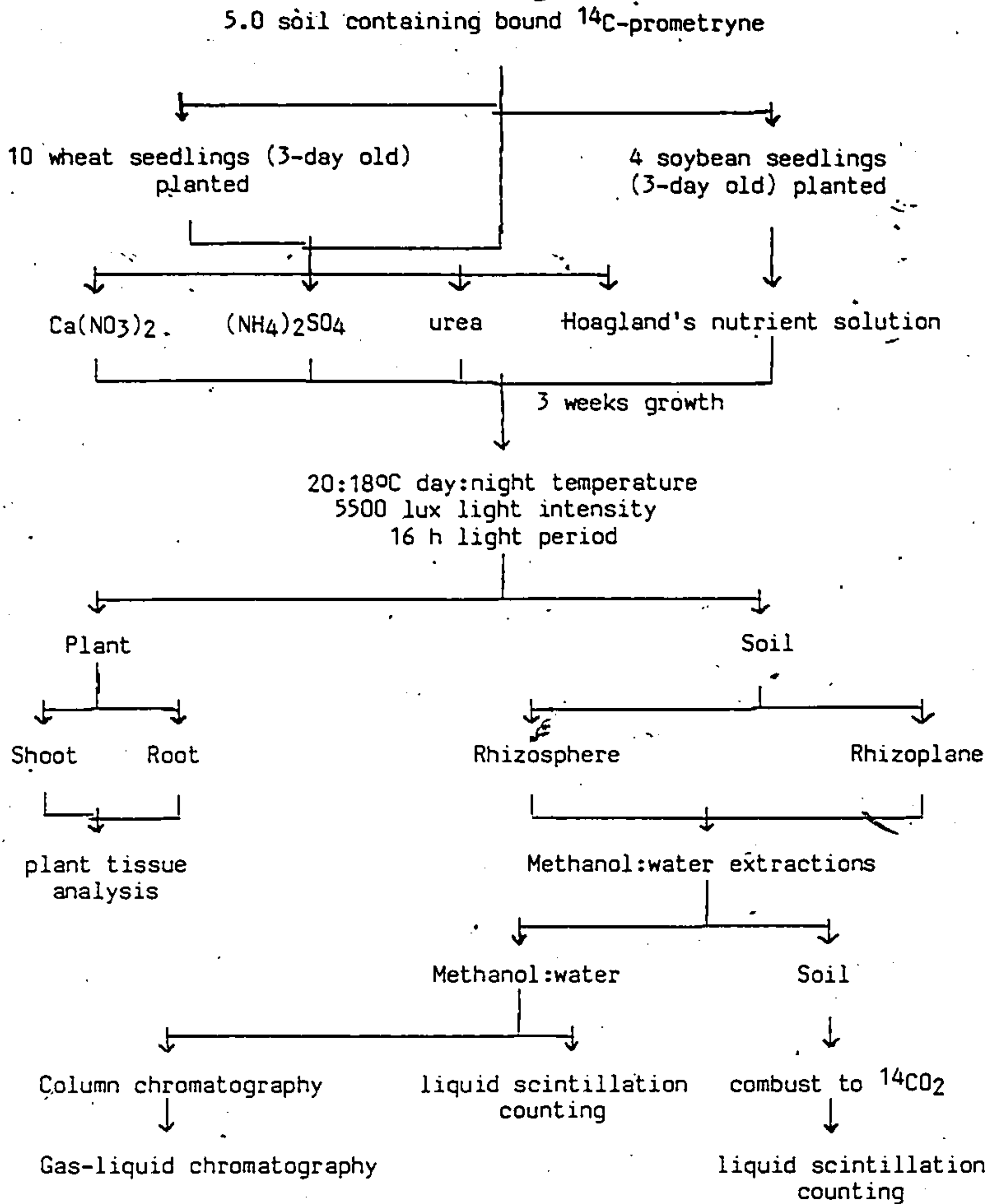


Fig. 15. Schematic Diagram for Plant Release and Uptake of Soil-Bound ^{14}C-Prometryne

though larger, soybean seedlings, were planted into each dish to yield six replicates for each fertilizer treatment. Four control sets containing bound ^{14}C residue soil and not containing any plants, each fertilized with one of the four different nitrogen sources, were also initiated. The wheat trials were then fertilized with either 1.0 mL of full strength Hoagland's nutrient solution, No. 2 or 8.0 μg of nitrogen g^{-1} of soil as $Ca(NO_3)_2$, $(NH_4)_2SO_4$ or urea. The soybean trials were fertilized only with the Hoagland's nutrient solution. Control plants grown in pesticide-free soil were used as indicators of background radioactivity levels. All trials were completed in triplicate.

The plants were then transferred into a controlled environment chamber under the conditions described above (section II.A.3.a). The plants were watered daily to maintain the moisture content at field capacity with deionized distilled water during the three week growth period.

2. Harvesting

At the end of the growth period, the plants in each set were harvested by removing the root and soil mass from their respective dishes. The soil mass was then vigorously shaken over clean sheets of paper and the soil fraction, representing the rhizosphere soil, was collected. As the mass was shaken, the plants were separated carefully to avoid root damage. Soil tightly adhering to the root surface was then

collected (rhizoplane soil). The soil was then stored at -10°C for further analysis. The plants from each dish were separated into shoot and root, freeze-dried for 4 h at -50°C, and homogenized by finely cutting the tissue. The plant tissues were then separately stored at -10°C for analysis of extractable and bound residues described later.

3. Soil Analysis

Both rhizosphere and rhizoplane soil samples were extracted with methanol and water as previously described (section II.A.4). The methanol:water extracts and residual soil were similarly processed as above (Section II.A.4).

4. Plant Analysis

The analysis of the radioactive residues in the plant tissues followed the procedure as shown in Fig. 16 (Khan 1980). Both the root and shoot tissues were thawed at room temperature and representative samples (200 mg) were removed for analysis. The tissues were extracted initially with 20 mL of dried chloroform by blending at high speed for 5 min with a polytron. This mixture was then filtered under suction and washed with chloroform. The chloroform extraction was repeated twice. The combined chloroform (non-polar fraction) extracts were concentrated and analyzed by liquid scintillation counting and gas-liquid chromatography (Varian Vista series 6000 GLC) after clean-up on an acidic aluminum oxide column described previously (section II.A.4).

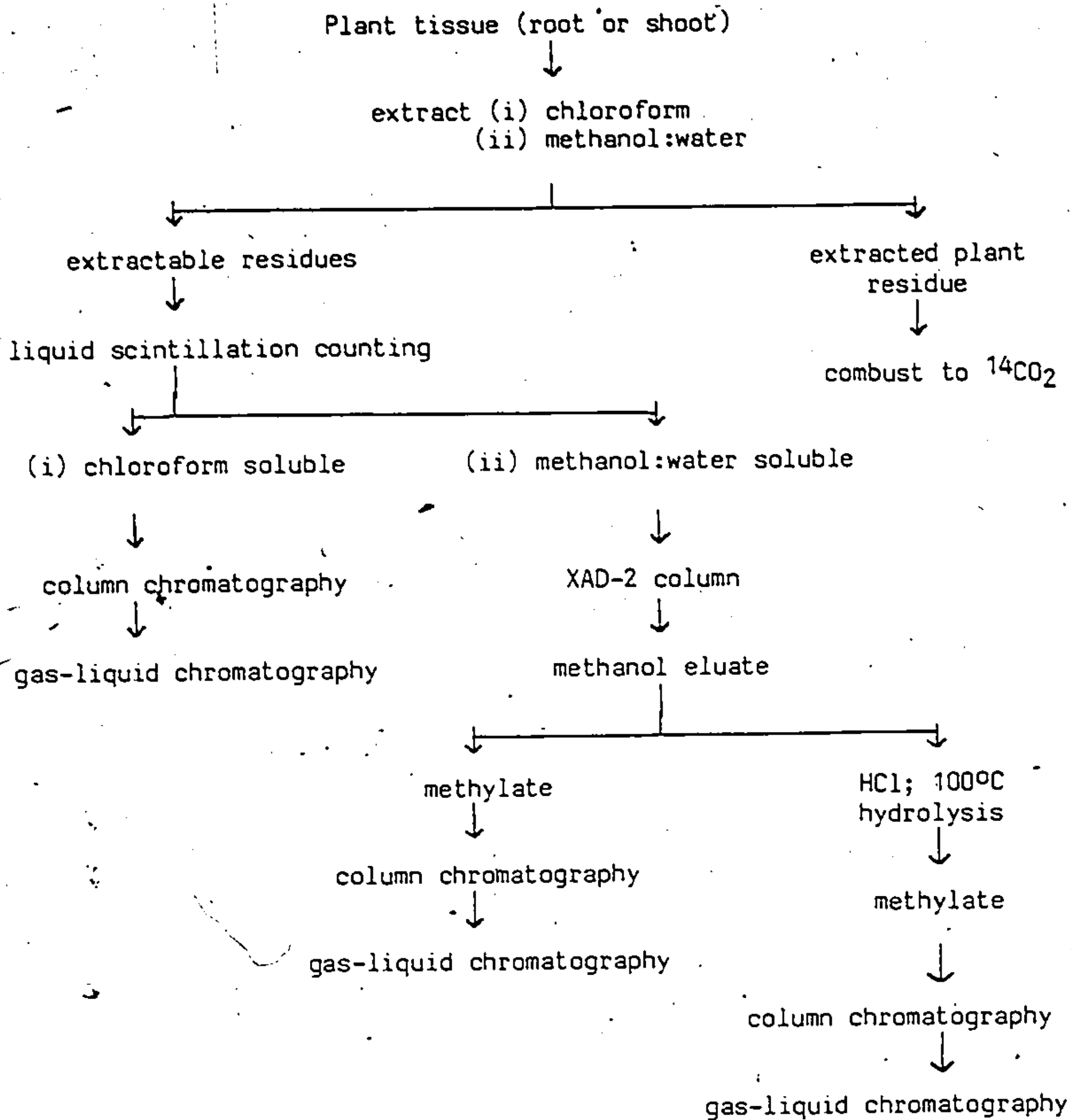


Fig. 16. Plant Tissue Analysis Procedure (from Khan 1980).

The chloroform insoluble-solid residue from the root or shoot tissue was then extracted with methanol (20 mL) for a total of three times and then finally once with 20 mL of water. The filtrates were combined and the methanol was removed on a rotary evaporator leaving a concentrated aqueous phase (polar fraction). The insoluble residue was freeze-dried and sample oxidized and the radioactivity measured by liquid scintillation counting. Because of the small quantity of tissue available, identification of the residues was not possible by using the high temperature distillation procedure.

The aqueous phase was then cleaned up on a XAD-2 (20-50 mesh) column (Khan 1980) by washing the adsorbed water phase with water first and then eluting with methanol. The methanol eluate was concentrated and an aliquot was removed for liquid scintillation counting. The remaining methanol was divided into two fractions for the identification of free and conjugated derivatives. One half was methylated with diazomethane and analyzed by gas-liquid chromatography. The other portion was hydrolyzed with concentrated HCl and then heated at 100°C for 4 h. The mixture was evaporated to dryness with a rotary evaporator, redissolved in methanol, methylated, and injected into the gas-liquid chromatograph.

III. RESULTS

A. PREPARATION OF SOIL-BOUND ^{14}C RESIDUES

Following the procedure of Khan and Hamilton (1980) for the preparation of soil-bound ^{14}C-prometryne (section II.A.4), 40.6% of the initial ^{14}C-prometryne inoculum had become bound in the soil after 121 days of incubation and this value increased to 53.7% after 475 days. The proportion of the bound nonpolar species (chloroform-soluble), which was subsequently identified as prometryne by the HTD method, quantification of the chloroform eluate, and gas-liquid chromatography, was 54.1 and 52.7% of the total bound ^{14}C after 121 and 475 days, respectively. Bound polar (methanol-soluble) species thus represented 20.9 and 23.0% of the total bound radioactivity after 121 and 475 days, respectively. Analysis of the polar fraction permitted the identification of the hydroxy derivative, hydroxyprometryne in the form of the methylated analog, prometone. The remaining radioactivity (25.0 and 24.3%) was thermally decomposed to $^{14}CO_2$ during the high temperature distillation procedure.

The column clean-up procedure was quite efficient in removing background impurities and in recovering the herbicide and its derivatives. By using spiked soil extracts, the percentage recovery of prometone, prometryne, and deisopropylprometryne were 100, 97, and 94%, respectively.

B. PROMETRYNE TOXICITY TO MACROPHYTES AND MICROPHYTES

1. Soybean Susceptibility

Treated plants exhibited a significant decrease in both fresh and dry weights compared with the controls (Fig. 17). Even at the lowest prometryne concentration tested (2.5 µg mL^{-1}), there was a 21.5 and 37.2% reduction in the fresh and dry weights, respectively (Fig. 17). (Probit plots for the weight analyses are in Appendix A and B).

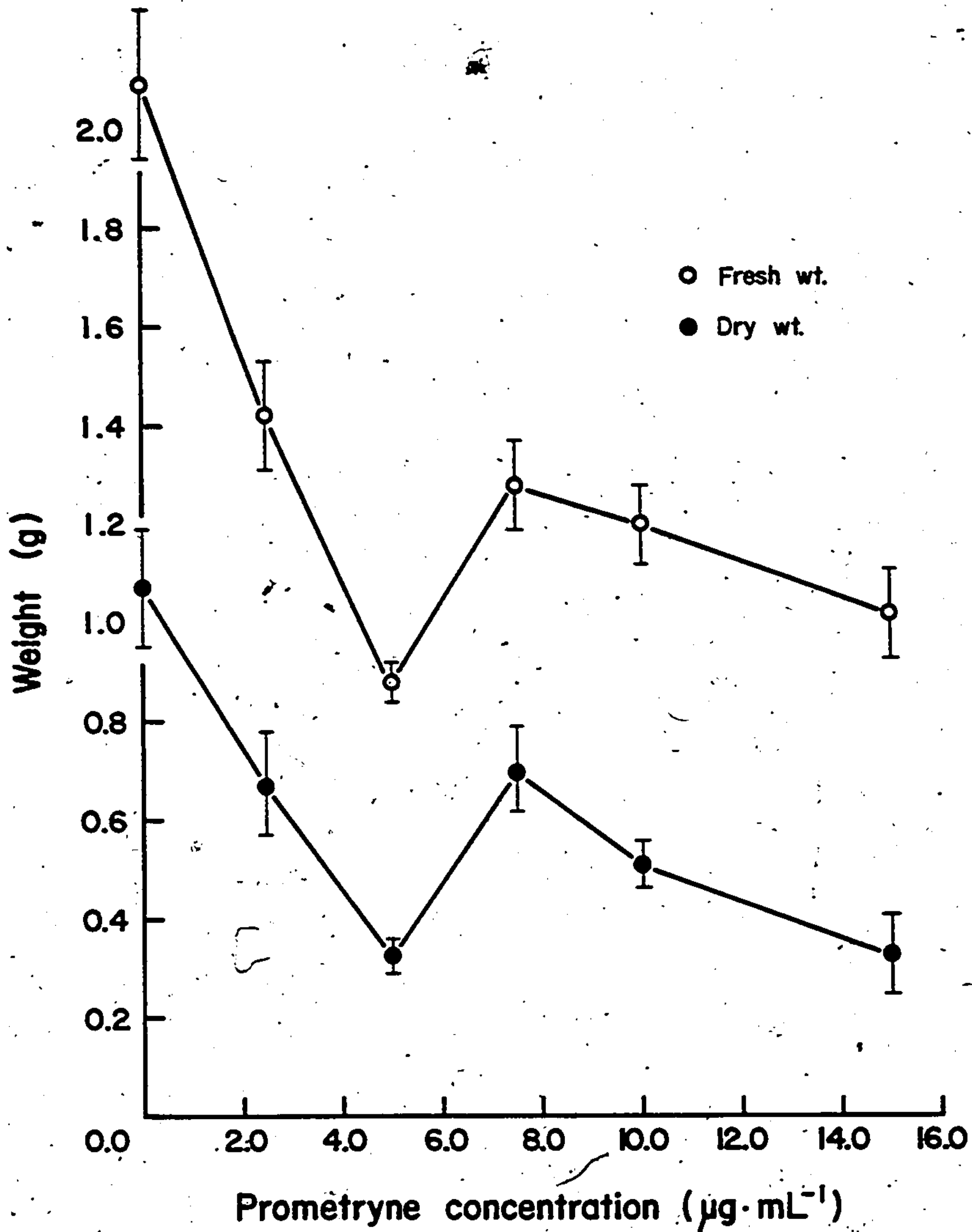
The decrease in the weights of the soybean plants, however, was not reflected in the protein analyses for any of the treatments. The Kjeldahl protein analysis indicated that there were wide fluctuations in the protein content from 10 to 20 mg g^{-1} total dry weight (Fig. 18). However, these protein values were not significantly at different the 95% level.

Protein contents as determined by the Hartree protein procedure, compared with Kjeldahl protein values, assayed considerably less protein in the plant tissues (Fig. 19). Soybean protein contents ranged from 3 to 8 mg g^{-1} total dry weight. However, as in the case of the Kjeldahl results, protein contents in prometryne treated soybeans were not significantly different from the control protein values (P=0.05).

Although the herbicide exposure had no effect on the protein content of soybean plants, exposure to prometryne for 2 weeks did have deleterious effects on its growth. Individual biomass was reduced significantly, even at the lowest concentration tested. In addition, morphological effects were observed. Prometryne exposure resulted in

Figure 17

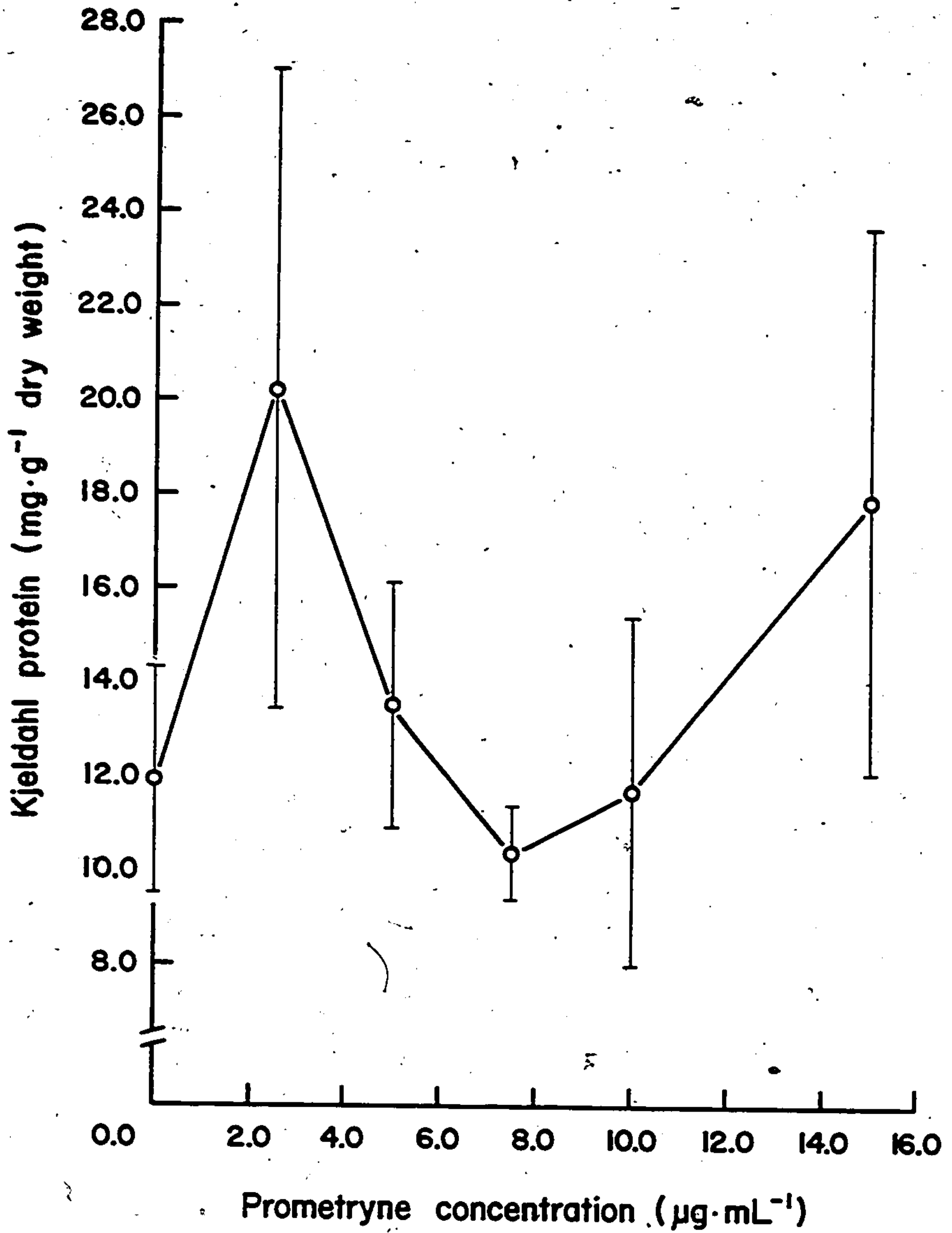
Effect of Prometryne on the Fresh and Freeze-Dried Weights of Soybean (*Glycine max* (L.) Merr. cv. Maple Presto). Mean ± S.E., n=6.



