Plant Growth-Promoting Rhizobacteria (PGPR) Enhanced

Phytoremediation of DDT Contaminated Soil

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis,

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ABSTRACT

Although the pesticide DDT has been banned from use in Canada for more than three decades, DDT still persists in Canadian farmlands at detectable levels. Much effort, such as incineration, thermal desorption, and bioremediation, has been used to remediate DDT contaminated soils, but so far it is either too expensive or impractically slow. In this study, a three-year period of field trials was performed to investigate phytoremediation of DDT contaminated soil.

In the field trials, millet, fall rye, sugar beet, potato, and pumpkin, treated with plant growth-promoting rhizobacteria (PGPR) were planted on two sites. As well, untreated plants were planted as a control. Plant growth, and 4,4'-DDT plus 4,4'-DDE concentrations in plant tissues and soil were monitored regularly. Comparing the plant growth between PGPR treated and untreated, PGPR significantly promoted the plant growth. On site 1, the root length and root weight of fall rye treated with PGPR were 16% and 44% greater, respectively, compared to the untreated plants. The root and shoot dry weights of millet treated with PGPR were 38% and 47% greater than those untreated plants. Root dry weight of sugar beet treated with PGPR was increased by 74% compared to untreated sugar beet. A significant effect of growth promotion was also observed in pumpkin and potato treated with PGPR.

Following plant growth, DDT detection in plants was performed. 4,4'-DDT and 4,4'-DDE were found in plant tissues of fall rye, millet, sugar beet, and pumpkin. The concentrations of 4,4'-DDT and 4,4'-DDE in fall rye roots were 0.61 and 0.59 μg/g, respectively. In pumpkin tissues at harvest, 4,4'-DDT and 4,4'-DDE concentrations were 0.67 and 1.64 μg/g in roots, 1.06 and 2.05 μg/g in the lower stems, and 0.2 and 0.32 μg/g in

the upper stems. The data indicated that it is feasible to phytoremediate DDT from contaminated soil.

In addition, 4,4'-DDT concentrations in soils with different plant species were determined. In millet plot on site 1, 4,4'-DDT concentration in rhizosphere soil dropped by 41% in 2006 compared to 4,4'-DDT concentration at t_0. In sugar beet plot on site 1, 28% of 4,4'-DDT dropped in rhizosphere soil in 2007. In pumpkin plot on site 1, 4,4'-DDT in rhizosphere soil was decreased by 22% in 2007. The results show that 4,4'-DDT concentration in rhizosphere soil was significantly lower than the initial level of DDT.

Based on the data of 4,4'-DDT in soils and plant tissues, a mass balance was constructed and calculated. The preliminary mass balance shows that the total amount that DDT decreased in rhizopshere soil approximately equals to the total amount of DDT accumulated in plant tissues. This indicates that phytoextraction is the mechanism of DDT phytoremediation. In addition, PGPR promoted plant growth and then enhanced the phytoremediation efficiency of DDT. Therefore, the research indicates that PGPR assisted phytoremediation has a great potential for remediation of DDT and other chlorinated aromatics from impacted soil.

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ABBREVIATION

ACC	1-aminocyclopropane-1-carboxylate
ANOVA	Analysis of variance
CEC	Commission for Environmental Cooperation
DCM	Dichloromethane
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethene
DDT	Dichlorodiphenyltrichloroethane
EC	Environment Canada
HPLC	High performance liquid chromatography
mAU	Milli-absorbance unit
min	Minute
ND	Near-root soil
OGGA	Ontario Ginseng Growers Association
PGPR	Plant growth-promoting rhizobacteria
RPM	Revolutions for minute
RS	Rhizosphere soil
TG-DDT	Technical grade DDT
TLC	Thin layer chromatography
TSB	Tryptic soy broth
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency

1 Introduction

Dichlorodiphenyltrichloroethane (DDT) is a chlorinated insecticide that has been widely used throughout the world. DDT consists of 2 isomers, 4,4'-DDT [1,1,1-trichloro-2,2-bis-(4-chlorophenyl) ethane] and 2,4-DDT [1,1,1-trichloro-2(2-chlorophenyl)-2-(4-chlorophenyl) ethane] (Figure1.1). DDT can be degraded to form one of 2 related compounds, DDE or DDD (Figure 1.1) (Corona-Cruz et al., 1999). Both forms of DDT and the related compounds were produced during its manufacture (USEPA, 1980). DDT was first synthesized in 1874 by a German chemist named Othmar Zeidler, but its insecticidal properties were not discovered until 1939 by a Swiss scientist named Paul Mueller who was the winner of 1948 Nobel Prize for his effort (Russel, 1955). Large scale industrial production was started in 1944 by Montrose Chemical Corporation in California (Singh, 1962). In developed countries, DDT was used extensively in agriculture as a general insecticide, and was also successfully used in exterminating insects that carried vector-borne diseases like typhus and malaria (EC, 1999). Although no longer used in the western world, many tropical countries still employ DDT to control malaria-infected mosquitoes and it remains one of the most effective and affordable insecticide available in the world (EC, 1999).

An ideal pesticide should persist long enough to control target organisms and then be degraded to inert or nontoxic products. Unfortunately, DDT was found to affect organisms across many kingdoms, and it is highly persistent in the environment (Nirwal, 2001). *Silent Spring* by Rachel Carson (1962) is widely accepted as launching the environmental
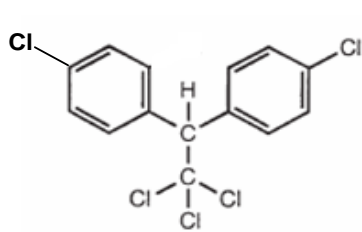
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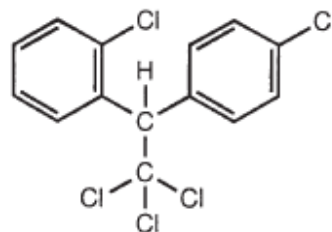
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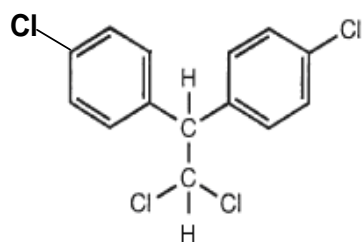
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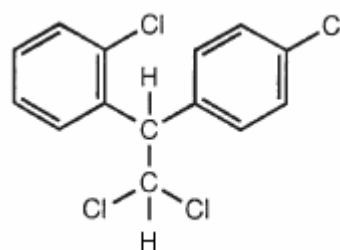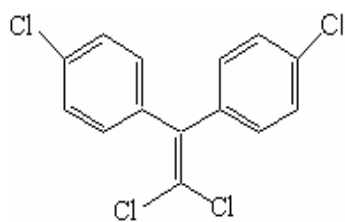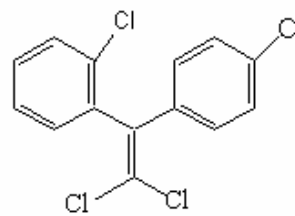
2,4-DDD

4,4'-DDE

2,4-DDE

Figure 1.1 Chemical structures of 4,4'-DDT, 2,4-DDT, 4,4'- DDD, 2,4-DDD, 4,4'-DDE, and 2,4- DDE.

movement in the west. The book claimed detrimental effects of pesticides on the environment. In particular, the author claimed that DDT had been found to cause thinner egg shells and result in reproductive problems and death. *Silent Spring* contributed to the ban of DDT in 1972 in the United States. The effects of DDT are not restricted to animals. Toxic effects on bacteria (Donato et al., 1997) and other eukaryotic microorganisms (Lal and Saxena, 1982) have been observed. Therefore, persistence of DDT in contaminated soil is of environmental concern. Research and development of remediation technologies for DDT has become a high priority.

In this chapter, the physical and chemical properties of DDT are described, which are related to the fate and persistence of DDT in our environment. Current remedial treatments, such as non-biological methods, bioremediation and phytoremediation, are introduced in detail. The objectives of this study are given at the end of the chapter.

1.1 Physical and chemical properties

DDT and its metabolites, DDD and DDE, are chlorinated aromatic hydrocarbons and are regarded as Persistent Organic Pollutants (POPs) (UNEP, 2001). They are chemically stable at room temperature and all highly persistent in the environment. DDT is subject to slow transformation in the environment. The major transformation products are DDD and DDE, both of which are more persistent than its parent compound.

Commercially available DDT is known as the technical grade DDT (TG-DDT) and is comprised of 4,4'-DDT (77.1%), 2,4-DDT (14.9%), 4,4'-DDE (4.0%), 2,4-DDE (0.1%), 4,4'-DDD (0.3%), 2,4-DDD (0.1%) and 3.5% unidentified products (WHO, 1989). TG-DDT is a non-flammable, odourless mixture that forms colorless crystals or a waxy solid at room temperature (Worthing and Hance, 1991). DDT and its related products are insoluble in water

and strongly lipophilic. As well, they are soluble in organic solvents such as acetone, xylene or petroleum distillates (Budavari et al., 1989). Hydrophobic chemicals are classified by an octanol-water partition coefficient (log K_{ow}) greater than 3.5; the log K_{ow} of DDT is 6 (Suntio et al., 1988).

There are three important physical properties related to the environmental behaviour of organic chemicals: vapour pressure, water solubility, and log K_{ow} (Paola et al., 2004). Table 1.1 shows these values for DDT, DDD, and DDE. All isomers of DDD and DDE have notably higher vapour pressures and water solubilities than DDT.

1.2 Fate and persistence of DDT in soil

The environmental fate of DDT and its related compounds has been well documented. DDT is slowly converted to DDE and DDD in soil. The half-life under field conditions for DDT ranges from 2 years (Lichtenstein and Schulz, 1959) to greater than 15 years (Keller, 1970). In flooded soils or slurries in laboratory tests, biodegradation of DDT spiked in soil is faster, with half-lifes estimated from 16 to 100 days (Castro and Yoshida, 1971). However, this is inconsistent with real half-life in slurries in the environment because DDT has been observed to persist for many years.

The fate and behaviour of DDT in soil is influenced by soil type, temperature, and the physiochemical properties of DDT (Nicholls, 1991). The physiochemical properties of DDT are the key factor in the fate of DDT in soil (Alexander, 1995). The high lipophilicity of DDT makes it adsorb strongly to organic matter in soil, thereby lowering bioavailability.

The adsorption of DDT by different types of soils has been tested. It was found that adsorption is lowest in sandy loam, followed by clay soil, and greatest in organic soil (EC, 1999). Adsorption of DDT has been related to organic matter in soil, and WHO (1989)

Table 1.1 Physical and chemical properties of DDT, DDD, and DDE
(Suntio et al., 1988; Worthing and Hance, 1991).

Property	4,4'-DDT	2,4-DDT	4,4'-DDE	2,4-DDE	4,4'-DDD	2,4-DDD
Molecular Weight	354.5	354.5	318.0	318.0	320.1	320.1
Melting Point (^{o}C)	108.5	74-74.5	88-90	88-90	109-110	76-78
Boiling Point (^{o}C)	185	N/A	N/A	N/A	N/A	N/A
Vapour Pressure[1] (mPa)	0.02	0.02	1	0.8	0.1	0.2
Water Solubility[1] (µg/L)	3	3	40	100	100	50
log K_{ow}	6.0	6.0	5.7	5.8	5.5	6.1

Note: [1] At 20 ^{o}C. N/A: Not Applicable.

reported that humic acids were the major source of adsorptive capacity for DDT. Therefore, DDT binds strongly to soil and is difficult to displace from sites of their application. Accordingly, Liechtenstein and Schulz (1959) found that the amount of DDT remaining from wetland soil was 1.4 times more than that remaining from a Miami silt loam. This is because wetland soil has more humic acids.

Another important factor in the persistence of DDT in soil is temperature. In tropical climates, volatilisation of DDT can account for their loss from soil surfaces (Suntio et al., 1988; Villa et al., 2006). High soil temperature, along with sunlight and atmospheric humidity were considered to be major factors responsible for dissipation of DDT by volatilisation (Samuel and Pillai, 1989). However, these factors have little effect on DDT absorbed below the soil surface. In the Arctic, the persistence of DDT is thus very long. Both low temperature and relatively low sunlight limit bioavailability of DDT (Falconer et al., 1995).

1.3 DDT levels in Canadian soils

DDT was produced in large scale after the World War II with the majority of production occurring in the United States. DDT was first registered and used in Canada in 1946 (CEC, 1997). DDT was never manufactured in Canada and most DDT products in Canada were imported from the US. DDT was used extensively in Canada to kill agricultural and forest pests. For example, in Ontario, DDT was sprayed on tobacco and vegetable crops to control cutworms and other pests (EC, 1999). DDT in Canada was applied by both aerial and land-based spraying operations (EC, 1999). Direct application, storage, and disposal of DDT resulted in the release of DDT to soil and other environmental components.

The data regarding DDT levels in Canadian soil, especially in northern Canada, such as

the Yukon and the Northwest Territories, is scarce (EC, 1999). However, soil samples collected in Ontario had DDT, DDD, and DDE levels ranging from 1.7 to 342 μg/g in the Niagara Peninsula (Hebert et al., 1994) and 0.17 to 1.3 μg/g in old urban parklands (OMEE and Ontario Ministry of Environment and Energy, 1993). DDT was also detected in agricultural soil in Ontario. In this study, 4,4'-DDT concentrations were around 0.6-1.2 μg/g in farm soils near Scotland, Ontario.

There are regulated limits for DDT residues and its related compounds in foods. For instance, DDT levels of 0.5-1.0 μg/g in eggs, fresh vegetables, and dairy products and 5 μg/g in fish are allowed in Canadian foods (CEC, 1997). These levels have been established based on the information from domestic and imported foods, and are periodically revised with updated available information.

1.4 Remediation of DDT

Many strategies have been advanced to remove DDT from soil. Conventional non-biological treatments for organochlorine contaminated soils include excavation and incineration, thermal desorption (Norris et al., 1997), microwave-enhanced thermal treatment (Kawala and Atamanczuk, 1998), and soil washing with surfactants and solvents (Parfitt et al., 1995). Although non-biological treatments are rapid and result in degradation and/or removal of DDT, they are very expensive. In addition, non-biological treatments may produce more toxic compounds during the process. Therefore, other technologies are needed to address DDT remediation.

1.5 Bioremediation of DDT

Bioremediation has become the focus of much research to develop safe and effective

methods to lower DDT levels in soil. The processes involved in bioremediation, such as

oxidation, hydroxylation, aromatic ring cleavage and dehalogenation, have been studied

extensively. In the laboratory, microbes that metabolize DDT, including aerobic bacteria,

anaerobic bacteria and fungi, have been isolated from soil, rotting wood, sewage and

activated sludge (Bumpust and Aust, 1987; Kamanavalli and Chandrappa, 2004; Rajiv et al.,

2001). DDT can be dechlorinated to DDD via reduction of an aliphatic chlorine with a

hydrogen atom (Figure 1.2). Another route for metabolism of DDT is oxidation. This can be

done by aerobic bacteria, which can metabolize DDT into DDE (Lipke and Kearns, 1959).

There are reports that DDE is susceptible to further aerobic microbial degradation (Figure

1.3). As well, several fungal genera have been reported to transform DDT via reductive

dechlorination (Bumpust and Aust, 1987).

The ability of different microbial strains to degrade DDT is variable. Hay and Focht

(1998; 2000) have demonstrated that *Pseudomonas acidovorans* strain M3GY could only

transform DDE to dihydroxy-DDE (Figure 1.3), while *Ralstonia eutropha* strain A5 could

transform not only DDT, but also DDE and DDD. Strain A5 can grow on biphenyl in the

presence of DDD and yield a distinct yellow product, which has been identified as

1,1-dichloro-2-(hydroxy-4-chlorophenyl)-2-(4-chlorophenyl) ethane by GC-MS analysis.

The concentration of DDT affects its aerobic degradation rate. Complete degradation has

been observed for up to 15 µg/mL of DDT. However, the inhibitory effects at higher

concentrations of DDT have been observed with a total loss of degradative ability at 50

µg/mL of DDT (Rajkumar and Manonmani, 2002). This is because high concentrations of

DDT are toxic to bacteria.

Field studies examining DDT degradation in soil are difficult due to changes in soil
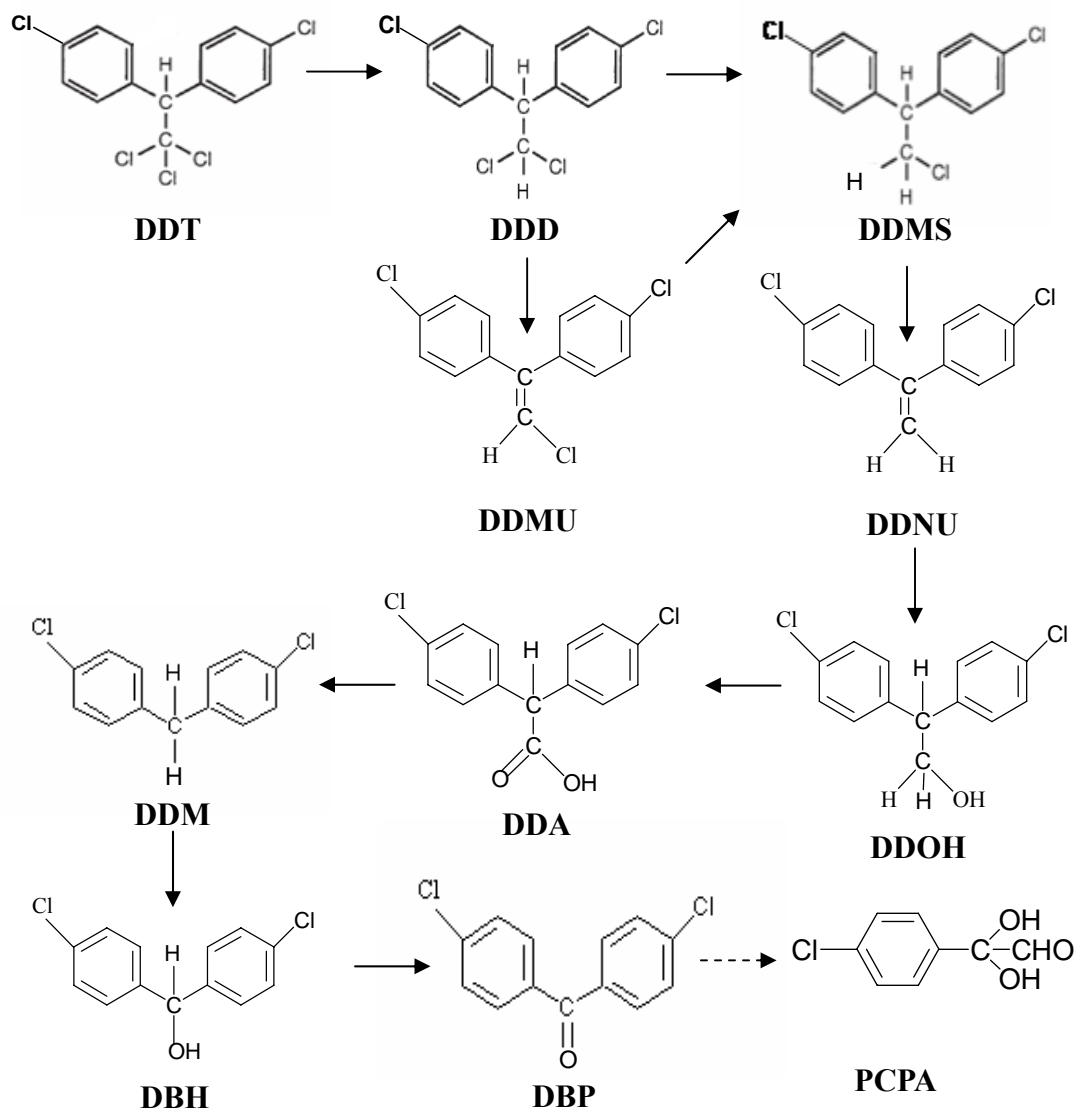
Figure 1.2 Proposed pathway for bacterial metabolism of DDT via reductive dechlorination and oxidation (Foght et al., 2001). DDMU: 1-chloro-2,2-bis(4-chlorophenyl)ethylene; DDMS: 1- chloro-2,2-bis(4-chlorophenyl)ethane; DDNU: 2,2-bis(4-chlorophenyl)ethylene; DDOH: 2,2-bis(4-chlorophenyl)ethanol; DDM: bis(p-chlorophenyl)methane; DDA: bis(4'-chlorophenyl)acetate; DBH: 4,4′-dichlorobenzhydrol; DBP: 4,4'-dichlorobenzophenone; PCPA: p-chlorophenylacetic acid. The dotted line indicates aerobic metabolism of DBP to PCPA.
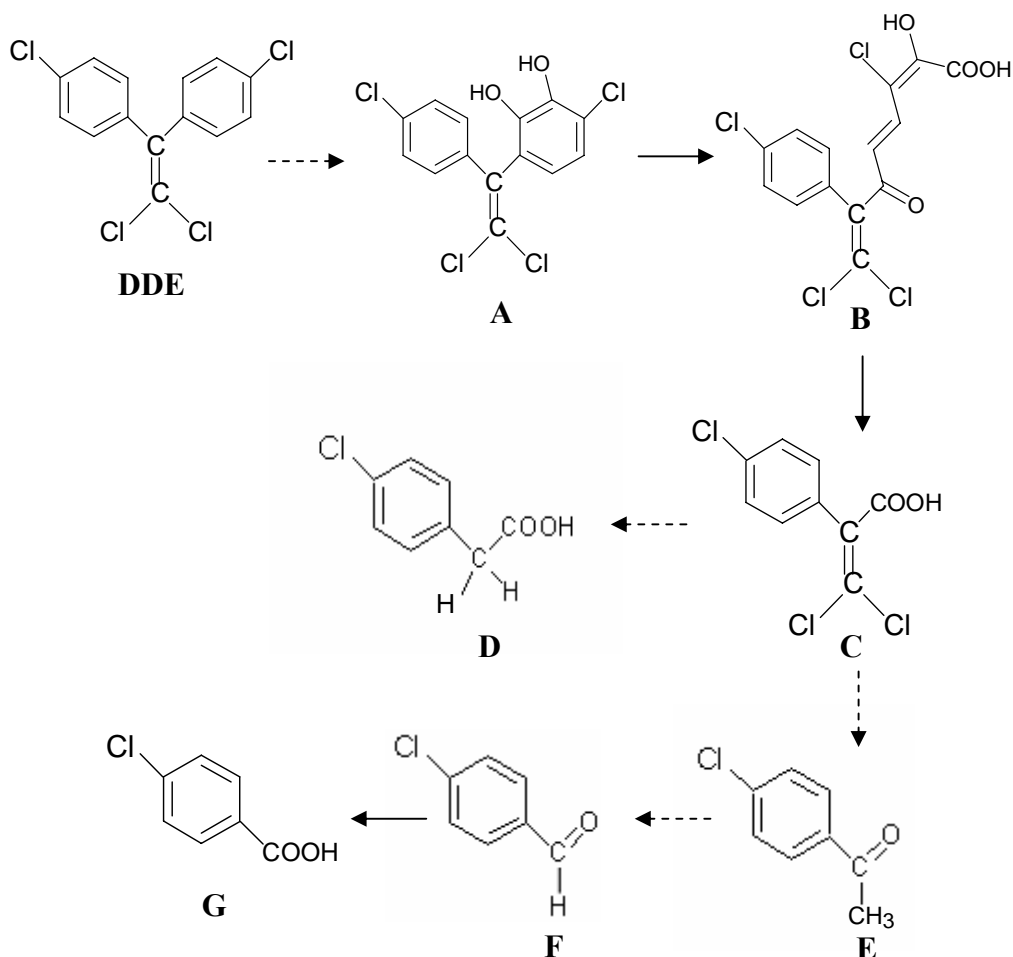
Figure 1.3 Proposed meta-ring cleavage pathway for degradation of DDE by *Pseudomonas acidovorans* M3GY (Hay and Focht, 1998). Metabolites C, D, and G were also produced by *Terrabacter sp*. DDE-1 incubated with DDE (Aislabie et al., 1999). Analogous pathways have been proposed for degradation of DDT (Nadeau et al., 1994) and DDD (Hay and Focht, 2000) by *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*) A5. Metabolite A: 1,1-dichloro-2-(dihydroxy-4-chlorophenyl)-2-(4-chlorophenyl)ethylene; B: 6-oxo-2-hydroxy- 7-(4-chlorophenyl)-4,8,8-trichloroocta-2,4-dienoic acid; C: 2-(4-chlorophenyl)-3,3-dichloropropenoic acid; D: 4-chlorophenylacetic acid; E: 4-chloroacetophenone; F: 4-chlorobenzaldehyde; G: 4-chlorobenzoic acid. Dotted lines indicate postulated steps in the pathway (Foght et al., 2001).

properties, mixed populations of soil microbes and climate. Both soil microorganisms and DDT are affected by soil properties and environmental conditions. Most studies have focused only on the biodegradation of DDT under controlled laboratory conditions.

DDT has generally been found to be persistent in the environment and is not easily biodegraded. There are a number of reasons for this. The low water solubility of DDT results in the poor bioavailability in soil (Alexander, 1995). The solubility of 4,4'-DDT in water is only 3 μg/L at 20°C (Table 1.1). In soil, DDT adheres strongly to soil particles, which affects the biodegradation rate of DDT. The chlorine substituents on DDT increase its persistence (Focht and Alexander, 1971) since few microbes have been isolated that can use DDT as a sole carbon and energy source (Golovleva and Skryabin, 1981).

Two general approaches have been tested to remediate contaminated soil (Atlas and Plilp, 2005): (1) soil can be excavated from the ground and be either treated or disposed of (*ex situ* treatment); (2) soil can be left in the ground and treated in place (*in situ* treatment). Because the cost of transporting the contaminated soil from the site of contamination to the site of treatment has been increasing with higher gasoline price, the whole cost of *ex situ* treatment is much higher than *in situ* bioremediation. *In situ* bioremediation, such as adding co-metabolic carbon sources to soils and introducing mixed populations of anaerobic bacteria (Corona-Cruz et al., 1999), or adding ligninolytic fungi (Hammel, 1995) and aerobic bacteria (Hay and Focht, 2000; Kamanavalli and Chandrappa, 2004) have been attempted. However, it is difficult to increase the natural attenuation rate of DDT due to low bioavailability of DDT in soil, and insufficient biomass of microbes in contaminated soils for degradation of contaminants.

1.6 Phytoremediation

The use of plants to remediate contaminated soil or water is known as phytoremediation. Phytoremediation can be defined as the process of utilizing green plants and their associated microorganisms, soil amendments, and agronomic techniques to absorb, accumulate, degrade, or render harmless environmental contaminants in the growth substrate (soil, water, and air) through physical, chemical or biological processes (Cunningham and Berti, 1993; Schwitzguebel, 2001). As an innovative technology, phytoremediation is gaining recognition as a cost-effective method of remediation of contaminated sites.

Phytoremediation includes the following distinct subtechnologies: phytoextraction, phytodegradation, phytovolatilization, and rhizodegradation. Phytoextraction refers to the use of plants to remove metals or organic chemicals from soil by accumulating them in harvestable parts of the plants (Singh and Jain, 2003). Metals and some organic compounds are taken up and/or translocated by plant tissues in a recoverable form. Then plants are harvested and disposed of by incineration or other recycling processes.

Phytodegradation involves processes beyond uptake and storage of contaminants in plant tissues. Contaminants are taken up from soil, metabolized in plant tissues and broken into less toxic or nontoxic compounds within the plant by compounds or enzymes produced by the plant (Burken et al., 2000; Meagher, 2000). Ekman et al. (2003) found that cytochrome P450, glutathione S-transferase, and the other two enzymes were possibly involved in plant degradation of 2,4,6,-trinitrotoluene (TNT) in *Arabidopsis*.

Phytovolatilization is another form of phytotransformation in which volatile chemicals or their metabolites are released into the atmosphere through the plant transpiration (Singh and Jain, 2003). This process has been observed in phytoremediation of petroleum hydrocarbons

(Macek et al., 2000).

Rhizodegradation, also referred to as rhizosphere biodegradation, enhanced rhizosphere biodegradation, and plant-assisted biodegradation, is the result of a plant root system releasing chemicals that enhance the biodegradation of organic contaminants by soil microorganisms in the rhizosphere (Germida et al., 2002). In the rhizosphere, soil redox conditions, organic matter, moisture, and other soil properties are affected by plant root exudates (Singh and Jain, 2003). Plants and microorganisms are involved directly and/or indirectly in the degradation of total petroleum hydrocarbons into products that are less toxic and less persistent in the environment (Carman et al., 1998).

The use of plants for removing organic or inorganic chemicals from contaminated soil has many advantages such as: growing plants is relatively inexpensive; large scale application is feasible because plants can be sowed over large areas; plants concentrate contaminants within their tissues, thus dropping the amount of waste; accumulated wastes within tissues can be easily reclaimed; rhizosphere activity is enhanced due to root exudates, thereby maintaining a healthy ecosystem; plants are helpful in lowering soil erosion and desertification (Saxena et al., 1999).

Unfortunately, phytoremediation also has limitations. Phytoremediation takes longer because plant growth rates and seasonality prolongs the remediation time compared with physical remediation technologies (Salt et al., 1995; USEPA, 2000). Since phytoremediation requires that contaminants be in contact with root zone of plants (Cunningham and Berti, 1993; USEPA, 2000), it is a challenge to extend the plant root system to contaminants or move contaminated media to the root zone of the plants. Nonetheless, phytoremediation has gained recognition as an environmentally friendly technology to clean up the environment.

More and more studies have been focusing on phytoremediation and/or the improvement of phytoremediation.

1.7 Plant growth-promoting rhizobacteria (PGPR) enhanced phytoremediation

To promote plant root growth to contact more contaminants in soil and to diminish contaminant and environmental stress on plant growth, plant growth-promoting rhizobacteria have been used in phytoremediation of creosote (Huang et al., 2004a; Huang et al., 2004b). There are two types of bacteria that provide some benefits to plant growth. One type forms symbiotic relationships, which involves the formation of nodules or other special structures on host plant roots. The other type found near, on or within plant tissues are free-living soil bacteria, which are generally referred to as plant growth-promoting rhizobacteria (PGPR) (Glick, 1995; Penrose and Glick, 2003; Persello-Cartieaux et al., 2003).

PGPR can promote plant growth and development by direct and indirect mechanisms. Indirect mechanisms include their ability to produce siderophores which chelate iron, making it unavailable to pathogens; the ability to synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, which suppress the growth of fungal pathogens; the ability to successfully compete with pathogens for nutrients or specific niches on the root; and the ability to induce systemic resistance (Bloemberg and Lugtenberg, 2001; Glick, 1995; Nelson, 2004; Persello-Cartieaux et al., 2003).

Direct mechanisms involve that fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and changes in phytohormone level (Glick, 2005). A good example is many PGPR contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which can break down ACC, the

precursor of ethylene, into **α**-ketobutyrate and ammonia (Figure 1.4). Because plants produce ethylene during chemical stress, PGPR containing ACC deaminase can promote plant growth by lowering ethylene levels (Glick 1995; 2005).

PGPR have been used in agriculture and horticulture. They increase germination rates, root growth, yield, leaf area, chlorophyll content, magnesium content, nitrogen content, protein content, hydraulic activity, tolerance to drought, shoot and root biomass, and delayed leaf senescence (Fages and Arsac, 1991). Recently, studies have shown three strains of PGPR to successfully remediate creosote from contaminated soil (Reed and Glick, 2005; Huang et al., 2004a). Plant properties including biomass, plant nutrition and health were improved by PGPR during phytoremediation. The PGPR, *Pseudomonas putida* UW3, was used in phytoremediation of creosote by three species of grass plants: Tall fescue, Kentucky bluegrass, and Wild rye (Huang et al., 2004a). Root biomass of all these plants significantly increased and germination of all three plant types increased dramatically in creosote-spiked soil. Creosote degradation from soil increased compared to the amount of creosote degraded in soil with plants not treated with UW3.

1.7.1 Phytoremediation of DDT

Phytoremediation can be applied to DDT contaminated soil. Many plants are able to take up and metabolize DDT from soil (Suresh and Ravishankar, 2004). In a greenhouse study, pumpkin and four other plants demonstrated the ability to take up DDT from soils (Lunney et al., 2004). At a high DDT concentration of 3.7 μg/g, pumpkins took up 6.7 μg/g dry weight to roots and shoots, while another species, zucchini, absorbed 5.3 μg/g of DDT. This success could be due to the large above-ground biomass and high volume of the fruits of these species. Similar results have been reported by White (2001). The phytoextraction of DDE by
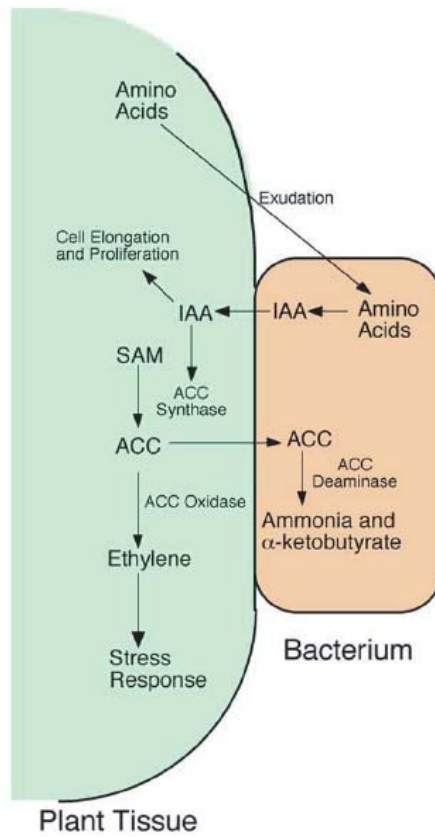
Figure1.4 A model depicting PGPR containing ACC deaminase promoting the plant root elongation (Glick et al., 1998).

cucumber and zucchini was investigated (Wang et al., 2004). Cucumber, which produces a fine and fibrous root system, accumulated more DDE than zucchini did under similar conditions.

In addition to the *Cucurbita* (pumpkin, zucchini, and cucumber), rye (*Secale cereale*) grown in Mexico was collected and investigated for the levels of organochlorine pesticides. Waliszewski et al. (2004) reported that 0.042 μg/g and 0.008 μg/g of 4,4'-DDT and 4,4'-DDE, respectively, were detected from rye roots growing in weathered soil though DDT has been banned in Mexico since 1999. In addition, alfalfa, ryegrass, and pole bean were planted in DDE contaminated soil (White, 2000). Significant decreases of DDE concentrations in the rhizosphere of alfalfa and ryegrass occurred. Concentrations of 4,4'-DDE in the roots of these three plants ranged from 0.025 to 0.103 μg/g of dry tissue. However, no measurable levels of DDE were translocated into the shoot system of any of the plants. The concentration of 4,4'-DDE in alfalfa roots was four times more than that in pole bean roots and was almost equal to the concentration of 4,4'-DDE in ryegrass roots.

The metabolism of DDT in plant tissue has been investigated. Phytoremediation of 4,4'-DDT and 2,4'-DDT in the aquatic plant elodea and the terrestrial plant kudzu was reported (Garrison et al., 2000). The results indicated that reductive dehalogenation of 2,4'- and 4,4'-DDT in plants produced the corresponding 2,4'- and 4,4'-DDD. However, DDE was not detected.

In the laboratory, hairy root cultures were tested for the ability to take up and transform DDT because of their fast growth and high metabolic activity (Suresh et al., 2005). A possible dehalogenation of DDT by the plant culture is shown in Figure 1.5. Suresh et al (2005) has reported that hairy root cultures of *Cichorium intybus* and *Brassica juncea* can
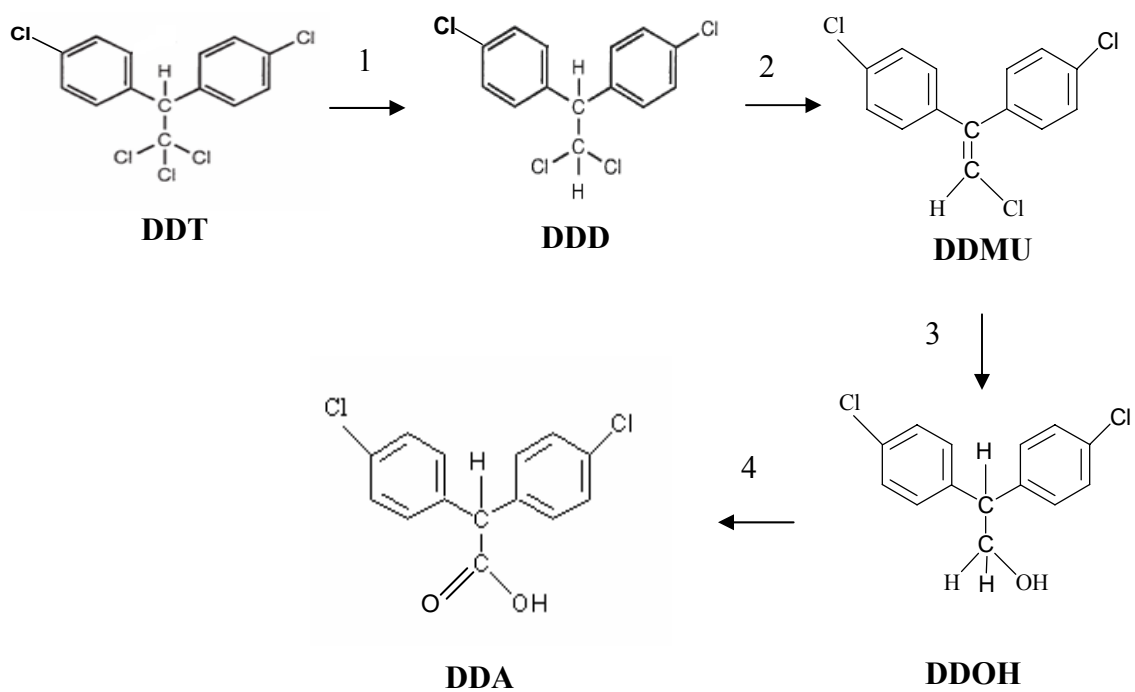
Figure1.5 Possible dehalogenation of DDT to further metabolites by plant cell cultures
(Suresh and Ravishankar, 2004).
DDMU: 1-chloro-2,2-bis(4-chlorophenyl)ethylene;
DDA: bis(4'-chlorophenyl)acetate;
DDOH: 2,2-bis(4-chlorophenyl)ethanol.
Enzymes:
1. Dechlorinase, 2. Dehydrochlorinase, 3. Dehydrogenase, 4. Dehydrogenase

degrade DDT. The degradation products, as analyzed by thin layer chromatograph (TLC), were DDD, DDE, 1-chloro-2,2-bis(4-chlorophenyl)ethylene (DDMU), and some unidentified products. It is important that plant roots can metabolize DDT, which implies phytoremediation can be used to remove and degrade DDT at contaminated sites.

Because DDT has been reported to be metabolized by plants or plant cell culture, some enzymatic reactions in plant cells appear to be involved in DDT transformation. In a study on accumulation, distribution, and transformation of DDT in *Phragmites australis* and *Oryza sativa*, Chu et al. (2006) spiked DDT into the enzyme extracts of different parts of the plants and then analyzed DDT every 30 min with GC-ECD. The results indicated that root extracts of *Phragmites australis* readily transformed DDT to DDD and DDE. They suggested that the metabolism of DDT by *Phragmites australis* was partly mediated by peroxidases and the plant P450 system.

Soil amendments have been tested to improve phytoremediation of DDT. White and Kottler (2002) reported that periodic amendments of citric acid to the soil during the growth of mustard, clover, and vetch yielded up to 40% increases in the concentration of 4,4'-DDE in root tissues under field conditions. Similar enhanced phytoextraction of weathered 4,4'-DDE by zucchini was observed when periodic additions of citric or oxalic acid were made during a 28-day growing period under greenhouse conditions (White et al., 2003). The addition of low molecular weight organic acids caused the partial dissolution of the soil structure through the chelation of inorganic structural ions, resulting in the release of organic carbon and metal ions and thus the subsequent enhanced desorption of DDE or other POPs from the soils. Also, low molecular weight organic acids increased microbial activity in soil. A similar report was made by Luo et al. (2006).

1.8 Objectives

It is clear that plants can take up DDT from DDT contaminated soil. A possible dehalogenation of DDT by plant cultures in the laboratory has been proposed. Amendments of organic acids to soil during plant growth have been proved to improve DDE phytoremediation, however, higher concentration of organic acids inhibits the DDE phytoextraction. In addition, the relationship between the amount of DDT decreased in soil and the amount of DDT accumulated in plant tissues has not been studied yet. In this study, several plant species will be utilized to study the phytoremediation of DDT contaminated soil. To improve DDT phytoremediation, PGPR will be used to investigate if PGPR can improve plant growth and increase phytoremediation of DDT. In addition, the mechanism of DDT phytoremediation will be investigated.

Two natural, non-pathogenic strains of *Pseudomonas putida*, UW3 and UW4, which promote plant growth in petroleum contaminated soil and enhance remediation efficiency of petroleum (Greenberg et al., 2007), will be used in this study. However, UW3 and UW4 each have different effects on different plants. Therefore, in this study, the effect of UW3, UW4, and the combination of UW3 and UW4 on candidate plants was investigated in the greenhouse. After that, the plants with the combination of UW3 and UW4 were planted in the field to study DDT removed from soil. Thus, the specific objectives of this thesis were to 1) investigate the effect of UW3, UW4, and the combination of UW3 and UW4 on plant growth in DDT contaminated soil in greenhouse and select an optimal PGPR/plant combination for field trials; 2) analyze the plant tissues to determine if and how much DDT is taken up and/or transported by tissues; 3) extract DDT in soils to determine if and how much DDT is removed from the soils; 4) perform the mass balance of DDT disappeared from the soils and

accumulated in the plant tissues to elucidate the possible mechanism(s) for DDT

phytoremediation.

2   Materials and Methods

2.1 Materials

Fall rye (*Secale cereale*), pumpkin (*Cucurbita pepo* L.cv. *Howden*), and potato (L. cv. *Superior*) seeds used in this project were purchased from the Ontario Seed Company (Waterloo, Ontario, Canada). Millet (*Pennisetum glaucum* L. cv.CFPM-101) and sugar beet (*Beta vulgaris* L.) seeds were purchased from Agriculture Environmental Renewal Canada Inc (Ottawa, Ontario, Canada). DDT and DDE (2,4-DDT, 4,4'-DDT, 2,4-DDE, and 4,4'-DDE) were purchased from the Restek Corporation (Bellefonte, Pennsylvania, USA). Two strains of PGPR, *Pseudomonas putida* UW3 and *Pseudomonas putida* UW4, were used to promote plant growth (Glick et al., 1995) .

All solvents used for extraction and analysis of DDT were HPLC grade. Acetone and hexane were obtained from Caledon Laboratories LTD (Georgetown, Ontario, Canada) and Fisher Scientific (Mississauga, Ontario, Canada), respectively, while acetonitrile was purchased from EMD Chemicals Inc (Gibbstown, New Jersey, USA).

2.2 Study site

We performed pilot trials on two sites. Site 1 was around 4,200 m^2 and site 2 was about 1850 m^2. The sites were selected by Ontario Ginseng Growers Association (OGGA) Board. The sites were near Scotland, Ontario (south of Brantford and north of Simcoe, Figure 2.1). Test site 1 was separated into two parts by a new Ginseng field. Site 1 was planted with fall rye and millet in 2005 (Figure 2.2); fall rye, millet, and sugar beet in 2006 (Figure 2.3); millet, sugar beet, and pumpkin in 2007 (Figure 2.4). The site 2 was a single plot. We planted

Figure 2.1 Geographic map of our field. The sites are near Scotland, Ontario (south of Brantford and north of Simcoe). Site 1 is located 410 Burford-Delhi Townline Rd Brant, Ontario, Canada; site 2 is in 595 Norfolk city 19 West Rd, Ontario, Canada.
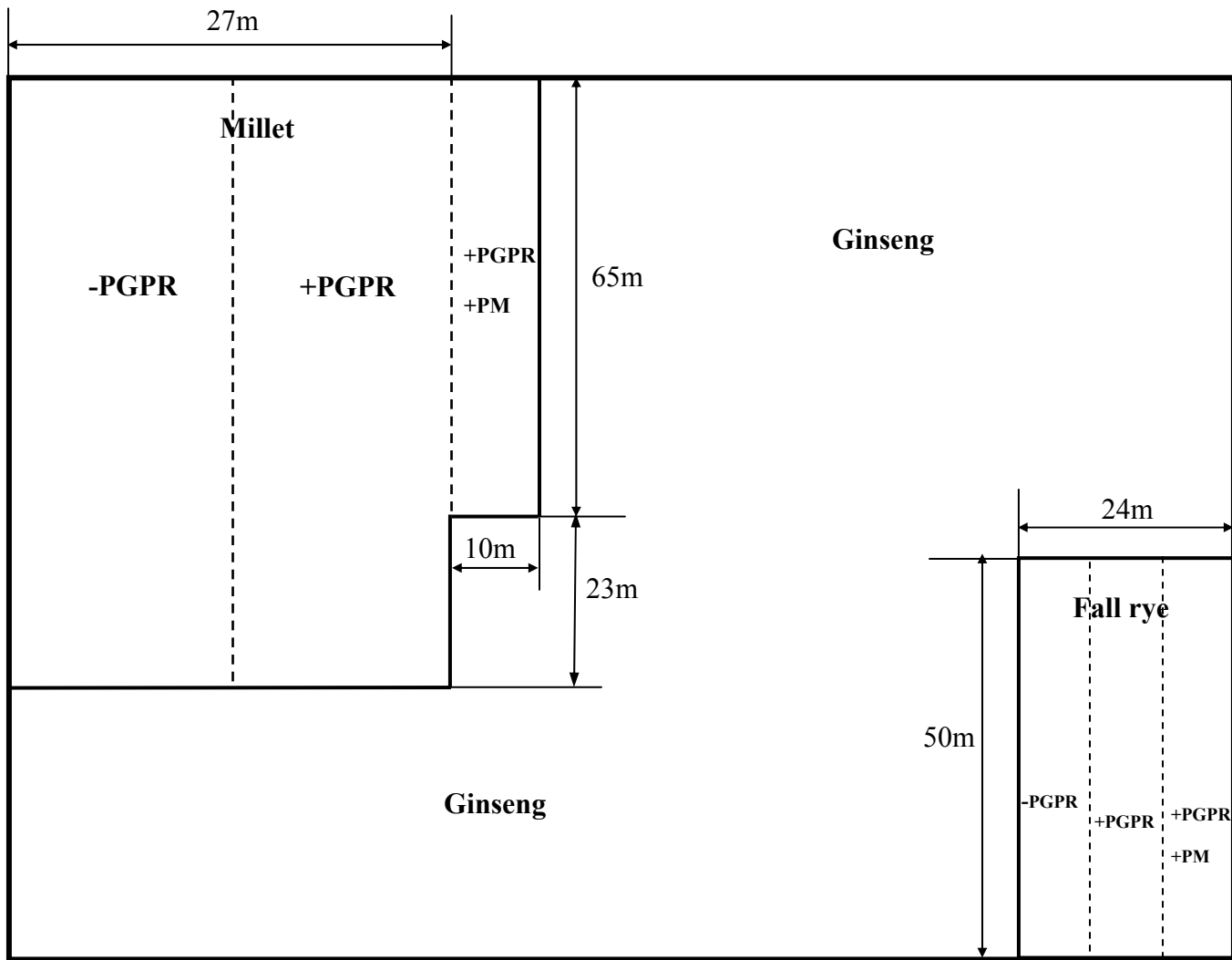
Figure 2.2 Planting map on site 1 in 2005. Millet and fall rye were planted on site 1. Different treatments of each plant were divided by dotted line. PM was peat moss.

24

27m

-PGPR   +PGPR

+PGPR
-PGPR

Sugar
beet

Ginseng

65m

Millet

+PGPR   -PGPR   10m

23m

24m

Fall rye

50m

-PGPR   +PGPR

Ginseng
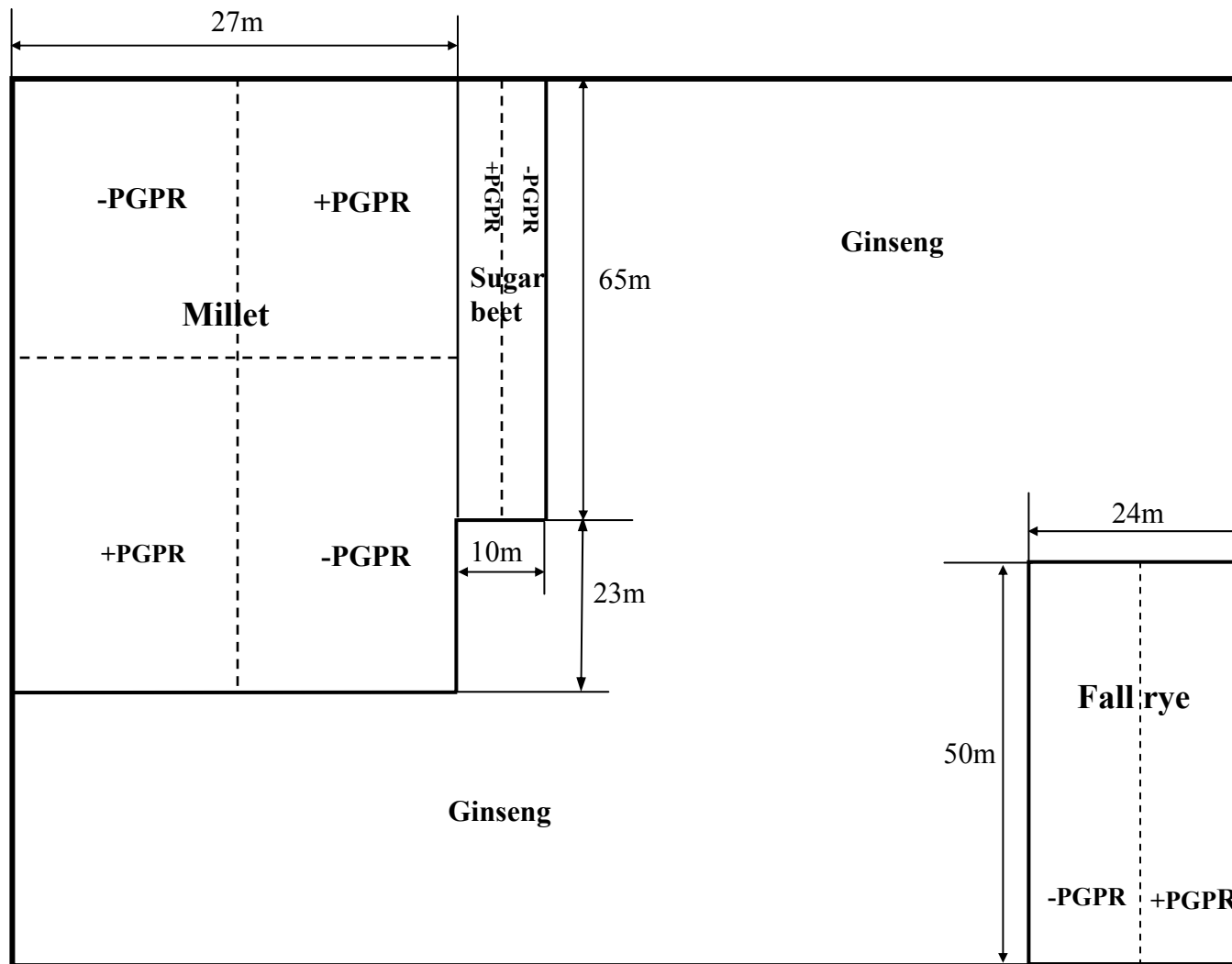
Figure 2.3 Planting map on site 1 in 2006. Millet, fall rye, and sugar beet were planted on site 1. Different treatments of each plant were divided by dotted line.

17m

10m

+PGPR

-PGPR

+PGPR

-PGPR

Pumpkin

Sugar beet

65m

Ginseng

24m

-PGPR +PGPR +PGPR -PGPR

10m

23m

Millet

50m

Ginseng

+PGPR

-PGPR

+PGPR

-PGPR
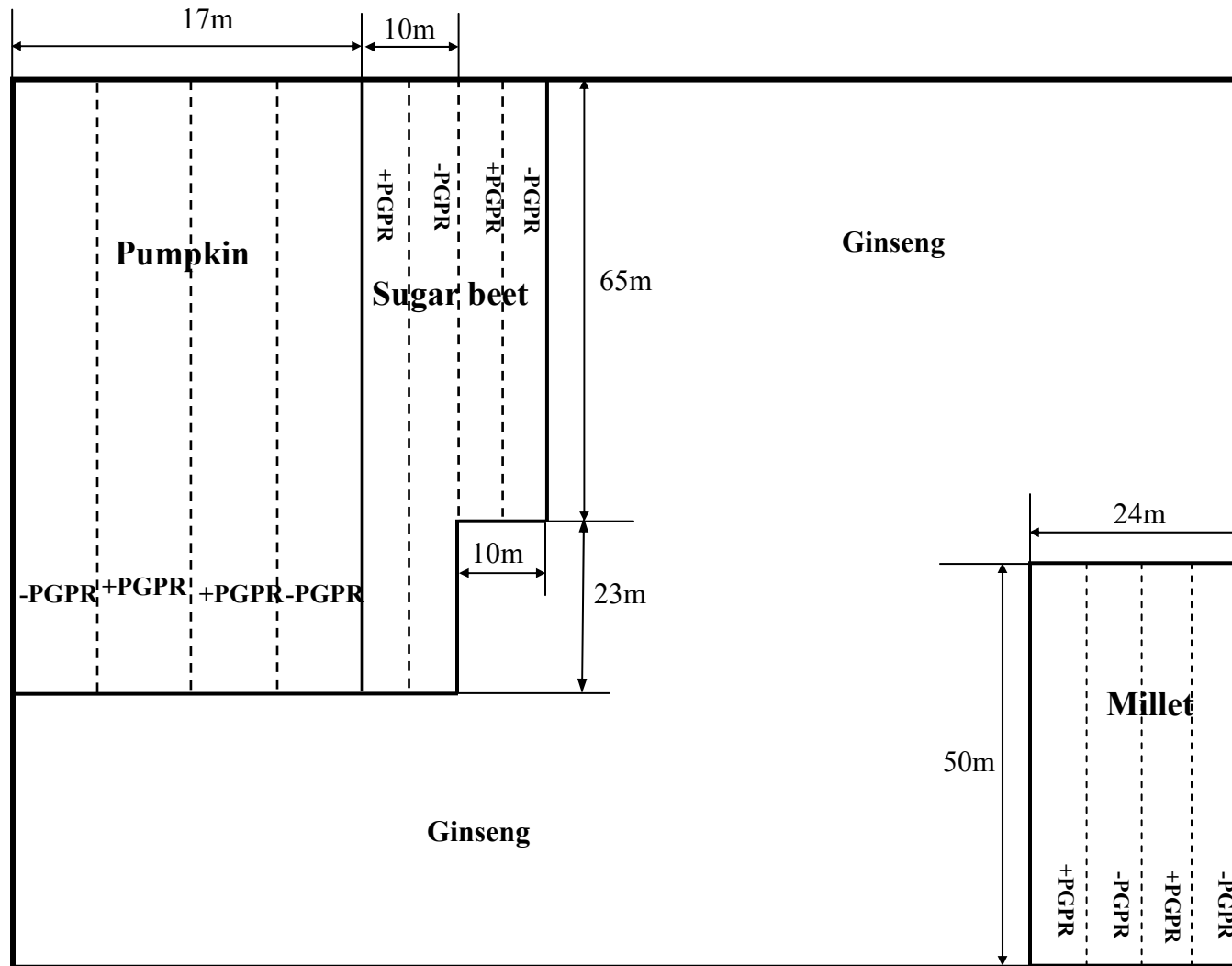
Figure 2.4 Planting map on site 1 in 2007. Millet, sugar beet, and pumpkin were planted on site 1. Different treatments of each plant were divided by dotted line.

one third of the site with fall rye, one third with millet, and one third with sugar beets in 2006 (Figure 2.5); one third of site with pumpkin, one third with sugar beets, and one third with millet and potato in 2007 (Figure 2.6). The level of DDT across the site 1 was initially assessed at 0.9 μg/g, with a fairly uniform distribution (0.6 to 1.2 μg/g), and 0.61 μg/g across the site 2, with a distribution 0.3 to 0.95 μg/g.

2.3 The treatment of seeds with PGPR

A Hege 11 Liquid Seed Treater (Wintersteiger Inc., Saskatoon, Canada) was used in our lab to coat seeds with PGPR using a procedure developed in-house. Briefly, 1 mL of UW3 or UW4 glycerol stock was incubated in 50 mL of Tryptic soy broth (TSB) at room temperature overnight. From this initial culture, 1 mL was used to inoculate another 50 mL of TSB to obtain a high cell density. The culture was centrifuged at $2516\times$ g (5000 RPM) and the cell pellet was washed twice with sterile deionized H_2O (dH_2O), and then resuspended in dH_2O. We adjusted the concentration of bacteria to get a final OD_{600} of 2.0. Next, a 1.5% (w/v) solution of methylcellulose (Sigma, Oakville, Ontario, Canada) was autoclaved and then added to the bacterial suspension to facilitate the adhesion of the PGPR to the seed and reducing bacterial loss due to dusting off. To this polymer/bacteria slurry, a blue commercial seed colorant (Color Coat Blue, Becker Underwood, Saskatoon, Canada) was added. The slurry was dispensed from a plastic disposable syringe onto a flanged disc attached to the driveshaft of Seed Treater, resulting in a fine spray evenly coating the spinning seeds. In addition to facilitating homogenous application of the slurry, the spinning process dried the seeds by the end of the treatment run.

In this study, seeds of millet, fall rye, pumpkin and sugar beet were all treated with PGPR
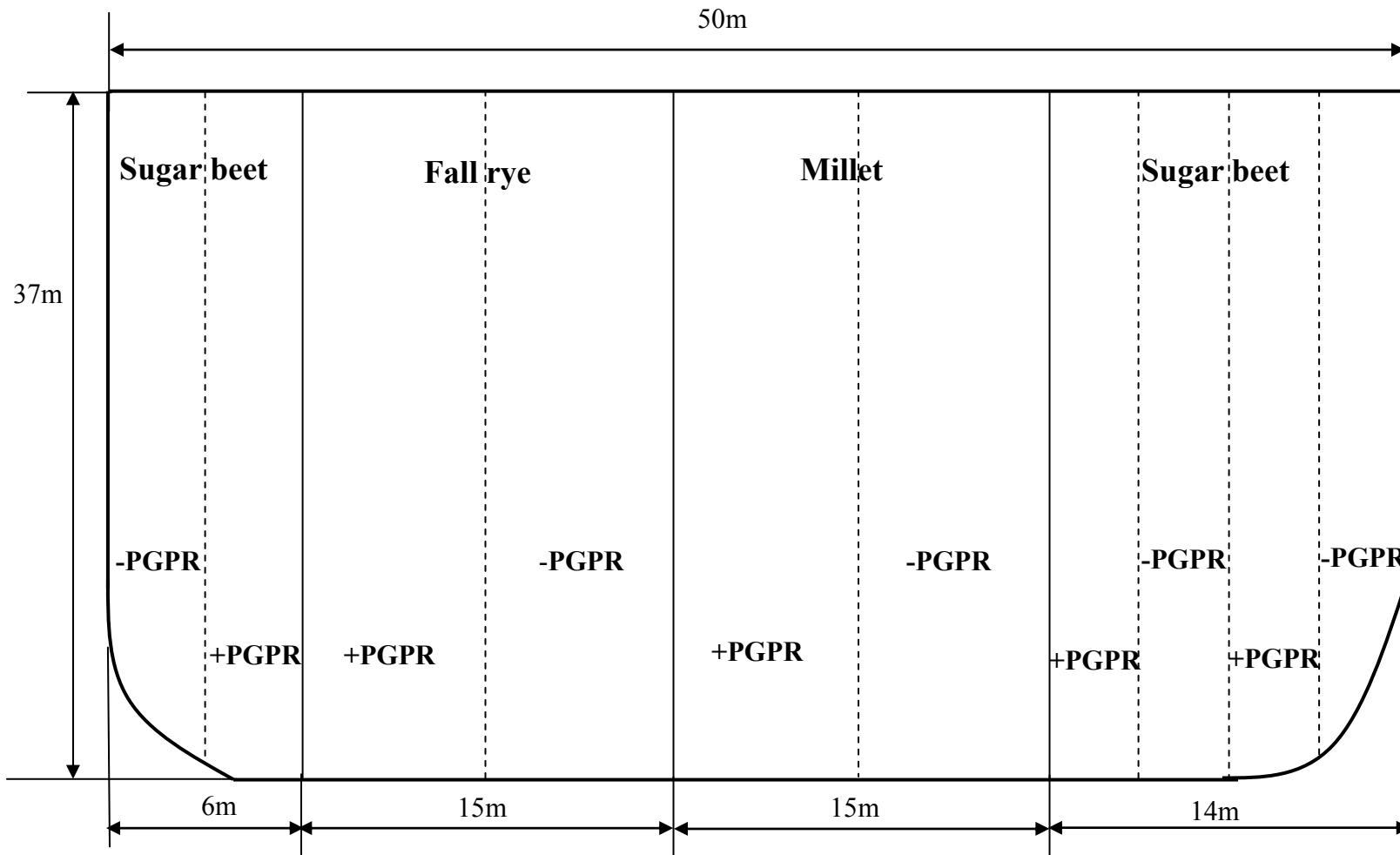
Figure 2.5 Planting map on site 2 in 2006. Millet, fall rye, and sugar beet were planted on site 2. Different treatments of each plant were divided by dotted line.
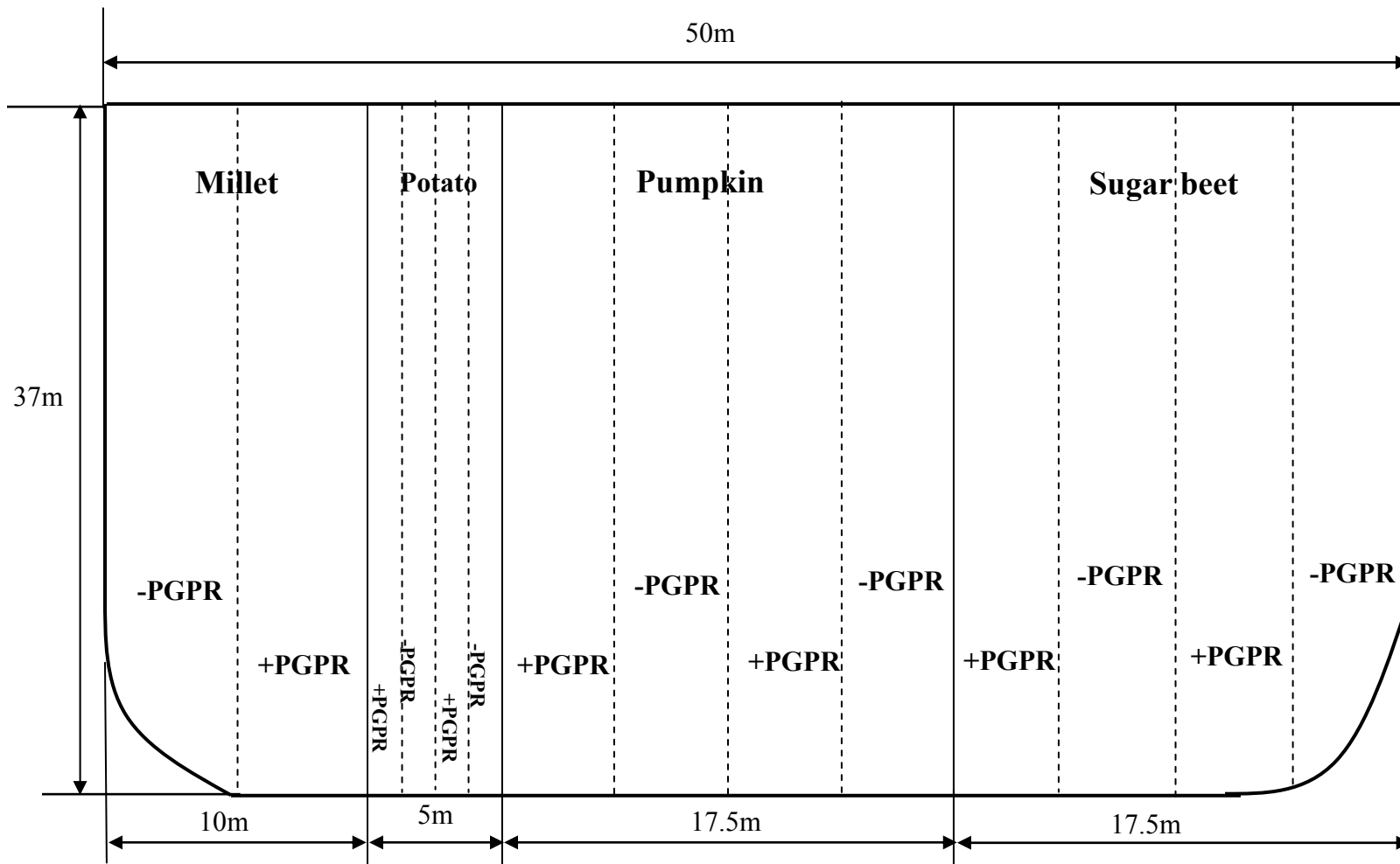
Figure 2.6 Planting map on site 2 in 2007. Millet, sugar beet, potato, and pumpkin were planted on site 2. Different treatments of each plant were divided by dotted line.

according to the above procedure. The exception to this process was potato which was treated by hand due to its large size. Before planting, one potato tuber was cut into 2-3 pieces with at least one "eye" on each piece. Each piece is about 6 cm long and around 4 cm in diameter (Figure 2.7). PGPR in dH_2O with an OD_{600nm} of 2.0 was sprayed onto the seeds, which were then covered with a thin layer of peat moss. Peat moss was used to protect the cutting from drying. Potato seeds without PGPR were also covered with a thin layer of peat moss.