Weight (g)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
Fresh wt.
Dry wt.
Prometryne concentration (µg·mL⁻¹)

Figure 18

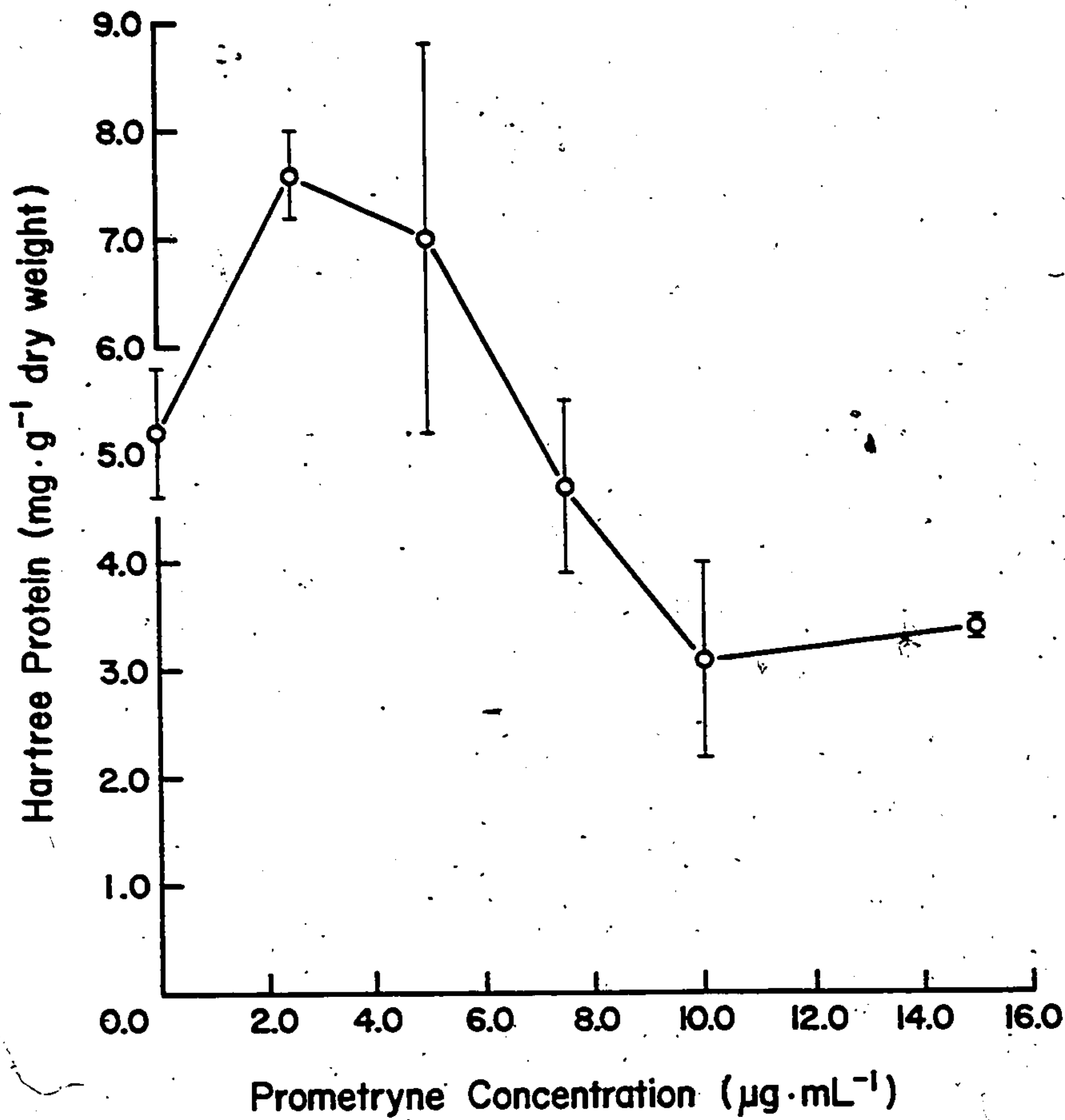
Effect of Prometryne on the Protein Estimates of Soybean (*Glycine max* (L.) Merr. cv. Maple Presto) as Measured by Kjeldahl Analysis. Mean ± S.E., n=6.



28.0
26.0
24.0
22.0
20.0
18.0
16.0
14.0
12.0
10.0
8.0
Kjeldahl protein (mg·g⁻¹ dry weight)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
Prometryne concentration (µg·mL⁻¹)

Figure 19

Effect of Prometryne on the Protein Content of Soybean (*Glycine max* (L.) Merr. cv. Maple Presto) as Determined by the Hartree Protein Assay. Mean ± S.E., n=6.



Hartree Protein (mg·g⁻¹ dry weight)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
Prometryne Concentration (μg·mL⁻¹)

stunted growth and chlorosis (Fig. 20), even at 2.5 μg mL^{-1}. Leaf development in the treated plants was severely restricted with eventual mortality of the plants.

2. Algae - Chlamydomonas segnis Ettl

At the end of the experimental period, corresponding to G_1 of the cell cycle, the synchronous cultures of C. segnis Ettl cells exposed to acetone (0.13% v/v) exhibited the same stepwise increase in cell numbers as the control cultures (Fig. 21). In both the control and treated sets, the initial concentration of 20 x 10^4 cells mL^{-1} doubled to 40.1 x 10^4 and 37.4 x 10^4 cells mL^{-1}, respectively. However, in all treatment sets with prometryne (0.75 - 12.0 μg mL^{-1}), the concentration of C. segnis Ettl cells remained unchanged at the initial inoculum level of 20 x 10^4.

Following 14 days of growth, the colony counts of washed and plated cells exposed to 12.0 and 3.0 μg mL^{-1} prometryne evidenced a 50% reduction in numbers and those exposed to 0.75 μg mL^{-1} prometryne showed a 23.4% reduction compared to the untreated controls (Table 8). Interestingly, in the control sets, only 35% of the cells in the original culture survived the distilled water washes to form colonies on the nutrient agar. However, in the 0.75 and 3.0 μg mL^{-1}, 35% of the cells remained viable, forming colonies on the nutrient agar.

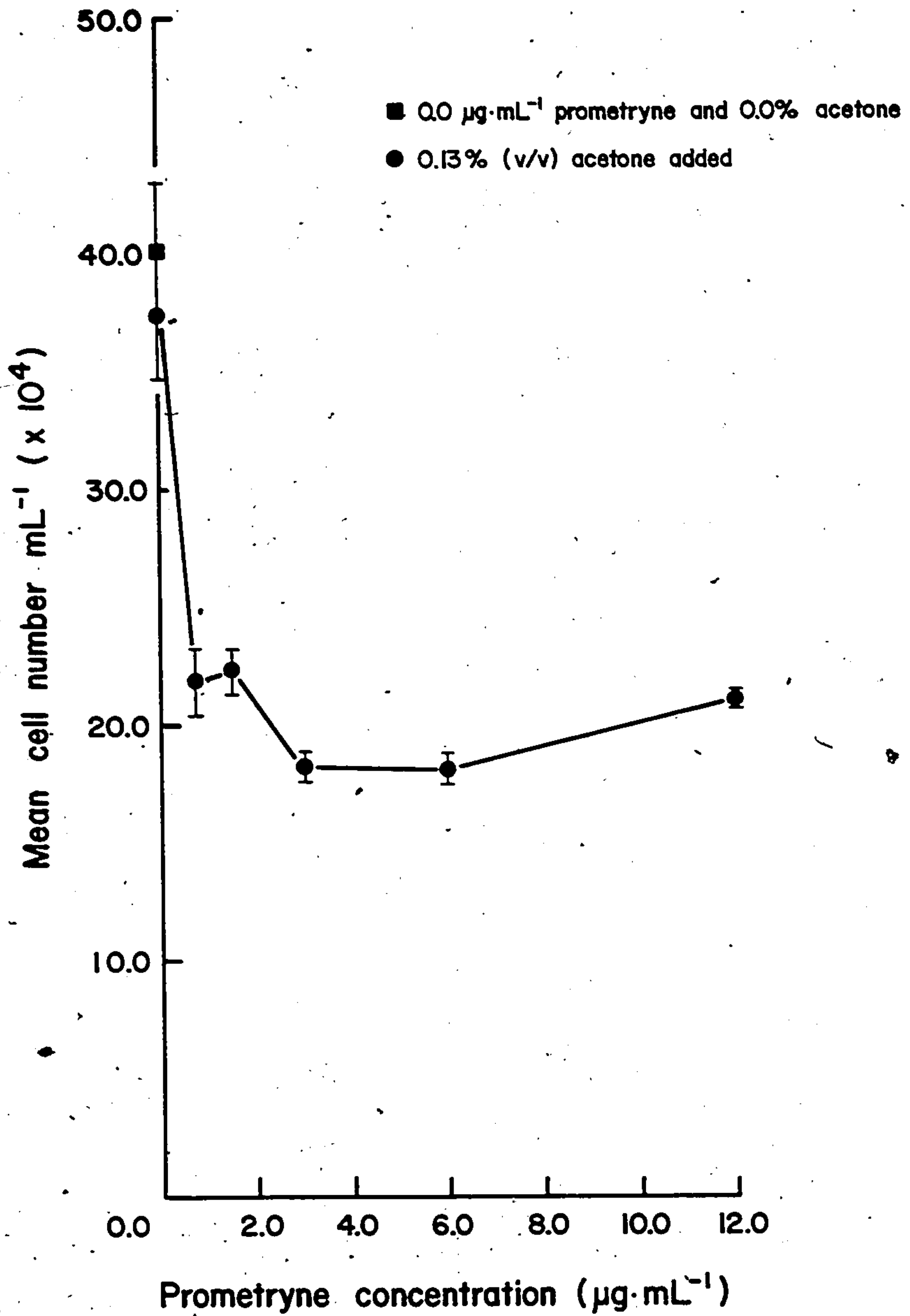
Figure 20

Effect of Prometryne on the Morphology of Soybean (*Glycine max* (L.) Merr. cv. Maple Presto).

COLOURED PICTURES
Images en couleur

Figure 21

Effect of Prometryne on the Synchronous Cultures of Chlamydomonas segnis Ettl After One Cell Cycle. Exposure was at the beginning of the S-phase (2 h before the dark cycle) and Initial Cell Numbers Were 20×10^4 Cells mL^{-1}. Mean ± S.E., n=6.



0.0 μg·mL⁻¹ prometryne and 0.0% acetone
0.13% (v/v) acetone added
Mean cell number mL⁻¹ (x 10⁴)
50.0
40.0
30.0
20.0
10.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Prometryne concentration (μg·mL⁻¹)

Table 8

Recovery of Chlamydomonas segnis to Prometryne Following Plating on Fresh Nutrient Agar*.

Treatment	No. of Colonies/ Plate	% of Inoculum	% Control
Control	1423 ± 60.3 a**	35 a**	-
Acetone Control	1505 ± 72.6 a	40 a	105.8
0.75 μg mL^{-1} Prometryne	1090 ± 132.2 b	50 a	76.6
3.0 μg mL^{-1} Prometryne	755 ± 111.9 c	41 a	53.0
12.0 μg mL^{-1} Prometryne	746 ± 86.9 c	35 a	52.4

* Mean ± standard error for 6 replicates

**Means followed by different letters are significantly different (P=0.05) from others in the same column as determined by one-way analysis of variance (ANOVA) and Student-Newman-Kuel's multiple range test.

3. Heterocyst Study - Anabaena spp.

Following treatment with acetone (0.25% v/v), the Anabaena population showed a trend toward increasing heterocyst frequency although this increase was not significantly different from the control sets at the 90% level (Fig. 21). An insignificant reduction in heterocyst frequency was observed in cultures treated with 0.1 μg mL^{-1} prometryne. However, at higher concentrations of prometryne (0.5 to 10.0 μg mL^{-1}), heterocyst frequency was significantly reduced by 65 - 93% (Fig. 22) at the 95% level. Probit analysis of heterocyst frequency is in Appendix C.

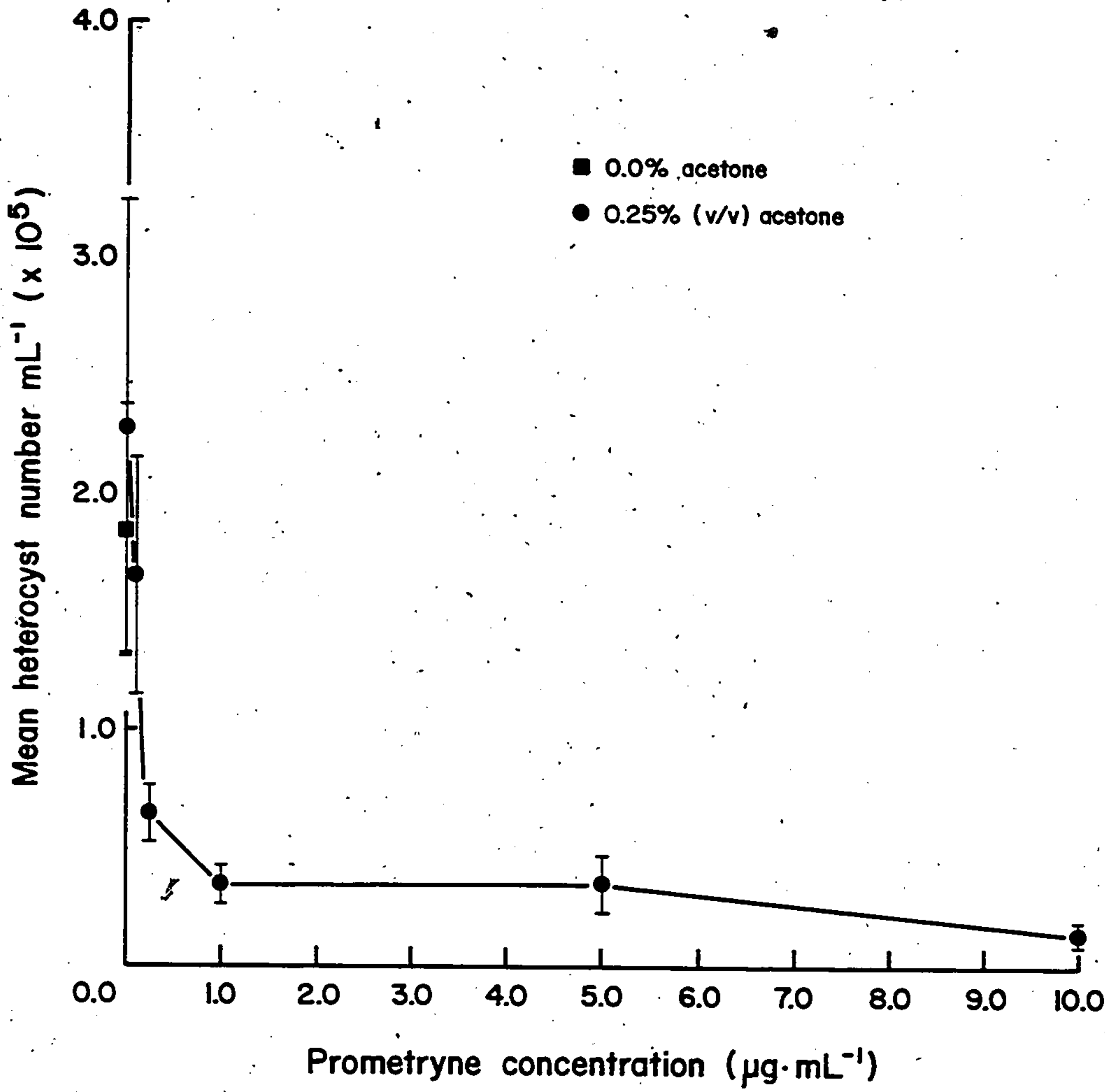
The effects of prometryne on Anabaena spp. persisted after the herbicide was removed from the media. When the cells were washed and then plated on nutrient agar, the prometryne-treated cultures continued to show a significant reduction in growth compared with the control and acetone treated sets (Fig. 23). Filamentous growth of Anabaena spp. was observed in the control and acetone treated plates, whereas the prometryne treated cultures showed little filamentous growth.

4. Rhizobium japonicum and Klebsiella pneumoniae

Apart from one exception, exposure of K. pneumoniae strain M5A1 and R. japonicum strain 61A76 to the range of prometryne concentrations (0.25 - 6.8 μg mL^{-1}) did not evoke significant effects on the generation time (Fig. 24 and 25). In most of the concentrations tested, the generation time remained at the control levels, that is, 0.78 h and 9.5 h for K. pneumoniae and R. japonicum, respectively. The single

Figure 22

Effect of Prometryne on the Heterocyst Frequency of *Anabaena* spp. After One Week of Growth. Mean ± S.E., n=6.



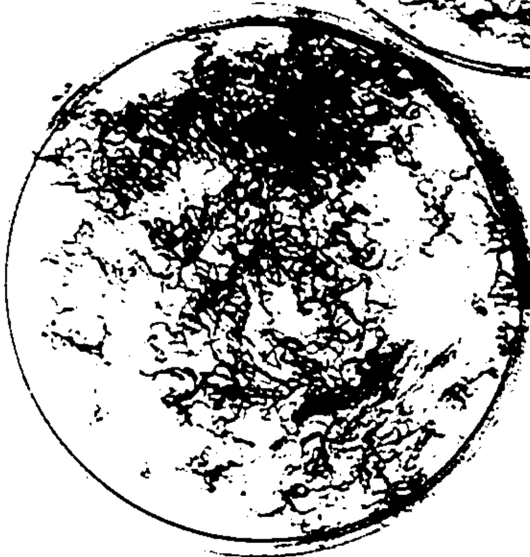
0.0% acetone
0.25% (v/v) acetone
Mean heterocyst number mL⁻¹ (x 10⁵)
4.0
3.0
2.0
1.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
Prometryne concentration (µg·mL⁻¹)

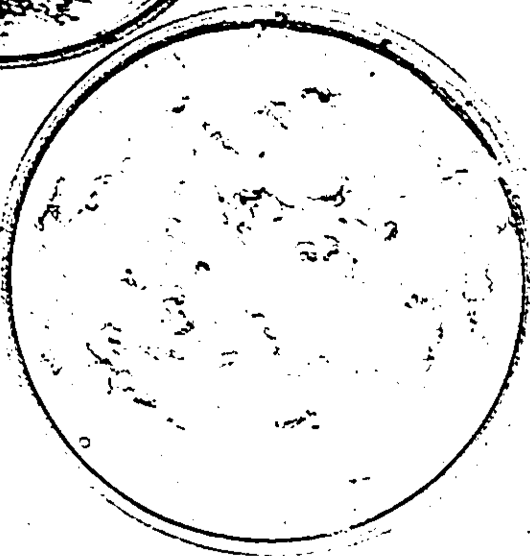
Figure 23

Recovery of *Anabaena* spp. From Prometryne Exposure Following Distilled Water Washes and Plating on Pesticide-Free Nutrient Agar. Note Decreased Filamentous Growth in Cultures Containing Cells Previously Exposed to Prometryne. A-Controls; B-Acetone-Treated; C-10 μg mL^{-1} Prometryne; D-1.0 μg mL^{-1} Prometryne; and E-0.1 μg mL^{-1} Prometryne.

A

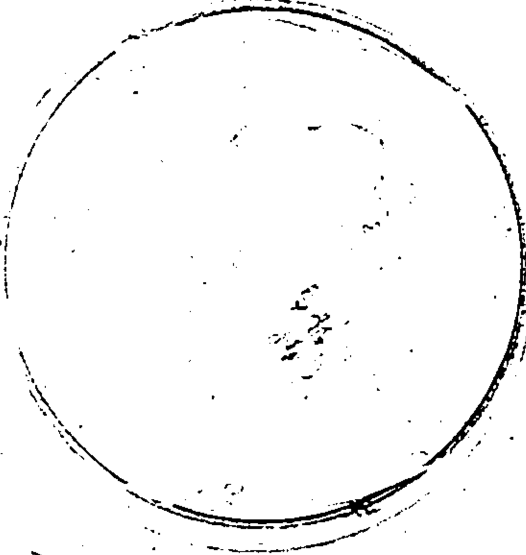
B

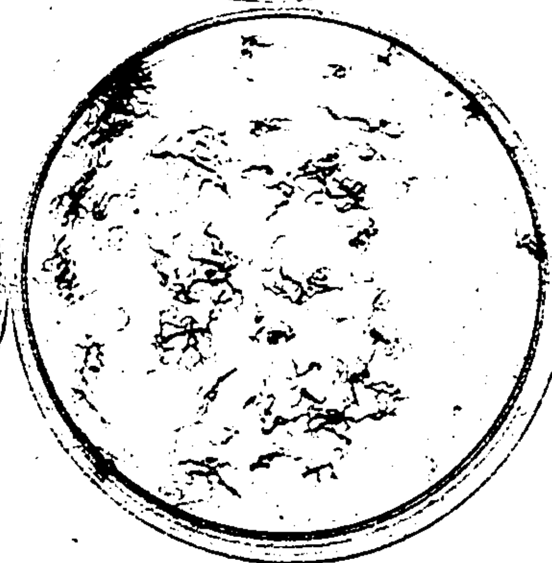




C

D





E

Figure 24

The Effect of Prometryne on the Generation Time of the Nitrogen-Fixing Bacteria, *Rhizobium japonicum* strain 61A76. Mean ± S.E., n=3. Note the Increased Generation Time at 6.8 μg mL^{-1} Prometryne.