2.4 Greenhouse trials for plant growth

Greenhouse trials were complementary to the field work and were done in the winter between field seasons. This allowed us to optimize conditions for DDT analysis and picking plant species and PGPR combinations. Before planting in the greenhouse, the seeds of fall rye, millet, and sugar beet, treated with either UW3, UW4 or a combination of UW3 and UW4, were planted in DDT contaminated soil from the field site 1 at a 4,4'-DDT concentration of around 0.8 μg/g. Seeds without any PGPR were planted under the same soil conditions.

Fall rye and millet were planted in a 6x6x6 cm^3 pot of 10 seeds/pot with quadruplicate replicates; sugar beets were planted 3 seeds/pot with a 13.5 cm diameter and a 20 cm depth in triplicate. Two of the 3 spouts of sugar beets/pot were removed after germination to allow enough space for growth. Root and shoot lengths and weights were measured to compare the effect of PGPR on plant growth.

2.5 Field tests for DDT remediation

Plots for each crop species were divided into two sections: section 1, untreated control seeds; section 2, seeds treated with PGPR (the combination of UW3 and UW4). The planting
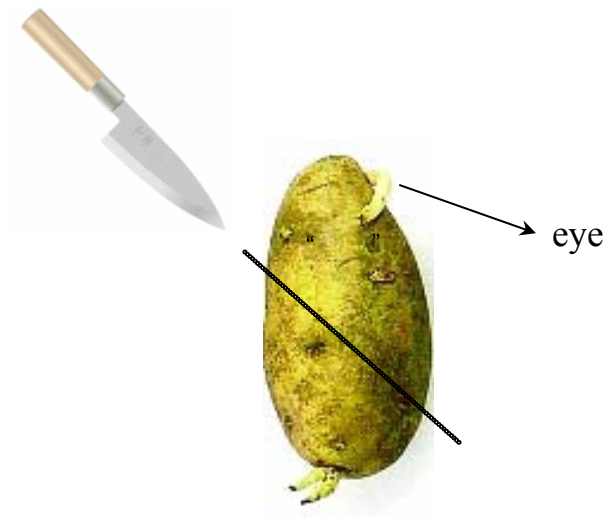
Figure 2.7 One potato cut into 2 pieces with at least one "eye" on each part.

designs are shown in Figures 2.2-2.6. Planting density was 68 lbs/acre for millet and fall rye, while for sugar beets planting density was 12 lbs/acre. The planting of millet, fall rye, and sugar beet was done using a 12 feet wide seeder. Pumpkin seeds were planted by hand in rows 3-3.5 m apart, with plants spaced 0.7-0.8 m apart in rows. 3-4 seeds were planted in a hole of 7-10 cm of depth. Potato seeds were planted in rows about 1 meter apart, with plants spaced 0.3-0.4 m apart in rows. Ditches were made by a small machine and potato seeds were planted in the ditches by hand.

2.6 Sampling methods for soil and plants in the field

Soil from top 20 cm of depth was collected using an auger, mixed well, and stored in glass jars. The soil samples were air dried for about 3 days at room temperature in a fume hood in the laboratory prior to being sifted through a 100-mesh sieve (Fisher Scientific, West Chester, Pennsylvania, USA) to remove rocks and biological debris. Samples were then stored in glass jars at $4^{o}C$. Whole plant samples were taken from the field and stored in plastic bags to be transported to the lab. In the lab, plant samples were then washed, patted dry using paper towels after which fresh weights of roots and shoots were measured. After fresh weight measurements, roots and stems were dried at about $50^{o}C$ for at least one week prior to measuring dry weights.

2.7 Method for DDT extraction from soil and plant tissue

The method for extracting DDT from soil was adapted from the US Environmental Protection Agency (USEPA, 1996). A minimum of 2 g dry weight of soil was mixed with 10 mL of 1:1 hexane-acetone and sonicated for 30 min using a Branson Sonifier 450 (output 3.5, 50% duty cycle) or a Branson Sonic Dismembrator Model 500 (0.5 second pulsed ON/OFF,

amplifier: 30%) (Branson Ultrasonic, Danbury, Connecticut, USA). The soil was allowed to settle and the supernatant removed to a clean glass test tube, This procedure was repeated twice more using 5 mL 1:1 hexane-acetone and the samples sonicated for 5 min each time. All supernatants were combined and centrifuged at $906 \times g$ (3000 RPM) for 20 min. After centrifugation, the supernatant was transferred to a new test tube and a gentle stream of ultra high-purity nitrogen gas (Praxair, Kitchener, Ontario, Canada) was introduced into the tube to evaporate the solvent. The sample residue was resuspended with 50 μL of dichloromethane (DCM, Fisher Scientific, Mississauga, Ontario, Canada) and 950 μL of acetonitrile using a vortex mixer and was transferred to a wide mouthed crimp top vial (Agilent Technologies, California, USA) for HPLC analysis.

The method for DDT extraction from plant tissue is described by Lino and Silveira (1997). It is similar to the extraction method of DDT from soil. Before extraction, dry plant tissue was cut into fine pieces by a blender (Black & Decker Corporation, Towson, Maryland, USA) followed by sieving plant tissues through a 18 mesh sieve (Fisher Scientific Company, West Chester, Pennsylvania, USA) to make the pieces as fine and sizable as possible. Then a minimum of 1 g dry weight of minced tissue was placed in an 18 mL glass scintillation vial with 10 ml of hexane added and the sample was sonicated for 30 min. Sonication settings were the same as for DDT extraction from soil described above. The supernatant was removed and collected in a separate test tube. This was repeated twice more with further 5 mL solvent added and the sample sonicated for 5 min each time. The supernatants were combined and centrifuged at $906 \times g$ (3000 RPM) for 20 min. After centrifugation, the supernatant was transferred to a new test tube and a gentle stream of nitrogen gas was introduced into the tube to concentrate the solvent to 3mL.

Due to the number of complex compounds present in plant tissues (e.g. lipids, flavonoids) that can confound HPLC analysis of DDT, the extract was cleaned using C18 SEP-PAK cartridge (3 mL) (Millipore, USA). The column was activated with 5 mL hexane without letting it dry. The concentrated hexane extract was transferred to the column and forced through the column with a plunger. The eluant was collected in a centrifuge tube and excess solvent evaporated with nitrogen gas. The pellet was dissolved using 950 μL acetonitrle and 50 μL DCM and analyzed by HPLC.

2.8 DDT analysis by HPLC

The HPLC analysis method for DDT was established in our lab. The DDT extracts were analyzed with a Shimadzu HPLC system equipped with a SPD-M10A photo-diode array detector, LC-10AD pumps, a SCL-10A System Controller, and SIL-10A autosampler (Shimadzu Corp., Kyoto, Japan). The analytical column was a C18 reverse-phase silica column (4.6 mm by 25 cm) (Supelco, Oakville, Ontario, Canada) equilibrated with acetonitrile/water (pH3.0) (70:30) as the mobile phase. The mobile phase was a linear gradient from 70% to 85% acetonitrile in 11 min and held at 85% acetonitrile for 7 min, then to 100% acetonitrile in 3 min and held at 100% acetonitrile for 5 min, and to 70 % acetonitrile in 5 min and held at 70% acetonitrile for 10 min. The flow rate was 1.0 mL/min. The spectrum of the effluent was scanned from 200 to 500 nm by the diode array detector. Peaks coming off the column were monitored at 235 nm. The chromatograms and spectra of standard 4,4'-DDT and 4,4'-DDE are shown in Figures 2.8 and Figure 2.9.

A stock solution of 1000 μg/mL of 4,4'-DDT was purchased from Restek (Bellefonte, Pennsylvania, USA) and stored at 4 °C. An aliquot of 20 μL of the stock solution was diluted in acetonitrile to make a 20 μg/mL solution to prepare dilutions for the standard curve. In a
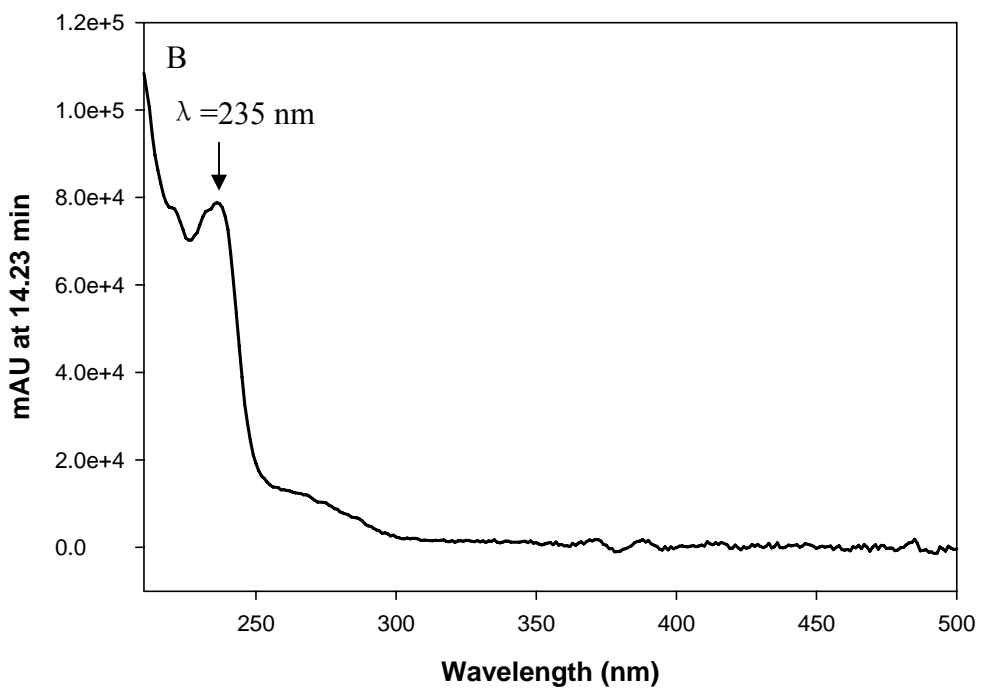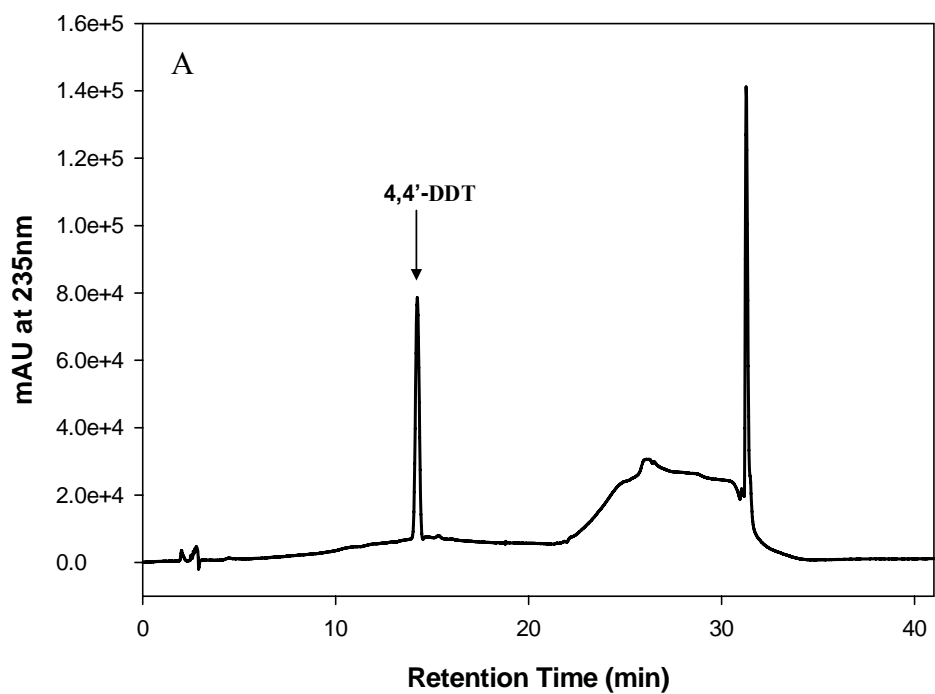
Figure 2.8 HPLC chromatogram (A) and spectrum (B) of standard 4,4'-DDT (4.0 μg/mL).
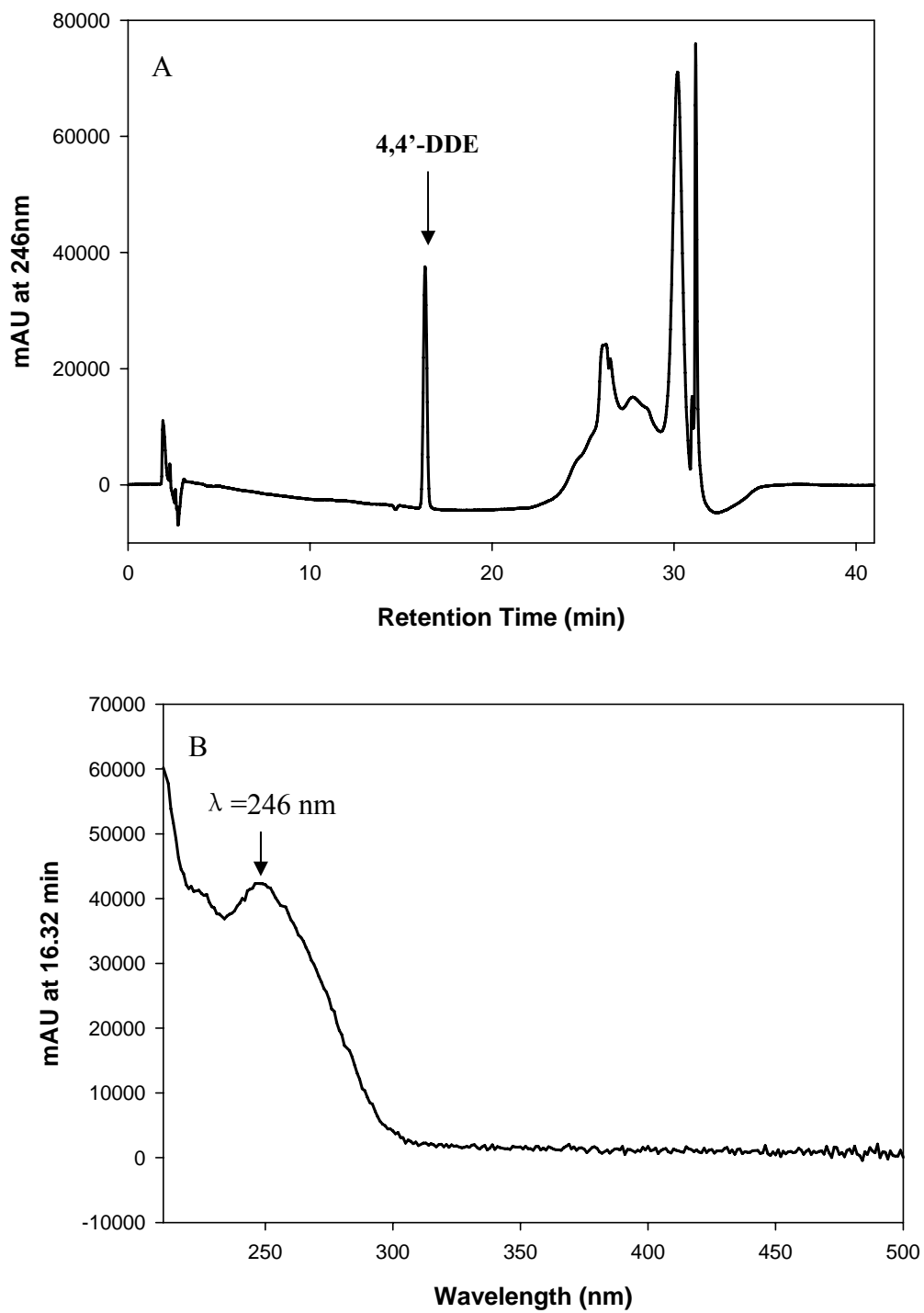
Figure 2.9 HPLC chromatogram (A) and spectrum (B) of standard 4,4'-DDE (2.0 μg/mL).

volume of 1.0 mL, known amounts of 4,4'-DDT were prepared from the 20 μg/mL solution, ranging from 0.2 to 4.0 μg/mL. The peak heights were plotted against the standard 4,4'-DDT concentrations to create a standard curve. All reported concentration values of 4,4'-DDT are expressed on a dry-weight basis of either soil or vegetation.

2.9 Statistical analysis

Biomass data of plant growth in greenhouse and field trials were analyzed by one-way analysis of variance (ANOVA) and the Dunnett post-hoc test ($P<0.05$) which compares plants treated with PGPR and the untreated plants (-PGPR). DDT concentrations in soils in the field tests were analyzed using one-way analysis of variance (ANOVA) and the Dunnett post-hoc test ($P<0.05$) which compares DDT concentrations in near-root soil or rhizosphere soil and DDT levels in bulks soil at t_0. The software used for one-way ANOVA was SYSTAT version 12.

3   Results

3.1 Plant growth in greenhouse

Greenhouse trials were complementary to the field work. This allowed optimization of conditions for DDT analysis and selecting plant species and PGPR combinations. Before planting in the greenhouse, seeds of fall rye, millet, and sugar beet were treated with either UW3, UW4 or a combination of UW3 and UW4. The seeds treated with PGPR were planted in DDT contaminated soil collected from the field site 1 at a 4,4'-DDT concentration of around 0.8 μg/g. The untreated seeds were planted under the same soil conditions.

In greenhouse trials, the growth of fall rye, millet, and sugar beet was investigated. After 30-day growth, biomass based on shoot and root length and weight was measured for fall rye and millet. Shoot length of millet varied with PGPR treatment. The longest average shoot length (31 cm) was observed with application of the combination of UW3 and UW4, followed by UW3 alone (27 cm) (Figure 3.1A). Little difference was observed in millet shoot length between UW4 treatment and untreated plants (Figure 3.1A). Root length of millet followed the same trend as shoot length with the longest roots detected in plants treated with the combination of UW3 and UW4 (Figure 3.1B). Comparing dry weights, shoot weight and root weight of millet treated with the combination of UW3 and UW4 were 218.06 g/m^2 and 70.83 g/m^2, respectively (Figure 3.1C and D). Millet treated with UW4 had 182.64 g/m^2 of shoot dry weight and 61.04 g/m^2 of root dry weight (Figure 3.1C and D). Millet treated with UW3 had more biomass than those treated with UW4, and the millet without PGPR treatment had the lowest shoot and root dry weights.
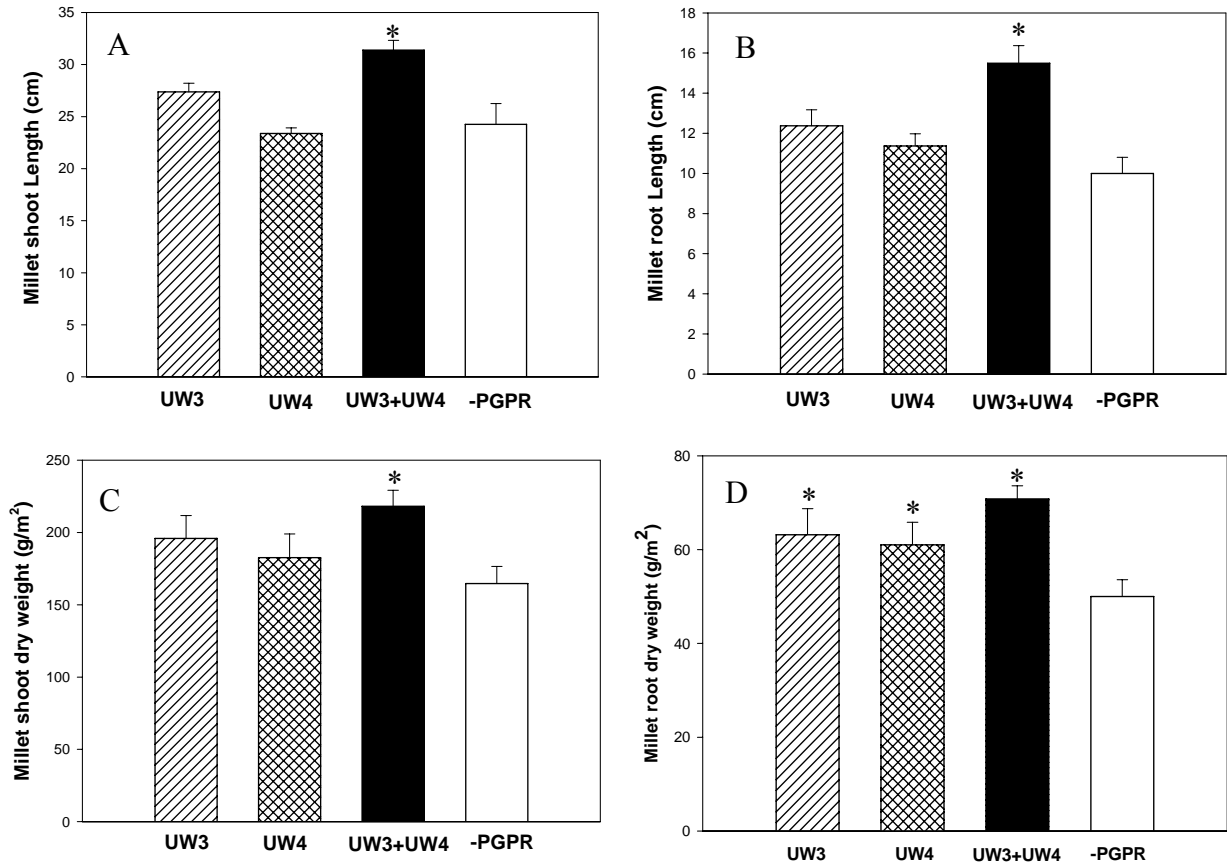
Figure 3.1 Effect of PGPR on shoot length (A), root length (B), shoot dry weight (C), and root dry weight (D) of millet treated with UW3, UW4, and the combination of UW3 and UW4, compared to the untreated plants in greenhouse trials. The growth period was 30 days. Results expressed as mean ± S.E., n = 8. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treatment (UW3, UW4, or UW3+UW4) and control (–PGPR) plants.

Shoot and root lengths and dry weights of fall rye with and without PGPR were determined to assess the effectiveness of PGPR treatment (Figure 3.2). The shoot and root lengths of fall rye treated with the combination of UW3 and UW4 were 27 cm and 8.75 cm, respectively. The dry weights of the shoots and roots in plants treated with PGPR were 150 g/m^2 and 31.6 g/m^2, respectively. Fall rye without PGPR treatment had shoot and root lengths of 22.5 and 6.5 cm, respectively, and shoot and root dry weights of 125 and 77.8 g/m^2, respectively. Thus, there were clear promotion effects of PGPR on fall rye growth.

PGPR effects on sugar beet are shown in Figure 3.3. The root length and fresh weight of sugar beet treated with the combination of UW3 and UW4 was 17.5 cm and 28.6 g, respectively. Sugar beet without PGPR treatment had root length and fresh weight of 12.5 cm and 19.0 g, respectively. Once again there were clear positive effects of PGPR on plant growth in DDT contaminated soil. Taken together, the above data indicated a positive effect of PGPR on plant growth and this could translate to better remediation of DDT.

3.2 Plant growth in the field

Given the results of improved growth in the greenhouse due to PGPR treatment, field trials were initiated. In 2005, millet and fall rye were planted on site 1. The level of DDT across the site 1 was initially assessed at 0.9 μg/g, with a fairly uniform distribution (0.6 to 1.2 μg/g). In the field, all PGPR treatments were a combination of UW3 and UW4. After three weeks, the plants had emerged and had a very healthy appearance. There was evidence at this point of a small PGPR effect (Figure 3.4). By October, the fall rye was about 0.5 m tall and the millet was approximately 2 meters tall. All of the plants, regardless of PGPR treatment, appeared strong and healthy (Figure 3.5).
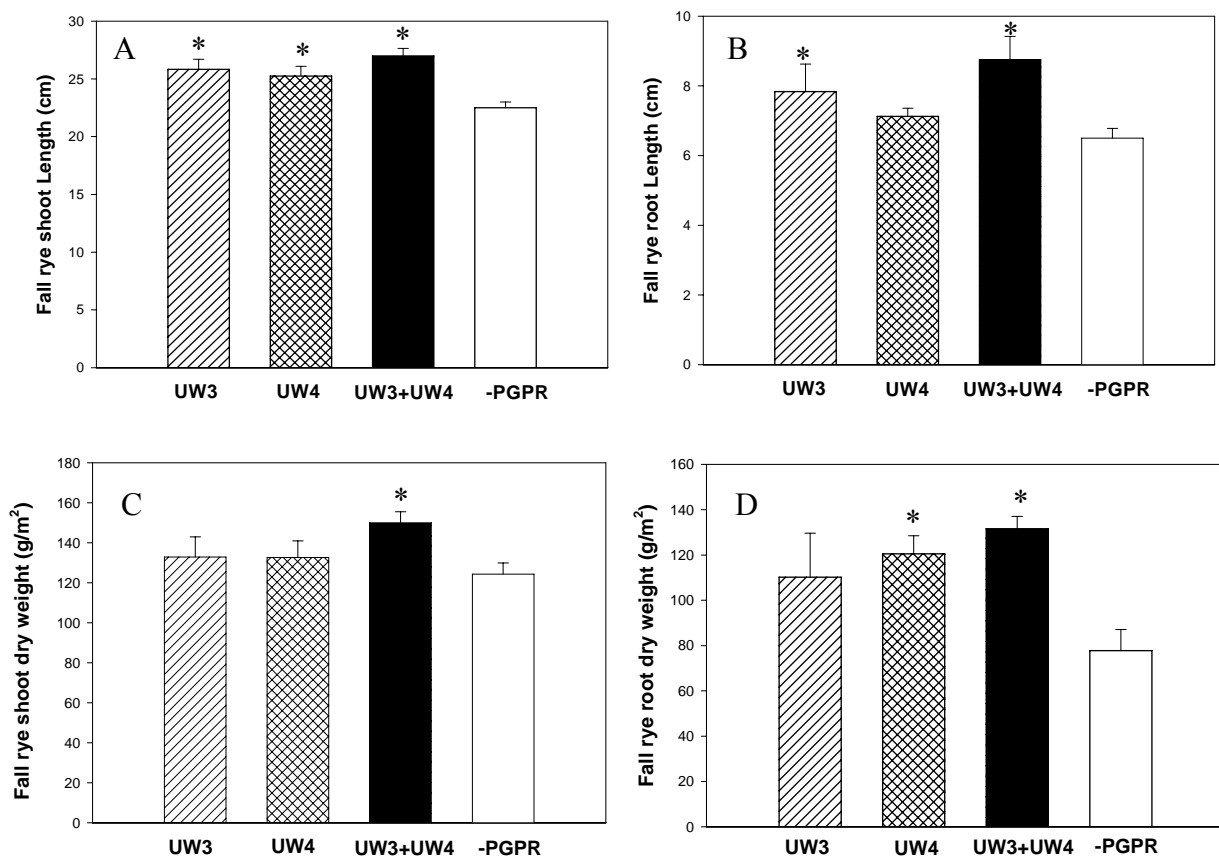
Figure 3.2 Effect of PGPR on shoot length (A), root length (B), shoot dry weight (C), and root dry weight (D) of fall rye treated with UW3, UW4, and the combination of UW3 and UW4 in greenhouse trials. The growth period was 30 days. Results expressed as mean ± S.E., n = 8. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treatment (UW3, UW4, or UW3+UW4) and control (–PGPR) plants.
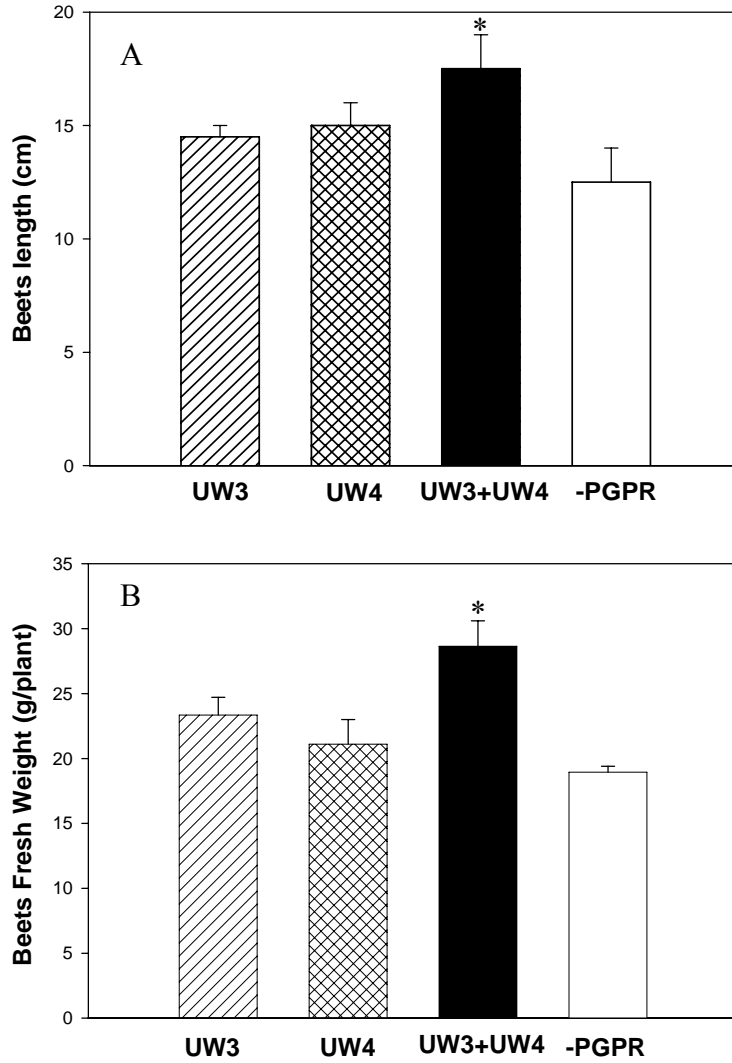
Figure 3.3 Effect of PGPR on root length (A) and fresh weight (B) of sugar beets treated with UW3, UW4, and the combination of UW3 and UW4 in greenhouse trials. The growth period was 60 days. Results expressed as mean ± S.E., n = 3. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treatment (UW3, UW4, or UW3+UW4) and control (–PGPR) plants.
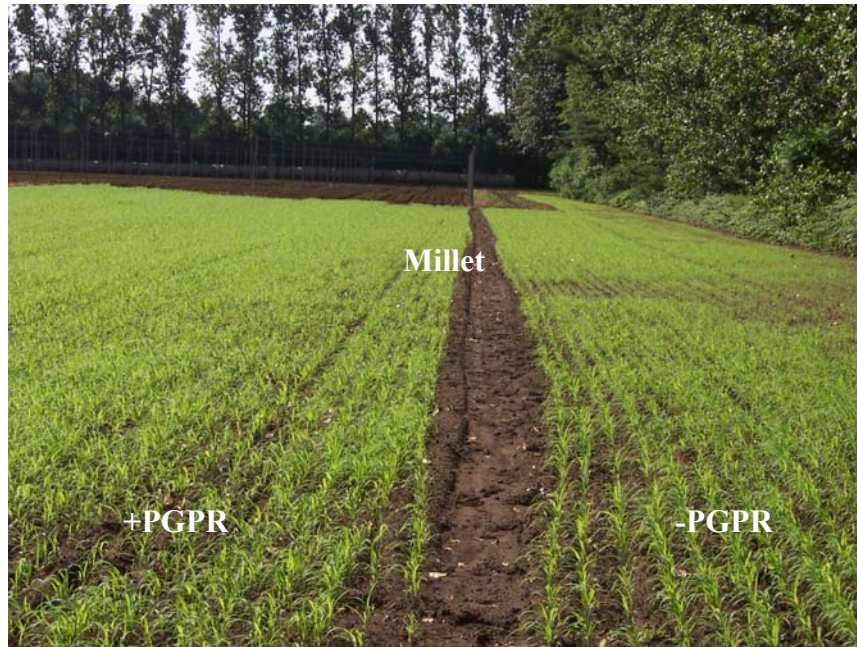
Figure 3.4 Millet and fall rye with and without PGPR on the site 1 three weeks after sowing in 2005.
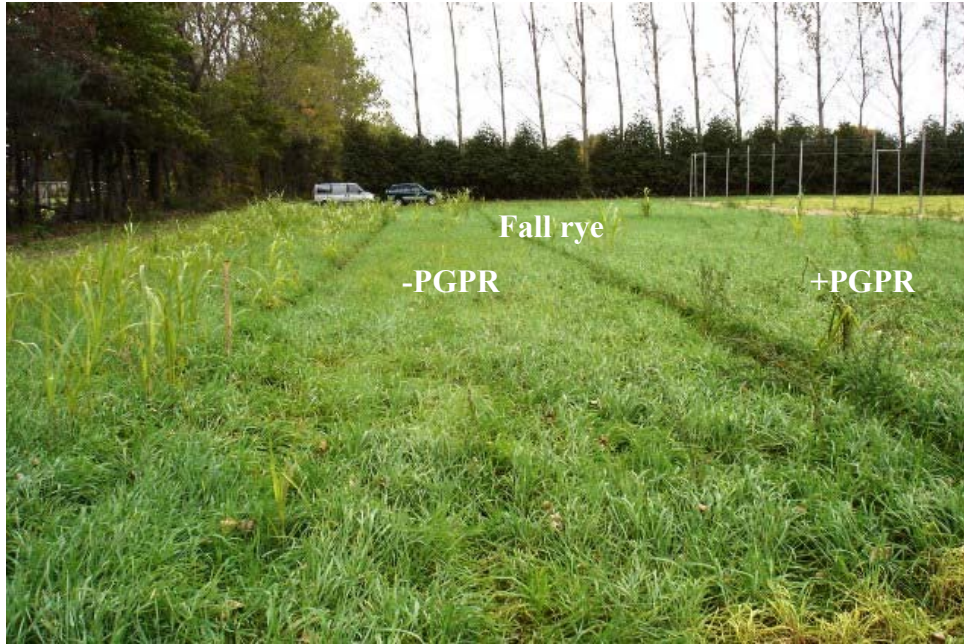
Figure 3.5 Millet and fall rye with and without PGPR on the site 1 three months after sowing. For height reference, the person in the pictures is 178 cm tall.

To prove a significant PGPR effect on the plant growth and DDT remediation in the field, two more seasons of DDT phytoremediation were done in 2006 and 2007. In 2006, millet, fall rye, and sugar beet were planted on site 1 and site 2. After three weeks, the plant growth was monitored. A small but consistent PGPR effect was observed (Figure 3.6). At harvest, the difference of plant growth between PGPR treatment and the untreated plants on site 1 was significant (Figures 3.7-3.9). The root length and root weight of millet treated with PGPR on the site 1 were 11% and 47%, respectively, greater than the untreated plants. On site 2, biomass of millet treated with PGPR followed the same trend as root length and weight on the site 1 (Figure 3.7). Fall rye treated with PGPR was larger than the untreated plants on site 1. The root length and root weight of fall rye treated with PGPR were 16% and 44% greater, respectively, compared to the untreated plants. However, the PGPR effect on fall rye at site 2 was not significant (Figure 3.8). Sugar beets at harvest were smaller than normal because they were planted in July, which is late in the season for planting sugar beets in Southern Ontario, Canada. Nonetheless, on site 1, sugar beets treated with PGPR were longer and bigger than untreated plants (Figure 3.9). Similar to fall rye on the site 2, no significant differences in root length and weight of sugar beet were observed between PGPR treated and the untreated sugar beets (Figure 3.9).

In 2007, millet, sugar beet, and pumpkin were planted on both sites. Potato was planted only on the site 2 because potato can adversely affect Ginseng growth, which was being grown on the farmland by site 1. After 70 days, plant growth was monitored at both sites. Comparing the length of millet treated with PGPR and without PGPR (Figure 3.10), it is clear that millet treated with PGPR was taller than the untreated plants. Biomass quantification of millet samples at harvest illustrated that the difference in millet growth
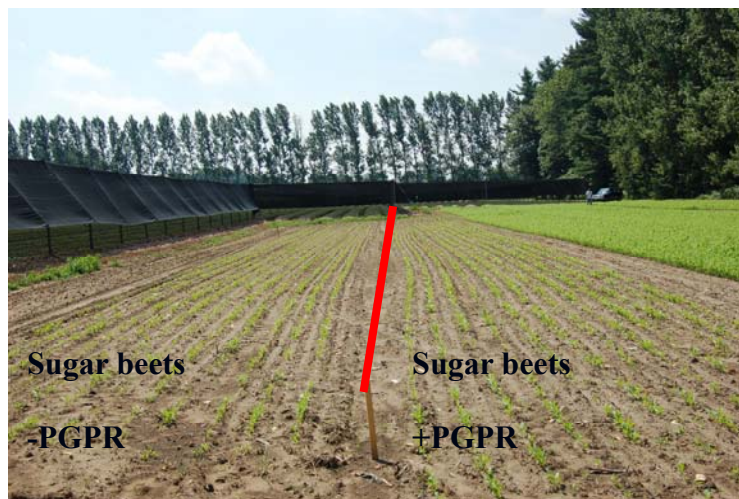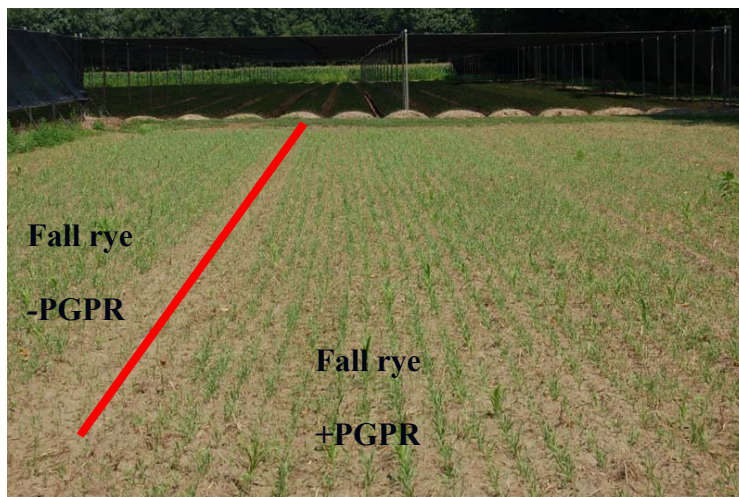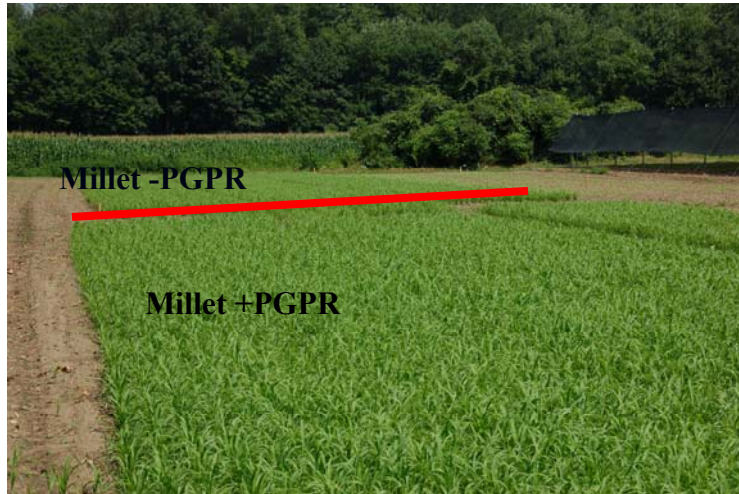
Figure 3.6 Millet, fall rye, and sugar beet with and without PGPR on site 1 three weeks after sowing in 2006.
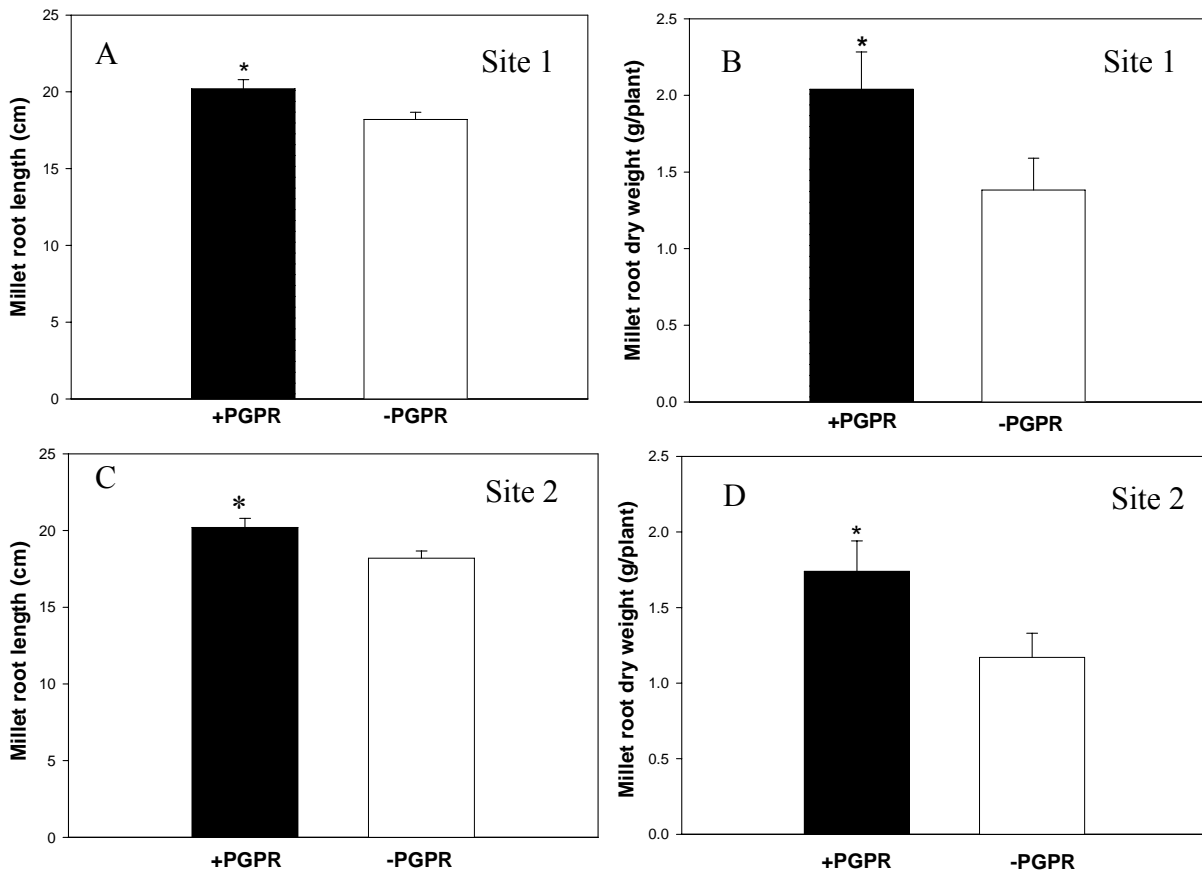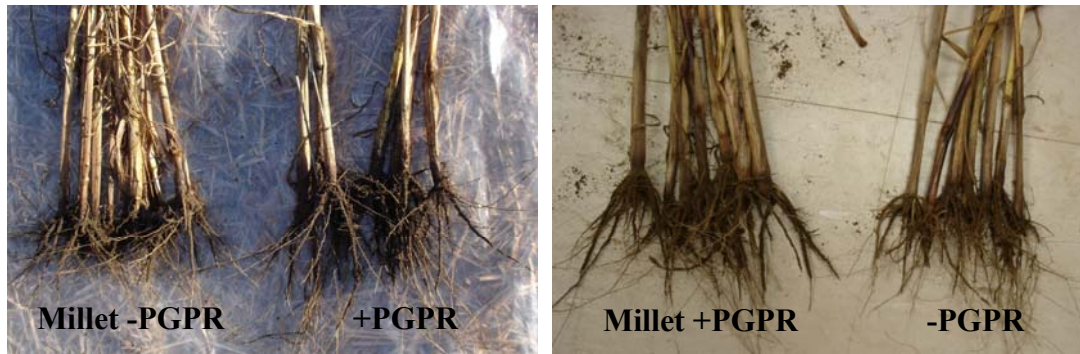
Figure 3.7 Effect of PGPR on millet samples on site 1 (top left) and site 2 (top right) at harvest in 2006. Root length (A) and dry weight (B) of millet on site 1 and root length (C) and dry weight (D) of millet on site 2 at harvest in 2006. Data represents mean ± S.E., n = 6. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treated plants and untreated plants. Site 1 was surrounding by tall trees. Site 2 was open without any shield.
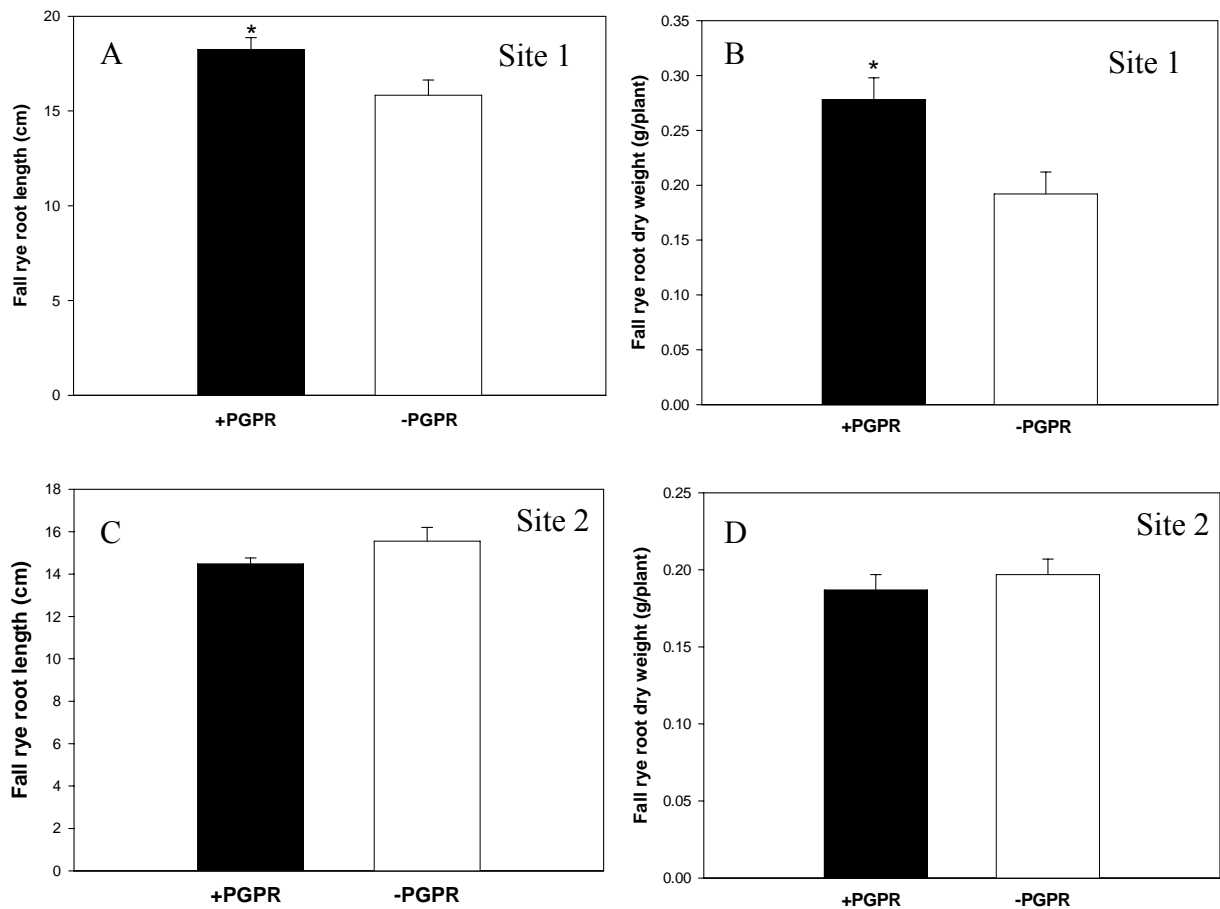
Figure 3.8 Effect of PGPR on root length (A) and dry weight (B) of fall rye on site 1 and root length (C) and dry weight (D) of fall rye on site 2 at harvest in 2006. The significant difference between plants treated PGPR and the untreated plants on site 1 can be observed, while PGPR effect was not obvious on site 2. Data represents mean ± S.E., n = 6. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treated plants and untreated plants. Site 1 was surrounding by tall trees. Site 2 was open without any shield.
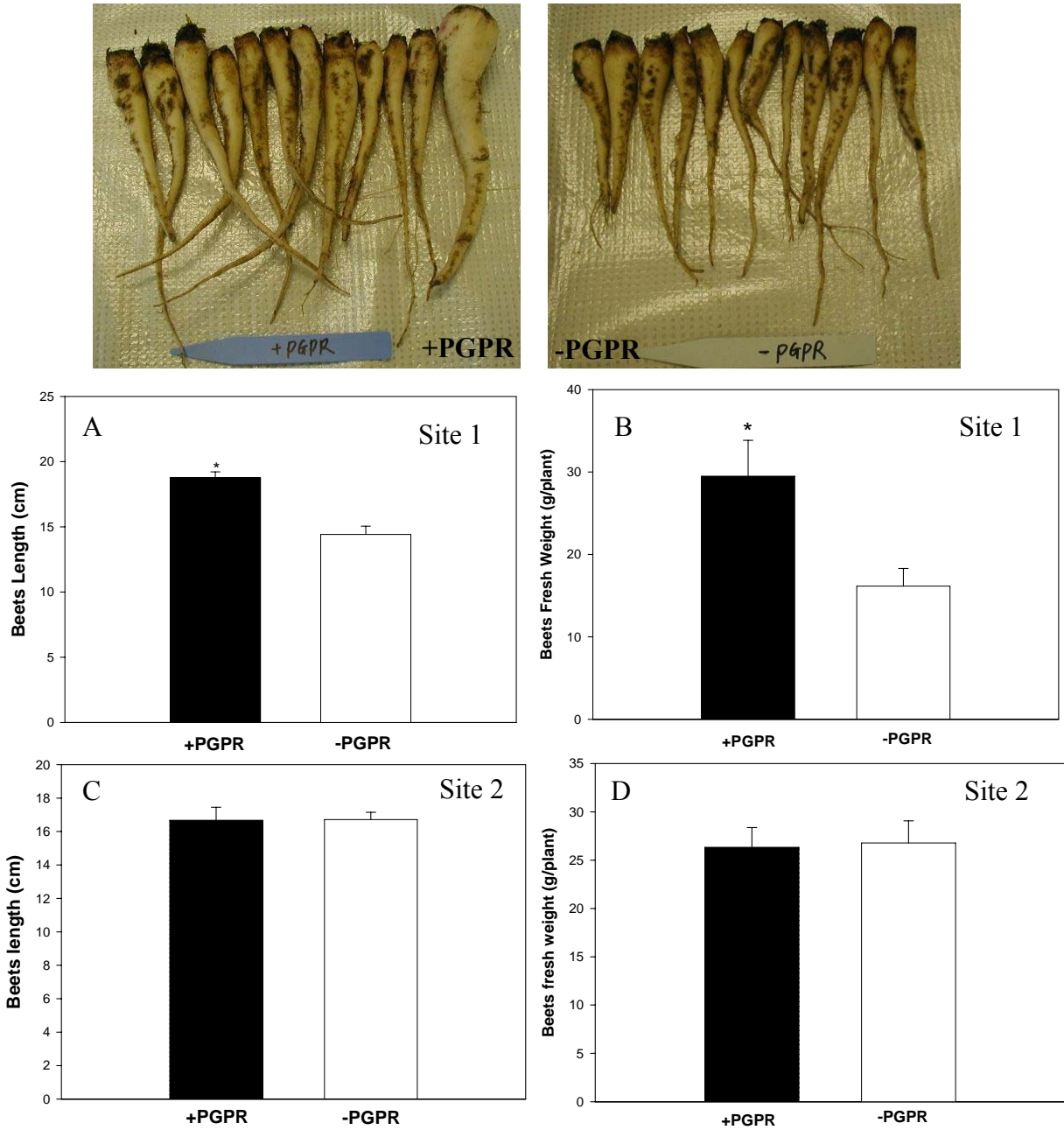
Figure 3.9 Sugar beets taken from the site 1 at harvest in 2006 (top pictures). Beet length (A) and weight (B) on site 1 and beet length (C) and weight (D) on site 2 at harvest in 2006. Data represents mean ± S.E., n = 10. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treated plants and untreated plants. Site 1 was surrounding by tall trees. Site 2 was open without any shield.
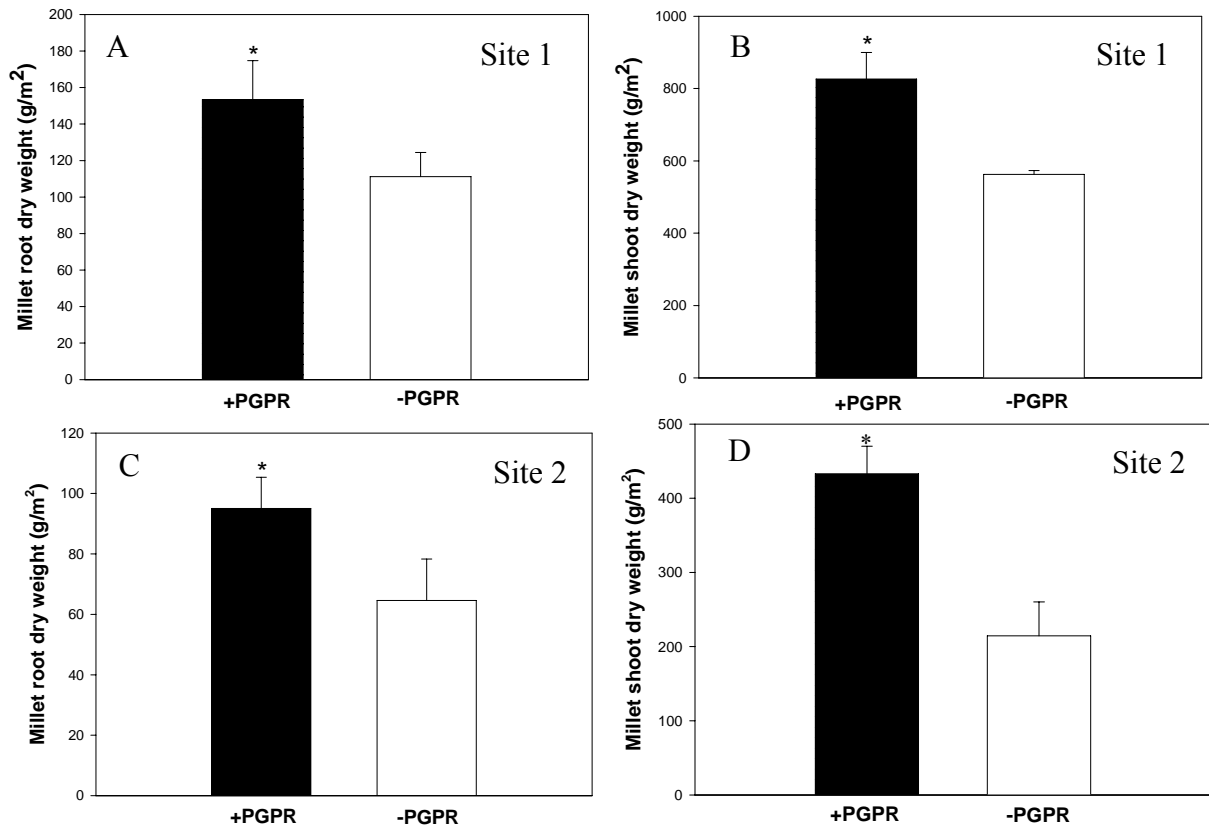
Figure 3.10 Millet samples collected on site 1 at 70-day growth (top left) and millet growing on site 1 at harvest (top right) in 2007. PGPR effect on root dry weight (A) and shoot dry weight (B) of millet on site 1 and root dry weight (C) and shoot dry weight (D) of millet on site 2 at harvest in 2007. Data represents mean ± S.E., n = 6. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treated plants and untreated plants. Site 1 was surrounding by tall trees. Site 2 was open without any shield.

between PGPR treated and the untreated plants was significant (Figure 3.10). A comparison of the biomass of millet treated with PGPR on the two sites, the root and shoot dry weights on site 2 were 153.33 and 826.67 g/m^2, which were significantly lower than those on site 1 (Figure 3.10). The untreated millet biomass followed the same trend.

In 2007, there was excessive weed growth on both sites, which had a negative effect on crop growth, especially on germination. This was likely due to the hot and dry weather conditions. On site 2, the sugar beets had poor germination (Figure 3.11). The planting area was covered with weeds. The dominant weed was identified as goosefoot (*Chenopodium album*) based on leaf and shoot appearance. Therefore, sugar beets were hard to collect at harvest, which meant it was not possible to compare biomass data between PGPR treated and untreated sugar beets on site 2. On site 1, sugar beets grew well enough even with the weeds (Figure 3.12). PGPR effects on sugar beet were observed 70-day after sowing (Figure 3.12). At harvest, biomass of sugar beet treated with PGPR compared to untreated plants grown was quantified (Figure 3.12). The average length of beets treated with PGPR was above 22 cm, while the length of untreated plants was less than 18 cm (Figure 3.12).

PGPR effects on pumpkin growth were also tested. The stem and root dry weights of pumpkin after 120 days growth were assessed (Figure 3.13). On the site 1, the stem and root dry weights of pumpkin treated with PGPR were 66.3% and 30.5% greater, respectively, than the untreated plants. On the site 2, pumpkin biomass of PGPR treated plants was also significantly greater than the untreated plants (Figure 3.13).

Potato was treated with PGPR as well. At 30-day after planting, weeds were dominant in the potato plots (Figure 3.14). However, it was clear that potato shoots treated with PGPR were taller than the untreated potatoes (Figure 3.14). Due to weeds, the growth of potatoes

Figure 3.11 Growth of sugar beet 20-day (top) and 60-day (bottom) after sowing on site 2. No sugar beet growth observed. All visible plants in pictures in the experimental plots were weeds.
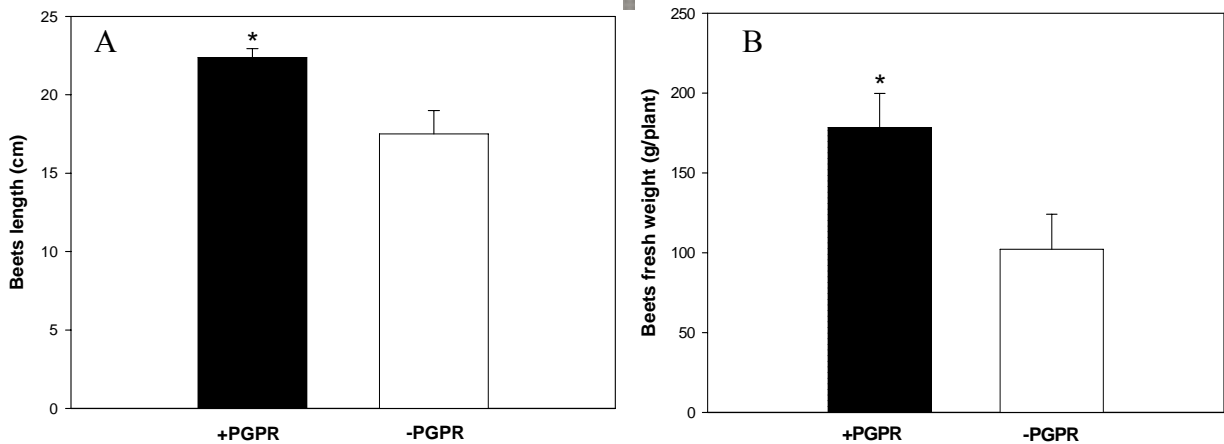
Figure 3.12 Growth of sugar beet 20-day after sowing on site 1 (top left). PGPR effect on sugar beets collected on site 1 at 70-day growth (top right) and at harvest (middle) in 2007. Beet length (A) and fresh weight (B) at harvest on site 1 are shown. Data represents mean ± S.E., n = 8. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treated plants and untreated plants.
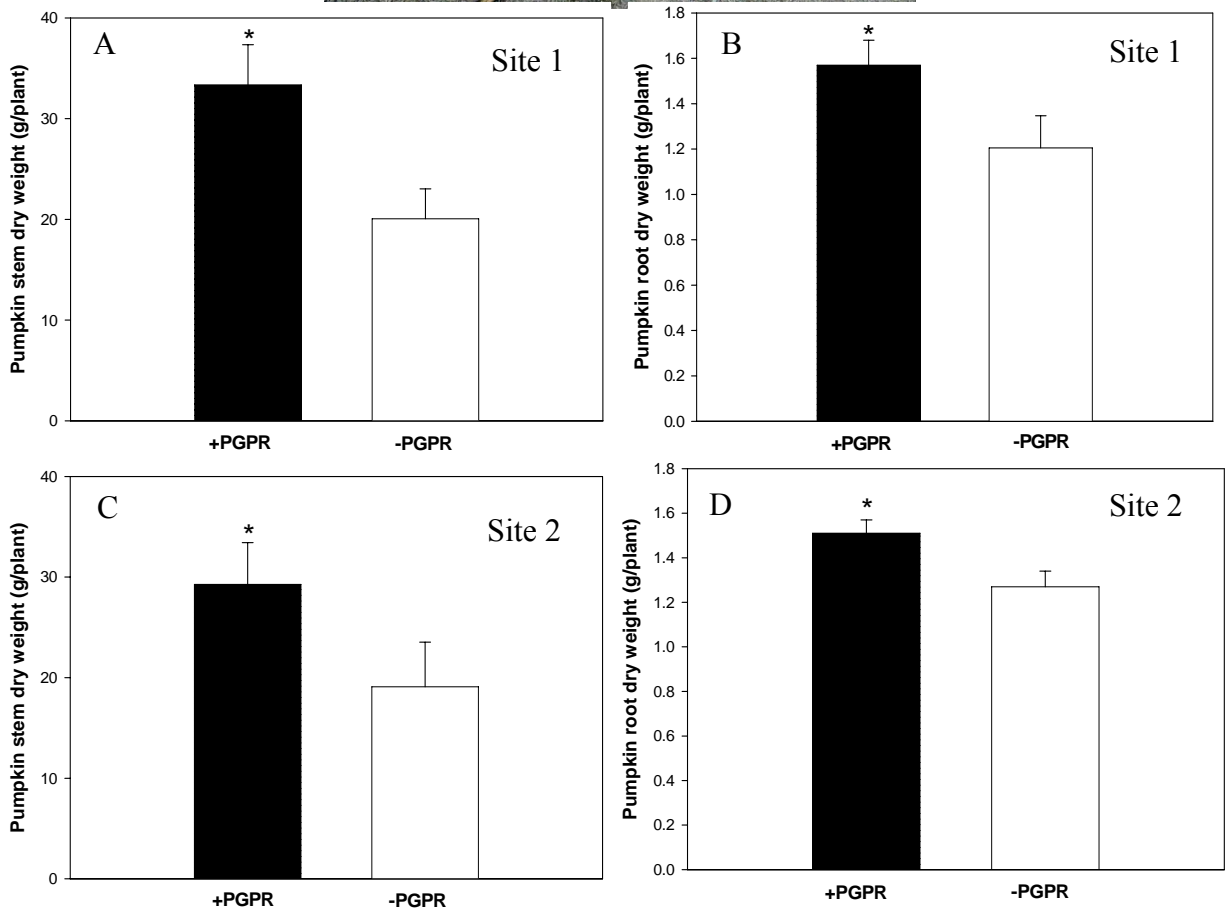
Figure 3.13 Pumpkin samples taken on site 2 at 40-day growth in 2007 (top). Effect of PGPR on dry weights of stems (A) and roots (B) of pumpkin on site 1 and the dry weights of stems (C) and roots (D) of pumpkin on site 2 at harvest in 2007. Error bars are standard error, n = 18 (site 1) and 7 (site 2). Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treated plants and untreated plants. Site 1 was surrounding by tall trees. Site 2 was open without any shield.
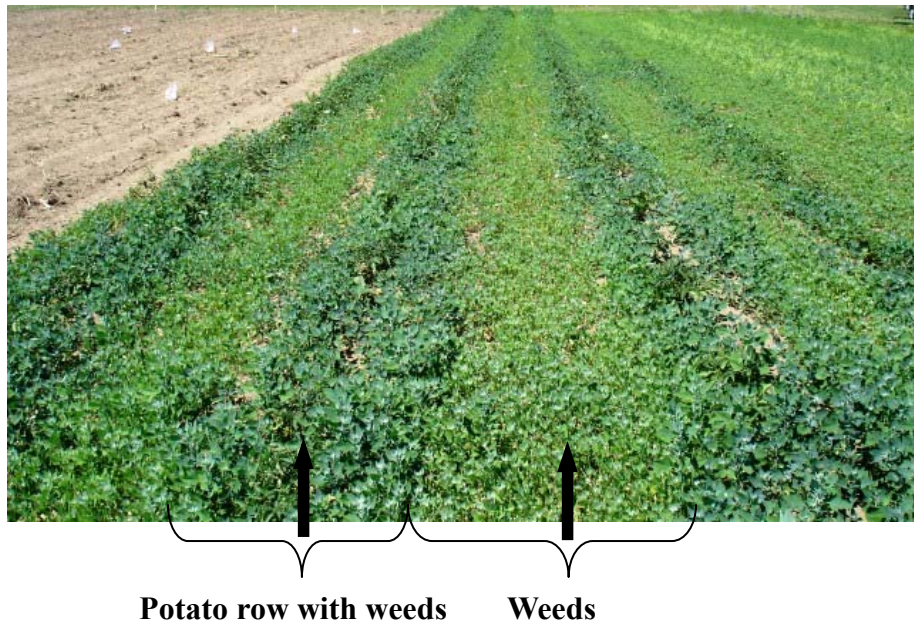
Figure 3.14 Growth of potatoes on site 2 at 30-day. Potato plots were overrun with weeds (top). Close-up picture showing concurrent growth of potatoes and weeds (bottom left). PGPR effects on potato shoots were observed (bottom right).

was sparse (Figure 3.15). All potato shoots senesced 80-day after sowing (Figure 3.15). Potato was harvested after 160 days of growth. It was difficult to find potato tubers owing to their sparse growth. Nonetheless, at harvest, the fresh weight of potato tubers treated with PGPR was 46% greater than the untreated plants (Figure 3.15).