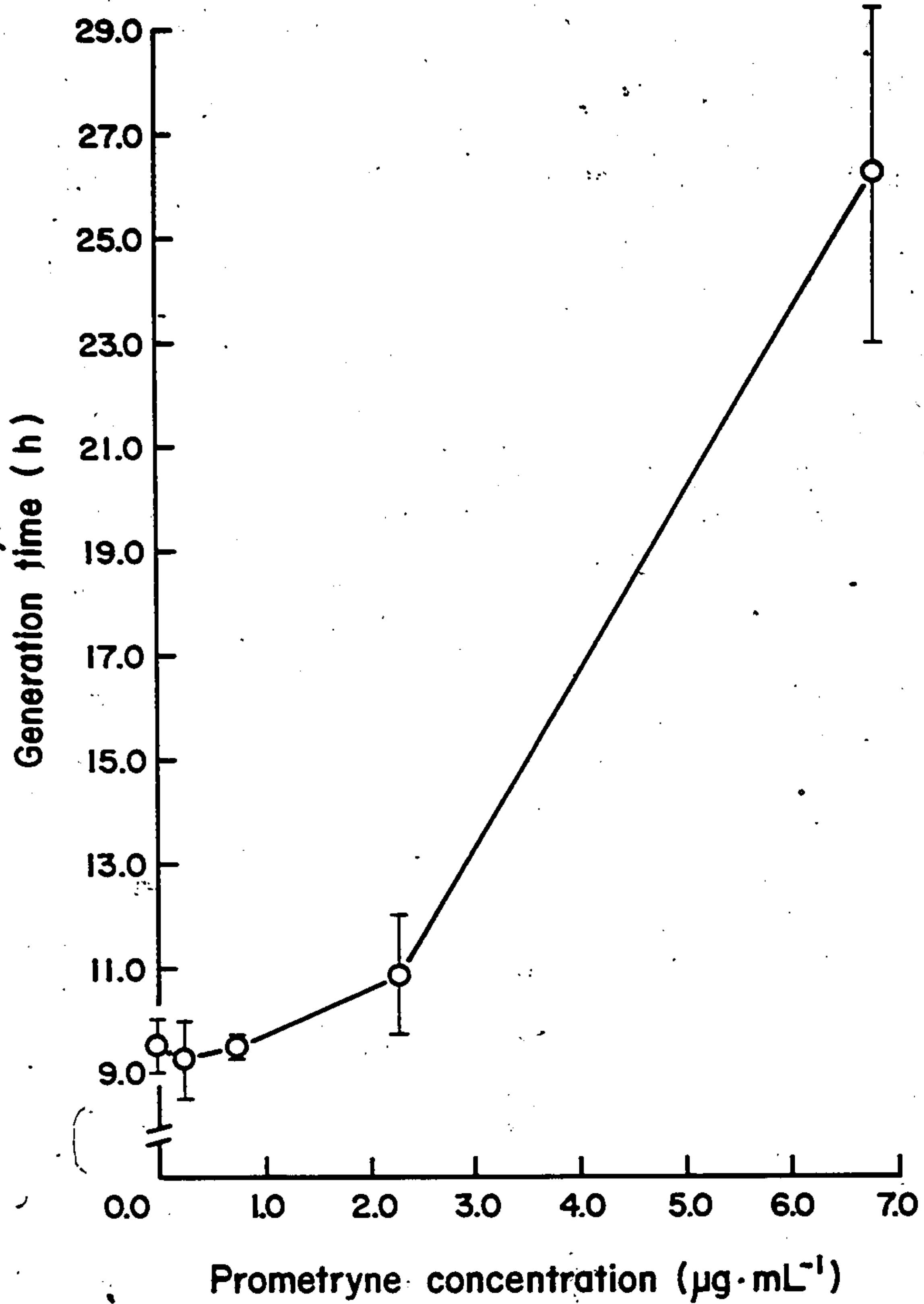
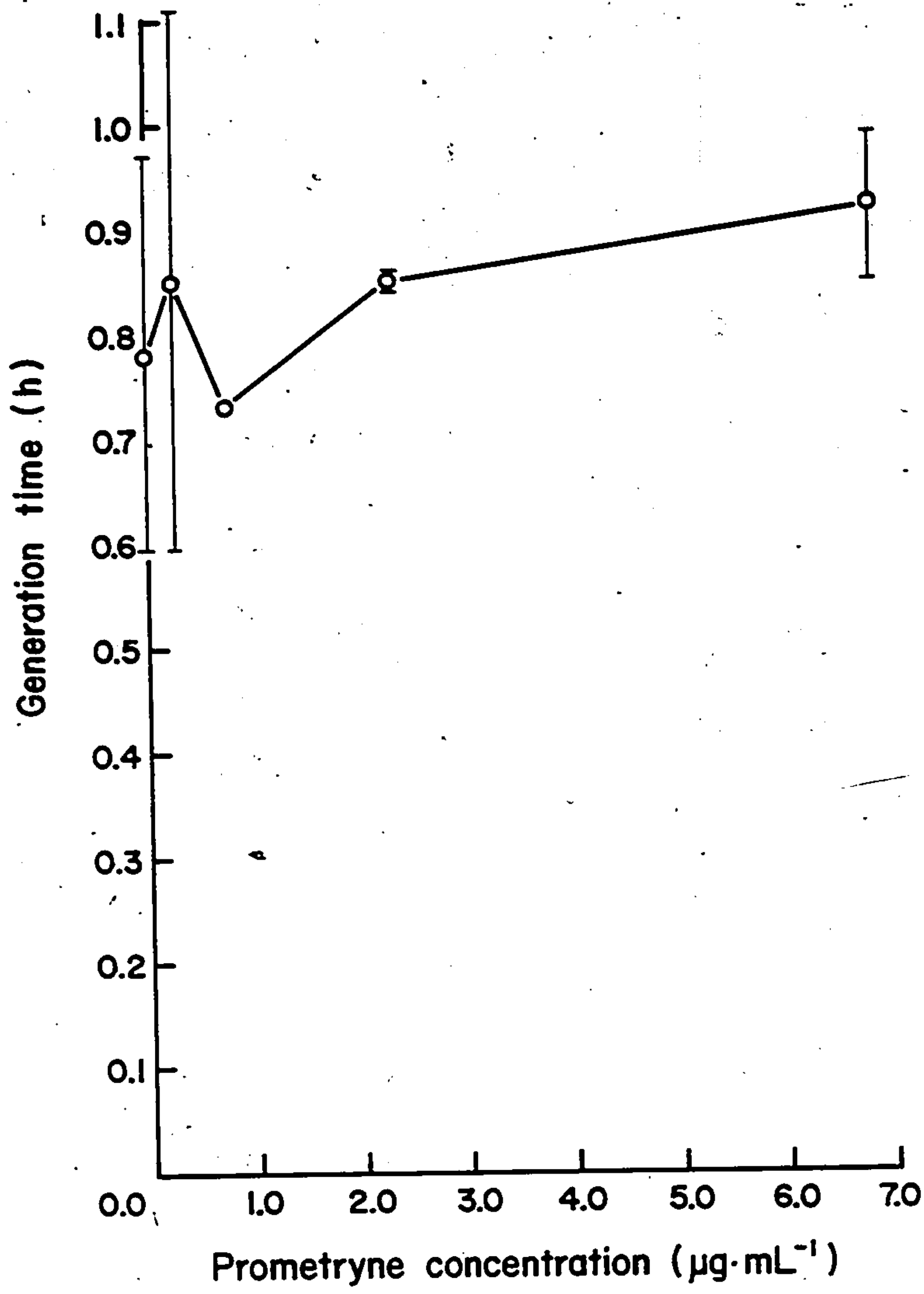

29.0
27.0
25.0
23.0
21.0
19.0
17.0
15.0
13.0
11.0
9.0
Generation time (h)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
Prometryne concentration (µg · mL⁻¹)

Figure 25

The Effect of Prometryne on the Generation Time of the Nonsymbiotic Nitrogen-Fixing Bacteria, Klebsiella pneumoniae strain M5A1. Mean ± S.E., n=3.



Generation time (h)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
Prometryne concentration (µg·mL⁻¹)

exceptions were the sets of *R. japonicum* treated with 6.8 µg mL^{-1} where a three-fold increase in the generation time from 9.5 h to 26.25 h was obtained.

C. ROOT ALTERATION OF SUBSTRATE pH

1. Hydroponic pH Changes

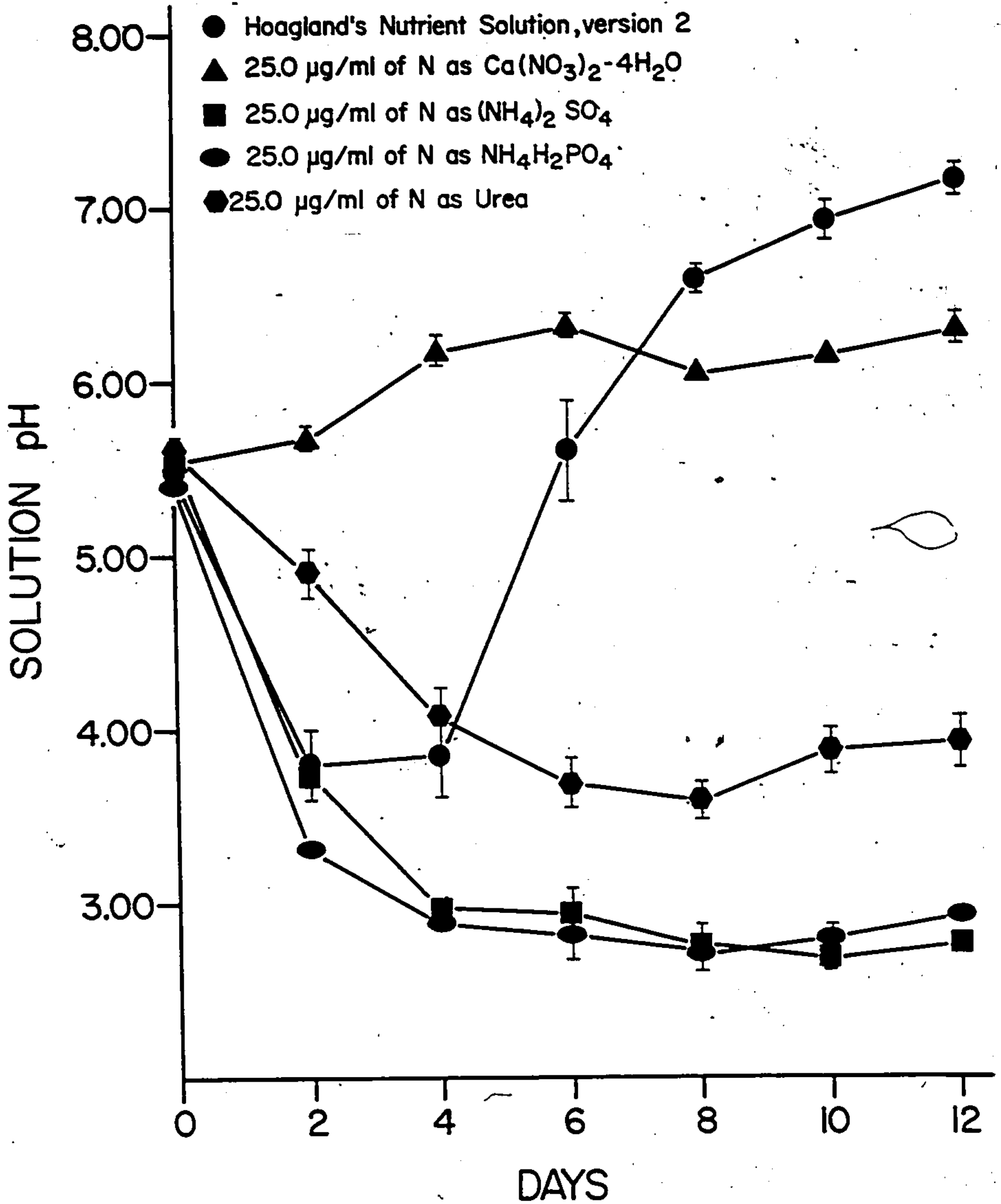
The source of nitrogen plays an important effect on the root's ability to alter the pH of the hydroponic solution (Fig. 26 and 27). Wheat plants grown in distilled water elicited a steady decrease in solution pH to pH 4.3 after 12 days (Fig. 27). However, for plants grown in Hoagland's nutrient solution, No. 2, there was an initial decrease to pH 3.8 after 2 days. Following this, there was a steady increase in solution pH to pH 7.2 (Fig. 26).

An immediate increase in pH was noted in the nitrate-fertilized wheat seedlings and no initial decrease in pH was apparent. The pH increased steadily during the 12 days monitored (Fig. 26). As the nitrate concentration increased, the final pH value after 12 days of growth was higher. At 25.0 µg mL^{-1} of nitrogen as nitrates, the final hydroponic pH was pH 6.3 whereas for 50 and 1000 µg mL^{-1}, the final values were pH 6.7 and 7.5, respectively (Fig. 26 and 27).

In the ammonium-fertilized seedlings, they produced an acidification of the solution irrespective of the source. Both $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ fertilized plants resulted in the same level of

Figure 26

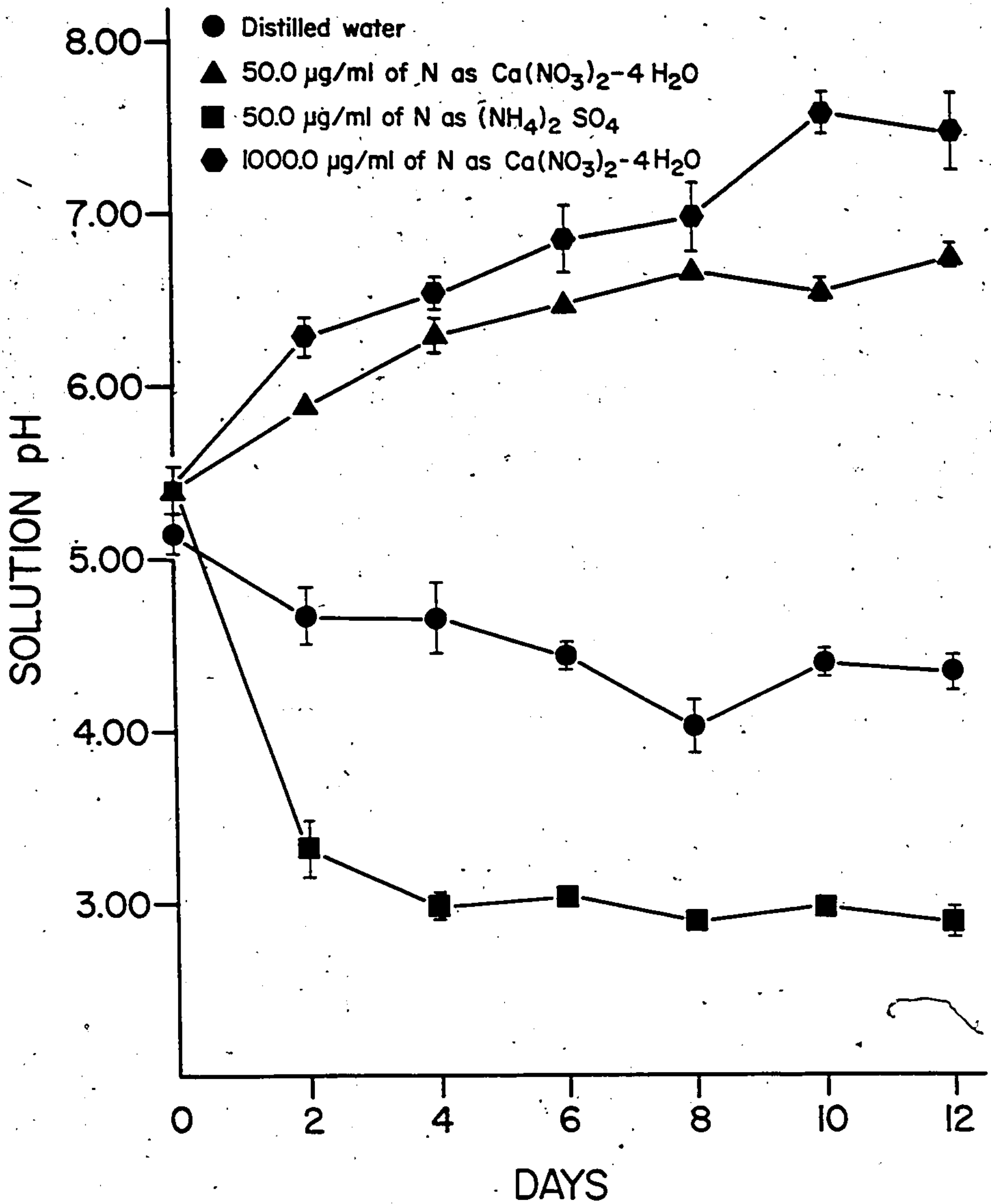
Wheat Root Induced Changes of Hydroponic Solution pH Fertilized with Different Nitrogen Sources. Mean ± S.E., n=6



Hoagland's Nutrient Solution, version 2
25.0 μg/ml of N as Ca(NO3)2-4H2O
25.0 μg/ml of N as (NH4)2 SO4
25.0 μg/ml of N as NH4H2PO4
25.0 μg/ml of N as Urea
8.00
7.00
6.00
5.00
4.00
3.00
SOLUTION pH
0
2
4
6
8
10
12
DAYS

Figure 27

Wheat Root Induced Changes of Hydroponic Solution pH Fertilized with Different Nitrogen Sources. Mean ± S.E., n=6



Distilled water
50.0 µg/ml of N as Ca(NO3)2-4 H2O
50.0 µg/ml of N as (NH4)2 SO4
1000.0 µg/ml of N as Ca(NO3)2-4 H2O
SOLUTION pH
8.00
7.00
6.00
5.00
4.00
3.00
0
2
4
6
8
10
12
DAYS

acidification, that is, pH 2.6 - 3.0 (Fig. 26). Even doubling the concentration of nitrogen as ammonium ions in $(NH_4)_2SO_4$ fertilized plants (Fig. 26) did not alter the final pH (2.9). Higher concentrations of ammonium were not tested due to the toxicity of these ions at high concentrations. Unlike the steady increase in solution pH for nitrate-fertilized plants, the most dramatic pH changes for ammonium-fertilized plants occurred within 2 days (Fig. 26 and 27).

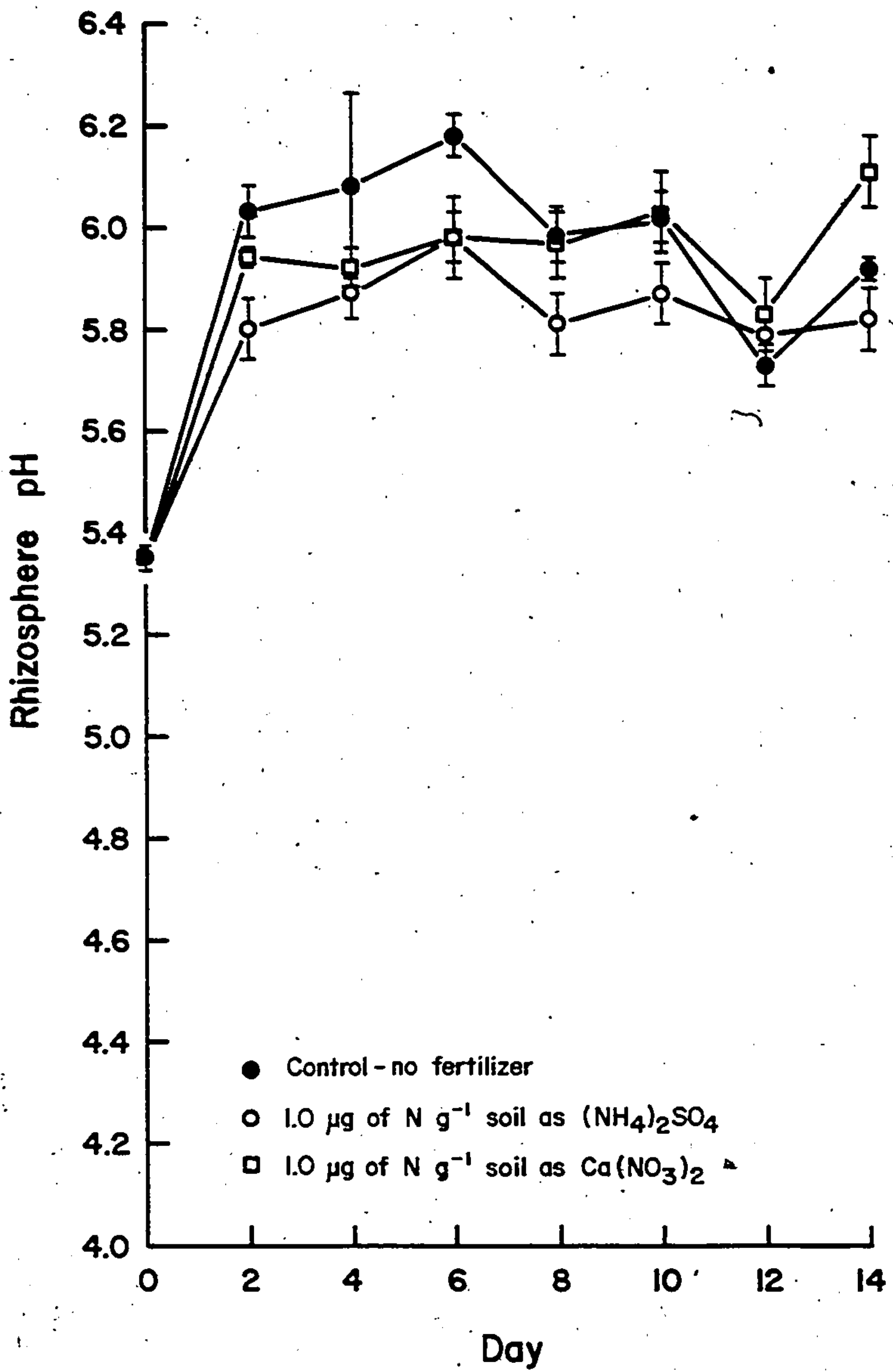
When the plants were provided with urea as the nitrogen source, there was a definite acidification of the media (Fig. 26). The pH of the solution after 12 days was pH 4.0 and the pattern of acidification was similar to that of plants grown in distilled water. However, the final pH was significantly different from the pH of the distilled water set (pH 4.3) at the 95% level of significance.