3.3 DDT detection in plant tissues

Because the plants grew very well both in the greenhouse and in the field, the next step was to determine if these plants can take up DDT from the impacted soil. In greenhouse tests, DDT detection in fall rye roots after 30 days growth was performed. The DDT peak was not observed in fall rye root extracts. The possible reason is that greenhouse tests can not run long enough to see the accumulation of DDT in plant tissues. Thus, it was decided to detect DDT in plant tissues from the field, which would be more feasible. The following results for DDT detection in plant tissues are from the field trials.

Roots and shoots of fall rye were collected, dried, and extracted prior to analysis by HPLC. In the 2005 season, 4,4'-DDT was present in the extracts from composite roots samples (Figure 3.16). This was observed again in the 2006 season (Figure 3.17). Due to the number of complex compounds present in fall rye roots, a standard of 4,4'-DDT was run to confirm the identification of the peak. According to the chromatograms and absorbance spectra (Figure 3.17), 4,4'-DDT was present in the fall rye roots extracts. In addition to the 4,4'-DDT, 4,4'-DDE was observed in roots extracts from the fall rye samples. The concentrations of 4,4'-DDT and 4,4'-DDE were 0.58 and 0.56 μg/g dry weight of roots, respectively. 4,4'-DDT was not observed in the extracts of fall rye shoots (Figure 3.17B). Duplicate fall rye composite samples were sent to the ALS laboratory group (Waterloo, ON) for analysis. 4,4'-DDT and 4,4'-DDE at 0.61 and 0.59 μg/g, respectively, were detected in

A
B

Potato rows at 40-day growth

Senescent potato shoots

C

Potato fresh weight (g/plant)

70
60
50
40
30
20
10
0

*

+PGPR   -PGPR
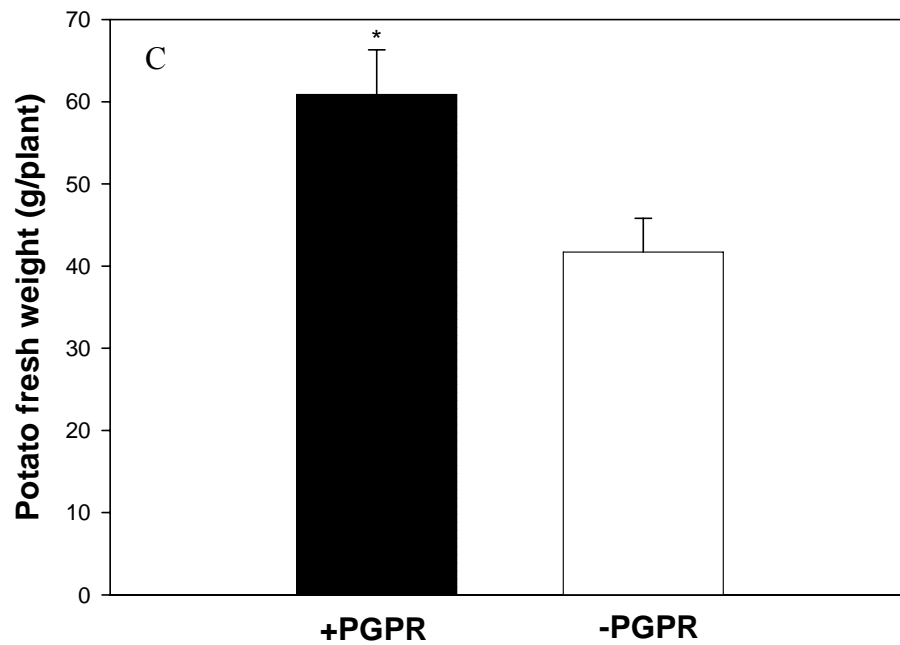
Figure 3.15 Potato at 40-day after weeds were mowed (A). All potato shoots senesced after 80 days sowing (B). Comparison of potato tubers at harvest between PGPR treated plants and the untreated plants (C). Data represents mean ± S.E., n = 7. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference between PGPR treated plants and untreated plants.
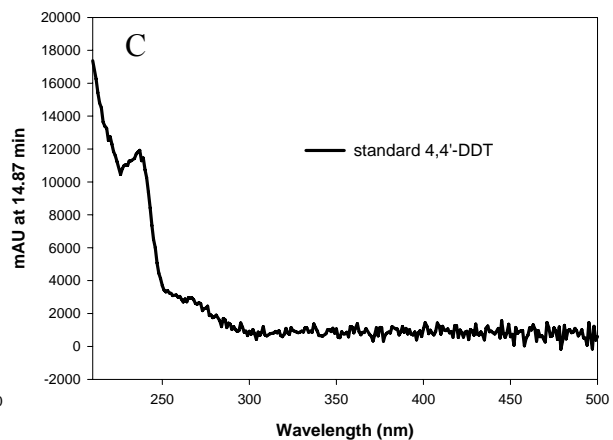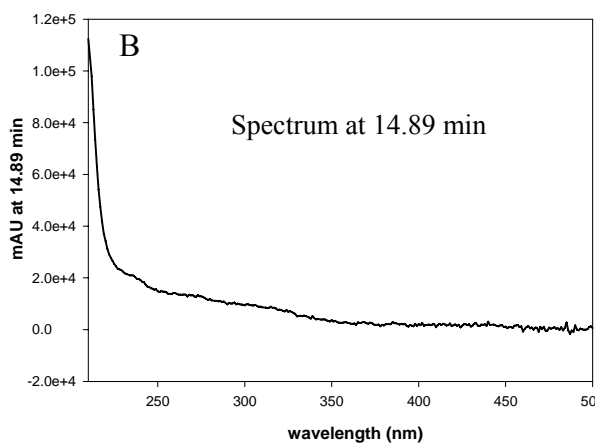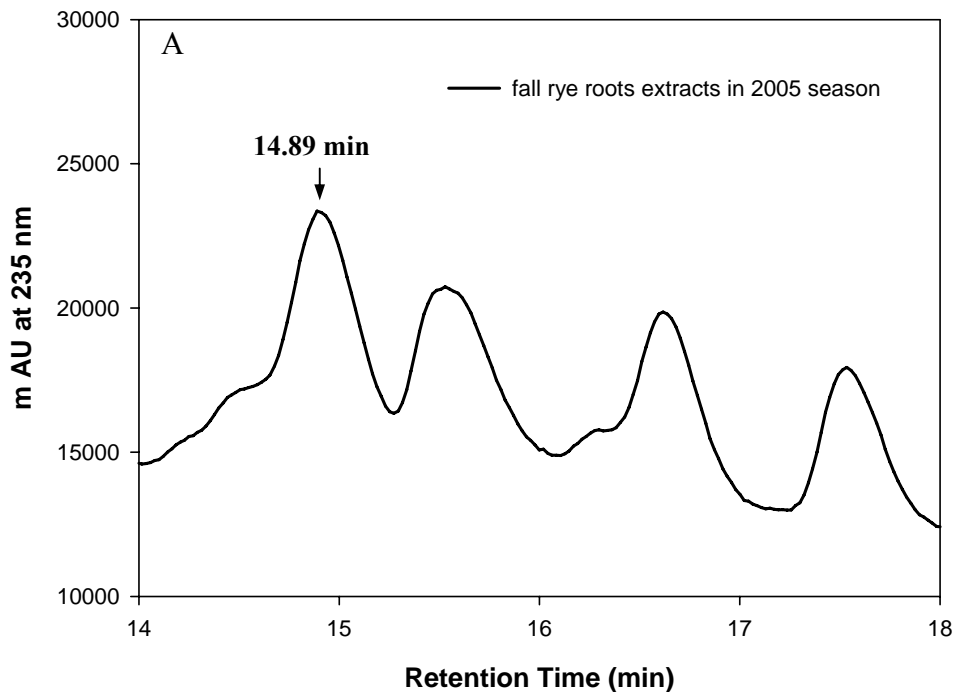
Figure 3.16 HPLC chromatogram of root extracts from fall rye composite samples (n=6) collected in 2005 season (A) and spectrum of the peak at 14.89 min from fall rye root extracts (B), identified as 4,4'-DDT by comparison to standard 4,4'-DDT spectrum (C).
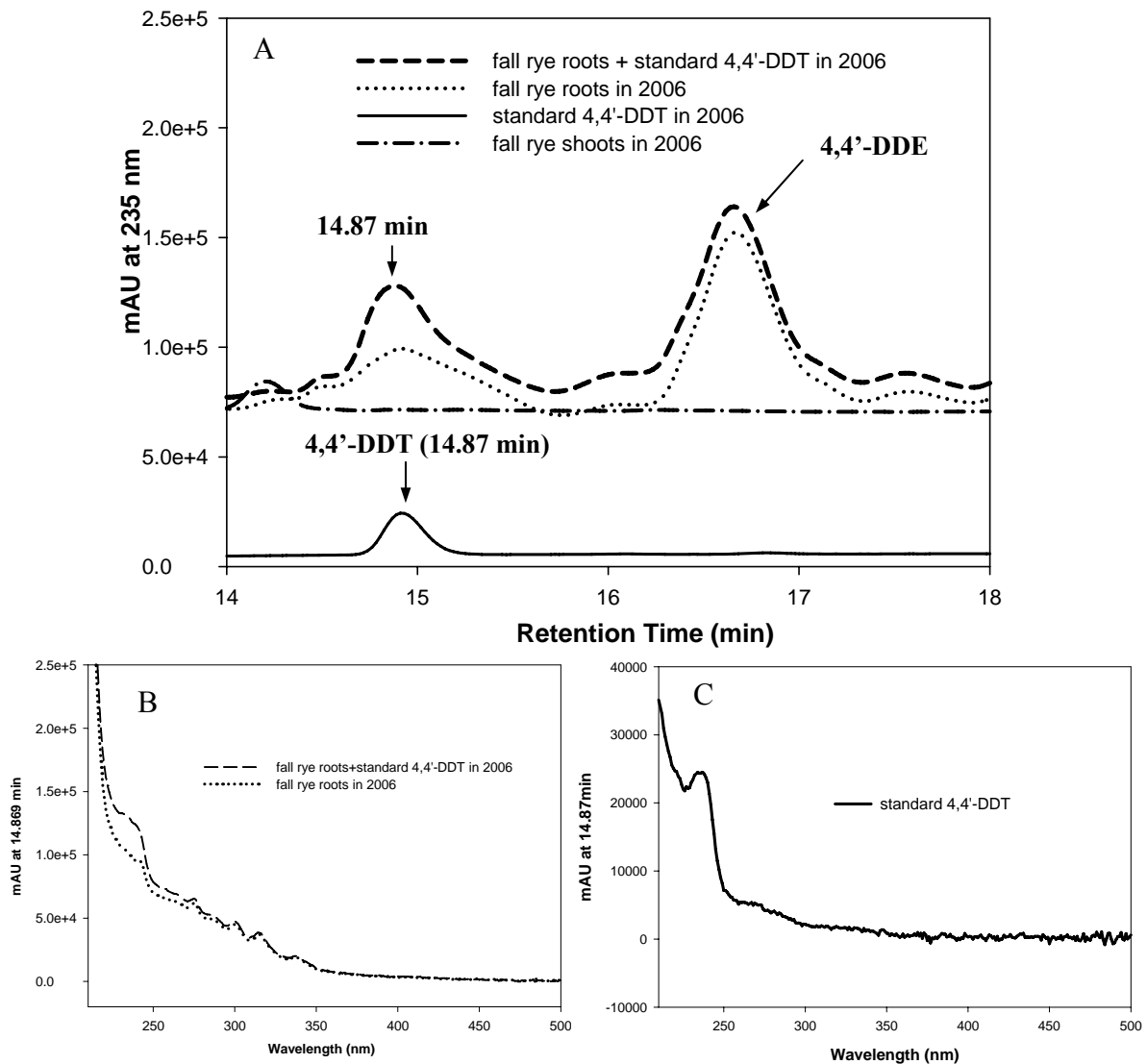
Figure 3.17 HPLC chromatogram of root and shoot extracts from fall rye composite samples (n=6) collected in 2006 season (A) and spectra of the peak at 14.87 min from fall rye root extracts (B) and standard 4,4'-DDT (C).

root extracts of fall rye from the ALS lab, which confirmed the in-house analytical results.

DDT detection in millet roots and shoots by HPLC was performed in 2007. In 2007, the detection of DDT in millet samples was performed twice. The first was after 70 days of plant growth (Figure 3.18). Comparing the chromatograms, peaks corresponding to 4,4'-DDT and 4,4'-DDE were detected from millet shoot extracts. The concentrations of 4,4'-DDT and 4,4'-DDE were 0.63 and 0.76 μg/g dry shoots, respectively. However, small peaks of 4,4'-DDT and 4,4'-DDE were detected in millet roots extracts, which corresponded to concentrations of 4,4'-DDT and 4,4'-DDE: 0.04 and 0.07 μg/g, respectively. The concentrations of DDT and DDE in millet shoots were much higher than the levels in millet roots. It seems not reasonable. The possible reason could be caused by the extraction method. The solvents used to extract DDT from plant tissues was hexane. If DDT strongly adhered to millet roots, the nonpolar solvent could be hard to extract DDT accumulated in millet roots.

A second analysis of DDT in millet samples was done at harvest. However, in millet shoot extracts, a small peak of 4,4'-DDT was observed and 4,4'-DDE peak was not present. Only small amount of 4,4'-DDT and 4,4'-DDE were observed from the extracts of millet roots (Figure 3.18). Note, the absorbance spectrum of the large HPLC peak from millet shoot extracts at 14.04 min did not match 4,4'-DDT spectrum of the 4,4'-DDT standard (Figure 3.18C). Although the compound corresponding to the peak at 14.04 min was not 4,4'-DDT, it may be related to DDT because its absorbance spectrum was similar to that of DDT standard.

DDT detection in sugar beet was performed for 2006 and 2007 seasons. In 2006 season, DDT detection in roots of sugar beet was done at harvest. To confirm the accurate detection of 4,4'-DDT, both root extracts and a 4,4'-DDT standard were run under the same HPLC conditions. A peak at 14.8 min was observed from the root extracts (Figure 3.19). The
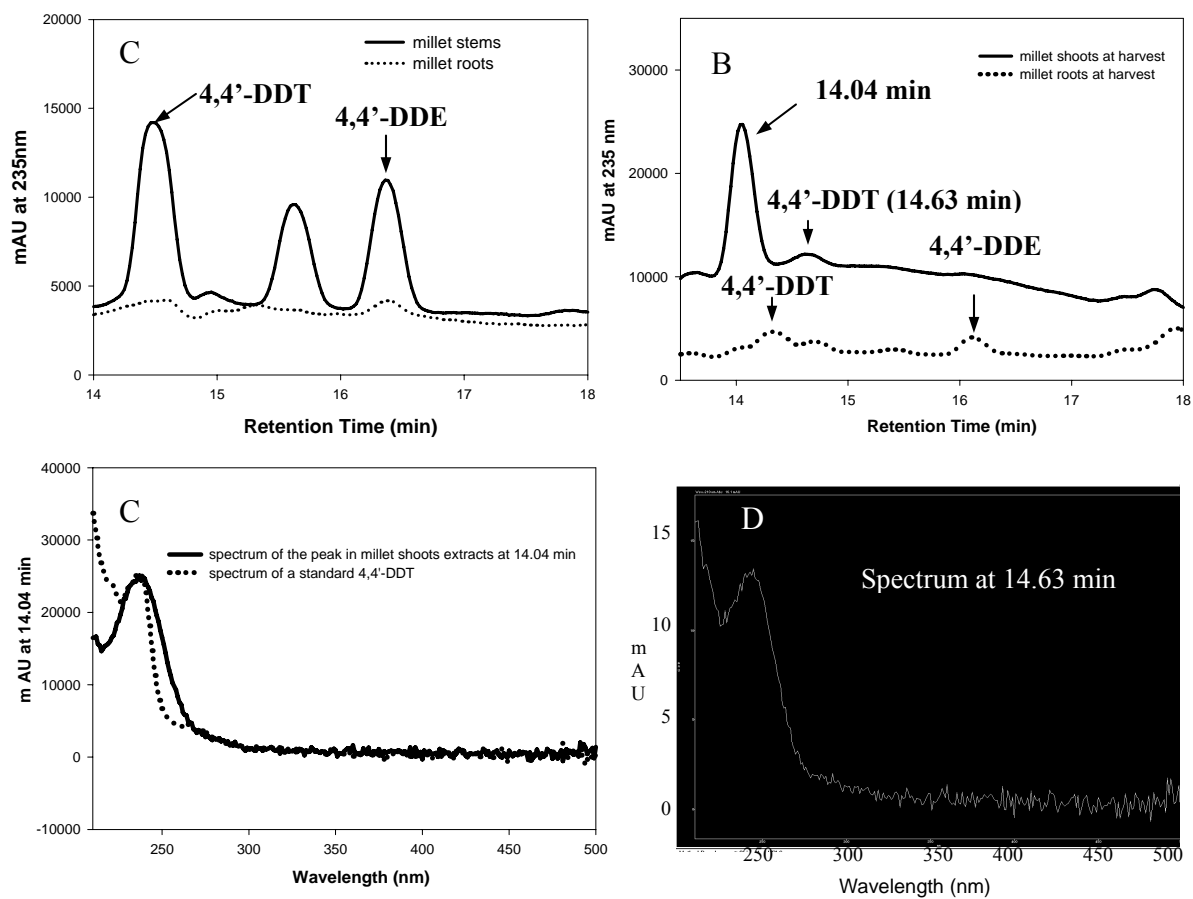
Figure 3.18 HPLC chromatograms of root and shoot extracts from millet composite samples (n=6) collected at 70-day growth (A) and at harvest (B) in 2007 season. The spectrum of the large peak in millet shoot extracts at 14.04 min is compared to a spectrum of standard 4,4'-DDT (C). The spectrum of the small peak of 4,4'-DDT in millet shoot extracts at 14.63 min (D).
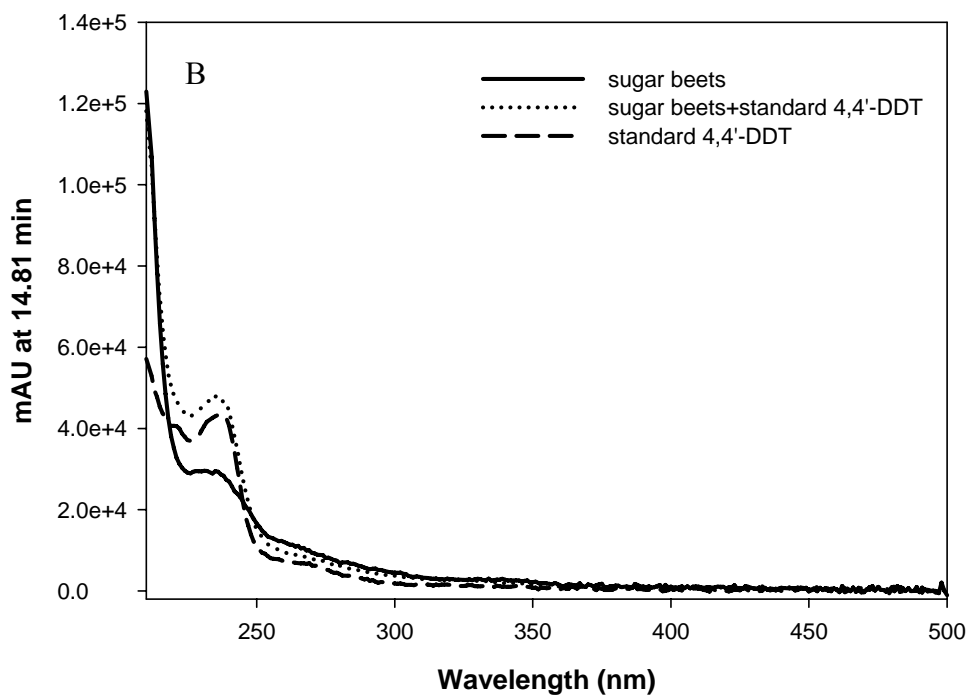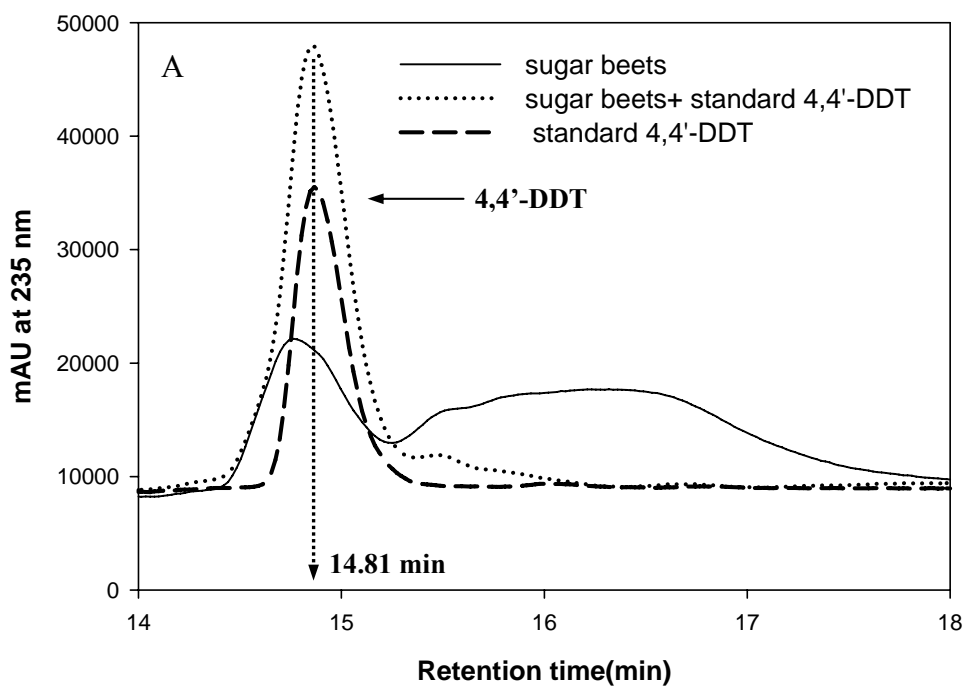
Figure 3.19 HPLC chromatograms of roots extracts of sugar beet at harvest in 2006 (A). Spectra of the peak at 14.81 min in sugar beets extracts and a standard DDT are shown to confirm the DDT peak (B).

absorbance spectra of sugar beet extracts and the standard 4,4'-DDT were compared at 14.8 min (retention time). This gave a positive identification for 4,4'-DDT extracted from the sugar beets (Figure 3.19). The concentration of 4,4'-DDT was 0.37 μg/g dry beets as shown in the chromatogram. No DDT was detected in sugar beet leaves.

In 2007 season, DDT detection in sugar beet was performed at 70-day growth and harvest, respectively. At the retention time between 14 min to 15 min, there were two peaks observed absorbance spectra of peaks at 14.27 and 14.96 min, 4,4'-DDT was not observed in the sugar beet extracts (Figure 3.20). As well, 4,4'-DDT peak can not be observed in the beets extracts collected at harvest (Figure 3.20).

Pumpkin tissues from the 2007 season were analyzed to determine if DDT or its metabolites were present. To extract DDT from pumpkin tissue, each pumpkin was divided into roots, stem 1, stem 2, and leaves and all were separately analyzed (see Figure 3.21). Stem 1 was the stem from above the roots until the part where the first leaf connects. The stem 2 was the rest of stem region of the plant. DDT analyses of pumpkin tissues were performed after 40-day growth and harvest.

Chromatograms of the extracts from stem 1 at 40-day and harvest are shown (Figure 3.22). At 40-day, 4,4'-DDT and 4,4'-DDE were detected in pumpkin stem 1 at concentrations of 0.31 and 0.70 μg/g dry tissue, respectively, while the concentrations of 4,4'-DDT and 4,4'-DDE were 1.06 and 2.05 μg/g at harvest. In pumpkin stem 2 at 40-day growth, 4,4'-DDT peak was difficult to distinguish due to interference from plant metabolites in the extracts from stem 2 (Figure 3.23). The absorbance spectra at 14.23 and 14.51 min are shown to confirm the 4,4'-DDT peak (Figure 3.23). Compared to the absorbance spectrum of standard 4,4'-DDT (Figure 2.9), the large peak at 14.23 min was not 4,4'-DDT. However, it
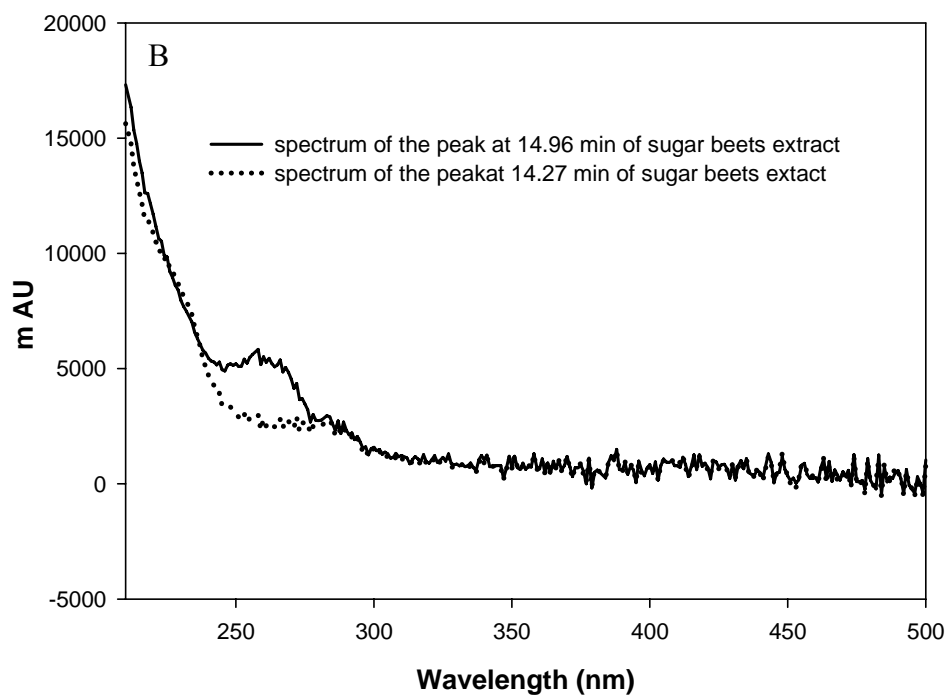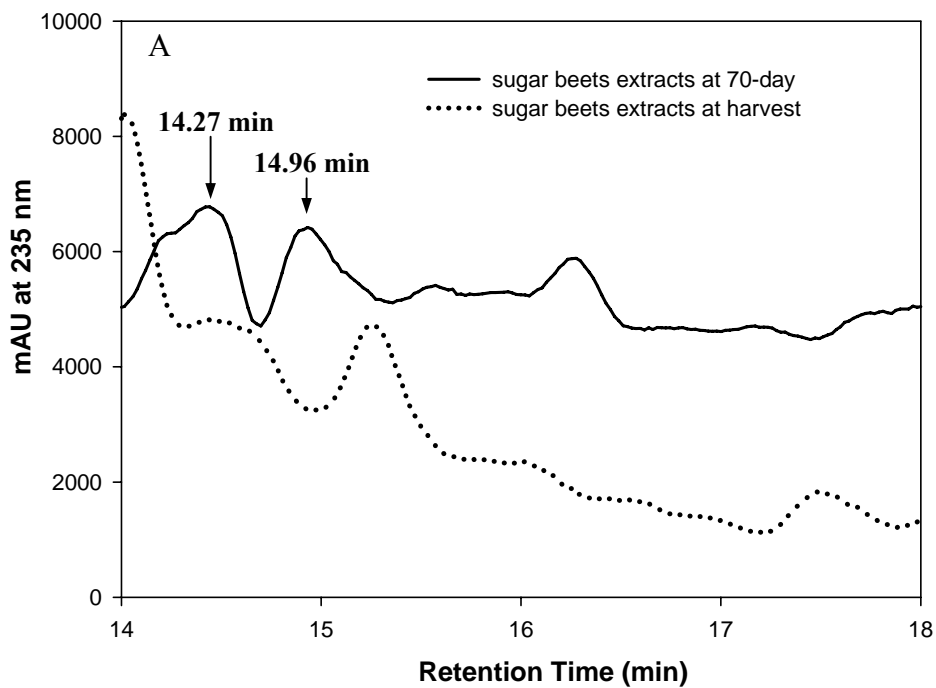
Figure 3.20 Chromatograms of sugar beets extracts at 70-day and at harvest (A). The spectra of the peaks at 14.27 and 14.96 min observed in the extracts from sugar beets collected at 70-day (B).
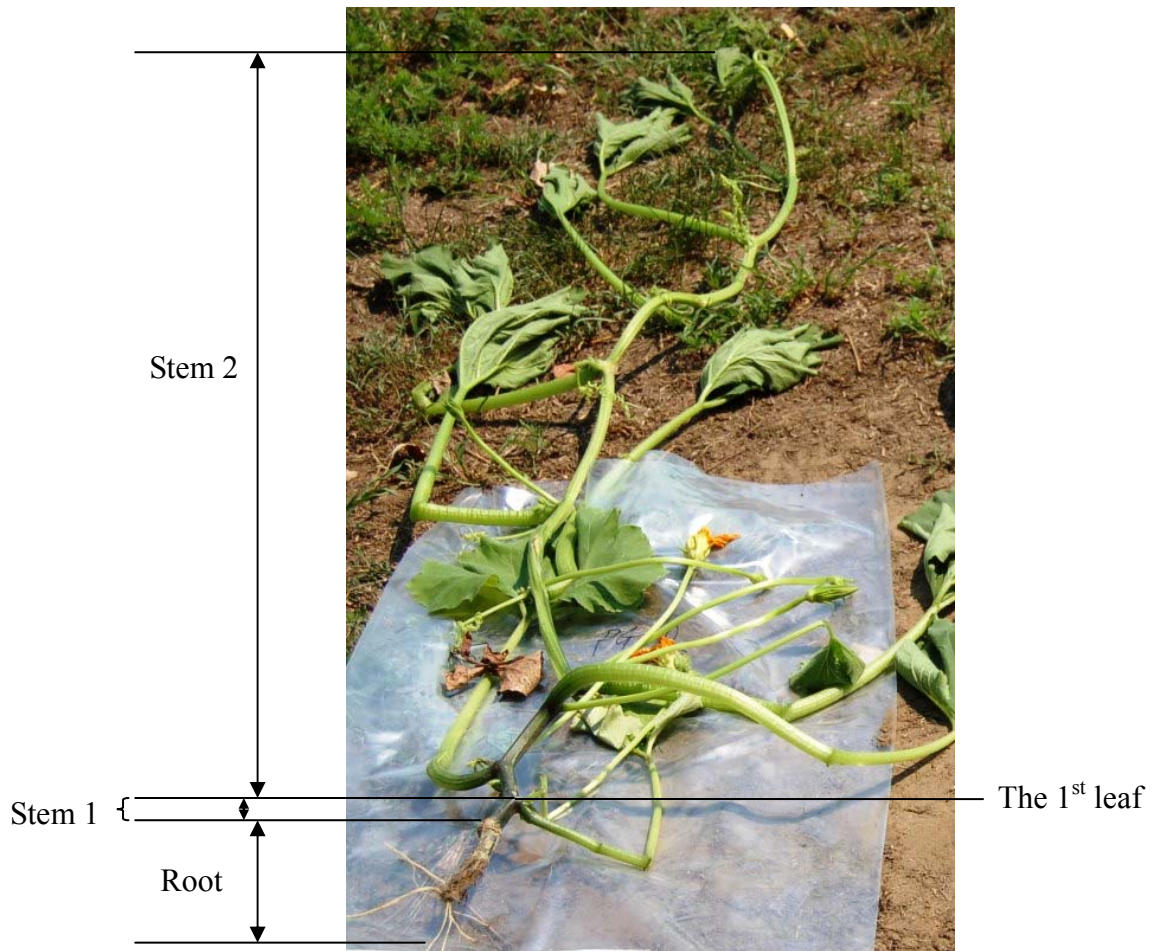
Figure 3.21 The graph of pumpkin parts analyzed by HPLC. Root, stem 1 and stem 2, leaves of pumpkin were analyzed separately. Stem 1 was defined as the part between the end of the root and the first leaf. Stem 2 was the rest of stem region of the plant.
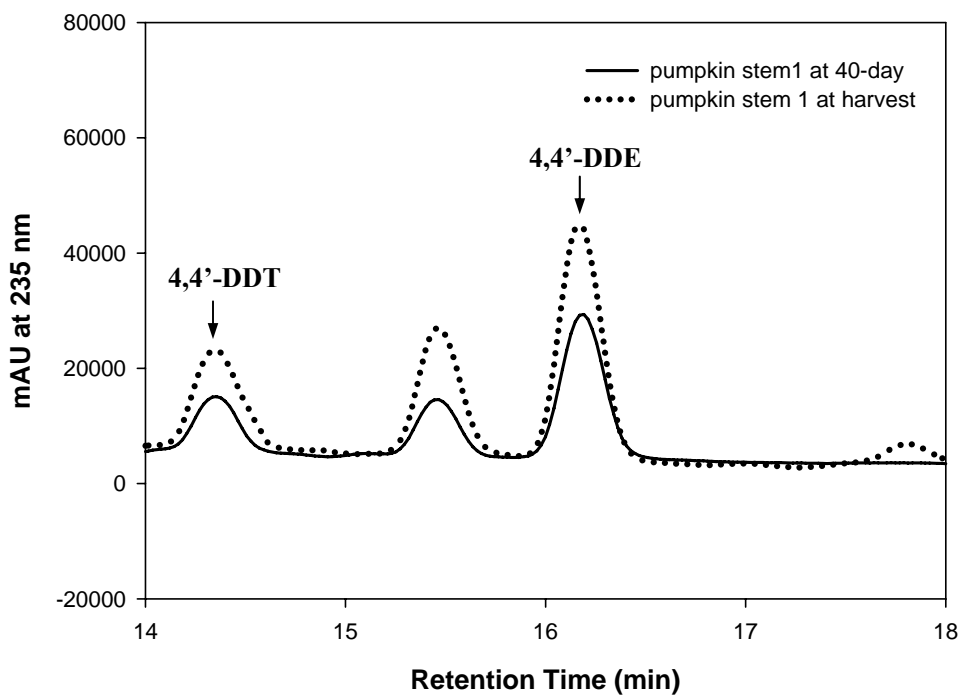
Figure 3.22 Comparison of HPLC chromatograms from extracts of pumpkin stem 1 collected at 40-day and harvest in 2007 season.
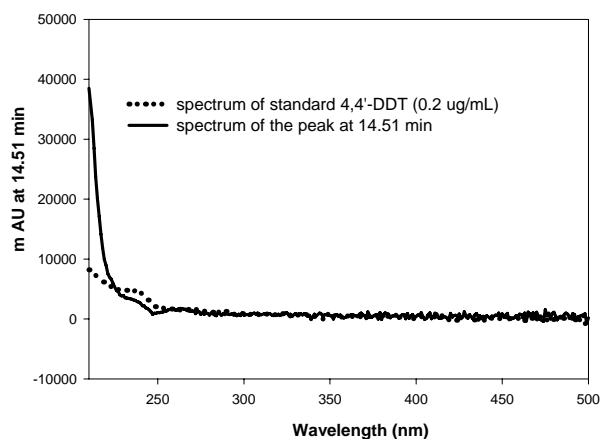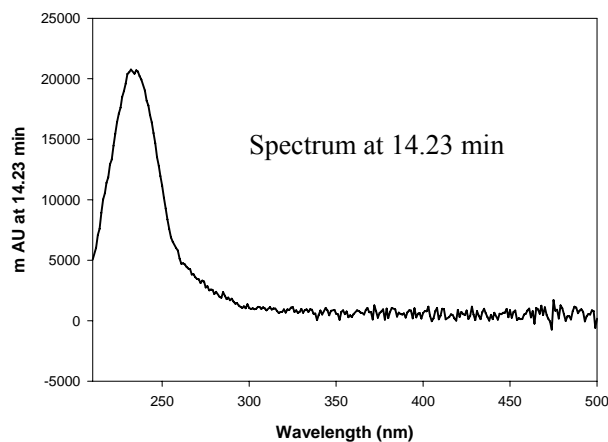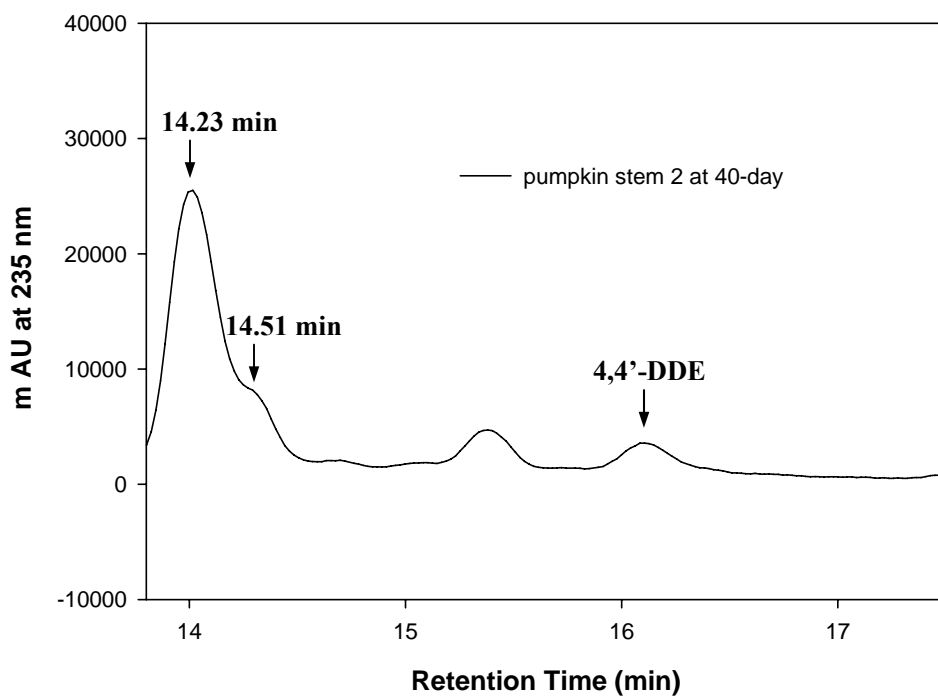
Figure 3.23 Chromatogram of the extracts of pumpkin stem 2 collected at 40-day in 2007 season (A). Spectra of the large peaks at 14.23 (B) and 14.51 min (C). The spectrum of standard 4,4'-DDT (0.2 μg /mL) is shown in the graph (C).

may be related to DDT because they have the same wavelength (235 nm) and similar absorbance spectral shape. Interestingly, compared to the absorbance spectrum of a standard 4,4'-DDT (0.2 μg/mL), the spectrum of the peak shoulder at 14.51 min was similar to the standard and was thus identified as DDT. The DDT concentration was estimated to be 0.1 μg/g (Figure 3.23). In addition, a small peak of 4,4'-DDE was observed in the extracts from pumpkin stem 2 collected at 40-day and the concentration of 4,4'-DDE was 0.2 μg/g (Figure 3.23). At harvest, 0.2 and 0.32 μg/g of 4,4'-DDT and 4,4'-DDE were observed in the extracts of stem 2 (Figure 3.24).

The DDT analysis in pumpkin leaves was performed 40 days after sowing. A chromatogram of the extracts from pumpkin leaves is shown in Figure 3.25. Due to the interference from pigments in leaves, it was difficult to locate the peaks of 4,4'-DDT and 4,4'-DDE in the HPLC chromatograms. For instance, although there was a large peak in the extract of leaves at the retention time of 14.27 min, it was not likely 4,4'-DDT based on its absorbance spectrum (Figure 3.25). While the peak at 14.51 min was likely 4,4'-DDT compared the absorbance spectrum (Figure 3.25C). Results from ALS laboratory show that the concentrations of 4,4'-DDT and 4,4'-DDE were both 0.03 μg/g dry leaves, which is below the detection limit of the HPLC used here.

Pumpkin roots collected at 40-day growing were also found to contain DDT and DDE as analyzed by HPLC and confirmed by ALS laboratory analysis. The concentrations of 4,4'-DDT and 4,4'-DDE in roots at 40-day growth were 0.32 and 1.3 μg/g, respectively (Figure 3.26). At harvest, 0.67 and 1.64 μg/g of 4,4'-DDT and 4,4'-DDE were detected from the root extracts of pumpkin.
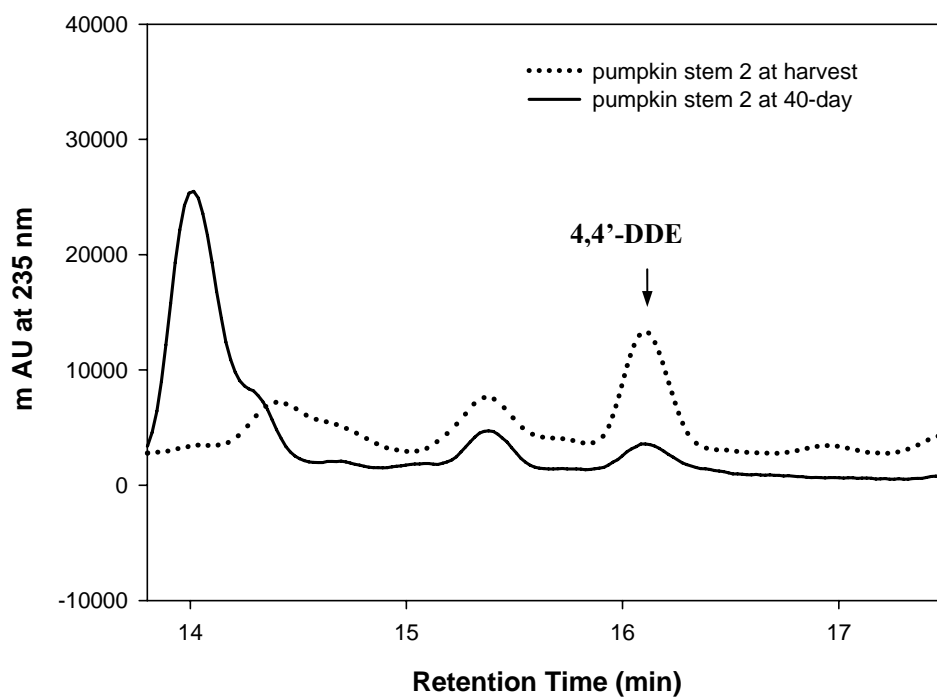
Figure 3.24 Comparison of chromatograms of the extracts from pumpkin stem 2 collected at 40-day and harvest.
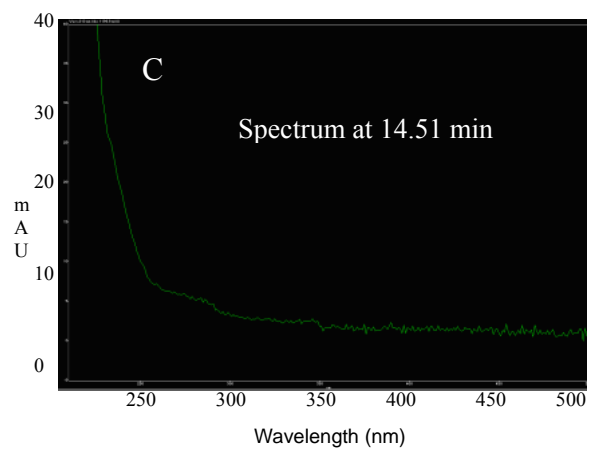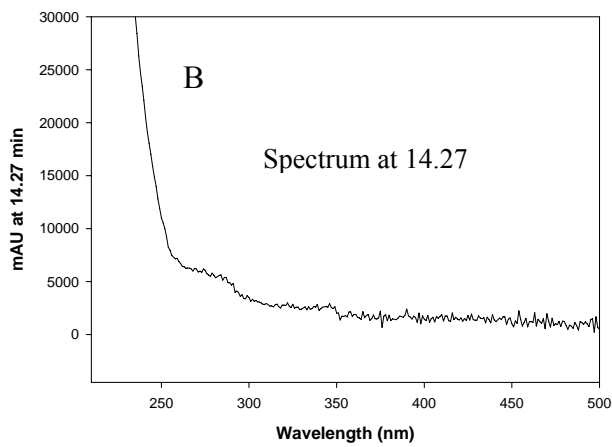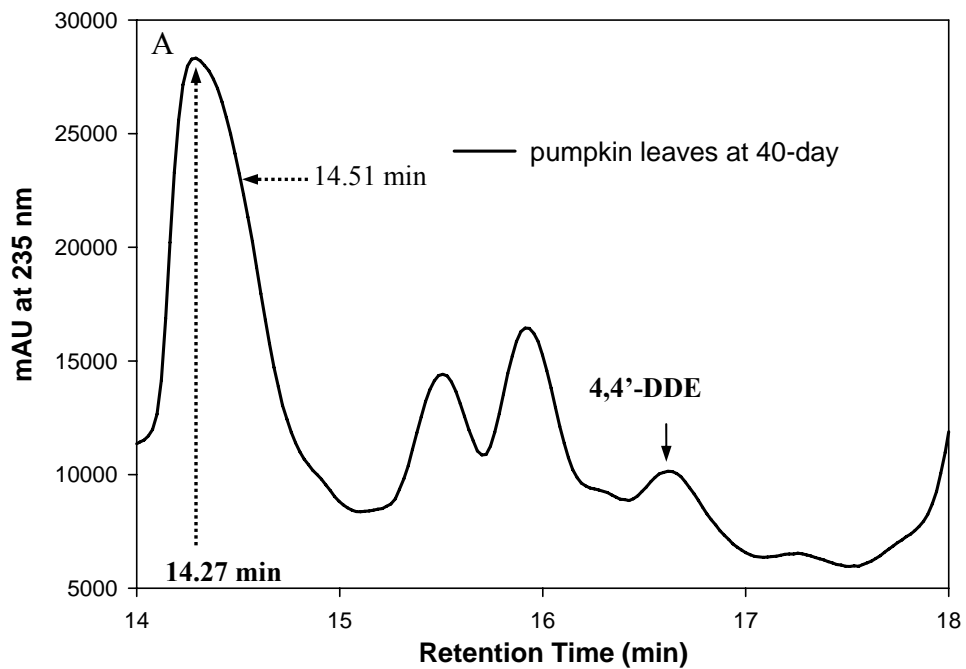
Figure 3.25 Chromatogram of pumpkin leaves extracts (A) and spectra of the large peak at 14.27 min (B) and 14.51 min (C).
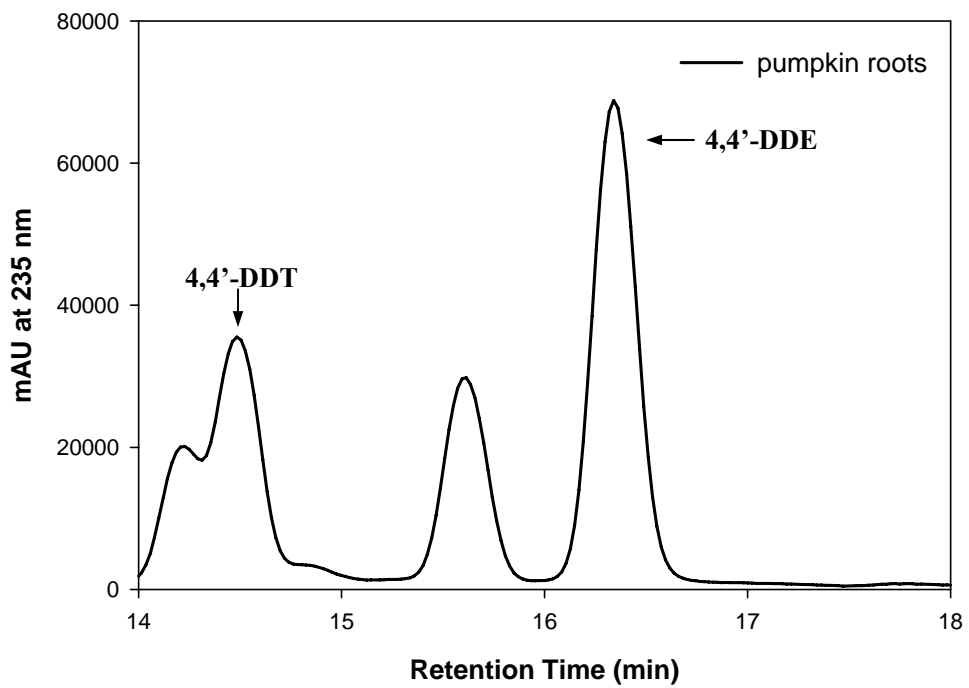
Figure 3.26 HPLC chromatogram of root extracts from pumpkin at 40-day growth.

The DDT analysis of pumpkin fruit was performed at harvest. However, neither DDT nor DDE were detected in the extracts from pumpkin fruits (Figure 3.27). The only peak (at 14.01 min) between 14 to 18 min was not 4,4'-DDT based on the following reasons. 4,4'-DDE is easier to be transported to fruits than 4,4'-DDT because DDE is much more soluble than DDT (Worthing and Hance, 1991). Because higher 4,4'-DDE concentration than 4,4'-DDT was observed in pumpkin stem 2 (Figure 3.24), 4,4'-DDE peak should be present if the peak at 14.01 min was 4,4'-DDT. However, the 4,4'-DDE peak was not observed in the extracts from pumpkin fruits (Figure 3.27). Therefore, the peak at 14.01 min was not 4,4'-DDT.

Due to the difficulty in analyzing DDT concentration in potatoes in our lab, samples were sent to ALS laboratory for determination. DDT was not detected in any of the samples tested.

To compare the detection of DDT in plant tissues in 2006 and 2007 seasons, the analytical results of 4,4'-DDT and 4,4'-DDE in plant tissues of fall rye, millet, sugar beets, pumpkin, and potato are summarized in Table 3.1

3.4 Changes in DDT concentrations in field site soil

Before planting, soil samples in the field were collected and background DDT concentrations in soil were analyzed. The results indicated that the concentration of 4,4'-DDT was much higher in the soil than the levels of its related isomers. Therefore, 4,4'-DDT concentration in the soil during the plant growth was determined.

In 2005, millet and fall rye were planted on the site 1. DDT concentration at t_0 was not determined. After 120 days of growth, 4,4'-DDT was extracted from near-root soil samples and blank soil samples. The soil from the vicinity of the plant roots was defined as near-root soil. Blank soil was the unplanted soil from areas adjacent to the experimental plots. The
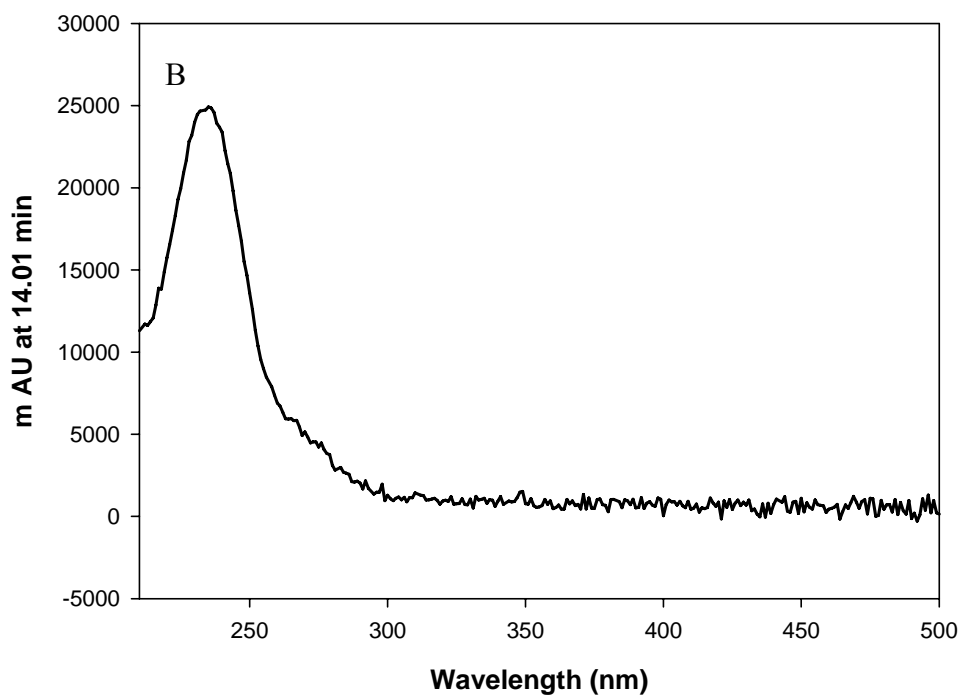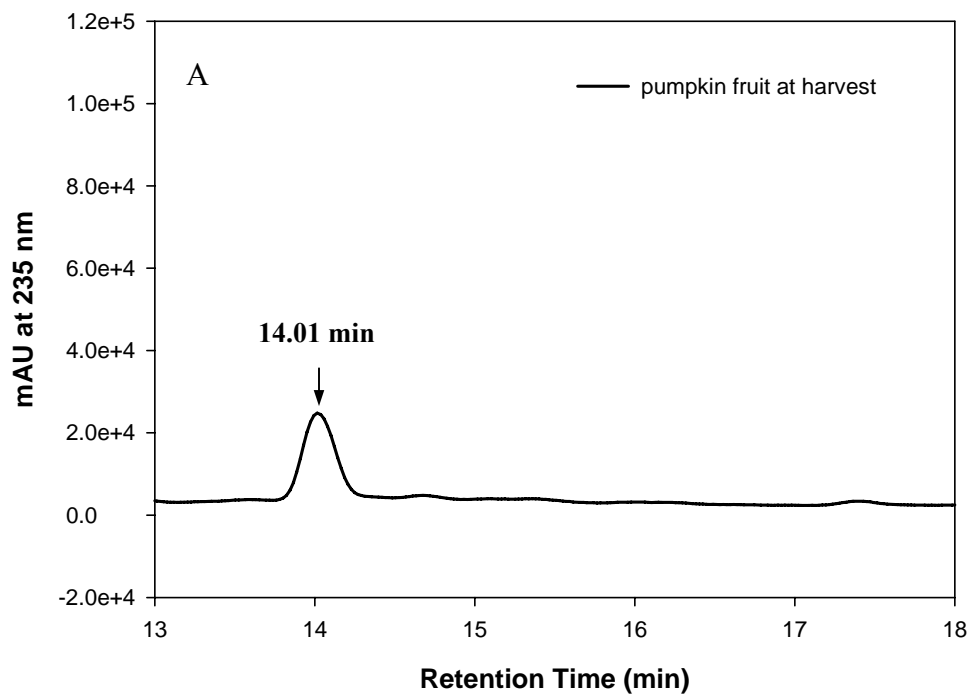
Figure 3.27 Chromatogram of the extracts from pumpkin fruit at harvest (A) and the spectrum of peak at 14.01 min (B).

Table 3.1 The detection of 4,4'-DDT (µg/g) and 4,4'-DDE (µg/g) in plant tissues in 2006 and 2007 seasons

			2006 season	2007 season	
				at 40-day (pumpkin) or 70 day (millet and sugar beet)	at harvest (all)
Fall rye	4,4'-DDT	Roots	0.61	Not planted	
		Shoots	ND		
	4,4'-DDE	Roots	0.59		
		Shoots	ND		
Millet	4,4'-DDT	Roots	Not analyzed	0.04	0.05
		Shoots	Not analyzed	0.63	0.04
	4,4'-DDE	Roots	Not analyzed	0.07	0.15
		Shoots	Not analyzed	0.76	ND
Sugar beet	4,4'-DDT	Roots	0.37	ND	ND
		leaves	ND	ND	ND
	4,4'-DDE	Roots	ND	ND	ND
		leaves	ND	ND	ND
Pumpkin	4,4'-DDT	Roots	Not planted	0.32	0.67
		Stem 1		0.31	1.06
		Stem 2		0.1	0.2
		Leaves		0.03	Not analyzed
		Fruit		Not analyzed	ND
	4,4'-DDE	Roots		1.3	1.64
		Stem 1		0.7	2.05
		Stem 2		0.2	0.32
		Leaves		0.03	Not analyzed
		Fruit		Not analyzed	ND
Potato	4,4'-DDT	tuber	Not planted	ND	ND
	4,4'-DDE	tuber		ND	ND

Note: ND means the concentration below the detection limit (0.02µg/g). The actual amount of DDT and DDE detected in per pumpkin plant is listed in Table 3.1A.

Table 3.1A Calculation of the actual amount of DDT detected in roots and stems of per pumpkin

	one pumpkin		
	Roots	Stem 1	Stem 2
Biomass (dry weight, g)	1.57	1.89	31.47
4,4'-DDT concentration (µg/g dry weight)	0.67	1.06	0.2
4,4'-DDE concentration (µg/g dry weight)	1.64	2.05	0.32
4,4'-DDT amount (µg)	1.05	2	6.29
4,4'-DDE amount (µg)	2.57	3.87	10.07

average concentrations of 4,4'-DDT in the blank soil around the millet and fall rye plots were 0.83 and 0.73 μg/g soil, respectively (Table 3.2). The average concentrations of 4,4'-DDT in the soil from the near-root soil samples of millet and fall rye were 0.60 and 0.55 μg/g soil, respectively (Table 3.2). One more sampling at fall rye site was done in April, 2006. But 4,4'-DDT concentration in the near-root soil was remained approximately constant compared to the DDT level in the near-root soil in December, 2005 (Figure 3.28).

In 2006, site 1 and site 2 were tested for phytoremediation of DDT. Before planting, the background concentrations of 4,4'-DDT in the experimental plots on both sites were determined. These were labelled as "T=0, bulk soil" (Figures 3.29 and 3.30). Bulk soil was defined as the soil in the planting plots before sowing the plants. To compare phytoremediation with natural attenuation, DDT concentrations in blank soil as a control were determined at t_0 and harvest. Blank soil was the unplanted soil from areas adjacent to the experimental plots. Millet, fall rye, and sugar beets were planted on both sites. At harvest, 4,4'-DDT concentrations in blank soil, near-root soil, and rhizosphere soil were analyzed separately. Near-root soil was defined as the soil from the vicinity of the plant roots in the experimental plots. Rhizosphere soil was defined as the soil that closely adhered to the root surfaces, and was obtained by gentle shaking and rubbing of the roots.

On site 1 where millet was grown, 4,4'-DDT concentrations in near-root soil and rhizosphere soil at harvest were 0.66 and 0.52 μg/g soil, respectively. The 4,4'-DDT concentrations in blank soil at t_0 and at harvest were 0.97 and 0.87 μg/g (Table 3.2), respectively. Compared to the 4,4'-DDT concentration (0.88 μg/g) in bulk soil at t_0, DDT dropped by 25% in near-root soil and 41% in rhizosphere soil (Figure 3.29A). Concentrations of 4,4'-DDT were decreased as well by 24% in near-root soil and 43% in rhizosphere soil on

Table 3.2 DDT concentrations (µg/g) in soils on site 1 during seasons 2005, 2006, and 2007

		2005 season		2006 season		2007 season	
		At t_0	At harvest	At t_0	At harvest	At t_0	At harvest
Fall rye	Blank soil		0.73±0.10/ 0.82[a]±0.05	0.84±0.09	0.73±0.05		
	Bulk soil			0.62±0.06		Not planted	
	Near-root soil		0.55±0.063/ 0.61[a]±0.047		0.49±0.05		
	Rhizosphere soil				0.43±0.026		
	% decrease (NR/RS)			**21/31**			
Millet	Blank soil		0.83±0.04	0.97±0.09	0.87±0.03	0.77±0.11	0.75±0.056
	Bulk soil			0.88±0.04		0.72±0.04	
	Near-root soil		0.6±0.056		0.66±0.04		0.5±0.07
	Rhizosphere soil				0.52±0.04		0.41±0.07
	% decrease (NR/RS)			**25/41**		**31/43**	
Sugar beet	Blank soil			1.02±0.03	1.04±0.04	0.99±0.05	1.01±0.03
	Bulk soil	Not planted		0.93±0.04		0.95±0.04	
	Near-root soil				0.83±0.042		0.88±0.02
	Rhizosphere soil				0.77±0.024		0.68±0.02
	% decrease (NR/RS)			**-/17**		**-/28**	
Pumpkin	Blank soil					1.05±0.096	1.08
	Bulk soil					0.88±0.03	
	Near-root soil	Not planted					0.75±0.03
	Rhizosphere soil						0.69±0.027
	% decrease (NR/RS)					**15/22**	

Note: The bold figures were the percentage of DDT decreased in near-root soil (NS) and rhizosphere soil (RS) compared to the DDT level in bulk soil at t_0. 0.73/0.82[a] was the 4,4'-DDT concentrations in blank soil in December 2005 / in April 2006; 0.55/0.61[a] was the 4,4'-DDT concentrations in near-root soil in December 2005 / in April 2006. "-" means DDT concentration in near-root soil did not significantly drop.
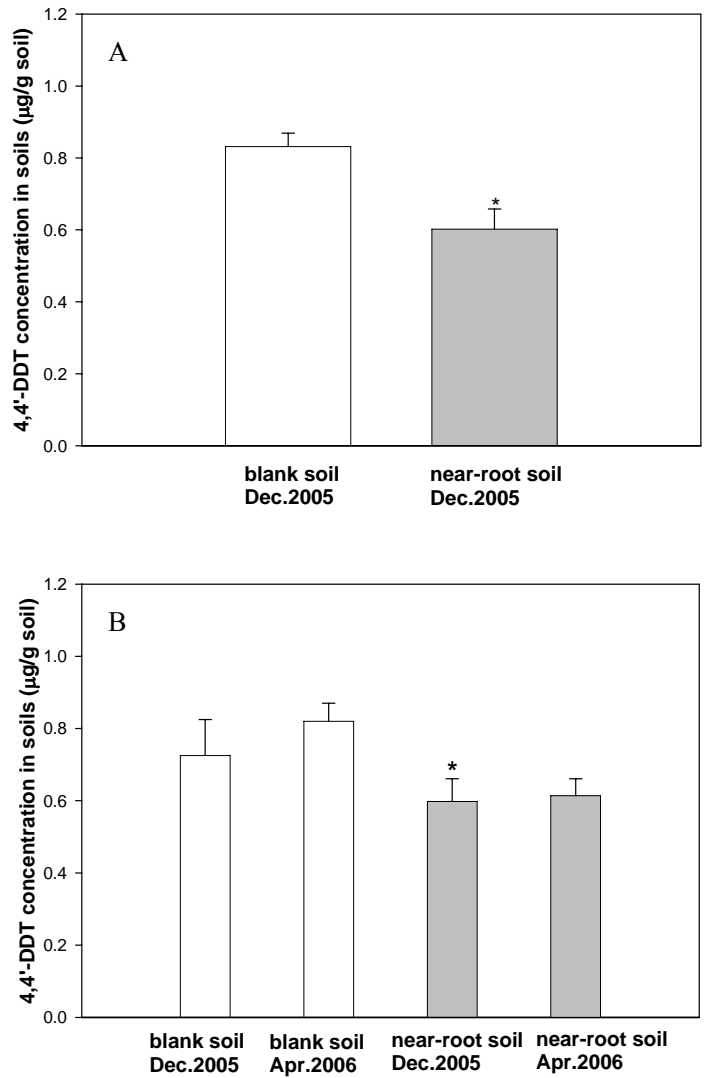
Figure 3.28 The concentrations of 4,4'-DDT in soil on site 1 in 2005 season. Soil samples collected from millet plots (A) and from fall rye plots (B). Blank soil was the unplanted soil from areas adjacent to the experiment plots, while near-root soil was the soil from the vicinity of the plant roots in the plots. Data represents mean ± S.E., n = 6. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference from the DDT level in blank soil.
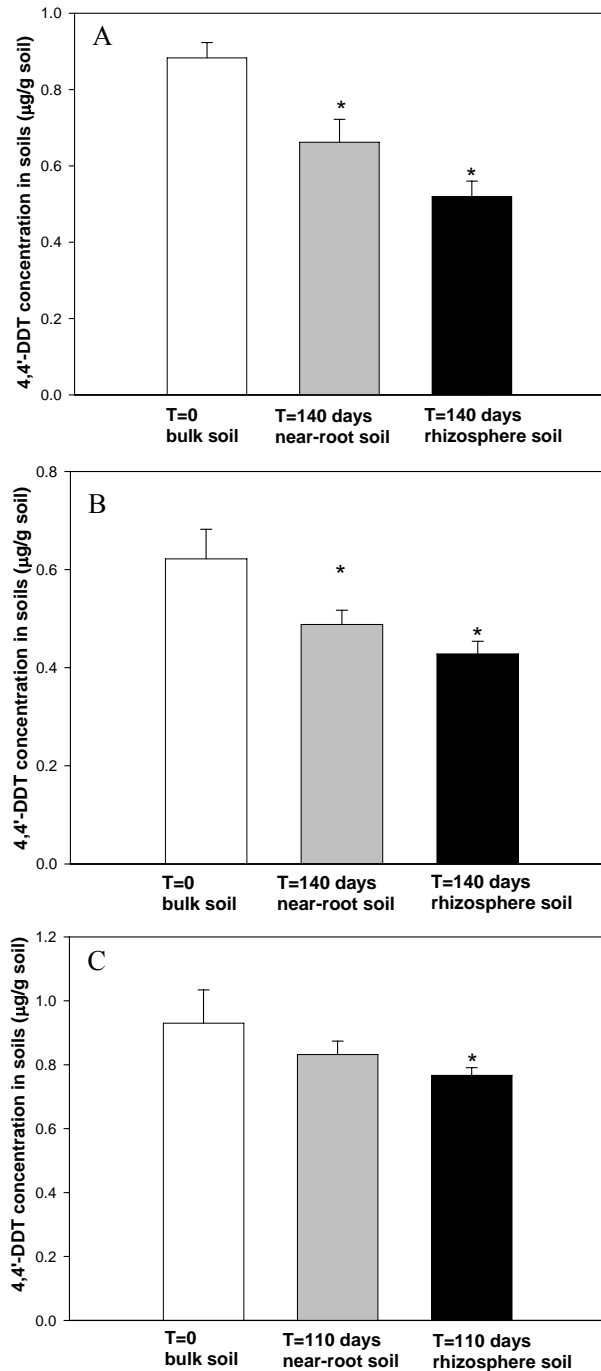
Figure 3.29 The concentrations of 4,4'-DDT in soil planted with millet (A), fall rye (B), and sugar beet (C) on site 1 in 2006 season. Bulk soil was the soil in the experiment plots before sowing, and data represents mean ± S.E., n = 8 (A), 14 (B), and 6 (C). Near-root soil was the soil from the vicinity of the plant roots in the plots, and data represents mean ± S.E., n = 16 (A and B) and 10 (C). Rhizosphere soil was the soil that closely adhered to the root surfaces, and was obtained by gentle hand shaking and rubbing of the roots, and data represents mean ± S.E., n = 16 (A and B) and 10 (C). Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference from the DDT level in bulk soil.
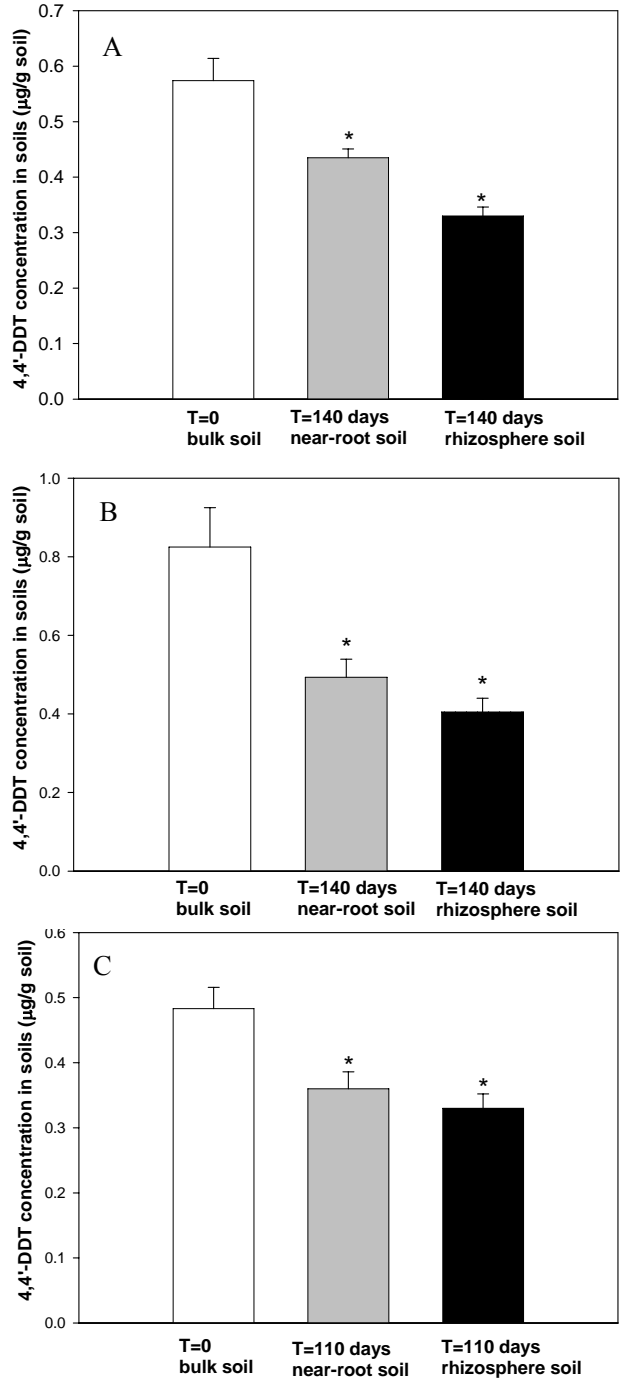
Figure 3.30 The concentrations of 4,4'-DDT in soil planted millet (A), fall rye (B), and sugar beet (C) on site 2 in 2006 season. Bulk soil was the soil in the experiment plots before sowing, and data represents mean ± S.E., n = 8 (A, B, and C). Near-root soil was the soil from the vicinity of plant roots in the plots, and data represents mean ± S.E., n =16 (A and B) and 12 (C). Rhizosphere soil was the soil that closely adhered to the root surfaces, and was obtained by gentle hand shaking and rubbing of the roots, and data represents mean ± S.E., n = 16 (A and B) and 12 (C). Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference from the DDT level in bulk soil.

on site 2 where millet was planted (Figure 3.30A).