2. pH Microelectrode Analysis

The addition of wheat plants to the soil significantly increased the pH from the initial value (Fig. 28). The wheat roots had alkalized the soil from pH 5.4 to values greater than pH 5.8 within two days. As well, the pH values fluctuated considerably during the growth period. Wheat seedlings grown without any augmented nitrogen source exhibited higher rhizosphere pH values than seedlings fertilized with either ammonium or nitrate salts. Ammonium fertilization appeared to lower the pH in the rhizosphere while nitrate fertilization had an intermediate effect. Generally, none of these differences were significant (P=0.05), and consequently, no major trend in rhizosphere pH relative to fertilization

Figure 28

Influence of Nitrogen Sources on Rhizosphere Soil pH With Time (pH Microelectrode Measurements). Mean ± S.E., n=6. (One way analysis of variance, P = 0.05).



Rhizosphere pH
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
0
2
4
6
8
10
12
14
Day
Control - no fertilizer
1.0 µg of N g⁻¹ soil as (NH4)2SO4
1.0 µg of N g⁻¹ soil as Ca(NO3)2

could be identified. After 14 days, the final values were pH 5.9, 5.8 and 6.1 for the control, ammonium-, and nitrate-fertilized plants, respectively.

3. Wheat Rhizosphere and Rhizoplane pH

In Table 9, soil pH values for the rhizosphere and rhizoplane, as measured from a soil suspension, are shown for ammonium- and nitrate-fertilized plants. As in the microelectrode measurements, when plants were grown in the soil, there was an increase in pH from the initial level of soil pH (5.4).

In each case, there was little difference in soil pH between the rhizosphere and rhizoplane. Control plants produced a rhizosphere and rhizoplane pH of 5.8. Similarly, the ammonium-fertilized plants gave values of pH 5.8 and pH 5.7. However, wheat seedlings fertilized with nitrate salts were able to increase the soil pH significantly. In the rhizosphere and rhizoplane, a pH of 6.3 was obtained.

4. Fertilizer Influence on Soil pH

There was no difference in the soil pH after the application of any of the nitrogen fertilizers (Table 10). The soil remained at pH 5.3 in all cases. As a result, the use of the fertilizers, by themselves, did not contribute significantly to any changes in pH.

Table 9

Rhizosphere and Rhizoplane pH of Wheat
(*Triticum aestivum* (L.) Merr. cv. Marquis) as Measured
by 1:1 (v/v) Suspension With 0.01 M $CaCl_2$

Treatment	Soil pH*	
	Rhizoplane	Rhizosphere
Control-No Fertilizer	5.84 ± 0.06 a**	5.78 ± 0.04 a
$Ca(NO_3)_2$	6.29 ± 0.10 b	6.34 ± 0.10 b
$(NH_4)_2SO_4$	5.74 ± 0.06 a	5.83 ± 0.10 a

* Mean ± standard error, n = 6
**Means followed by different letters are significantly different (P=0.05) as determined by one-way analysis of variance (ANOVA) and Student-Newman-Kuel's multiple range test.

Table 10

Influence of Nitrogen Fertilizers on the Organic Soil pH

Fertilizer	Nitrogen Source	Soil pH*
Control - No Fertilizer	-	5.35 ± 0.02 a**
Hoagland's Nutrient Solution	NH_4^+, NO_3	5.35 ± 0.02 a
$Ca(NO_3)_2$	NO_3	5.30 ± 0.02 a
$(NH_4)_2SO_4$	NH_4	5.33 ± 0.02 a
Urea	H_2NCONH_2	5.33 ± 0.02 a

* Mean ± Standard Error, n = 6
**Means followed by different letters are significantly different (P=0.05) as determined by one-way analysis of variance (ANOVA) and Student-Newman-Kuel's multiple range test.

5. Root Mediated Changes in Agar

In Table 11, localized pH changes around the wheat seedling roots were recorded weekly for each fertilizer treatment. In addition, root mediated changes in pH for soybeans fertilized with Hoagland's nutrient solution, No. 2, were also reported.

The control plates did not show any colour change from the initial plates when the indicator solutions were added. They remained constant at pH 5.5. When wheat seedlings were grown under axenic conditions, considerable colour changes of the pH indicator solution were evidenced, representing changes in the agar pH (Fig. 29 and 30). After the first week, plants fertilized with Hoagland's nutrient solution, No. 2, displayed an initial decrease from pH 5.5 to pH 5.0, followed by a subsequent increase to pH 9.5 in the remaining two weeks of the experiment. Wheat plants grown in the agar augmented by nitrate salts, demonstrated no initial acidification but only a steady increase in pH. After three weeks, pH 8.5 was obtained by these plants. These patterns of pH change appear consistent with the hydroponic solution pH changes described earlier (section III.C.1).

Similarly, a rapid acidification of the agar media was observed for wheat seedlings grown in an ammonium-fertilized media. This acidification to pH 4.0 occurred within the first week of growth and the level was maintained in the remaining weeks. Urea fertilization, however, produced no major change in pH during the three weeks the roots were monitored.

Table 11

Root Induced Changes in Agar pH With Time as Determined by pH Indicator Solutions (pH ± 0.05)

Nitrogen Source		Week 1	2	3
Control-No Plants	-	5.5	5.5	5.5
Wheat				
Hoagland	NH_4^+, NO_3^-	5.0	8.0	9.5
$Ca(NO_3)_2$	NO_3^-	6.5	7.5	8.5
$(NH_4)_2SO_4$	NH_4^+	4.0	4.0	4.0
Urea	H_2NCONH_2	5.5	5.5	5.5
Soybean				
Hoagland	NH_4^+, NO_3^-	5.5	5.2	4.5

Figure 29

Control Agar Plate After Three Weeks Without Plants.

Universal pH Indicator Solution Added.

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Images en couleur

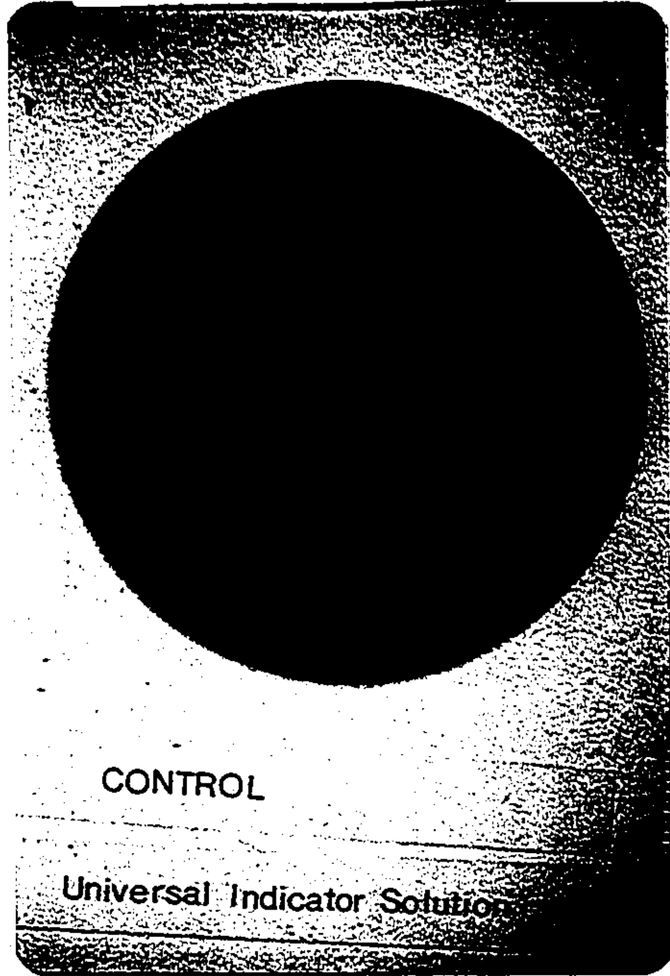
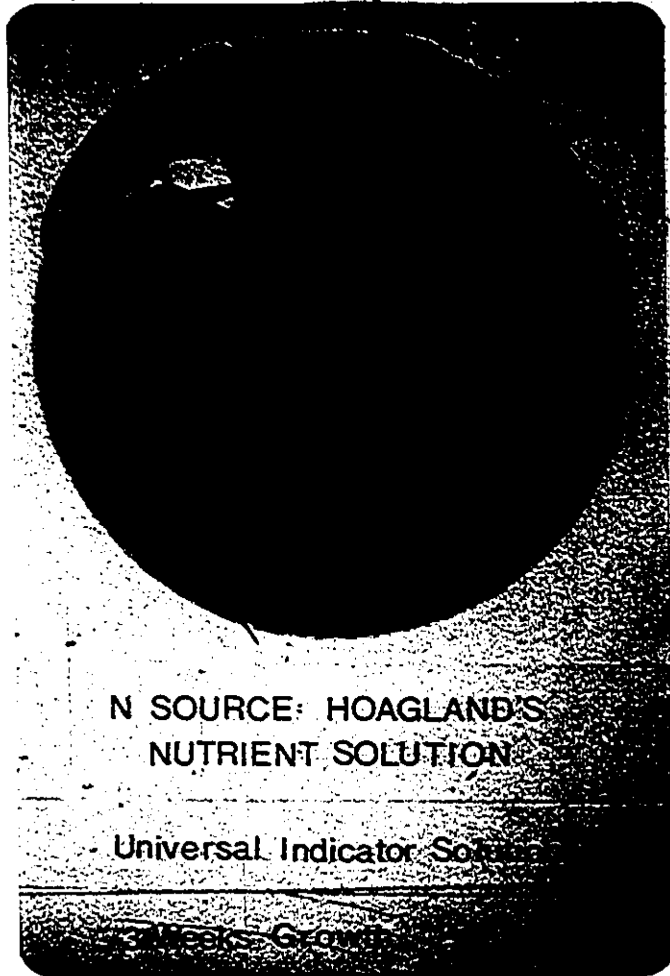


Figure 30

Hoagland's Nutrient Plate With Wheat Seedling After Three Weeks of Growth. Universal pH Indicator Solution Added. Note that the open space in the agar in the upper left is due to displacement of the agar by plant growth.

COLOURED PICTURES
Images en couleur



Unlike the wheat seedlings, soybean seedlings grown in agar augmented with Hoagland's nutrient solution, did not alkalize the media during three weeks of growth. Rather, the roots slowly acidified the media so that after three weeks of growth, the pH remained at pH 4.5.

D. EFFECT OF CHANGE IN pH ON THE RELEASE OF SOIL-BOUND ^{14}C-PROMETRYNE RESIDUES

Altering the pH of the soil containing bound residues with buffers (pH 4 to 8) and incubation for two weeks led to a differential release in the soil-bound radioactivity (Fig. 31). The released radioactivity was significantly minimized at pH 4.9 while significant increases or decreases from the original soil pH of 5.4 resulted in more extractable radioactivity. At pH 4.9, 15.7% of the total bound ^{14}C was extractable and 25.2% was released at pH 8.0. Similarly, 24.1% of the bound ^{14}C was released when the soil was incubated at pH 4.0.

Gas chromatographic analysis of the chloroform eluate (nonpolar fraction) from the cleanup of these extracts indicated that only prometryne was found in this fraction. The hydroxy analogue, hydroxyprometryne, which was derivatized to prometone, was the only product identified in the methanol eluate (polar fraction).

In most cases, the majority of the released material was the parent herbicide (Fig. 32 and 33). Prometryne represented 65 - 72% of the released radioactivity whereas hydroxyprometryne constituting the

Figure 31

Release of Soil-Bound ^{14}C as a Function of the pH of the Incubated Soil. Mean ± S.E., n=6

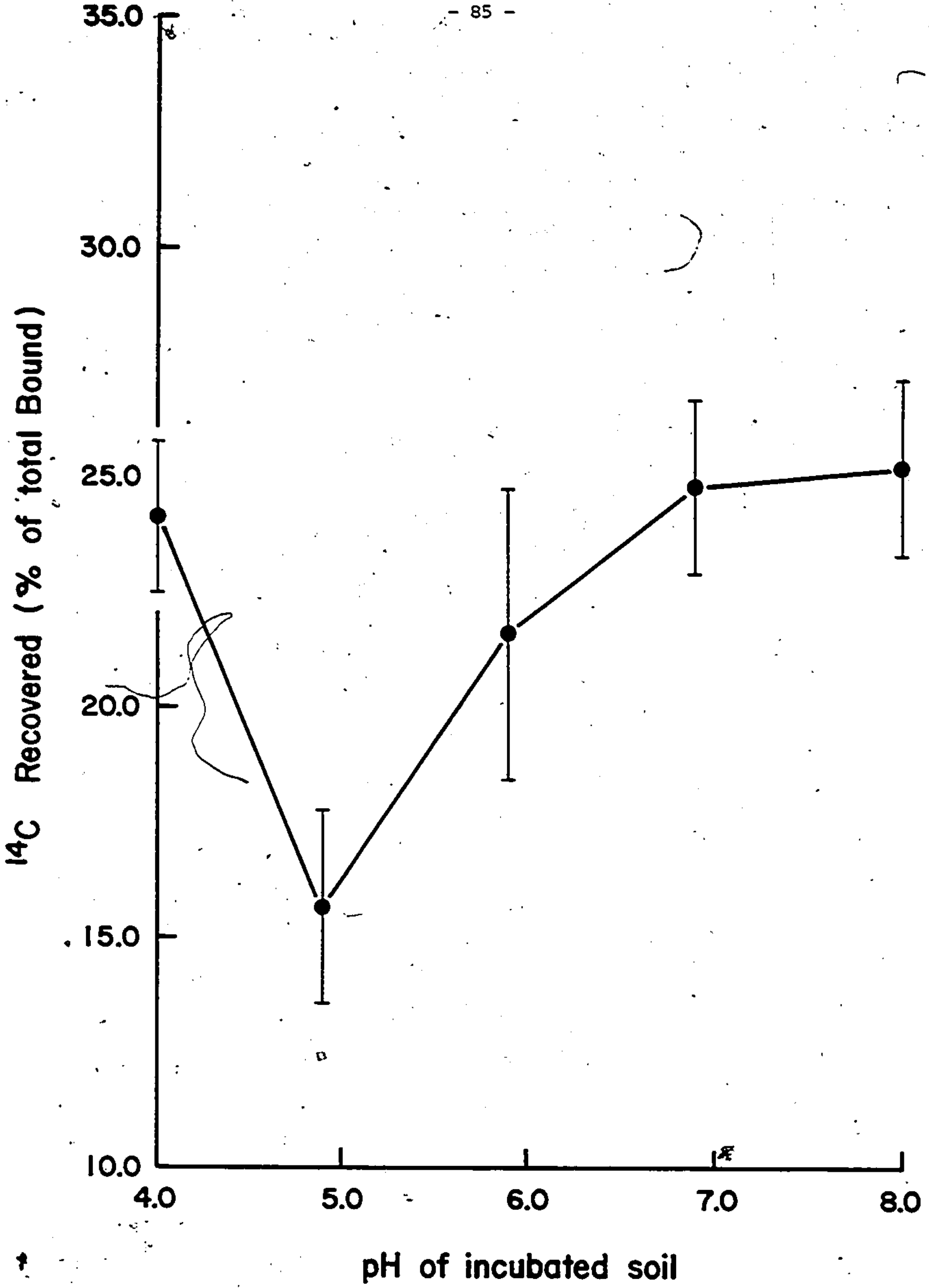

35.0
30.0
25.0
20.0
15.0
10.0
¹⁴C Recovered (% of total Bound)
4.0
5.0
6.0
7.0
8.0
pH of incubated soil

Figure 32

The Proportion of Released ^{14}C-Prometryne and ^{14}C-Hydroxyprometryne from Soil Containing Bound ^{14}C as a Function of the pH of the Incubated Soil. Mean ± S.E., n=6.

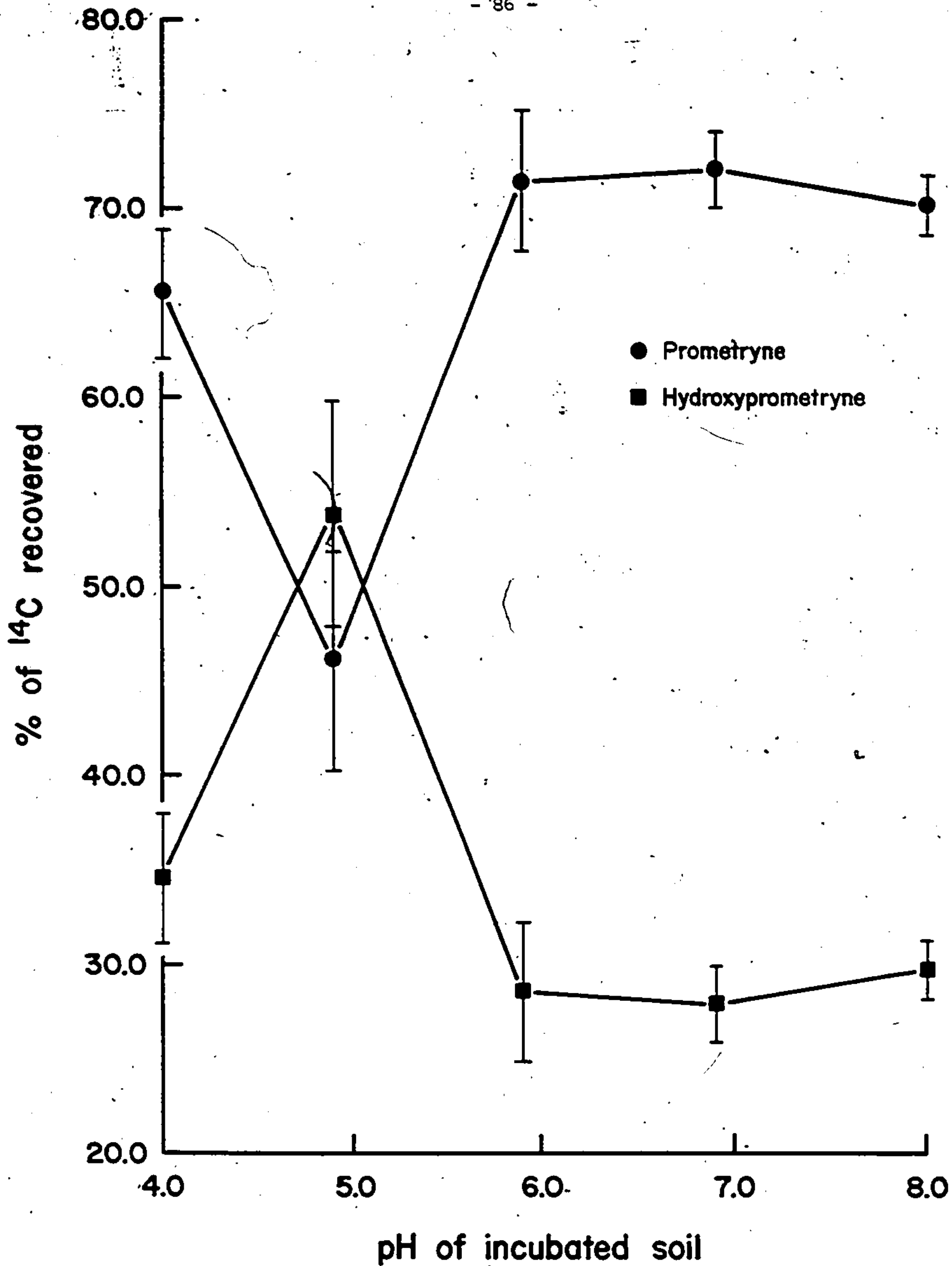
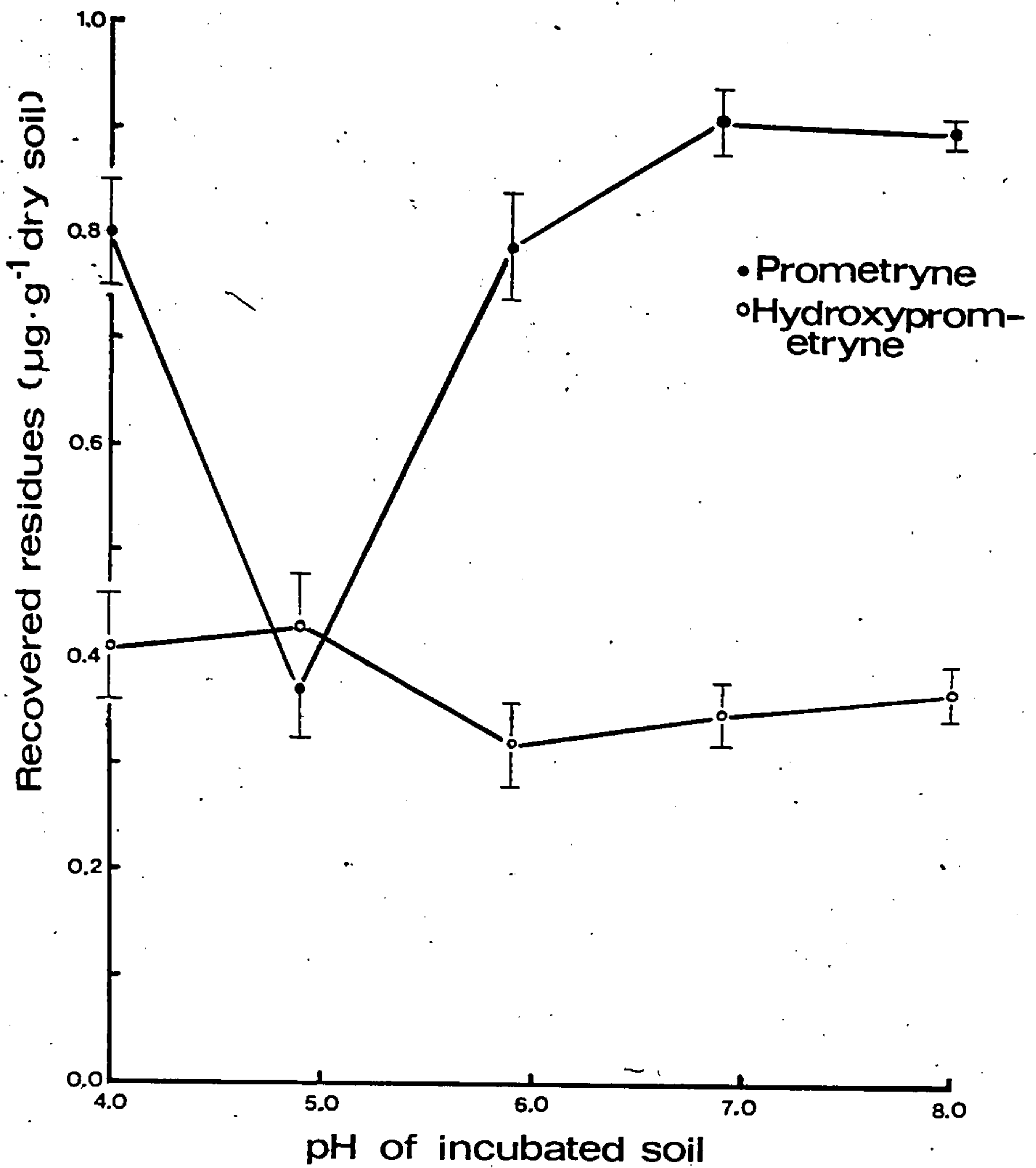

80.0
70.0
60.0
50.0
40.0
30.0
20.0
% of ^{14}C recovered
Prometryne
Hydroxyprometryne
4.0
5.0
6.0
7.0
8.0
pH of incubated soil

Figure 33

Relative Amounts of Recovered ^{14}C-Prometryne and ^{14}C-Hydroxyprometryne from Soil Containing Bound ^{14}C Incubated at Various pH. Mean ± S.E., n=6.