For fall rye grown on site 1, 0.49 and 0.43 µg/g of 4,4'-DDT were determined in near-root soil and rhizosphere soil at harvest (Table 3.2). Compared to the 4,4'-DDT level in bulk soil at t_0, 4,4'-DDT concentrations dropped in the near-root soil and the rhizosphere soil by 21% and 31%, respectively (Figure 3.29B). On site 2, 4,4'-DDT concentrations in bulk soil at t_0, rhizosphere soil, and near-root soil, were 0.83, 0.49, and 0.41 µg/g, respectively (Table 3.3). Compared to the 4,4'-DDT levels in bulk soil at t_0, 4,4'-DDT decreased in near-root soil and rhizosphere soil by 41% and 51%, respectively (Figure 3.30B).

For sugar beets, 4,4'-DDT concentrations in near-root soil and rhizosphere soil on the site 1 at harvest were 0.83 and 0.77 µg/g, respectively (Table 3.2). 4,4'-DDT concentration decreased by 17% in the rhizosphere soil compared with the bulk soil at t_0, while 4,4'-DDT concentration in the near-root soil was not significantly changed (Figure 3.29C). On site 2, 4,4'-DDT concentrations were diminished by 25% and 31% in near-root soil and rhizosphere soil, respectively (Figure 3.30C).

In 2007, bulk soil samples were taken from the planting areas to determine 4,4'-DDT concentrations in soil at t_0. These were labelled as "T=0 bulk soil" (Figures 3.31 and 3.32). As well, blank soil, the unplanted soils from areas adjacent to the experimental plots, were collected to determine DDT levels in blank soil at t_0. Millet and sugar beet were planted on both sites because they are easier to harvest than fall rye. In addition, pumpkin was grown because it was reported to take up halogenated organic contaminants (White, 2002; Lunney et al., 2004; Whitfield Aslund et al., 2007). Thus, pumpkin was planted on both sites to investigate its capacity for phytoremediation of DDT. In addition, potato was planted on the site 2.

Table 3.3 DDT concentrations (μg/g) in soils on site 2 during seasons 2006, and 2007

		2006 season		2007 season	
		At t_0	At harvest	At t_0	At harvest
Fall rye	Blank soil	0.85±0.1	0.61±0.05		
	Bulk soil	0.83±0.1			
	Near-root soil		0.49±0.046	Not planted	
	Rhizosphere soil		0.41±0.035		
	% decrease (NR/RS)	**41/51**			
Millet	Blank soil	0.67±0.03	0.61±0.039	0.69±0.085	0.64±0.06
	Bulk soil	0.58±0.04		0.64±0.08	
	Near-root soil		0.44±0.016		0.59±0.06
	Rhizosphere soil		0.33±0.02		0.4±0.04
	% decrease (NR/RS)	**24/43**		**-/37.5**	
Sugar beet	Blank soil	0.54±0.042	0.48±0.028		
	Bulk soil	0.48±0.033			
	Near-root soil		0.36±0.026	Not germinated	
	Rhizosphere soil		0.33±0.022		
	% decrease (NR/RS)	**25/31**			
Pumpkin	Blank soil			0.71±0.04	0.69±0.06
	Bulk soil			0.59±0.05	
	Near-root soil	Not planted			0.57±0.04
	Rhizosphere soil				0.48±0.03
	% decrease (NR/RS)			**-/19**	
Potato	Blank soil			0.76±0.01	0.68
	Bulk soil			0.86±0.03	
	Near-root soil	Not planted			0.82±0.04
	Rhizosphere soil				0.7±0.03
	% decrease (NR/RS)			**-/19**	

Note: The bold figures were the percentage of DDT decreased in near-root soil (NS) and rhizosphere soil (RS) compared to the DDT level in bulk soil at t_0. "-" means DDT concentration in near-root soil did not significantly drop.
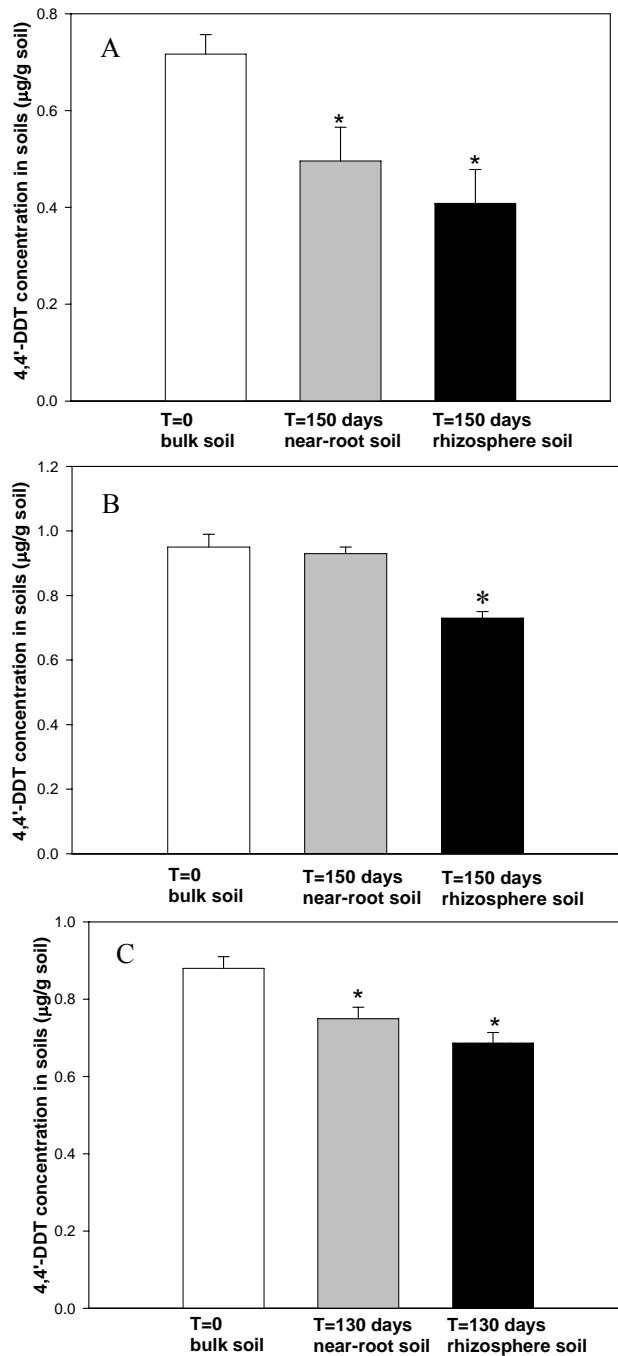
Figure 3.31 The concentrations of 4,4'-DDT in soil planted millet (A), sugar beet (B), and pumpkin (C) on site 1 from the 2007 season. Bulk soil was the soil in the experiment plots before planting. Data represents mean ± S.E., n=16 (A, B, and C). Near-root soil was the soil from the vicinity of plant roots in the plots. Data represents mean ± S.E., n=12 (A and C) and n=10 (B). Rhizosphere soil was the soil that closely adhered to the root surfaces, and was obtained by gentle hand shaking and rubbing of the roots. Data represents mean ± S.E., n=12 (A and C) and n=10 (B). Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference from the value of bulk soil.
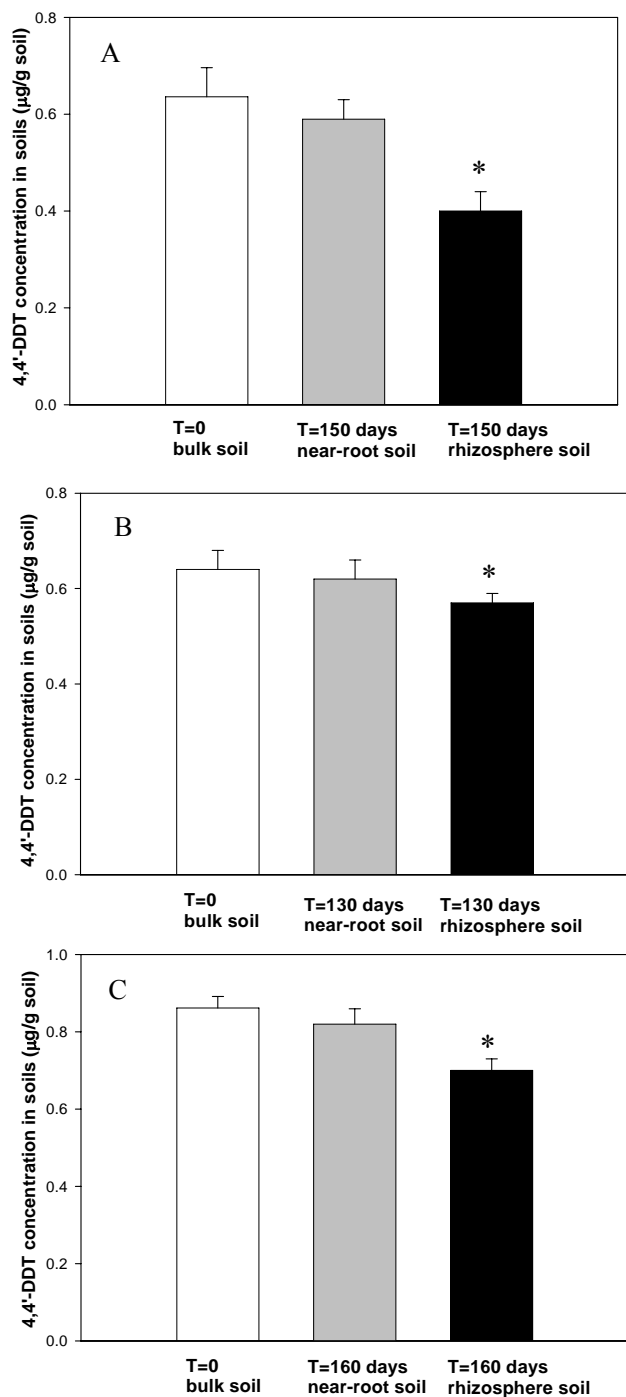
Figure 3.32 The concentrations of 4,4'-DDT in soil planted millet (A), pumpkin (B), and potato (C) on site 2 from the 2007 season. Bulk soil was the soil in the experiment plots before planting. Data represents mean ± S.E., n = 8 (A) and 12 (B and C). Near-root soil was the soil from the vicinity of the plant roots in the plots. Data represents mean ± S.E., n = 8 (A) and 12 (B and C). Rhizosphere soil was the soil that closely adhered to the root surfaces, and was obtained by gentle hand shaking and rubbing of the roots. Data represents mean ± S.E., n = 8 (A) and 12 (B and C). Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference from the value of bulk soil.

At harvest, 4,4'-DDT concentrations in blank soil, near-root soil, and rhizosphere soil were analyzed. For millet grown on site 1, 4,4'-DDT concentration in near-root soil and rhizosphere soil at harvest were 0.50 and 0.41 μg/g, respectively (Table 3.2). Compared to the DDT level in bulk soil at t_0, 4,4'-DDT concentrations in the near-root soil and rhizosphere soil dropped by 31% and 43%, respectively (Figure 3.31A). A similar trend was observed in the rhizopshere soil on site 2 where millet was grown, while the 4,4'-DDT concentration in near-root soil was not statistically lower compared to the 4,4'-DDT level in bulk soil at t_0 (Figure 3.32A).

Sugar beets did not germinate on the site 2 likely due to inhibitory effects of weed growth. On site 1, 4,4'-DDT concentrations in near-root soil and rhizosphere soil at harvest were 0.88 and 0.68 μg/g, respectively (Table 3.2). 4,4'-DDT concentration decreased in the rhizosphere soil by 28%, while 4,4'-DDT concentration in the near-root soil did not drop significantly compared to the initial level of 4,4'-DDT (Figure 3.31B).

On site 1 where pumpkin was grown, 4,4'-DDT concentrations of 0.75 and 0.69 μg/g were determined in near-root soil and rhizosphere soil at harvest. The 4,4'-DDT concentrations in blank soil were 1.05 μg/g at t_0 and 1.08 μg/g at harvest (Table 3.2). Compared to the concentration of 0.88 μg/g in the plots at t_0, 4,4'-DDT in the near-root soil and rhizosphere soil was decreased by 15% and 22%, respectively (Figure 3.31C). For pumpkins on site 2, compared to DDT level in the plots at t_0, 4,4'-DDT concentration in near-root soil did not significantly decrease, while DDT concentration in rhizosphere soil decreased by 19% (Figure 3.32B).

For potato on site 2, 4,4'-DDT concentrations were 0.86 μg/g in bulk soil at t_0, 0.7 μg/g in rhizosphere soil, and 0.82 μg/g in near-root soil (Table 3.3). Compared to the initial level in

the bulk soil at t_0, 4,4'-DDT concentration in the near-root soil was not changed and about 19% of the 4,4'-DDT in the rhizosphere soil was remediated (Figure 3.32C).

The average concentrations of DDT in bulk soil and blank soil at t_0 and in blank soil, near-root soil, and rhizosphere soil at harvest on site 1 and site 2 in 2005 through 2007 are summarized in Tables 3.2 and 3.3, respectively. The 4,4'-DDT concentration decreased in near-root soil and rhizosphere soil was compared to the 4,4'-DDT level in bulk soil at t_0. All concentrations were the average of DDT in soils expressed mean ± S.E.

To compare the DDT phytoremediation, 4,4'-DDT concentrations in the experimental plots at t_0 and the end of both of the 2006 and 2007 seasons were quantified (Figures 3.33 and 3.34). There were three experimental plots on site 1 in 2006 and 2007. On site 1, sugar beet was planted in the first plot for both 2006 and 2007. 4,4'-DDT concentrations in the plots were 0.93 µg/g at t_0 in 2006, 0.77 µg/g at the end of 2006 season, and 0.68 µg/g at the end of 2007 season (Figure 3.33A). In the second plot on site 1, millet was planted in 2006 and pumpkin was planted in 2007. 4,4'-DDT concentrations at t_0 both 2006 and 2007 were 0.88 µg/g. 4,4'-DDT levels were 0.52 µg/g at the end of 2006 season and 0.69 µg/g at the end of 2007 season (Figure 3.33B), which means DDT concentration dropped by 22% after two seasons of phytoremediation. In the third plot, fall rye was planted in 2006 and millet was planted in 2007. 4,4'-DDT concentrations at t_0 were 0.62 µg/g in 2006 and 0.66 µg/g in 2007. At the end of season, 4,4'-DDT concentrations were 0.43 µg/g in 2006 and 0.52 µg/g in 2007 (Figure 3.33C).

As well, there were three experimental plots on site 2 in 2006 and 2007. Sugar beet was planted in the first plot both for 2006 and 2007 seasons, however, no growth was observed likely due to excessive weed growth. In the second plot on site 2, millet was planted in 2006
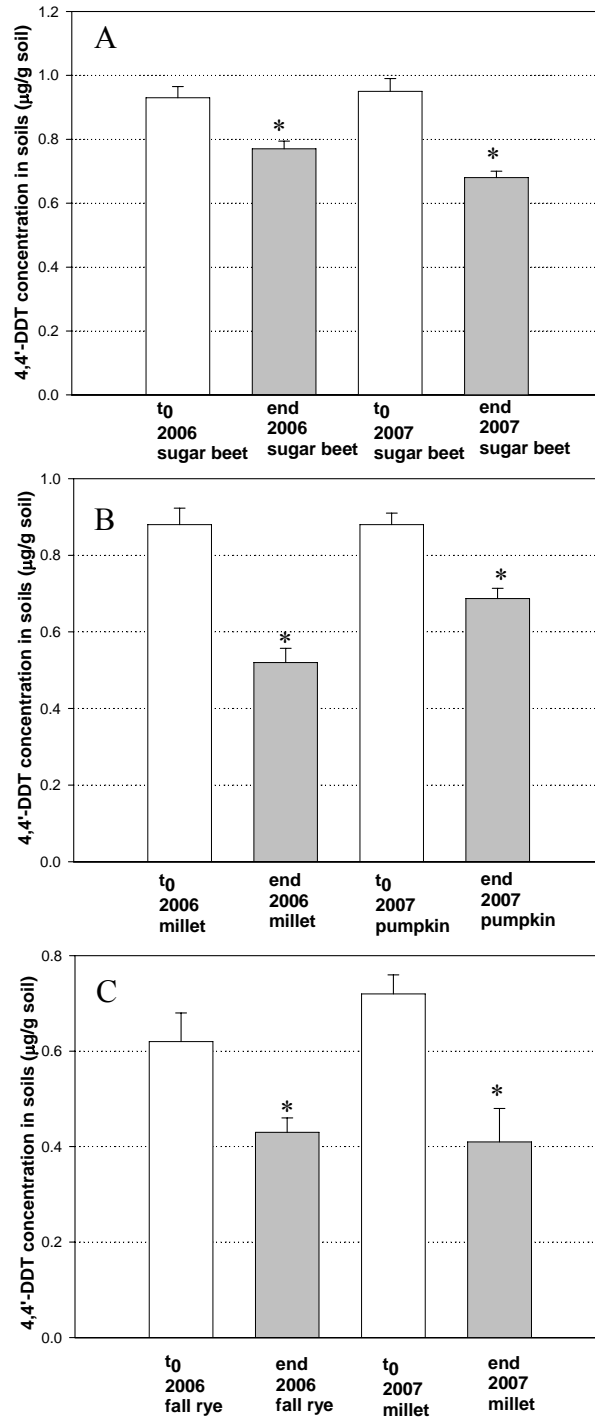
Figure 3.33 The concentrations of 4,4'-DDT in three experiment plots on site 1 at t_0 and the end of season in 2006 and 2007. Sugar beet was planted in the first plot both in 2006 and 2007 (A). In the second plot, millet was planted in 2006 and pumpkin was planted in 2007 (B). In the third plot, fall rye was planted in 2006 and millet was planted in 2007 (C). Data represents mean ± S.E., n=12. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference from the DDT levels at t_0.

and pumpkin was planted in 2007. 4,4'-DDT concentrations at t_0 in 2006 and 2007 was

around 0.58 μg/g, while 4,4'-DDT concentrations dropped to 0.33 μg/g at the end of 2006

and 0.48 μg/g at the end of 2007 (Figure 3.34A). There was a 17% of DDT remediation

observed in this plot at the end of 2007. In the third plot, fall rye was planted in 2006, while

millet was planted in 2007. 4,4'-DDT concentration at t_0 was 0.64 μg/g, which decreased to

0.4 μg/g at the end of 2007 (Figure 3.34B).

Since the total average concentrations of 4,4'-DDT in bulk soil at t_0 and in rhizosphere

soil at the end of seasons were significantly different, the comparison of 4,4'-DDT

concentrations in bulk soil at t_0 and in rhizosphere soil at harvest between 2006 season and

2007 season was performed. On site 1, the total average concentrations of 4,4'-DDT in bulk

soil at t_0 were 0.81 μg/g in 2006 and 0.85 μg/g in 2007 (Figure 3.35A). At the end of seasons,

the total average concentrations of 4,4'-DDT in rhizosphere soil were 0.57 μg/g in 2006 and

0.59 μg/g in 2007. Compared to the initial level of 4,4'-DDT in 2006, 4,4'-DDT

concentration in rhizosphere soil dropped by 30% at the end of season 2006. While compared

the initial levels of 4,4'-DDT between 2006 season and 2007 season, 4,4'-DDT

concentrations were not changed. Because plant tissues were not removed from the soil at

harvest in 2006, it is possible that 4,4'-DDT accumulated in plant tissues could be back to

soil, which then resulted in the increase of 4,4'-DDT in bulk soil at t_0 in 2007. This trend was

also observed on site 2 (Figure 3.35B). Therefore, it is necessary to remove all plant parts

from soil at harvest.

3.5 PGPR effect on DDT phytoremediation

Since each plant species was divided into two sections: untreated seeds and seeds treated

with PGPR, the following results for the DDT remediation are based on each plant species
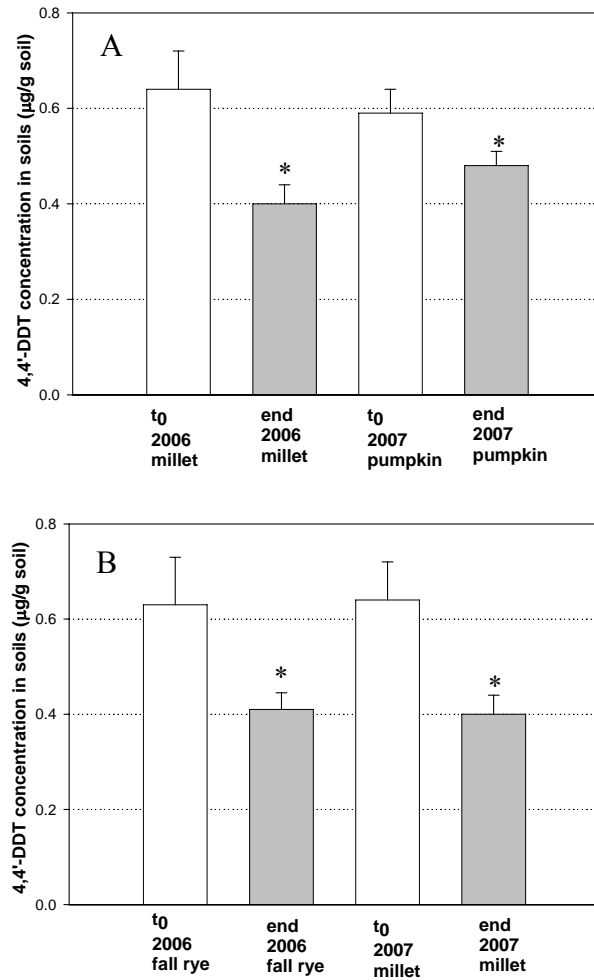
Figure 3.34 The concentrations of 4,4'-DDT in two of three experiment plots on site 2 at t_0 and the end of season in 2006 and 2007. In the second plot, millet was planted in 2006 and pumpkin was planted in 2007 (A). In the third plot, fall rye was planted in 2006 and millet was planted in 2007 (B). Data represents mean ± S.E., n=12. Data was analyzed using one-way ANOVA with Dunnett post-hoc test ($P<0.05$). * indicates the significant difference from the DDT levels at t_0.
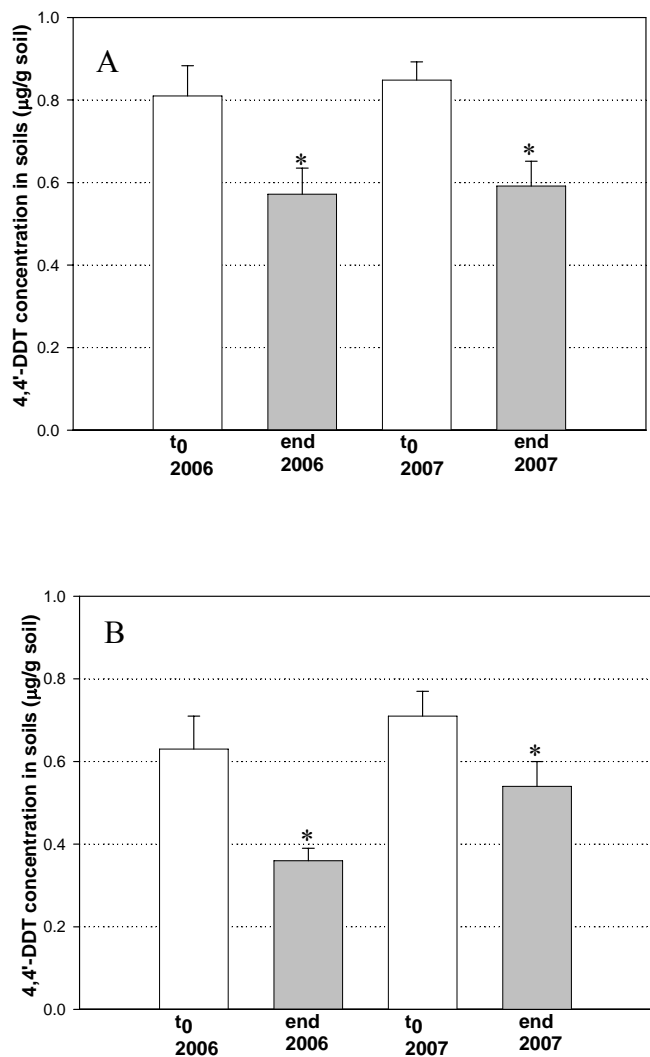
Figure 3.35 The concentrations of 4,4'-DDT on site 1 (A) and site 2 (B) at t_0 and the end of season in 2006 and 2007. Comparison of the average concentrations of 4,4'-DDT in bulk soil at t_0 and in rhizosphere soil at the end of season both in 2006 and 2007. Data represents mean ± S.E., n=36. Data was analyzed using one-way ANOVA with Dunnett post-hoc test (P<0.05). * indicates the significant difference from the DDT levels at t_0.

treated with and without PGPR. Tables 3.4 and 3.5 list the average concentrations of DDT in blank soil and bulk soil at t_0, blank soil at harvest, and near-root soil and rhizosphere soil at harvest with different plant species with and without PGPR treatment. This was done for the 2006 and 2007 seasons. In addition, the percentage of DDT decreases in near-root soil and rhizosphere soil compared to the bulk soil at t_0 are listed in the tables.

In the 2006 season, for fall rye treated with PGPR on site 1, the 4,4'-DDT concentrations in near-root soil was not statistically different, but dropped by 20% in rhizosphere soil. 4,4'-DDT concentrations in near-root soil and rhizosphere soil grown untreated fall rye decreased by 28% and 36%, respectively, compared to bulk soil at t_0. The same trend was observed for fall rye on site 2.

However, this trend was not the same for millet grown on both sites in 2006. On site 1, 4,4'-DDT concentrations both in near-root soil and rhizosphere soil grown of millet treated with PGPR dropped by 30% and 37.5% compared to bulk soil at t_0. The 4,4'-DDT levels decreased in near-root soil and rhizosphere soil of untreated plants by 28% and 43%, respectively. However, the converse trend was observed on site 2. On site 2, DDT concentrations in near-root soil and rhizosphere soil grown millet treated with PGPR dropped by 25% and 41%, both of which were greater than for the untreated plants. A similar trend was observed with millet at both sites in 2007 season (Tables 3.4 and 3.5). These results indicated that PGPR effects on growth can not be directly related to the percentage of DDT decreased from soils. However, more biomass achieves higher phytoremediation. PGPR can enhance the phytoremediation of DDT by promoting plant growth.

Table 3.4 DDT concentrations (µg/g) in soils grown plant species treated with PGPR and untreated on site 1 in seasons 2006 and 2007

		2006 season		2007 season	
		+PGRP	-PGPR	+PGRP	-PGPR
Fall rye	Blank soil (T_0/Tn)	0.84±0.09/ 0.73±0.11	0.84±0.09/ 0.73±0.11		
	Bulk soil	0.50±0.092	0.72±0.068		
	Near-root soil	0.46±0.051	0.52±0.025	Not planted	
	Rhizosphere soil	0.40±0.044	0.46±0.025		
	% decrease (NR/RS)	**-/20**	**28/36**		
Millet	Blank soil (T_0/Tn)	0.97±0.06/ 0.87±0.033	0.97±0.06/ 0.87±0.033	0.77±0.105/ 0.75±0.04	0.77±0.105/ 0.75±0.04
	Bulk soil	0.80±0.046	0.96±0.047	0.76±0.059	0.68±0.07
	Near-root soil	0.56±0.081	0.69±0.073	0.49±0.097	0.52±0.12
	Rhizosphere soil	0.50±0.047	0.55±0.066	0.43±0.10	0.39±0.116
	% decrease (NR/RS)	**30 /37.5**	**28/43**	**36/44**	**23.5/43**
Sugar beet	Blank soil (T_0/Tn)	1.02±0.026/ 1.04±0.037	1.02±0.026/ 1.04±0.037	0.99±0.096/ 1.01±0.096	0.99±0.096/ 1.01±0.096
	Bulk soil	0.93±0.035	0.950	0.94±0.053	0.954±0.052
	Near-root soil	0.88±0.04	0.76±0.076	0.89±0.029	0.91±0.035
	Rhizosphere soil	0.792±0.033	0.73±0.029	0.74±0.03	0.62±0.018
	% decrease (NR/RS)	**-/15.1**	**20/23.2**	**-/21.3**	**-/35**
Pumpkin	Blank soil (T_0/Tn)			1.05±0.096/ 1.08±0.096	1.05±0.096/ 1.08±0.096
	Bulk soil			0.90±0.042	0.86±0.036
	Near-root soil	Not planted		0.72±0.056	0.78±0.022
	Rhizosphere soil			0.70±0.042	0.67±0.038
	% decrease (NR/RS)			**20/21.7**	**9.3/22.1**

Note: The data of DDT average concentrations represents mean ± S.E.. The bold figures were the percentage of DDT decreased in near-root soil (NR) and rhizosphere soil (RS) compared to the DDT level in bulk soil at t_0. T_0 and Tn mean the soil samples collected before planting and at harvest, respectively. "-" means DDT concentration in near-root soil did not significantly drop.