1.0
0.8
0.6
0.4
0.2
0.0
Recovered residues (μg·g-1 dry soil)
• Prometryne
∘Hydroxyprom-
etryne
4.0
5.0
6.0
7.0
8.0
pH of incubated soil

radioactivity (28 - 35%). No significant difference (ANOVA and SNK multiple range test, P=0.05) in the proportion of prometryne to hydroxyprometryne was observed from one pH value to another, except at pH 4.9. In this case, the amount of residues in the extractable fraction was 46% prometryne and 54% hydroxyprometryne. Thus, there was a significant pH effect, not only on the release of the soil-bound "prometryne" residues, but also on the proportion of the released derivative and parent herbicide.

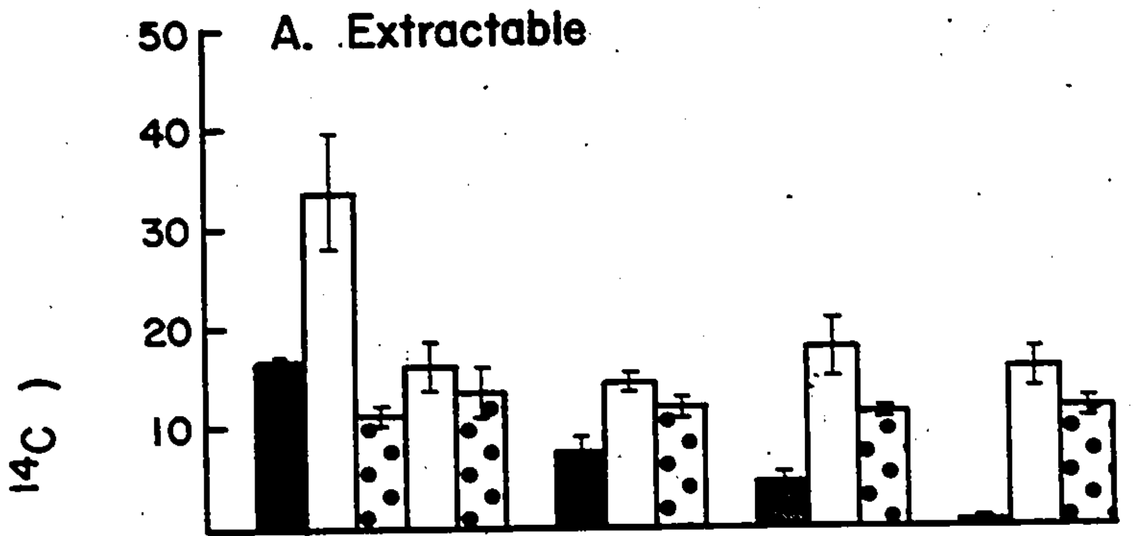
E. RELEASE OF SOIL-BOUND ^{14}C-PROMETRYNE BY PLANTS

1. Extractable ^{14}C-Prometryne in the Rhizosphere and Rhizoplane Soils

The release of bound ^{14}C residues in soil was found to be elicited by the addition of the nitrogenous fertilizers (Fig. 34). In the trials without any plants, there was a release of soil-bound radioactivity with the addition of the fertilizer solutions. Soil fortification with Hoagland's nutrient solution, No. 2 released 16.7% of the soil-bound radioactivity while 7.5% and 4% was extractable following nitrate and ammonium fertilization, respectively. Following the application of urea as the nitrogen source, only 0.4% was extractable. There was no significant difference (P=0.10) in the amount of radioactivity remaining bound (71 to 99%) in the soil for each of the fertilizer treatments (Fig. 33).

Figure 34

Distribution of Bound and Extractable ^{14}C in the Rhizosphere (RS) and Rhizoplane (RP) Soils Following Three Weeks of Plant Growth. The Plants were Fertilized with Different Nitrogen Sources. Mean ± S.E., n=6.



B. Bound

^{14}C Recovered (% of total bound ^{14}C)

100
90
80
70
60
50
40
30
20
10

no plants
soybean
wheat
no plants
wheat
no plants
wheat
no plants
wheat

RP RS RP RS
Hoagland's Nutrient Solution

RP RS
$Ca(NO_3)_2$

RP RS
$(NH_4)_2SO_4$

RP RS
Urea

However, when plants were grown in soil containing the bound radioactivity, a different situation was established. In the rhizosphere of soybeans, which were fertilized with Hoagland's nutrient solution, No. 2, 10.8% of the originally bound radioactivity was extractable with methanol; this was similar to the level of extractability in the Hoagland's nutrient solution of the controls that contained no plants. However, there was a three-fold increase in extractable radioactivity (33.7%) in the soybean rhizoplane. In addition, compared to the controls, a significant decrease in bound radioactivity remaining in the rhizoplane soil after methanol extractions was noted; no such difference was observed in the rhizosphere fraction (Fig. 34).

In most of the wheat trials, compared to their respective controls (where no plants were present), nitrogen fertilization, whether as nitrate, ammonium, or urea, resulted in significant (P=0.05) increases in extractable radioactivity from both the rhizosphere and rhizoplane fractions (Fig. 34). The most significant increase was in the rhizoplane and rhizosphere of urea-fertilized wheat where extractable levels of 15.9 and 12% were obtained compared to 0.4% in the urea controls. Nitrate-fertilized wheat seedlings released 14.3 and 11.8% of the bound radioactivity from the rhizoplane and rhizosphere respectively, compared to control levels of 7.5%, while 18.1 and 11.4% were extracted from the rhizoplane and rhizosphere soils of ammonium-fortified wheat. These levels were higher than the 4.3% extracted in the ammonium controls without plants.

No significant difference (P=0.05) between rhizosphere and rhizoplane extractable residues was observed in the wheat fertilized with the Hoagland's nutrient solution, No. 2. In all the other treatments, there were significantly higher levels of extractable radioactivity in the rhizoplane compared to the rhizosphere (P=0.10).

Generally, after 3 weeks of growth of wheat, there was considerably less ^{14}C remaining bound in the wheat rhizoplane soil (P=0.05) compared to the rhizosphere fraction. In the rhizoplane of wheat fertilized with the Hoagland's nutrient solution, No. 2, 60.4% of the initially bound radioactivity still remained unextractable, while 91.8% remained bound in the rhizosphere. For nitrate fertilization, 75.6 and 90.7% of the initially bound radioactivity remained bound in the rhizoplane and rhizosphere fractions, respectively compared to 99.4% in the control. Ammonium-fertilized wheat seedlings left 66.0 and 90.7% bound in the rhizoplane and rhizosphere soils, respectively, in comparison to 88.8% remaining bound in the unplanted ammonium control.

Compared to the control soils, there was no significant difference (P=0.05) in the amount of radioactivity that remained bound in the rhizoplane and rhizosphere soils after the growth of urea-fertilized wheat seedlings. In the urea contorls without any plants, 74% of the initially bound radioactivity remained bound after 3 weeks. Wheat growth for 3 weeks resulted in 65 and 92% of the originally bound radioactivity remaining unextractable in the rhizoplane and rhizosphere. The rhizoplane contained more bound radioactivity than the rhizosphere (P=0.10).

2. Plant Uptake of Released ^{14}C-Prometryne

Uptake of the released ^{14}C-prometryne residues by the individual plants was very low, irrespective of fertilizer treatment or plant species. In each case, approximately 1% of the previously soil-bound radioactivity was taken up by each plant (Table 12). There was no significant difference in uptake at the 95% level.

3. Partitioning of ^{14}C-Prometryne in Plant Tissues

In all four wheat-fertilizer treatment sets (Fig. 35), there was an equal distribution of radioactivity between the root and shoot tissues (approximately 50%:50% root:shoot). No significant difference (P=0.10) was obtained in root-shoot distribution in any of the treatments. However, significant differences (P=0.05) were observed for soybean seedlings. 75.5% of the total plant radioactivity was found in the shoot tissues leaving 24.5% in the roots.

Gas-liquid chromatographic analyses of both the wheat and soybean extracts identified only the parent herbicide, prometryne, in the chloroform-soluble fraction. In the methanol-soluble fraction, hydroxyprometryne was identified in the hydrolyzed fraction with little detected in the unhydrolyzed fraction. Insufficient plant tissue was obtained to extract and identify the plant bound residues by high temperature distillation technique.

Table 12

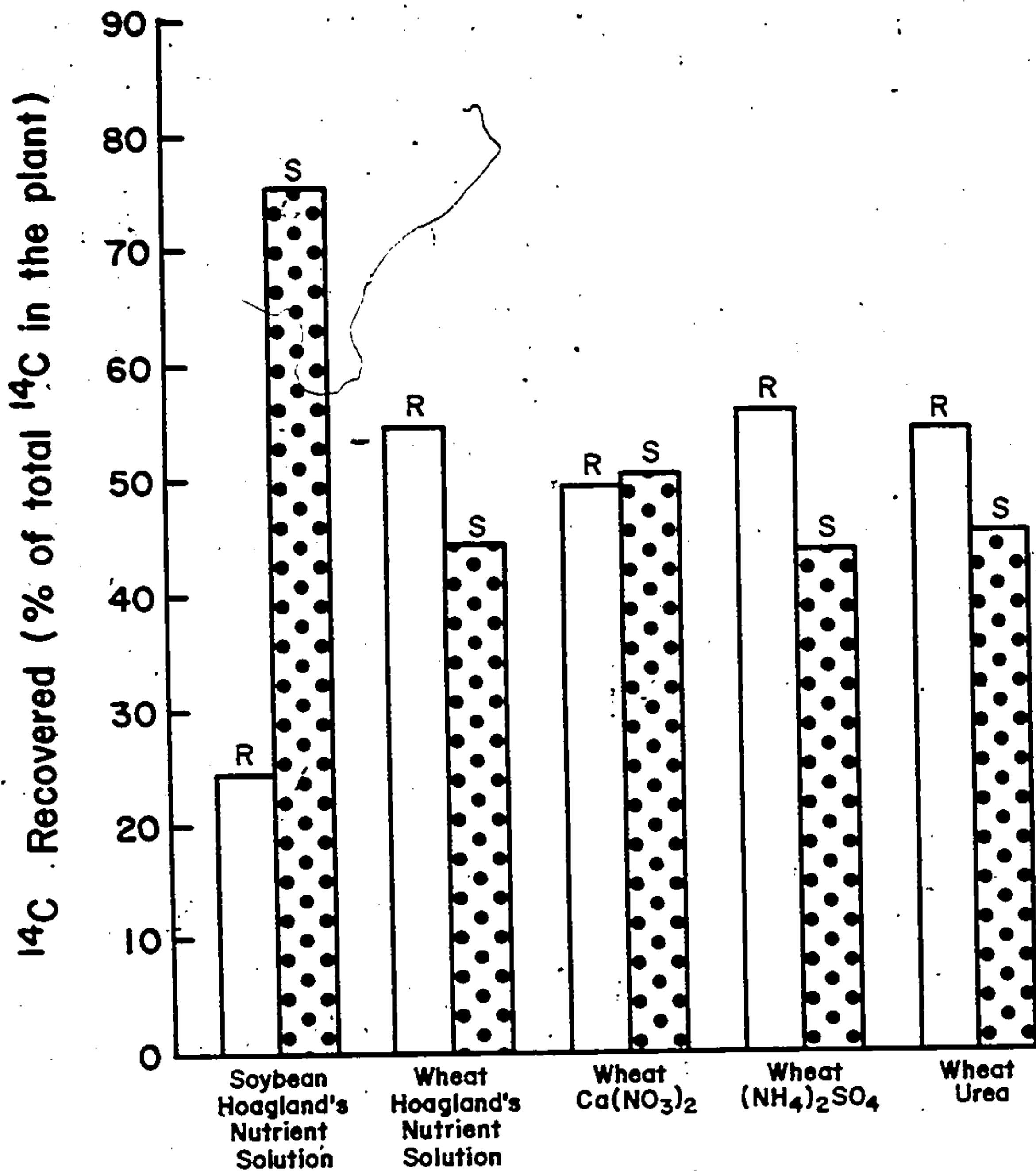
Plant Uptake of Soil-Bound ^{14}C After
3 Weeks of Growth

Treatment	% of Total Soil-Bound ^{14}C (per plant basis)* (dry weight basis)
Wheat	
Hoagland's Nutrient Solution	0.94 ± 0.14 a**
$Ca(NO_3)_2$	1.23 ± 0.18 a
$(NH_4)_2SO_4$	1.11 ± 0.14 a
Urea	1.27 ± 0.38 a
Soybean	
Hoagland's Nutrient Solution	1.09 ± 0.28 a

* Mean ± Standard Error, n = 6
**Means followed by different letters are significantly different (P=0.05) as determined by one-way analysis of variance (ANOVA) and Student-Newman-Kuel's multiple range test.

Figure 35

Distribution of ^{14}C Between Root (R) and Shoot (S) Tissues of Wheat and Soybean. Plants were Grown for Three Weeks in Soil Containing Bound ^{14}C Residues and Fertilized with Different Nitrogen Sources.



^{14}C Recovered (% of total ^{14}C in the plant)
90
80
70
60
50
40
30
20
10
0
R
S
Soybean Hoagland's Nutrient Solution
Wheat Hoagland's Nutrient Solution
Wheat $Ca(NO_3)_2$
Wheat $(NH_4)_2SO_4$
Wheat Urea

a. Root Tissues

In the wheat root tissues (Fig. 36), the chloroform soluble fraction (ie. prometryne) represented 20 to 25% of the total radioactivity in the wheat while 17 to 20% was found in the polar fraction (methanol:water soluble). In addition, 11 to 15% of the total plant radioactivity remained unextractable in the plant tissues for the four fertilizer treatment sets. There was no significant difference (P=0.05) in the partitioning or radioactivity in the wheat root tissues for any of the fertilizer treatments.

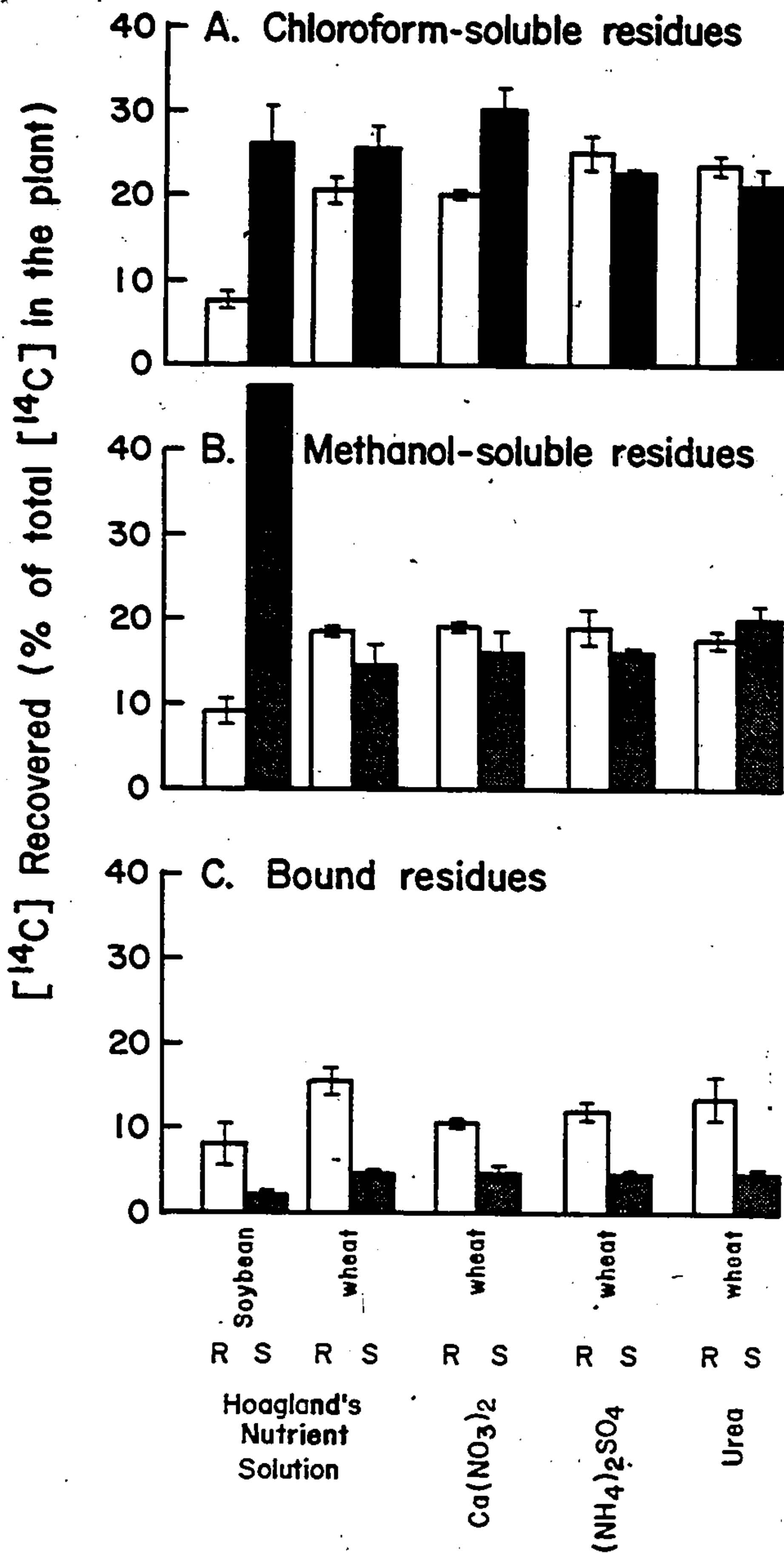
However, in soybeans, less radioactivity was partitioned into the root tissues (24.5%). Within the root tissues, 7.4, 8.9, and 8.1% of the total plant radioactivity was found in the chloroform, methanol, and bound fractions, respectively. No significant difference (P=0.10) was observed between these three fractions.

b. Shoot Tissues

In the wheat fertilized with Hoagland's nutrient solution, No. 2 and with nitrate salts, there was a significant increase in the proportion of chloroform-soluble (ie. prometryne) radioactivity in the shoots compared to the root tissues (Fig. 36). In both of these cases, in the root tissues, 20% of the total plant radioactivity was soluble in chloroform whereas in the shoots of the Hoagland- and nitrate-fertilized plants, chloroform-soluble residues constituted 26.1 and 29.7%, respectively. Both values are significantly higher (P=0.05) than those

Figure 36

Partitioning of ^{14}C in the Tissues of Wheat and Soybean Into Chloroform- and Methanol-Soluble and Bound Residues. R-Root Tissues; S-Shoot Tissues. Mean ± S.E., n=6.



A. Chloroform-soluble residues
B. Methanol-soluble residues
C. Bound residues
[14C] Recovered (% of total [14C] in the plant)
0
10
20
30
40
Soybean
wheat
wheat
wheat
wheat
R S
R S
R S
R S
R S
Hoagland's Nutrient Solution
Ca(NO3)2
(NH4)2SO4
Urea

obtained from the root. In the ammonium- and urea-fertilized wheat seedlings, no significant change in the quantity of ^{14}C-prometryne residues in the shoots was noted at the 95% level.

In soybean shoots, however, there was a three-fold increase in the amount of ^{14}C-prometryne compared to the root tissues (P=0.05) indicating a higher rate of transport of the herbicide to the shoot tissues than observed in the wheat. The chloroform fraction of shoot tissues represented 26.2% of the total plant radioactivity compared to 7.4% in the root tissues.

In the shoot tissues, the partitioning of the radioactivity in the polar fraction (methanol:water soluble) was the same as in the root in most cases. In wheat shoot tissues, methanol:water were able to extract 15 to 20% of the total plant radioactivity. Urea fertilization of wheat, compared to other fertilizer treatments, was the only case which showed a significant increase (P=0.05) in the quantity of hydroxyprometryne in the shoot.

Similarly, in soybeans, there was a five-fold increase in the hydroxy derivative from 8.9% in the root to 47.4% in the shoot. This level of radioactivity in the methanol:water fraction was also significantly higher (P=0.05) than any of the wheat fractions.

The proportion of the total ^{14}C bound to shoot tissues was lower than the level observed in the roots. For wheat, approximately 4.3% was biologically incorporated and unextractable by solvents. Binding of

radioactivity was lower in the soybean shoots (P=0.05), representing only 1.9% of the total radioactivity in the plant.

IV. DISCUSSION

A. PREPARATION OF SOIL-BOUND ^{14}C RESIDUES

In the resolution of the thesis, a major emphasis was placed upon the actual production of soil-bound ^{14}C-prometryne residues and their potential for subsequent recycling. Consequently, it was important to maximize the levels of binding of the herbicide in the soil. In previous studies, it was demonstrated that the binding of ^{14}C-prometryne to soil increased rapidly to a steady level within 150 days (Khan and Hamilton 1980). By duplicating these procedures and analyses in the present set of experiments, it was observed that the soil contained 40.6% and 53.7% bound (nonextractable) radioactivity after 121 and 475 days, respectively. The level of binding is consistent with those found previously where 43% became bound after 150 days (Khan and Hamilton 1980) and increased to 57.4% after one year of soil incubation (Khan and Ivarson 1981; Khan 1982a).

Similar time course studies have shown that the binding of pesticides to organic soils was not a phenomenon exclusive to prometryne, although there may be differences in the pattern and level of binding obtained. For example, a variety of trifluoromethanesulfonanilide pesticides and their analogues rapidly bind and reach equilibrium levels (20 to 40% of the amount of ^{14}C applied) within 2 to 3 weeks (Bandal et al. 1976).

Recovery of ^{14}C-parathion as bound residues after one to five soil applications reached maximal levels of 20 to 40% in four weeks. However, given ten applications, the same levels were obtained within one week (DeAndrea et al. 1982).

Until recently, the nature of the soil-bound species could not be evaluated. In 1980, Khan and Hamilton, using a high temperature distillation procedure, found the presence of prometryne and its 2-hydroxy analogue, hydroxyprometryne, as the bound species in the incubated soil. In the present set of experiments, the parent herbicide, prometryne, was the only species present in the chloroform-soluble (non-polar) fraction and thus, represents most of the radioactivity that was bound to the soil (approximately 50% of the total ^{14}C bound). The remaining sequestered radioactivity was found in the methanol-soluble (polar) fraction and only hydroxyprometryne was identified in this fraction. However, the presence of the mono-N-dealkylated product namely, 2-(methylthio)-4-amino-6-(isopropylamino)-s-triazine, the di-N-dealkylated product of prometryne, that is, 2-(methylthio)-4,6-diamino-s-triazine, and unidentified methanol-soluble materials was also determined as bound species in previous studies (Khan and Ivarson 1981); Khan 1982a). The inability to determine the presence of these analogues in this project may be due to the lack of sufficient materials in the samples. The lack of reference standards prevented the testing of recoveries of all analogues.

The presence of the parent herbicide as a bound species may indicate that soil binding may not only be a chemical binding process but also include some physical interactions. In past studies, binding has been

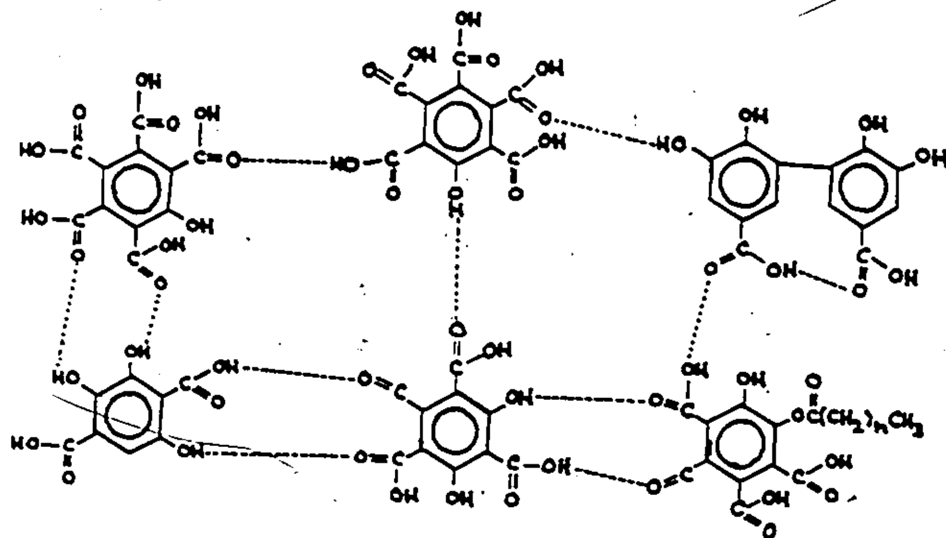
envisioned as a chemical stabilizing reaction between phenolic hydroxyls in the humic substances and free aromatic amino groups of the pesticide or its metabolites (DeAndrea *et al*. 1982; Helling and Krivonak 1978a; Katan *et al*. 1976). The binding of 3,4-dichloroaniline, a major degradation product of several herbicides including propanil, diuron, and linuron, to humus was postulated to involve covalent binding of the chloroaniline and quinone groups of soil humus (Saxena and Bartha 1983a,b; You *et al*. 1982). Other anilines have been shown to cross-couple with quinone sites of humic substances (Bollag *et al*. 1983).

Triazine herbicide binding to humic constituents was envisioned to involve ionic bonds (Hayes 1970; Sullivan and Fellbeck, Jr. 1968), hydrogen bonds (Stevenson 1972), electron donor-acceptor complexes (Muller-Wegener 1977), covalent bonds (Hayes 1970; cf. Stevenson 1972) and free humic radical interactions (Dunigan and McIntosh 1971; Stevenson 1972). However, the presence of the unaltered parent herbicide, prometryne, itself as a major bound species, implies that physical binding in addition to chemical binding, may play a major role in the formation of soil-bound residues (Khan 1982a).

Schnitzer and Khan (1972) theorized that humic substances are composed of "building blocks" (benzenecarboxylic and phenolic acids) held together by hydrogen bonds, van der Waal's forces, and pi bonding. This "open" and flexible structure consists of holes or voids of different dimensions that can trap or fix organic and inorganic compounds that fit into these holes (Fig. 37). Based on these conceptualizations,

Figure 37

A Proposed Partial Chemical Structure for Fulvic Acid (from Schnitzer and Khan 1972).



soil-binding may also involve the "adsorption of the pesticide or derivatives on the external surfaces and entrapment in the internal voids of a molecular sieve type arrangement" (Khan 1982a). Consequently, the formation of soil-bound ^{14}C-prometryne residues appears consistent with previous findings and theories.

B. PROMETRYNE TOXICITY TO MACROPHYTES AND MICROPHYTES

1. Soybean Susceptibility

The herbicidal action of prometryne was quite apparent in the growth of soybean. Considerable decreases in the wet and dry weights of the plants was obtained following treatment with 2.5 μg mL^{-1} prometryne. This reduction appears to be related to the phytotoxic interference of photosynthesis by triazine herbicides (Ebert and Dumford 1976).

Triazine herbicides are known to block the photosynthetic electron transport system at the level of photosystem II of the Hill reaction (Ashton and Crafts 1981). This blockage appears to be at the second electron carrier, called "B", which is unable to be reduced by electrons released by the primary electron acceptor, "Q" (Pfister and Arntzen 1979). The herbicide binding site is thus postulated to be a receptor protein of "B" (Arntzen _et al_. 1979; Gardner 1981; Pfister _et al_. 1979, 1981).

Consequently, reductions in the soybean weights following prometryne treatment may be related to a decrease in the reducing power produced by the light reactions. The lack of reducing power, therefore, would inhibit carbon dioxide fixation (Beruter and Temperli 1970; Sikka and Davis 1969) and lead to less photosynthate available to the plant. Since less photosynthate is available, carbohydrate concentrations in the plants would be expected to decrease due to metabolism to CO_2 or metabolic conversions to amino acids and proteins (Bolher-Nordenkampf 1979). A 15% reduction in the concentration of sugars in spinach leaves were observed by Singh et al. (1972b) following foliar application of simazine. Similar reductions in carbohydrates in pea and corn seeds were also apparent. Singh et al. (1972a) found a comparable decrease in the total carbohydrate content in Phaseolus vulgaris along with a concomitant increase in protein content in plants treated with sublethal concentrations of triazine herbicides.

Plant damage and death by triazine herbicides does not appear to be primarily due to the lack of photosynthate available to the plant, but rather to a photo-oxidative process through the formation of free radicals as detailed by Bolhar-Nordenkampf (1979) and Ashton et al. (1963).

Two major photo-oxidative schemes are described, both mediated by atrazine. The first involves an incomplete blockage of electron transport at photosystem II coupled with a complete inhibition of carbon dioxide fixation. This results in an excess of electrons at photosystem I. To compensate for the surplus of electrons, free radicals are

produced with oxygen which can photo-oxidize the leaf tissues. The plant is able to protect itself, initially, by photorespiration which activates superoxide dismutase to form hydrogen peroxide, and thus oxidizes glyoxylate to carbon dioxide. This protection seems to involve use of the carotenes, fatty acids, and photorespiration. Most of the stored carbohydrates are photorespired or metabolized to amino acids and proteins. Eventually, reserves are depleted and the protective mechanisms are gone, leaving the photo-oxidation to occur. Pigment photo-oxidation occurs first, followed by leaf defects, and eventual irreversible damage (Bolhar-Nordenkampf 1979). The weight decreases in the prometryne-treated soybeans and variability in protein content may, therefore, be partially due to the photorespiratory protective mechanisms.

Giannopolitis and Ayers (1978) propose a similar scheme except for a different role of the free radical. With no electron transfer, the photoexcited chlorophyll molecules are unable to channel all of their energy into electron transport, but rather partly reacts with oxygen (bleaching) and partly with an unsaturated fatty acid, removing a hydrogen atom (Packer and Heath 1968). This begins a cyclic peroxidation chain where the hydroperoxides formed can attack chlorophyll and other components. The photo-oxidation (bleaching and lipid peroxidation) occurs after the supply of NADPH is depleted since NADPH functions as the reducing potential in an enzymatic cyclic epoxidation process in the carotenoid system (Giannopolitis and Ayers 1978). Lipid peroxidation can result in increased membrane permeability and electrolyte leakage (Crowley and Prendeville 1980) as well as decreased input of ATP

required for membrane integrity which is necessary for active transport (Moreland and Hilton 1976).

In these experiments, fluctuations in the protein content of the soybeans were apparent although such deviations in protein were not significant. These results were apparent in both the Kjeldahl nitrogen and Hartree protein assay procedures although the Kjeldahl nitrogen assay measured 2 to 5-fold higher protein contents.

Increases in protein content with triazine treatment noted in the present study may be attributable to several causes. However, the most likely is that the higher protein content is due to the reduction in dry weight of the plant without a corresponding decrease in protein or nitrogen content (Monson et al. 1971; Singh et al. 1972a; Tweedy et al. 1971; Vergara et al. 1970). Another possible explanation for the different protein results is that the assay procedures may detect or measure the herbicide or the metabolites, particularly with the Kjeldahl nitrogen assay. Since prometryne contains nitrogen, the Kjeldahl analysis may be sensitive to this form of nitrogen. In a preliminary test, samples of the herbicide were subjected to the analysis and the test was able to assay almost all of the nitrogen present in the molecule (91%). Consequently, the effects of prometryne on the Kjeldahl protein may be the result of decreasing plant material and increasing quantities of prometryne to assay. The Hartree protein assay, used in this study, demonstrated the same type of fluctuations in the protein contents of the prometryne-treated plants as the Kjeldahl nitrogen assays. However, these fluctuations may have been due to the reduction

in dry weight of the plants. In addition, the reaction of the Folin-phenol reagent used in this assay is variable with different proteins (Lowry, _et al_. 1951).

Whitenberg and Flippen (1977) argue that prometryne does not directly affect protein synthesis but rather acts on some cofactor(s) or intermediate(s) essential for their synthesis. A possible candidate is adenosine triphosphate (ATP) which is essential for some steps in protein synthesis. Prometryne has been demonstrated to block oxidative phosphorylation in soybean mitochondria (McDaniel and Frans 1969). This blockage will result in lower levels of ATP available for protein synthesis. Whitenberg and Flippen (1977) further suggest that protein effects are due to accelerated senescence due to prometryne's blockage of the photosynthetic pathways.

In any case, in the present study prometryne was demonstrated to be extremely toxic to soybeans for the concentrations tested. Reductions in plant weight, incomplete development, and chlorosis in the soybeans presently represent the onset of necrosis and senescence in the plant.

2. Algae - _Chlamydomonas segnis_ Ettl

Addition of acetone (0.13% v/v) to synchronous cultures of _C. segnis_ Ettl did not affect the population growth, or division and release of autospores. Consequently, any changes in the population growth of the _C. segnis_ Ettl cultures when treated with prometryne could not be attributed to the organic solvent acetone.

C. segnis Ettl cells in all treatment sets exhibited a 100% inhibition of cell division. That this was an algistatic effect (and not algicidal) was illustrated when the cells were plated on nutrient agar. Compared to the control values, the colony counts of the treated cultures were 50% lower, indicating the degree of inhibition of cell division. However, relative to the number of cells inoculated per plate, the proportion of treated cells able to divide and form colonies was higher (at the 90% level of significance) compared to the controls (50% vs. 35%) although not significantly higher at the 95% level. Subsequent recovery by a segment of the population may have been the result of cellular recovery to prometryne exposure coupled with the release and germination of autospores.

The acute algistatic effects of prometryne on C. segnis Ettl are consistent with the chronic algal effects detailed in the literature and with the biochemical action of the herbicide, as detailed above. Davis et al. (1976) observed that prometryne inhibited the growth of Chlorella pyrenoidosa, as measured by chlorophyll content, at concentrations greater than 0.024 µg mL^{-1} (10^{-7} M) and it was stimulatory at lower concentrations. Similar results were obtained by Hawxby et al. (1977). Chlorella pyrenoidosa and Chlorella vulgaris were shown to be inhibited by atrazine (Leoppky and Tweedy 1969). Chlamydomonas reinhardi and Chlamydomonas eugametos were also sensitive to atrazine. The sensitivity of these green algae to prometryne and atrazine was attributed to the inhibitory effects of these herbicides on photosynthesis (Davis et al. 1976; Hawxby et al. 1977; Loeppky and Tweedy 1969). Ashton et al. (1966) also found that atrazine hindered cell development in Chlorella

vulgaris. They hypothesized that this may have been due to a lack of sufficient energy or failure of the cells to attain a critical cell volume. In the present study, the inability of C. segnis Ettl to complete cell division, when exposed to prometryne during the S-phase of the cell cyle, would appear to implicate some dysfunction of macromolecular synthesis as detailed above for soybean.

3. Heterocyst Study - Anabaena spp.

Acetone (0.25% v/v) evoked a slight, though not statistically significant, stimulation in heterocyst formation in Anabaena. Other studies have shown that acetone in concentrations less than 0.6% (v/v) stimulated photosynthetic activity in Anabaena spp. while nitrogen fixation was stimulated with concentrations less than 0.4% (v/v) (Stratton et al. 1980; Stratton and Corke 1981). It has been theorized that the increase in these physiological functions may have been the result of acetone-induced membrane damage and consequently increase membrane permeability (Stratton et al. 1980). Similar mechanisms may be responsible for the increase in heterocyst frequency.

In the present study, prometryne severely affected the formation of heterocysts in Anabaena spp. Heterocyst frequency was reduced by 10 to 93% in the prometryne treated cultures (0.1 - 10.0 μg mL-1) compared to the controls. An indirect correlation between prometryne concentration and heterocyst frequency was obtained. This influence may have been due to the combined nitrogen in the prometryne molecule which could inhibit heterocyst formation like ammonia (Tyagi 1975) or to the

algicidal effects of the herbicide itself. When the cultures were plated on nutrient agar, all of the treated cultures showed reduced filamentous growth when compared to the controls. This appears to indicate that prometryne affected morphogenetic events including heterocyst formation. Hawxby et al. (1977) observed that prometryne decreased photosynthetic activity and increased the generation time of A. variabilis. Atrazine was shown to be equally toxic to Anabaena spp. by blocking the photolysis of water and interfering with primary electron transfer (Hawxby et al. 1977; Stratton and Corke 1981). As a result of these studies, prometryne, like other triazine herbicides, is able to exert different herbicidal effects on the growth of the three, taxonomically very different photosynthetic species, soybeans, C. segnis Ettl, and Anabaena spp.

4. Rhizobium japonicum and Klebsiella pneumoniae

The effects of prometryne on the growth of the nonphotosynthetic nitrogen-fixing bacteria were less pronounced. Klebsiella pneumoniae strain M5A1 was not affected by 0.25 to 6.8 μg mL^{-1} of prometryne while the highest does of prometryne (6.8 μg mL^{-1}) alone, deleteriously affected Rhizobium japonicum strain 61A76. The doubling time increased from 9.5 h in the control sets to 26.25 h and this may signal potential cause for concern in areas annually treated with prometryne. Lower concentrations of prometryne did not affect the generation time. Rhizobium spp. has been previously shown to be affected by 10 μg mL^{-1} of prometryne or simazine (Kaszubiak 1966). Effects of prometryne may be due to its influence on respiratory processes

(McDaniel and Frans 1969; Thompson *et al.* 1974, 1969) or its partial ability to replace uracil in RNA or thymidine in DNA as demonstrated for *E. coli* (Temperli *et al.* 1966). The difference in threshold concentrations may be explained by the use of different culture media and differences in strain tolerance. Differential susceptibility of *Rhizobium* and other bacteria to herbicides is known to be pH dependent (Balicka 1969; Kaszubiak 1966), species (Gillberg 1971), and strain dependent (Tu 1982).

The relative insensitivity of the gram negative *R. japonicum* and *K. pneumoniae* to the low concentrations of prometryne may be due to the "relative nonpolarity in nature of the herbicide, and thus may be unable to penetrate the bacterial cell in sufficient quantities or to inhibit any membrane or cell wall surface activities" (Breazeale and Camper 1972). This may not be the case as prometryne has been found to penetrate *E. coli* cells (Temperli *et al.* 1966) and atrazine sorbed by *Acinetobacter* spp., *Cytophaga* spp., and *Pseudomonas fluorescens* (Geller 1979). However, although these two species were relatively insensitive to the herbicide (based on changes in growth rates), it is evident that the high concentration of prometryne (6.8 µg mL^{-1}) may potentially affect some metabolic processes to elicit increased generation times.

C. ROOT ALTERATION OF SUBSTRATE pH

1. Hydroponic pH Changes

The major aim of these studies was to demonstrate the root's ability to alter the pH of the surrounding substrate, particularly in the

extremely localized regions of the rhizosphere and rhizoplane. Initial studies were carried out in hydroponic solutions to determine the types of pH response that could be elicited under different nitrogen fertilization regimes. The ability of plants to increase the pH of the surrounding solution when provided with nitrate salts and decrease under ammonium fertilization is well documented (Nightingale 1937). For example, tomato plants were shown to increase the solution pH from pH 5.5 to pH 6.5 and decrease to pH 4.0 when provided with nitrate and ammonium salts, respectively. Urea fertilization led to an intermediate decrease, to pH 4.5 (Kirkby and Mengel 1967). Corn roots in nitrate solutions after eight days increased the solution pH to pH 7.1 and decreased to pH 4.2 in ammonium-fertilized solutions (Blair et al. 1970). The pH alterations of the hydroponic solution by the wheat plants used in the present studies appear consistent with such findings.

The uptake of the nitrogen species and subsequent extrusion of counter-ions follows the basis of maintenance of electro-neutrality across membrane surfaces (Donnan 1924). As anions are absorbed across the plant membrane, as in the case with nitrate salts, the uptake is balanced by either an equivalent uptake of cations or release of equivalent amounts of anions, such as hydroxyl and/or bicarbonate (Kirkby and Mengel 1967; Walker 1960). In most cases, however, a corresponding equivalent uptake of cations (eg. K^+) is the major process present while anion exchange is less likely (Mengel 1971). The actual reason the pH of the solution rises is due to the diffusion from the root of hydroxyl and/or bicarbonate ions which are produced by nitrate reduction (Mengel 1971; Raven and Smith 1976).

Ammonium uptake from the nutrient solution results in an acidification of the media, as detailed here, and by previous workers. As before, cation (NH_4^+) uptake is compensated by an extrusion of protons into the surrounding media in order to maintain electroneutrality across the membrane surface (Hedley et al. 1982; Jenny and Overstreet 1939; Nye 1981). This results in the decline of pH in the media. The rapid decrease in pH can be attributed to the rapid absorption of monovalent ions such as NH_4^+ (Jacobson et al. 1960).

In order to fully appreciate the role that ions play in the alteration of pH in the solution, a distinction between physiological acidity or alkalinity of a salt is required. This is dependent upon which ion of the salt, cation or anion, is most rapidly absorbed (Moore 1971). In general, monovalent ions are more rapidly absorbed than di- or polyvalent ions, whether they be cations or anions (Hagen and Hopkins 1955; Jacobson et al. 1957, 1960; Leggett and Epstein 1956; Maas 1969; Moore et al. 1961a,b; Stenlid 1957). Therefore, in the nitrogen sources used, calcium nitrate would be physiologically alkaline since calcium ions enter slowly relative to the nitrate ions. Ammonium sulfate would be physiologically acid corresponding to the imbalance in cation-anion absorption rates. Ammonium phosphate appears to be physiologically acid as ammonium sulfate. This acidity may be due to the dependency of anion uptake on cation absorption (Dejaergere et al. 1980).

Ion uptake, both anionic (nitrate) and cationic (ammonium), has been postulated to be an electrogenic process (Bowling and Dunlop 1978; Bowling 1980; Hanson 1978; Raven and Smith 1980). Generally, protons are

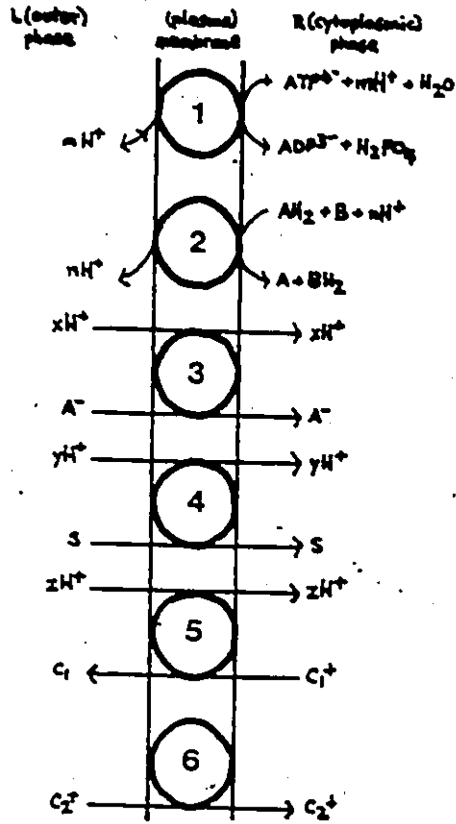
extruded across the membrane surface through either a translocating ATPase or oxido-reduction, creating an electropotential across the root membrane. This electropotential provides the energy required to absorb ions as shown in Fig. 38. For the most part, anion uptake occurs as the proton and anion pass through a proton-coupled symport. Cation uptake, on the other hand, proceeds through a uniport so that the proton remains on the outside to maintain electroneutrality (Raven and Smith 1980). This viewpoint is consistant with the findings observed in the hydroponic solutions followed in the present study.

Also, the intermediate results of urea can be viewed consistent with this theory. Urea, being virtually unchanged, would be expected to be physiologically neutral and cause no change in pH. However, a significant reduction in pH was observed although not as great as obtained with the ammonium fertilized wheat seedlings. This drop in pH may be due to the original proton extrusion from the ATPase or the oxido-reduction.

In the case of Hoagland's nutrient solution, the rise in pH on subsequent days indicate increases in anion uptake relative to cation uptake. This increased anion uptake may be the result of cationic depletion or to pH effects on cation absorption. As the pH decreases, the protons are believed to compete with cations for absorption sites, such as the uniports (Moore 1971). In addition, protons can be damaging to the roots. It has been pointed out that these protons increase cell permeability and allow constituents to leak out (Jacobson *et al*. 1957).

Figure 38

Some Components of the 'H^+' Chemiosmotic Hypothesis (from Raven and Smith 1980). (1) H^+-Translocating ATPase; (2) H^+-Translocating Oxidoreduction; (3) H^+-Coupled Anion (A^-) Symport; (4) H^+-Coupled Neutral Solute (S) Symport; (5) H^+-Coupled Cation (Na^+, Ca^{2+}, Represented as C_1^+) Antiport; (6) Cation (K^+, NH_4^+, Represented as C_2^+) Uniport.


phase
(plasma)
phase
1
2
$AH_2 + B + nH^+$
nH^+
$A + BH_2$
xH^+
xH^+
3
A^-
A^-
yH^+
yH^+
4
S
S
zH^+
zH^+
5
6

Since cation absorption is suppressed by the low pH, anion uptake is unaffected and begins to rise relative to the cation uptake. Consequently, the pH will increase as more anions are taken up.

Another concern raised was the pH values observed with Hoagland's nutrient solution, No. 2. There was an initial drop in pH after 2 to 4 days, indicating an excessive cation uptake relative to anions. Similar observations were made by Hoagland and Boyer (1940). The specific ions involved in the increased uptake of cations relative to anions at this stage is difficult to differentiate due to the complex nature of the nutrient solutions. However, if emphasis is focused on nitrogen sources, since plants have a high demand for nitrogen, the rapid initial decrease in pH may be attributed to the ammonium ions which pass through the uniport in order to compensate for proton extrusion by the ATPase or oxido-reduction. Ammonium uptake would then be considered to be a faster process than nitrate uptake during this early period.

The initial drop in pH after 2 to 4 days for Hoagland's nutrient solution, No. 2, may also be attributed to the presence of ammonium ions which prevent nitrate absorption (Doddema et al. 1978; Firth 1972; Pate 1973) although such inhibition is not definite (Schrader et al. 1972; Smith and Thompson 1971). There are many conflicting reports as to whether ammonium ions actually inhibits nitrate reductase levels or the assimilation of nitrates into organic nitrogen (Minotti et al. 1969; Radin 1975; Shen 1969). It has been suggested, however, that ammonium (NH_4^+) or the high acidity due to ammonium ion uptake causes changes

in membrane permeability which allows for nitrate absorption (Minotti et al. 1969).

2. Soil pH Changes

Although wheat roots are able to alter the pH of the hydroponic solutions, similar pH control in the soil was required to be demonstrated in the further resolution of these experiments. The importance of extruded ions (protons, hydroxyl, and bicarbonate) becomes more apparent in the soil where they would not be dissipated but rather remain near the root due to the static nature of the solid media (Moore 1971). However, in this study, definite localized changes in pH could not be conclusively demonstrated by either microelectrode or soil solution analysis. Monitoring the rhizosphere pH with the pH microelectrode produced erratic pH values for all three experimental sets. Likewise, pH measurements of soil suspensions found no difference in the pH between rhizosphere and rhizoplane soils. However, there was an increase in pH for plants fertilized with nitrates compared to the distilled water controls.

Contrary to previous work, localized pH changes could not be demonstrated in these studies. Smiley (1974) observed that various plants were able to increase the rhizosphere soil pH under nitrate fertilization and decrease with ammonium salts with the most dramatic results in the Gramineae. Similar results were found by other workers for corn (Boero and Thien 1979; Miller et al. 1970; Soon and Miller 1977), wheat (Smiley 1979) and soybean (Riley and Barber 1969, 1971).

However, in the present study, the lack of demonstratable localized pH changes in the rhizosphere and rhizoplane soils requires an

explanation. Nye (1981) presents some evidence for the lack of observable rhizosphere and rhizoplane pH changes. Essentially, plant induced pH changes in the soil are dependent upon several factors which Nye (1981) was able to model. These include time and buffering capacity. In general, changes in pH at any distance from the root varies logarithmically with time and linearly with soil buffering capacity (Nye 1981). Boero and Thien (1979) found that rhizosphere pH changes were inversely correlated with cation exchange capacity. A Ustoll soil with a cation exchange capacity of 18.9 meq per 100 g demonstrated the lowest change in rhizosphere pH of 0.1 pH units compared to 1.0 pH unit changes in soils with cation exchange capacities of 9.9 and 5.7 meq per 100 g. In the organic soil used in this study, the cation exchange capacity was 163 meq per 100 g (Khan, personal communication) indicative of a high buffering capacity so that measurable changes in soil pH in this study may not have been apparent.

Even the addition of the nitrogenous fertilizers to the soil did not measurably affect the soil pH although there are reports to the contrary. Pierre _et al_. (1971) noted that the addition of NH_4NO_3 decreased the soil pH by 0.9 pH units (T.E.C. = 22 to 26 meq/100g). In general, the use of nitrogenous fertilizers, particularly those which contain ammonium, has been demonstrated to acidity soils (Cairns 1968; McCoy and Webster 1977; Perl _et al_. 1982). Calcium nitrate, on the other hand, had no effect on the soil acidity (Cairns et al. 1980). The ability of ammonium sulfate to acidify the soil is probably due to its rapid oxidation to nitric and sulfuric acids (van Breeman et al. 1982).

3. Agar pH Changes

The studies on agar have confirmed that the pH changes observed in the hydroponic growth solution were localized around the root. As found in the hydroponic growth study, the type of nitrogenous fertilizer available to the plants influenced the root's ability to change the pH of the agar (Weinberger and Yee 1984). Ammonium and nitrate salts in the agar media will, respectively, result in decreases and increases in the pH of the agar surrounding the root. Urea, as the uncharged species, was observed to be physiologically neutral, thereby indicating that proton extrusion may not have played an important role in the acidification of the hydroponic solution pH by the wheat roots.

Soybean, grown in agar supplemented with Hoagland's nutrient solution, No. 2, however, displayed an acidification of the media, even after three weeks. This was completely different from the wheat which was grown on the same media. The acidification indicates that there is a higher uptake of cations relative to anions. However, the reasons for the slow acidification of the agar media still need to be elucidated in future work.

These experiments have shown that plant roots are able to alter the pH of the surrounding substrate. The changes in pH are related to the maintenance of electroneutrality across the root membrane during ion uptake which depends upon the nitrogenous fertilizer supplied.

D. EFFECT OF CHANGE IN pH ON THE RELEASE OF SOIL-BOUND ^{14}C-PROMETRYNE RESIDUES

The pH changes that occur in the area surrounding the plant roots under different nitrogen fertilizer regimes may have a profound impact on the release of the soil-bound prometryne. This impact can be visualized in that significant alterations in soil pH can increase the release of these residues into the soil solution. The process by which such pH changes can elicit the release of the soil-bound residues may involve changes in the structural framework of humic materials.

It has been suggested that in the formation of bound residues, the pesticide or metabolites are firmly retained by organic matter (humic fractions) by adsorption on the external surfaces and/or entrapment in the internal voids of a structural lattice (Khan 1982a). Physical measurements have shown a relatively "open" and flexible structure of humic materials perforated by voids of varying dimensions that can trap or fix organic compounds that fit into the voids, provided the charges are complementary (Schnitzer and Khan 1972; Fig. 37). Changes in this structure may result in the release of the bound species. It has been shown by various physical and chemical methods that both the shape and size of humic materials are strongly affected by pH, cation valence, and ionic interactions (Choudhry 1981; Schnitzer and Khan 1972; Schnitzer and Kodama 1975). At low pH, the humic substances are dispersed into fibrous networks with voids of varying sizes. As the pH increases, this network breaks down to form an organized lamellar structure with decreasing sizes in the voids and increasing dispersion (Chen and Schnitzer 1976; Schnitzer and Kodama 1975). Of particular interest are

these holes or voids. In order to trap or bind the pesticide molecule, the molecule must be able to fit into the holes, provided that the electrostatic charges in the holes and on the compound are complementary (Schnitzer and Kodama 1975). Based on this model of humic structure, release of the soil-bound prometryne residues may be the result of changes in the size of the voids and changes in the electrostatic charges in the holes or of the pesticide itself. Small changes in pH appear to yield little disruption of the humic structure and a minimal release of the bound residues. Large scale pH changes are likely responsible for massive disruption of the network, thereby increasing the amount of extractable residues.

This disruption may result in a release of both bound species as demonstrated in our study. The recovery of prometryne and hydroxyprometryne in the extracts was in the same proportions as originally bound. A small decrease in pH resulted in little effects on the internal voids and the decreased release of prometryne noted may have been the result of steric hinderances in that the voids did not become large enough for prometryne to become uncaged.

E. RELEASE OF SOIL-BOUND ^{14}C-PROMETRYNE BY PLANTS

1. Extractable ^{14}C-Prometryne in the Rhizosphere and Rhizoplane Soils

With the introduction of nitrogenous fertilizers to the soil containing bound residues, significant release of these residues is

elicited. It appears that the humic lattice structure is sensitive to ionic interactions (Choudhry 1981; Schnitzer and Kodama 1975). With the addition of the fertilizer solutions, the ions can interact with the humic structures to disrupt the bonds between the humic structural units in the polymer. Therefore, the more ions added to the soil, the greater the ionic interaction, and subsequently, the greater the release of soil-bound residues. As a result, fertilization with Hoagland's nutrient solution, No. 2, would be expected to release the largest amount of soil-bound residues due to its high ionic constituents (Hoagland and Arnon 1938) and urea would have a negligible effect largely due to its non-ionic nature.

The results of this study confirms such theories although urea was able to release a small amount of the bound radioactivity. Urea was also found to be able to displace humic-bound 3,4-dichloroaniline residues (Saxena and Bartha 1983a,b). Urea-induced release of soil-bound residues may be ascribed to its weakly basic nature and its ability to disrupt hydrogen bonding through the accommodation of charges in its molecule (Morrison and Boyd 1976; Saxena and Bartha 1983a,b).

When plants were grown in soil with the bound residues, there was no significant alteration in the amount of extractable radioactivity when fertilized with Hoagland's nutrient solution, No. 2. The interaction of plant growth and the other nitrogenous fertilizers, however, resulted in significiant increases in released residues in the rhizosphere. The increase in extractable radioactivity may have been due to an increase in soil microbial activity. It has long been recognized that the

rhizosphere effect, where microbial populations in the soil surrounding the root are significantly higher than in the bulk soil, was a common phenomenon (Clark 1949), especially for gram-negative bacteria (Alexander 1977; Kleeberger 1983). Gram-negative Pseudomonas-type bacteria dominate in the rhizosphere of plants while gram-positive Arthrobacter, Nocardia, and Bacillus are more common in root-free soils (Coleman et al. 1978). As well, recent studies have shown that release of bound ^{14}C-prometryne may be affected by soil microorganisms. After 22 days of incubation with a microbial inoculum, 27% of the bound radioactivity was extractable from soil with bound ^{14}C-prometryne (Khan and Ivarson 1981). In a similar experiment, different physiological microbial groups were able to release 23.5 to 27.1% of the bound ^{14}C (Khan and Ivarson 1982). Therefore, the introduction of plants into the soil microcosm may stimulate microbial activity and alter the balance of bacterial types, which may then elicit increased release of bound residues.

Bacterial release of such residues, as well as pH effects, may also be responsible for the similarities in the extractable radioactivity in the wheat rhizoplane and rhizosphere. Coupled with the plant uptake of the released residues, all these factors may contribute to the insignificance of displaced residues.

In the soybean rhizoplane, however, there was a two-fold increase in extractable residues compared to the control without any plants. Differences in pesticide release in the rhizoplane of soybean compared to wheat may be the result of the differences observed in their root

mediated pH changes. As observed previously, wheat roots in Hoagland's nutrient solution, No. 2 increased the pH of the media following an initial acidification. Soybeans, on the other hand, produced only slight acidification in the agar media. Disruption of the soil matrices to release the bound residues would be expected to be higher in the wheat roots because of its wide range of pH variation. But, such was not the case in this study.

Higher extractability of bound residues in the soybean rhizoplane soil may possibly be attributed to differences in the microflora between wheat and soybean roots. Wheat root environments are mainly characterized by Enterobacter, Klebsiella, and Pseudomonas bacteria (Haahtela et al. 1983; Kleeberger et al. 1983). Although Enterobacter and Klebsiella have been isolated from legume roots, such as soybean (Evans et al. 1972), rhizobia populations predominate (Robert and Schmidt 1983; Tuzimura and Watanabe 1962). Differences in the constituent microbial populations may, therefore, be responsible for differences in the release of residues in the rhizoplane.

A concern raised by such research is that the root and its surrounding soil environment is a complex matrix of multifarous interactions between roots, microflora, and soil. Therefore, simplification of this system to a few measurable parameters may be experimentally, but not ecologically, valid. Events relating to the release of the soil-bound ^{14}C-prometryne residues by pH changes mediated by the root may be masked by the complex interactions that are present in the microcosm. Even with such limitations, the primary

hypothesis was validated in that there was a significant release of previously "bound" herbicide residues, and this was especially confirmed in the soybean trials. The release of formerly bound residues may be potentially available for interactions with other components of the ecosystem.

Potential interactions may include exerting its herbicidal effects on the crop species or soil microflora, as detailed above, uptake into crops, and mineralization by the microflora. Microbial mineralization of pesticides to carbon dioxide has been responsible for the inability to recover all of the pesticide inoculum. Khan and Hamilton (1980) attributed the loss of radioactivity in the soil-^{14}C-prometryne incubation flasks to microbial degradation and volatilization. More importantly, diazinon and parathion mineralization (13 - 18% of the added insecticide) was more extensive in the rhizosphere of bush bean plants than in the controls lacking plants (5 - 8%) (Hsu and Bartha 1979). Parathion mineralization has also been observed in rice and pearl millet. The amount of $^{14}CO_2$ evolved was dependent on the age of the plant and the root oxidase activity (Reddy and Sethunathan 1983). Carbon dioxide evolution from the rhizosphere decreased as the plants age and the root oxidase activity decreased.

2. Plant Uptake of Released ^{14}C-Prometryne

The crop species, itself, is not an isolated entity in the microcosm but may also interact with the unbound pesticide residues. These residues may be absorbed by the plants and translocated into the plant

tissues. In the present experiments, the crops were able to absorb approximately 1% of the soil-bound radioactivity.

Oat plants have previously been shown to extract 0.53% of soil-bound ^{14}C and incorporate this into the tissues after 3 weeks of growth (Khan 1980). Similar uptake of bound pesticide residues by other plant species have been noted. Absorption of soil-bound ^{14}C-cypermethrin, in 6 weeks, ranged from 0.14 to 0.58% for wheat plants (Roberts and Standen 1981) while ryegrass was able to take up 0.23% of the bound ^{14}C-hydroxymonolinuron (Haque et al. 1982). Higher uptake levels (0.46 - 1.07%) were found for soybeans after 10 weeks for bound dinitroaniline residues (Helling and Krivonak 1978b); for maize (2.4%) of bound methabenzthiazuron residues after 29 days (Fuhr and Mittelstaedt 1980); and in 14 days for oats (5.1%) of ^{14}C-parathion residues (Fuhremann and Lichtenstein 1978). In the present study, the uptake levels of bound ^{14}C-prometryne by wheat and soybeans appears consistent with such findings.

Absorption of prometryne by the root tissues, like most triazine herbicides, is through a passive process (Sheets 1961; Shone and Wood 1974; Sikka and Davis 1968). Root absorption of triazine herbicides is considered to be biphasic, viz., there is an initial rapid uptake where the herbicide moves into the apoplast followed by a relatively slow continuous phase (Vostral et al. 1970). The initial rapid sorption has also been attributed to partitioning of the chemical into the lipophilic constituents of root solids up to an equilibrium concentration (Briggs et al. 1982) and this is generally viewed as a physical sorptive

process. Briggs *et al.* (1982) further suggests that the lipophilic nature of root solids does not vary widely among plant species, thereby implying that the lipophilicity of the pesticide is the determining factor of root uptake. If such is the case, this may account for the lack of variation observed in the ^{14}C-prometryne uptake between wheat and soybean in this study. However, this view is still conjectural.

3. Partitioning of ^{14}C-Prometryne in Plant Tissues

Within the plant, herbicide entry into shoot tissues is apoplastic (Ashton and Crafts 1981). Although there was no difference in uptake of the herbicide by wheat and soybean, there was a considerable increase in the levels of radioactivity in soybean shoots (75%) compared to wheat shoots (50%), reflecting differences in the rates of herbicide transport. In most studies with triazine herbicides, there was a higher proportion of the residues located in the shoots. Mayer *et al.* (1981), using ^{14}C-dimethametryn, found that paddy rice had accumulated 5.2% of the initial soil applied radioactivity into shoot tissues and 0.6% in the roots. Similar levels were observed for atrazine in corn (Jachetta and Radosevich 1981) and simazine in fir trees (Gile *et al.* 1980). For prometryne, Khan (1980) found 69.8% of the total plant radioactivity in the oat shoots and the remaining in the roots. Pea plants have been found to transport approximately 70% of the absorbed prometryne into shoots (Eshel *et al.* 1975). Similarly, Sikka and Davis (1968) found similar partitioning of radioactivity in ^{14}C-prometryne exposed soybeans where 23% of the radioactivity was found in the roots and 77% in the shoots. This rapid movement and accumulation of the herbicide

into shoot tissues is significant since phytotoxicity is enhanced as the herbicide levels increase at the active sites of herbicide action (Eshel et al. 1975).

Differences in metabolism are also apparent between the two crop species. The 2-hydroxylation pathway was the only degradative process identified in these two species. In most cases, the parent herbicide, prometryne, represented about 20 to 30% of the radioactivity in the tissues with soybean roots being the only exception (8%) but this may have been due to the high level of radioactivity accumulated in the shoot. Such levels are significantly higher than levels observed by Khan (1980) for oat plants but this may be attributed to differences in the metabolism by the plant. The different levels of prometryne present in the tissues, particularly in the wheat, may reflect different abilities to degrade prometryne (Montgomery and Freed 1964).

Although there was no difference between fertilizer treatments in wheat metabolism of the herbicide, there was a significant difference between wheat and soybean, reflecting differences in herbicide mobility and metabolism. Hydroxylation of the 2-methylthio group of prometryne occurred in both crop species and the product was conjugated to an unknown moiety. Upon hydrolysis, the conjugated product was separated to a hydroxy analogue which was identified by methylation with diazomethane (Ashton and Crafts 1981; Khan 1980). In wheat tissues, this metabolic pathway was approximately the same in root and shoot tissues, viz., similar levels of radioactivity were obtained in the methanol:water soluble fraction. However, in soybean tissues, approximately 50% of the

total plant radioactivity was in the form of the conjugated hydroxy analogue in the shoots. High levels of conjugated products were similarly noticed in 3-week old oats (Khan 1980). The conjugated species were not identified in this project but previous workers, using atrazine, have postulated the presence of a glutathione conjugate (Lamoureux et al. 1973, 1972, 1970). As well, Smith and Wilkinson (1974) have postulated an N-glucoside conjugation pathway for metribuzin in soybeans.

More importantly, however, was the fact that the soil-bound prometryne residues had been absorbed and then become bound again in the plant tissues. Although analysis of the plant bound residues was not conducted in the present study, Khan (1980) found that the mono-N-dealkylated compound, 2-methylthio-4-amino-6-(isopropylamino)-s-triazine, and traces of the di-N-dealkylated metabolite, 2-methylthio-4,6-diamino-s-triazine, were present as the bound species. It was further shown that these residues had become bound in the lignin fraction of the plant tissues. Lignin incorporation of pesticide residues have been considered for nitrofen in wheat and rice straw. Approximately 30% of the radioactivity was found in the lignin of these plant tissues after treatment with ^{14}C-nitrofen (Honeycutt and Adler 1975). The pesticide moiety is believed to be incorporated covalently into the lignin polymer as postulated for chloroaniline (Still et al. 1981) and dieldrin (Stratton, Jr. and Wheeler 1983). In addition, some of the bound ^{14}C in the radishes exposed to ^{14}C-dieldrin was also found in the protein fraction (Stratton, Jr. and Wheeler 1983). Protein and carbohydrate binding of prometryne residues in plant tissues have also been observed (Khan 1980, 1982).

Binding in soybean and wheat tissues was considerably lower in the shoots than in the root tissues. In addition, binding levels, particularly in shoot tissues, are lower than values reported for oats (Khan 1980). Such differences may reflect species differences which may include metabolic differences or the action of the herbicides at active sites of these sensitive plants. These results support previous work, that binding differs with the variety of pesticides and crops (Sonobe et al. 1982). If binding plays a detoxication mechanism in plant tissues as suggested (Chin et al. 1973; Mallipudi and Fukuto 1981), herbicidal action cannot be elicited if high levels of binding occur in the sensitive tissues. Binding in root tissues would then be expected to be higher since these tissues are not the active site of the herbicide.

V. SUMMARY AND CONCLUSIONS

The results of the project demonstrate that the release of soil-bound ^{14}C-prometryne residues was a pH-dependent event. Changes in pH can affect the structure of humic materials in the soil which can uncage the bound residues and release them into the soil solution. Large changes in pH can cause major release of all bound residues while small deviations may be responsible for disproportionate release of the pesticide and its derivatives.

Such pH changes may have important implications in agricultural systems because plant roots are able to effect localized pH changes in the rhizoplane and rhizosphere. This phenomenon can be manipulated by

the application of different nitrogenous fertilizers and was shown to be dependent on the crop species.

However, under laboratory conditions, the release of the bound residues by crop species may involve more interactive physiological factors other than pH alone. The lack of a relationship between fertilizer treatments, and subsequently rhizosphere pH, with the release of bound residues may implicate more complex interactions in the soil microcosm. Such interactions may offer areas of concern in the field of soil-bound pesticide residues, particularly with the potential effects that these soil residues may have on soil microbial activity.

The release of bound residues into the soil solution and the role of nitrogenous fertilizers in this process may have serious implications for present-day cropping and fertilizing practices. With the release, the possibility exists that herbicidal activity may be exerted into non-target species and that these compounds may be incorporated into biological constituents. However, further studies must be carried out into the effect that these remobilized "bound" residues may have on non-target crop species and on the soil microflora and microfauna.

The fact that nitrogen-fixing organisms were placed under conditions of chronic rather than acute stress, when exposed to prometryne, and that this in some cases affected heterocyst formation in Anabaena and generation time in R. japonicum elicits some concern as to the effect of pesticide residues, particularly of soil-bound residues, on natural

nitrogen cycles. Similarly, prometryne effects on C. segnis signals concern for the maintenance of population levels of stressed cells and possible effects on carbon fixation via photosynthesis. Yearly application of pesticides in the soil may potentially augment residual pools in the soil, especially the soil-bound residues, and thus, as this study indicates, there should be concern for potential effects of such compounds on ecologically important natural cycles. Further studies will be required on the effects of soil-bound residues on these soil interactions.

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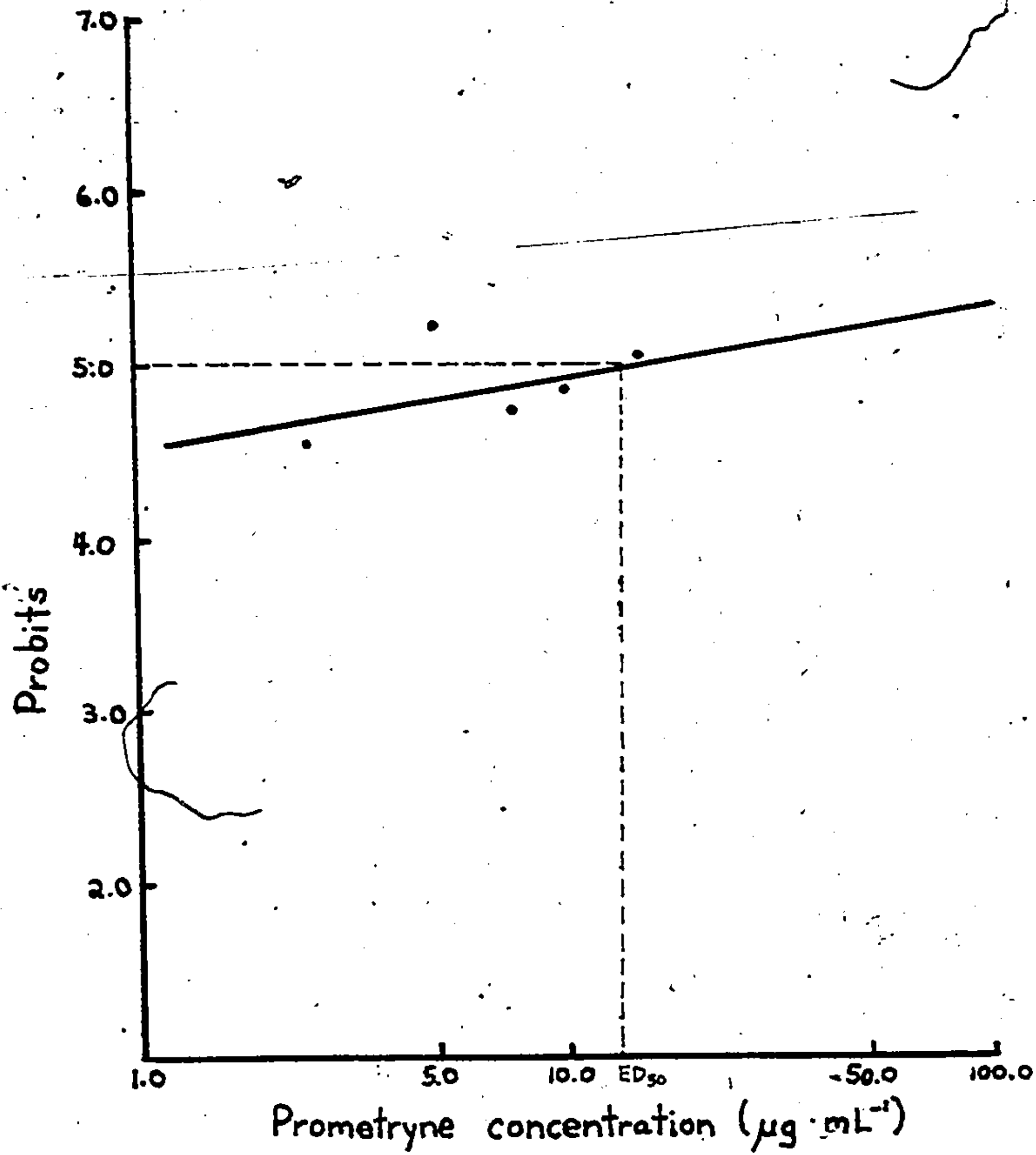
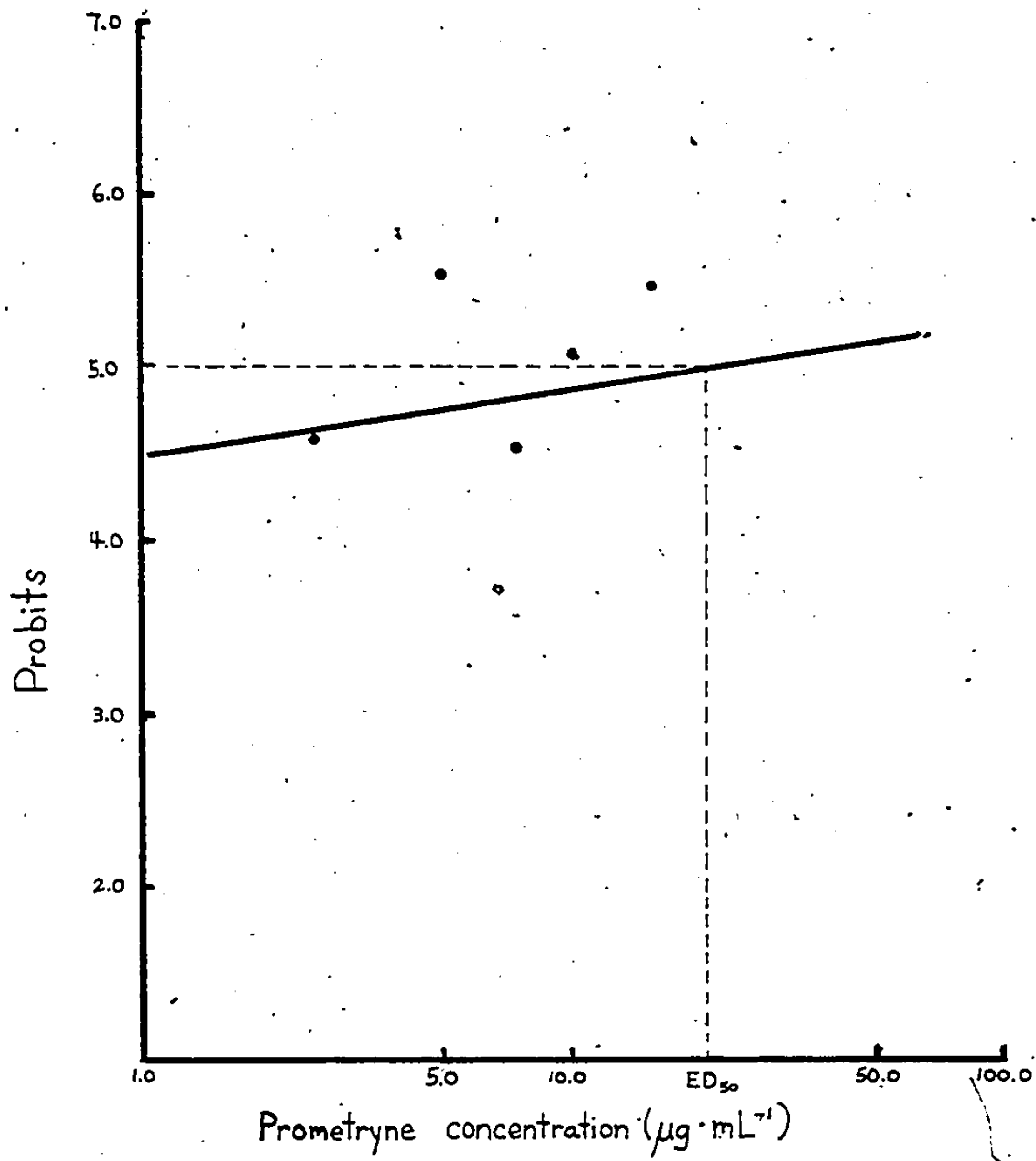
Appendix A

Probit Plot of Soybean Fresh Weights Against Prometryne Concentration


7.0
6.0
5.0
4.0
3.0
2.0
Probits
1.0
5.0
10.0
ED50
50.0
100.0
Prometryne concentration (µg · mL⁻¹)

Appendix B

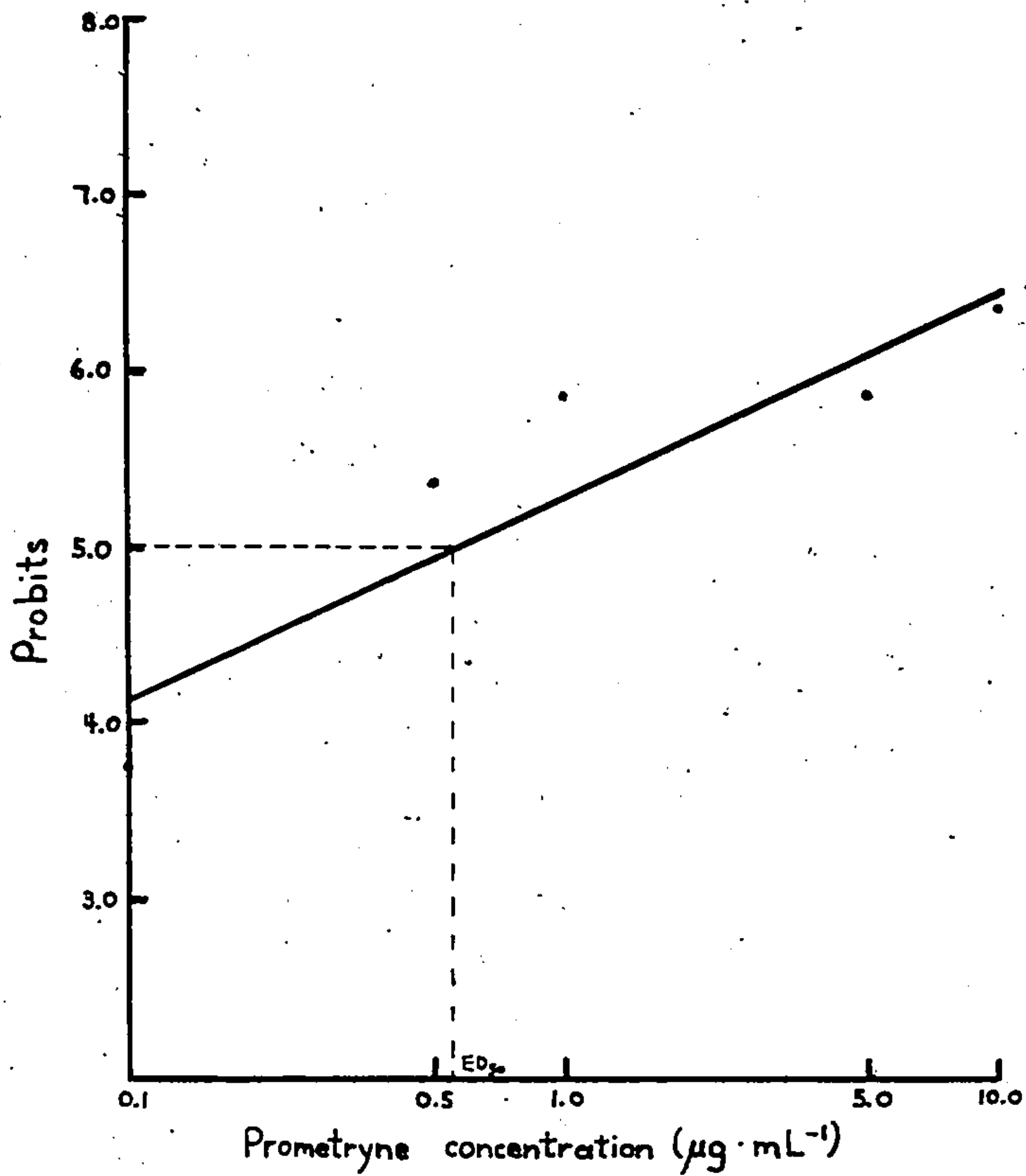
Probit Plot of Soybean Freeze-Dried Weights Against Prometryne Concentration.



7.0
6.0
5.0
4.0
3.0
2.0
Probits
1.0
5.0
10.0
ED50
50.0
100.0
Prometryne concentration (μg·mL⁻¹)

Appendix C

Probit Plot for <u>Anabaena</u> spp. Heterocyst Frequency Against Prometryne Concentration.


8.0
7.0
6.0
5.0
4.0
3.0
Probits
ED50
0.1
0.5
1.0
5.0
10.0
Prometryne concentration (μg · mL⁻¹)

RÉSUMÉ

Des plantules de fèves de soya (*Glycine max* (L.) Merr. cv. Maple presto), une algue verte (*Chlamydomonas segnis* Ettl), une cyanobactérie (*Anabaena* spp.), et deux bactéries pouvant fixer l'azote (*Klebsiella pneumoniae* souche M5A1 et *Rhizobium japonicum* souche 61A76) furent exposées séparément à des concentrations de 0,25 - 15,0 µg mL^{-1} de l'herbicide *s*-triazine, prométryne. Les paramètres de croissance observés ont inclus le poids frais et le poids sec (fève de soya), le contenu total de protéine (fève de soya), le nombre cellulaire (*C. segnis* Ettl), la fréquence d'hétérocystes (*Anabaena* spp.), et le temps de génération (*K. pneumoniae* et *R. japonicum*).

Une réduction du poids frais et du poids sec fut apparente chez les plantules de fève de soya traitées avec l'herbicide (2,5 - 15,0 µg mL^{-1} de prométryne). Le contenu total de protéine, mesuré par le test de Hartree pour les protéines et par le test de Kjeldahl pour l'azote, fut variable chez les plantules traitées. Les plantules de fève de soya traitées ont montré un pauvre développement foliaire et de la chlorose.

Une réduction de la population chez *C. segnis* Ettl et de la fréquence d'hétérocystes chez *Anabaena* spp. fut notée après une exposition à l'herbicide (0,25 - 12,0 µg mL^{-1}). Une "récupération" complète fut obtenue chez les cultures lavées de *C. segnis* Ettl et inoculées sur des plaques d'agar nutritif ne contenant aucun pesticide. Une "récupération" semblable n'a pu être obtenue chez les cellules lavées d'*Anabaena* spp.

K. pneumoniae fut relativement insensible à l'herbicide, cependant le temps de génération chez *R. japonicum*, lorsqu'exposée à 6,8 µg mL^{-1} de prométryne, fut augmenté de 3 fois comparativement aux cultures non-traitées (26,25 h vs. 9,5 h).

A l'aide de cultures axéniques de plantules sur un milieu gélosé, les auteurs ont établi la relation entre l'azote utilisé comme élément nutritifs (nitrate, ammonium et urée) et les changements du pH de la rhizosphère. Il y a une relation entre le pH de la rhizosphère et les éléments nutritifs; cette relation est influencée par la source d'azote dans le milieu de culture. Après 3 semaines de croissance, les plantules de blé fertilizées avec la solution nutritive n° 2 de Hoagland produisent un pH de 8,5 à 9,5. Une alcalinisation comparable s'observe avec les plantes fertilisées au nitrate. Un pH inférieur à 4,0 est obtenu dans la rhizosphère si les plantes sont fertilisées avec des sels d'ammonium, tandis qu'on n'observe aucun changement significatif avec les plantules de blé pour lesquelles l'urée est la seule source d'azote. Ces résultats concordent avec le concept d'équilibre dans l'absorption des cations et des anions, avec le maintien de l'électro-neutralité à travers la surface des racines, ainsi qu'avec l'absorption par des cotransporteurs et les principes chimio-osmotiques.

La remobilisation des résidues de ^{14}C-prométryne liés au sol fut affectée par le pH du sol, par les traitements avec engrais (avec ou sans plantules) et par les espèces cultivées, soit le blé (*Triticum aestivum* (L.) Merr. cv. Marquis) et la fève de soya. De grands changements dans le pH du sol, de pH 5,4 à pH 4,0 et pH 8,0, ont résulté

en une libération de 24 - 25% de la radioactivité liée, pendant que de plus petits changements, de pH 5,4 à pH 4,9, ont libéré seulement 16% des résidus liés au sol. De plus, les changements de pH ont résulté en des différences dans la facilité d'extraction de la prométryne et de son dérivé hydroxylé, l'hydroxyprométryne. Plus de prométryne fut obtenu après de grands changements de pH, à partir du pH 5,4.

Des traitements avec engrais, à un pH 5,4, ont aussi conduit à la remobilisation du ^{14}C-prométryne lié au sol. L'utilisation de source d'azote ionique (NO_3^- et NH_4^+) a libéré 4,0 - 16,7% des résidus liés. Des quantités négligeables (0,4%) de radioactivité extraite furent détectés suivant des traitements à l'urée. Ces différences cependant ne furent pas apparentes en présence du système racinaire des plants de blé. Les plants de blé, poussant dans chacun des traitements à l'engrais ont résulté en une remobilisation d'environ 15% de la radioactivité liée totale.

La remobilisation fut spécifique aux espèces végétales, particulièrement dans le rhizoplan. Les racines de fève de soya ont provoqué une plus grande libération de radioactivité liée (36,6%) dans le rhizoplan que les racines de blé (10%). Cependant, le pourcentage d'absorption des dérivés remobilisés fut semblable chez les deux espèces (1% de la radioactivité liée totale). Le transport et le métabolisme de ces résidus furent aussi spécifiques aux espèces.