Table 3.5 DDT concentrations (µg/g) in soils grown plant species treated with PGPR and untreated on site 2 in seasons 2006 and 2007

		2006 season		2007 season	
		+PGRP	-PGPR	+PGRP	-PGPR
Fall rye	Blank soil (T_0/Tn)	0.85±0.1/ 0.61±0.055	0.85±0.1/ 0.61±0.055	Not planted	
	Bulk soil	0.65±0.075	1.0±0.14		
	Near-root soil	0.37±0.042	0.62±0.025		
	Rhizosphere soil	0.33±0.051	0.48±0.022		
	% decrease (NR/RS)	**43/49**	**38/52**		
Millet	Blank soil (T_0/Tn)	0.67±0.03/ 0.61±0.055	0.67±0.03/ 0.61±0.055	0.72/ 0.73	0.72/ 0.73
	Bulk soil	0.63±0.034	0.51±0.067	0.80±0.054	0.53±0.075
	Near-root soil	0.47±0.025	0.40±0.01	0.65±0.033	0.48±0.07
	Rhizosphere soil	0.37±0.022	0.30±0.01	0.51±0.03	0.34±0.05
	% decrease (NR/RS)	**25/41**	**22/40**	**19/36**	**-/36**
Sugar beet	Blank soil (T_0/Tn)	0.54±0.026/ 0.48±0.02	0.54±0.026/ 0.48±0.02	Not germinated	
	Bulk soil	0.5±0.051	0.47±0.046		
	Near-root soil	0.37±0.034	0.35±0.044		
	Rhizosphere soil	0.35±0.026	0.31±0.036		
	% decrease (NR/RS)	**26/30**	**26/34**		
Pumpkin	Blank soil (T_0/Tn)	Not planted		0.71±0.04/ 0.69	0.71±0.04/ 0.69
	Bulk soil			0.61±0.096	0.57±0.035
	Near-root soil			0.62±0.07	0.53±0.03
	Rhizosphere soil			0.48±0.06	0.49±0.03
	% decrease (NR/RS)			**-/21**	**-/14**
Potato	Blank soil T_0/Tn)	Not planted		0.76/0.68	0.76/0.68
	Bulk soil			0.87±0.021	0.86±0.049
	Near-root soil			0.76±0.056	0.87±0.034
	Rhizosphere soil			0.64±0.032	0.76±0.029
	% decrease (NR/RS)			**13/26**	**-/12**

Note: The data of DDT average concentrations represents mean ± S.E.. The bold figures were the percentage of DDT decreased in near-root soil (NR) and rhizosphere soil (RS) compared to the DDT level in bulk soil at t_0. T_0 and Tn mean the soil samples collected before planting and at harvest, respectively. "-" means DDT concentration in near-root soil did not significantly drop.

3.6 Mass balance of DDT following phytoremediation

Because DDT observed to be removed from the soil and DDT plus DDE were found in the plant tissues, it is possible to perform a mass balance for remediation. If the amount taken up by plants is similar to the amount lost from soil, this would indicate phytoremediation of DDT is limited to phytoextraction. It is necessary to accurately determine the absolute amount of DDT and DDE in different plant tissues to assess the uptake and translocation of DDT from soil. 4,4'-DDT and 4,4'-DDE concentrations in plant tissues were shown in Table 3.1. The data related to pumpkin tissue was the most complete data set. Thus, pumpkin was taken as the model system to determine the mass balance of DDT and DDE between plant and soil following plant growth.

In this study, the total soil volume impacted by the plants in the field was hard to assess. However, on the site with pumpkins, a small plot with 50x50 cm^2 was isolated and all pumpkin plant samples in this plot were collected at harvest. The average length of the taproots was 12 cm. However, the roots do not reach all the soil in this isolated plot. Specifically, the shape of the soil around the roots system is like a pyramidal shape with a volume of one third the area of the base times the height. Therefore, based on the real amount of soil around the root systems in this plot, the approximate dimension of $\frac{1}{3}$x10x10x12 cm^3 (400 cm^3) was estimated to contain the roots system of pumpkin samples in this plot. The density of the soil was determined to be 1.07 g dry soil/cm^3. Therefore, a mass of 428 g dry soil corresponds to the volume displaced by the root system of pumpkin. The biomass data of roots and stems of all pumpkins collected from the 50x50 cm^2 plot at harvest is shown in Table 3.6. The average concentrations of 4,4'-DDT and 4,4'-DDE in roots and

Table 3.6 Biomass of pumpkin tissues, volume of soil around the root system, and concentrations of DDT and DDE in pumpkin tissues and soils collected from the small plot

	At harvest			At t_0
	Roots	Stem 1	Stem 2	
Dry weight of biomass (g)	4.8	4.49	126.11	
4,4'-DDT extracted (µg/g)	0.67	1.06	0.2	
4,4'-DDE extracted (µg/g)	1.64	2.05	0.32	
Concentrations (µg/g) of 4,4'-DDT/4,4'-DDE in near-root soil		0.75/0.32		
Concentrations (µg/g) of 4,4'-DDT/4,4'-DDE in rhizosphere soil		0.70/0.28		
Concentration (µg/g) of 4,4'-DDT/4,4'-DDE in bulk soil at t_0				0.88/0.38
Soil density (g/cm^3)		1.07		
Volume of soil involved in the root system (cm^3)		400		
Dry weight of the soil (g)		428		

stems of pumpkin and soils in this plot are also listed in the table. The amount of DDT and/or

DDE in fruits of pumpkin at harvest was small enough to be ignored. Therefore, the

calculation of total plant dry weight will be based on only the roots and stems of pumpkin.

Note, the adventitious roots of pumpkin extending from the large tap roots were hard to

collect completely at sampling. Thus, the biomass data of pumpkin roots listed in Table 3.6 is

very conservative.

It is assumed that the disappearance of DDT and DDE in soil was caused by the uptake of

plants. Thus, mass balance means that the decrease of DDT and DDE amount in soil equals

to the increase of DDT and DDE amount in plant tissues. Mass balance is expressed as the

following equation:

$$\Sigma(DDT+DDE)_{\text{decreased in soil}} = \Sigma(DDT+DDE)_{\text{accumulated in plant tissues}}$$

Based on the data in the Table 3.6, total amount of DDT and DDE remediated from the soil

was:

$$[(0.88-0.70) + (0.38-0.28)] \, \mu g/g * 428 \, g = 119.8 \, \mu g$$

While the total amount of DDT and DDE accumulated in pumpkin tissues was:

$(0.67+1.64) \, \mu g/g * 4.8 \, g+ (1.06+2.05) \, \mu g/g * 4.49g + (0.32+0.2) \, \mu g/g *126.11g =93.3 \, \mu g$.

Compared the above results, the total amount of DDT and DDE taken by pumpkin

roughly equals to the amount of DDT and DDE disappeared from the soil. Thus, it appears

that DDT phytoremediation is logically limited to phytoextraction. Nonetheless, slightly

more DDT and DDE was removed from the soil than was accumulated in the plants. This

difference could have been metabolism in the plants perhaps explaining the unidentified

DDT-like compounds found in plants at the end of the growth seasons.

4　Discussion

The results showed that the combination of UW3 and UW4 promoted the growth of millet, fall rye, and sugar beet in both greenhouse and field trials. As well, the growth of pumpkin and potato in the field was enhanced by the combination of UW3 and UW4. The field data indicate that 4,4'-DDT concentrations in the rhizosphere soil were significantly less than 4,4'-DDT level in bulk soil at t_0 taken from the same experimental plots. Meanwhile, DDT and/or DDE were detected in plant tissue extracts from fall rye, millet, sugar beet, and pumpkin. Based on the data for DDT determined in soils and plant tissues, a mass balance was elicited to elucidate the possible mechanism(s) of DDT phytoremediation. This research raises interesting questions and conclusions on PGPR effects on plant growth, DDT remediation, phytoextraction of chlorinated aromatics, and possible mechanism(s) of DDT phytoremediation.

4.1 PGPR effect on plant growth

To be successful in phytoremediation, it is important first to test if the selected plants can grow in contaminated soils. As shown in Figures 3.1 to 3.3, PGPR treated and untreated millet, fall rye, and sugar beet, germinated and grew well in the greenhouse on soil collected from DDT contaminated sites. The growth of the plants treated with the combination of UW3 and UW4 was improved relative to the untreated plants. The healthy growth of plants was further confirmed in field trials (Figures 3.4 to 3.6). The field data show that plants treated with PGPR grew much better compared to the untreated plants. On site 1, the root length and root weight of fall rye treated with PGPR were 16% and 44%

greater, respectively, compared to the untreated plants (Figure 3.8). The root and shoot dry weights of millet treated with PGPR were 38% and 47% greater than those untreated plants (Figure 3.10). A significant effect of growth promotion was observed for sugar beet treated with PGPR (Figure 3.12).

A model describing the role of PGPR in promoting plant root growth was proposed by Glick et al. (1998). The PGPR, UW3 and UW4 used in this study, were shown to contain ACC deaminase activity. This enzyme can degrade ACC to ammonia and α-ketobutyrate. ACC is a precursor of ethylene in plant tissues. Ethylene is important for seed germination during the early phase of plant growth, but high levels of ethylene inhibit root elongation. Under stressed conditions, more ethylene is produced by plants. Therefore, PGPR containing ACC deaminase can promote plant growth by lowering ethylene levels (Glick 1995; 2005). In addition, most root-promoting bacteria synthesize indole-3-acetic acid (IAA), an auxin which stimulates plant growth (Patten and Glick, 2002).

PGPR promotional effects on plant growth are more evident under stressed conditions. The field data from this work supports this model. For instance, on site 1, root and shoot dry weights of millet treated with PGPR were 38% and 47% greater, respectively, compared to untreated plants. While on site 2, the root and shoot dry weights of millet treated with PGPR were 46% and 101% greater than untreated plants (Figure 3.10). Thus, the response of millet to PGPR on site 2 was greater than on site 1. It was possibly caused by the different environmental conditions between site 1 and site 2. Site 1 is located in a Ginseng growth area surrounded by tall trees, which provides warm and moist conditions for Ginseng growth. The plant species grown on site 1 benefited from such good conditions. Site 2 is an open field without any shield. Moisture in the soil therefore evaporates faster, which makes the

soil drier and creates a drought stress on plants. Interestingly, the biomass of millet without PGPR treatment was 111.20 g/m^2 of roots and 562.53 g/m^2 of shoots on site 1 (Figure 3.10). While on site 2, the dry weights of roots and shoots of untreated millet were 64.6 and 214.57 g/m^2, respectively (Figure 3.10). These were 42% and 62% less than those untreated millet on site 1. This indicates there indeed was more stress on site 2 and PGPR treatment alleviated the stress on plants, resulting in a greater PGPR response.

PGPR effects on roots versus shoots of the plants varied and were plant species dependent. Pumpkin shoots responded more to PGPR than roots as indicated by increased biomass by 66% in shoots and 30% in roots (Figure 3.13), whereas sugar beet roots had a greater response to PGPR than their leaves. Biomass of beet tubers increased by 75% and leaves increased by 60% after PGPR treatment (Figure 3.12). The fact that pumpkin shoots and sugar beet roots accumulated DDT will ease of harvest, and positive responses to PGPR, make their plants excellent choices for DDT phytoremediation as well as for other chlorinated aromatic compounds.

4.2 DDT concentration decreases in soils

The determination of DDT concentrations in soil demonstrated that the concentration of 4'4-DDT in the rhizosphere soil at harvest was significantly less than the initial level of DDT. The 4,4'-DDT concentrations in the rhizosphere soil collected from millet roots on site 1 dropped by 41% in season 2006 and 43% in season 2007, respectively (Table 3.2). On both sites where fall rye was grown in the season 2006, 4,4'-DDT concentrations in the rhizosphere soil decreased by 31% on site 1 and 51% on site 2 (Table 3.2). 4,4'-DDT concentrations in the rhizosphere soil from sugar beet and pumpkin were significantly lowered as well.

The decrease of DDT in rhizosphere soil could be due to rhizodegradation. Rhizodegradation, also called rhizosphere biodegradation, is the result of a plant root system releasing chemicals that enhance the biodegradation of organic contaminants by soil microorganisms in the rhizosphere (Germida et al., 2002). For instance, the concentration of total petroleum hydrocarbons (TPH) dropped significantly in rhizosphere soil (Greenberg et al., 2007). On a TPH contaminated site, more than 60% remediation of TPH was realized after a three-year period of treatment with ryegrass and tall fescue. The possible mechanism is TPH degradation in the rhizosphere. Generally, natural attenuation of TPH is unacceptably slow for a TPH remediation process. Plants treated with PGPR promote the degradation of TPH by enhancing the rhizosphere in contaminated soils. The rhizosphere supports complex microbial communities via root exudates (Bais et al., 2006), These microbes along with excreted enzymes from roots may directly degrade some soil contaminants such as diesel (Kim et al., 2006). Thus, the rhizodegradation seems to result in significant decrease of TPH in soil (Greenberg et al., 2007).

However, it is not likely that the DDT decrease in the rhizosphere soil is due directly to microbial activity or degradation by enzymes. DDT persists in the soil for at least 30 years. If microbial activity or enzymatic degradation in the rhizosphere had contributed to the loss of DDT from soil, DDT would not persist for so long in farm soils.

The decrease of DDT in rhizosphere soil is possibly due to the direct uptake and accumulation of DDT by plants. Weathered DDT is not readily mobilized through the soil to the plant tissues because of its lipophilicity. However, the detection of DDT in plant tissues suggests that the DDT mobility from the soil particles to plant tissues does occur. The reason is possibly that DDT mobility could be improved due to plant effects. This plant effect, one

of the rhizosphere effects, results from exudates released from the roots (Walker et al., 2003).

Root exudates include both low molecular compounds and high molecular compounds. Low

molecular weight compounds, such as amino acids, organic acids, and various other

secondary metabolites, account for much of the diversity of root exudates. Proteins and

mucilage (polysaccharides) belong to high molecular weight compounds and compose a

large proportion of the root exudates by mass, but they are less diverse (Walker et al., 2003).

Based on the studies, root exudates with low molecular weight play an important role in

interactions between plants and soil microbes (Bais et al., 2006; Rovira, 1965; Walker et al.,

2003).

A large range of root exudates are secreted into the soil by roots, which inevitably results

in changes of soil biochemical and physical properties. This can enhance biological activities

as well as the mobility of organic pollutants in the soil. White et al. (2003) investigated seven

organic acids on desorption of weathered DDE from soil. DDE contaminated soil where

zucchini seedlings were grown after 28 days were periodically amended with organic acids

in the irrigation water. The control consisted of DDE contaminated soil planted with the

same age of zucchini, but water with nothing in the irrigation. The results showed plants

amended with organic acids removed much more DDE from soil than the vegetation watered

with only water. The authors believed that low molecular weight organic acids caused a

partial dissolution of the soil structure through the chelation of inorganic ions, potentially

enhancing bioavailability of persistent organic pollutants in soil.

Because DDT bioavailability can been improved with plant growth, phytoextraction may

be a key mechanism for DDT remediation in rhizosphere soil. After a series of field

experiments, White (2000; 2001) found that DDE levels significantly declined in the

rhizosphere soil planted with alfalfa, ryegrass, zucchini, and pumpkin. In addition, DDT or DDE were found in the tissues of pumpkin and zucchini (White, 2001; Lunney et al., 2004). The data indicated that the decline of DDT or DDE concentrations in rhizosphere soil was related to plant uptake of DDT or DDE into their tissues. The same trend was observed in this study. DDT concentrations in rhizosphere soil significantly dropped, and DDT was observed in plant tissues of fall rye, millet, sugar beet, and pumpkin. Therefore, phytoextraction occurred and this was related by mass balance to the decrease of DDT in soil.

4.3 DDT uptake by plants

It is important to confirm if DDT can be accumulated in plant tissues following plant growth. The fact that DDT was found in plant tissues proves that plants can take up DDT from soil. The concentrations of 4,4'-DDT and 4,4'-DDE observed in fall rye roots were 0.61 and 0.59 μg/g, respectively (Table 3.1). Even higher concentration of 4,4'-DDT and 4,4'-DDE were found in roots and stems of pumpkin at harvest (Table 3.1). The data on DDT detected in plant tissues suggest that phytoextraction is a key mechanism for DDT remediation in soil.

The ability to take up DDT is plant dependent. The concentrations of 4,4'-DDT and 4,4'-DDE found in pumpkin tissues were 0.67 and 1.64 μg/g in roots, respectively, and 1.06 and 2.05 μg/g in the lower stems, respectively (Table 3.1). DDT and/or DDE concentrations in plant tissues of fall rye, millet, and sugar beet were lower than in pumpkin tissues. One of the possible reasons why different plants have different abilities to take up DDT may be the quantity and composition of root exudates produced by different roots system. This could cause differences in DDT bioavailability. Several aromatic acids in the exudates of cucumber

were detected by Pramanik et al. (2000). Whereas several di- and tricarboxylic acids, including malate, succinate, and citrate, were detected in the vascular exudates of two *Curcurbita* species (Richardson et al., 1982), the same genus as pumpkin. The differences in organic acids in exudates among these plant species could play an important role in phytoremediation of DDT from soil.

The uptake of DDT by pumpkin and zucchini was reported by Lunney et al. (2004). The authors compared the ability of five plant species (pumpkin, zucchini, alfalfa, tall fescue, and ryegrass) to extract DDT from soil. They found the highest concentrations of DDT accumulated in pumpkin. The concentration of DDT was much higher in pumpkin stems than in roots, which was consistent with the finding of this study. The authors contended that the successful uptake is due to the large biomass and high transpiration volume of these species. In addition, White (2002) found that the concentration of DDE accumulated in pumpkin stems was around 63 times more than in fruits. In this study, DDT concentration was not detectable in pumpkin fruits. The possible reason is that the lipophilicity of pumpkin stems is not helpful to transport DDT to fruits.

DDT and DDE were found in fall rye roots in this study (Figure 3.17). This agrees with the investigation by Waliszewski et al (2004). Fall rye produces a fine and fibrous root system, which can absorb and accumulate weathered DDT from soil. In addition, millet, a common rotation crop plant in Canada, was selected for remediation in this study. DDT and DDE were indeed detected in shoots and roots of millet (Figure 3.15). Although there are no previous reports on millet taking up organochlorine pesticides, it might be due to the character of millet shoot which is like a tube to absorb much more nutrient and water from the soil. DDT with other nutrient was taken up to millet shoots. Finally, sugar beet is a root

crop and easily to harvest. In this study, sugar beet was expected to be able to accumulate DDT in beets from soil. The DDT was detected in beets extracts (Figure 3.19).

Although DDT can be accumulated in plant roots, the distribution of DDT accumulation in plant tissues was species-specific. More DDT and DDE were detected in shoots than in roots of millet, while DDT and DDE were only found in fall rye roots. DDT and DDE were mostly observed in stems and roots of pumpkin. DDT was not detectable in sugar beet leaves, but was found in the beets.

Pumpkin has large biomass and high volume of the fruits, which requires nutrient, particularly water, from soil for the large leaves or fruits. Due to the rhizosphere effects, DDT bioavailability may have been enhanced by pumpkins. Thus, DDT was able to be transported to roots and then up to the shoots. This hypothesis agreed with the investigation reported by White (2002). That author found pumpkin stems were the major location for DDE localization.

Conversely, DDT was mainly accumulated in roots of fall rye. Fall rye produces a strong hairy-root system that allows DDT be accumulated in roots. Although hairy-roots are good at taking up DDT from soil, it is hard to remove all roots from soil at harvest, which is a limitation for fall rye used in phytoremediation. This is especially true since there is little DDT transport to the shoots. As expected, DDT was only found in roots of sugar beet. Moreover, the roots of sugar beet are easier to harvest, which makes DDT phytoremediation more practical.

It was very interesting to find that DDT accumulated in millet shoots 70-day after sowing had decreased to a small amount at harvest, and DDE accumulated in millet shoots 70-day after sowing had apparently disappeared at harvest (Table 3.1). It is possible that DDT could

have been metabolized. Interestingly, chromatograms of millet shoot extracts at harvest showed a peak at retention time of 14.04 min with an absorbance spectrum peak at 235 nm (Figure 3.18), which is similar to DDT. In addition, the shape of absorbance spectrum was similar to that of DDT standard. However, the wrong retention time and not quite right spectrum indicate that it is not DDT. Thus, the compound corresponding to this peak might be a metabolite of DDT. It could be a phenolic metabolite of DDT because its shorter retention time than DDT indicates a more polar compound and as well, the changes to the absorbance spectrum are consistent with hydroxylation of an aromatic ring. Additional characterization of the compound using MS spectra analysis should be performed in future studies.

The degradation of DDT by plant enzymes has been reported. In the laboratory, Suresh et al (2005) has reported that hairy root cultures of *Cichorium intybus* and *Brassica juncea* can degrade DDT. The degradation products were DDE and other unidentified compounds. Chu et al (2006) investigated that root extracts of *P. australis* readily transformed DDT to DDD and DDE. They suggested that peroxidases and the plant P450 system were involved in the metabolism of DDT by plants. This is again consistent with the finding here that late in the growth season the above DDT-like compound was observed.

4.4 Mass balance

To elucidate possible mechanism(s) of DDT remediation from soil, especially in rhizosphere soil, and accumulation in plant tissues, it was important to perform a mass balance. Mass balance describes the relationship between 4,4'-DDT and/or 4,4'-DDE disappearance from the soil and 4,4'-DDT and/or 4,4'-DDE accumulation in plant tissues.

According to the calculation based on the model system of pumpkin, total amount of

4,4'-DDT and/or 4,4'-DDE decreased in the rhizosphere soil from the sampling plot was approximately 120 μg, while the total amount of 4,4'-DDT and/or 4,4'-DDE accumulated in pumpkin tissues collected from this same sampling plot was 93.3 μg. Thus, the amount of DDT and DDE disappearing from soil was approximately equals to the amount of DDT and DDE accumulated in plant tissues.

It is important to note that the assessment of mass balance based on the field data is very rough. The volume of soil affected by plant is hard to calculate, which could result in an overestimation of total amount of DDT and DDE that was remediated from soil. In addition, not all pumpkin roots could be collected in the field, which leads to the total amount of DDT and DDE accumulated in roots less than the real amount of DDT and DDE in the roots. Although DDT and/or DDE were not detectable in leaves and fruits of pumpkin due to the detection limit, the real amount of DDT should not be neglected due to large biomass of the fruit. And also, the metabolism of DDT inside tissues could possibly occur. However, the preliminary mass balance indicates that phytoextraction is the primary mechanism to explain the DDT decrease in soil in this study.

4.5 PGPR assisted phytoremediation of chlorinated aromatics

In this study, although the accumulation of DDT in plant tissues is not directly related to PGPR treatment, the fact that PGPR promoted plant growth and the total amount of DDT and DDE decreased from soil equals to the total amount of DDT and DDE accumulated in plants, strongly suggest that PGPR can improve DDT remediation by increasing plant biomass. As an analog to DDT, the mechanism of PCBs phytoremediation has been studied recently. PCBs are a class of organic chemicals with 1 to 10 chlorine atoms attached to biphenyl. Due to the similar structures, PCBs and DDT share many properties, such as low

solubility in water, strong adsorption to soil particles, and extremely slow biological degradation. Therefore, PCBs and DDT may have the same mechanism for their phytoremediation. Interestingly, in a field trial of phytoremediation of PCBs performed by Whitfield Aslund et al. (2007), pumpkin, sedge, and tall fescue, were planted at a historically contaminated site. At harvest, PCBs were found in plant tissues of all plant species. These three plant species demonstrate the potential to phytoextract PCBs from soil. Thus, PGPR can assist phytoremediation of chlorinated aromatics if plants treated with PGPR are used.

4.6 Conclusions

Plant species used in this study can grow well in DDT contaminated soil and can accumulate DDT and DDE in their tissues. The distribution of DDT in tissues is plant dependent. DDT concentration in soil significantly dropped. The total amount of DDT and DDE accumulated in plant tissues equals to the total amount of DDT and DDE decreased in soil. PGPR treatment can enhance DDT remediation by promoting plant growth and increasing plant biomass.

REFERENCES

Aislabie J, Foght J, Saul D. 1999. Aromatic hydrocarbon-degrading bacteria from soil near Scott Base, Antarctica Polar Biology 23(3):183-188.

Alexander M. 1995. How toxic are toxic chemicals in soil. Environmental Science and Technology 29(11):2713-2717.

Atlas RM, Plilp J, editors. 2005. Bioremediation: applied microbial solutions for real-world envionmental cleanup. Washington D.C.: ASM Press. 366 p.

Bais H, Weir T, Perry L, Gilroy S, Vivanco J. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57:233-266.

Bloemberg GV, Lugtenberg BJJ. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Current Opinion in Plant Biology(4):343-350.

Budavari SO, Neil MJ, Smith A, Heckelman PE. 1989. The merk index: an encyclopedia of chemicals, drugs and biologicals. Rahway, New Jersey.: Merk and Company, Inc.

Bumpust AJ, Aust DS. 1987. Biodegradation of DDT [1,1,1-Trichloro-2,2-Bis(4-Chlorophenyl)Ethane] by the white rot fungus Phanerochaete chrysosporium. Applied and Environmental Microbiology:2001-2008.

Burken J, Shanks J, Thompson P. 2000. Phytoremediation and plant metabolism of explosives and nitroaromatic compounds. In: Spain J, Hughes J, Knackmuss H, editors. Biodegradation of Nitroaromatic Compounds and Explosives. Washington D.C.: Lewis. p 239-275.

Carman E, Crossman T, Gatliff E. 1998. Phytoremediation of no. 2 fuel-oil contaminated soil. Journal of Soil Contamination 7:455-466.

Carson R. 1962. Silent Spring. Boston: Houghton Mifflin.

Castro TF, Yoshida T. 1971. Degradation of organochlorine insecticides in flooded soils in the Philippines. Journal of Agriculture and Food Chemistry 19:1168-1170.

CEC. 1997. History of DDT in North America to1997. Commission for Environmental Cooperation.

Chu W, Wong M, Zhang J. 2006. Accumulation, distribution and transformation of DDT and PCBs by phragmites australis and Oryza sativa L.:II. Enzyme study. Environmental Geochemistry and Health 28:169-181.

Corona-Cruz A, Gold-Bouchot G, Gutierrez-Rojas M, Monroy-Hermosillo O, Favela E. 1999. Anaerobic-aerobic biodegradation of DDT (dichlorodiphenyl-trichloroethane) in soils. Bulletin of Environmental Contamination and Toxicology 63:219-225.

Cunningham SD, Berti WR. 1993. Remediation of contaminated soil with green plants: an overview. In Vitro Cellular & Developmental Biology 29:207-212.

Donato MM, Jurado AS, Madeira VMC, Antunes-Madeira MC. 1997. Bacillus stearothermophilus as a model to evaluate membrane toxicity of a lipophilic environmental pollutant (DDT). Archives of Environmental Contamination and Toxicology 33(2):109-116.

EC. 1999. Canadian soil quality guidelines DDT (Environmetal effects). National Guidelines and Standards Office,Environmental Quality Branch, Environment Canada,Ottawa.

Ekman D, Lorenz W, Przybyla A, Wolfe L, Dean J. 2003. SAGE analysis of transcriptome responses in Arabidopsis roots exposed to 2,4,6-Trinitrotoluene. Plant Physiology

133:1397-1406.

Fages J, Arsac JF. 1991. Sunflower inoculation with Azospirillum and other plant growth promoting rhizobacteria. Plant and Soil 137:87-90.

Falconer RL, Bidleman TF, Gregor DJ. 1995. Air-water gas exchange and evidence for the metabolism of hexachlorocyclohexanes in Resolute Bay. N. W.T. The Science of the Total Environment 160/161: 65-74.

Focht DD, Alexander M. 1971. Aerobic co-metabolism of DDT analogues by Hydrogenomonas sp. Journal of Agriculture and Food Chemistry 19:20-22.

Foght J, April T, Biggar K, Aislabie J. 2001. Bioremediation of DDT-Contaminated Soils: A Review. Bioremediation Journal 5(3):225-246.

Garrison WA, Nzengung AV, Avants KJ, Ellington JJ, Jones JW, Rennels D, Lee NW. 2000. Phytodegradation of p,p'-DDT and the enantiomers of o,p-DDT. Environmental Science and Technology 34:1663-1670.

Germida JJ, Frick CM, Farrell RE. 2002. Phytoremediation of oil-contaminated soils. In: Violante A, Huang PM, Bollag JM, Gianfreda L, editors. Soil mineral-organic matter-microorganism interactions and ecosystem health. Amsterdam Boston : Elsevier. p 169-186.

Glick BR. 1995. The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology 41:109-117.

Glick BR. 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiology Letters 251:1-7.

Glick BR, Karaturovíc DM, Newell PC. 1995. A novel procedure for rapid isolation of plant growth-promoting pseudomonads. Canadian Journal of Microbiology 41:533-536.

Glick BR, Penrose D, Li J. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. Journal of Theoretical Biology 190:63-68.

Golovleva LA, Skryabin GK. 1981. Microbial degradation of DDT. In: Leisinger T, Cook AM, Hutter R, Neusch J, editors. Microbial Degradation of Xenobiotics and Recalcitrant Compounds. London: Academic Press. p 287-292.

Greenberg B, Huang X-D, Gerhardt K, Glick BR. 2007. Field and laboratory tests of a Multi-Process Phytoremediation System for decontamination of petroleum and salt impacted soils. Proceedings of the ninth international in situ and on-site remediation symposium: Batelle Press.

Hammel KE. 1995. Mechanisms forpolycyclic aromatic hydrocarbon degradation by ligninolytic fungi. Environmental Health Perspectives 103(Suppl 5):41-43.

Hay AG, Focht DD. 1998. Cometabolism of 1,1-dichloro- 2,2-bis(4-chlorophenyl)-ethylene by Pseudomonas acidovorans M3GY grown on biphenyl. Applied and Environmental Microbiology 64:2141-2146.

Hay AG, Focht DD. 2000. Transformation of 1,1-dichloro-2,2-(4-chlorophenyl)ethane (DDD) by Ralstonia eutropha strain A5 FEMS Microbiology Ecology 31:249-253.

Hebert CE, Weseloh OV, Kot L, Glooschenko N. 1994. Organochlorine contaminants in a terrestrial food web on the Niagara Peninsula, Ontario, Canada, 1987-89. Archives of Environmental Contamnation and Toxicology 26:356-366.

Huang X, Alawi Y, Penrose D, Glick BR, Greenberg B. 2004a. Responses of three grass species to creosote during phytoremediation. Environmental Pollution 130:453-463.

Huang X, Alawi Y, Penrose D, Glick BR, Greenberg B. 2004b. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from

contaminated soils. Environmental Pollution 130:453-463.

Kamanavalli M, Chandrappa NZH. 2004. Biodegradation of DDT by a Pseudomonas species Current Microbiology 48:10-13.

Kawala Z, Atamanczuk T. 1998. Microwave-enhanced thermal decontamination of soil. Environmental Science and Technology 32:2602-2607.

Keller E. 1970. The DDT story. Chemistry 43:8-12.

Kim J, Kang S-H, Min K-A, Cho K-S, Lee I-S. 2006. Rhizosphere microbial activity during phytoremediation of diesel-contaminated soil. Journal of Environmental Science and Health Part A 41:2503-2516.

Lal R, Saxena DM. 1982. Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. Microbiology Review 46(1):95-127.

Lichtenstein E, Schulz K. 1959. Persistence of some chlorinated hydrocarbon insecticides as influenced by soil types, rate of application, and temperature. Journal of Economic Entomology 52:124-131.

Lino CM, Silveria IN. 1997. Extraction and clean-up methods for the determination of organochlorine pesiticide residues in medicinal plants. Journal of Chromatogaphy A 769:275-283.

Lipke H, Kearns CW. 1959. DDT dechlorinase. I. Isolation, chemical properties, and spectrophotometric assay. Journal of Biological Chemistry 234:2123-2128.

Lunney IA, Zeeb AB, Reimer JK. 2004. Uptake of weathered DDT in vascular plants: potential for phytoremediation. Environmental Science and Technology 38:6147-6154.

Luo L, Zhang S, Shan X, Zhu Y. 2006. Oxalate and root exudates enhance the desorption of

p,p'-DDT from soils. Chemosphere 63(8):1273-1279.

Macek T, Mackova M, Kas J. 2000. Exploitation of plants for the removal of organics in environmental remediation. Biotechnology Advances 18:23-34.

Meagher RB. 2000. Phytoremediation of toxic elements and organic pollutants. Current Opinion of Plant Biology 3:153-162.

Nadeau LJ, Menn FM, Breen A, Sayler GS. 1994. Aerobic degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by Alcaligenes eutrophus A5. Applied and Environmental Microbiology 60:51-55.

Nelson L. 2004. Plant growth promoting rhizobacteria (PGPR): Prospects for New Inoculants.

Nicholls PH. 1991. Organic contaminants in soils and groundwaters. In: Jones KC, editor. Organic contaminants in the environment. New York: Elsevier Applied Science. p 87-132.

Nirwal AS. 2001. Environmental behavior and fate of dichlorodiphenyltrichloroethane (DDT) residues in a terrestrial arctic ecosystem Kingston: Queen's University. 1-124 p.

Norris GH, Al-Dhahir Z, Birnstingl J, Ciu S, Plant SJ, Mayell P. 1997. A case study of the management and remediation of soil contaminated with polychlorinated biphenyls. In: Thomas RNYaHR, editor. Geoenvironmental Engineering, Contaminated Ground: Fate of Pollutants and Remediation. London,UK: Thomas Telford Publishing. p 473-483.

OMEE, Ontario Ministry of Environment and Energy. 1993. Ontario Typical Range of Chemical Parameters in Soil, Vegetation, Moss Bags and Snow. Queen's Printer for Ontario.

Paola G, Ester P, Battaini F. 2004. Ranking and classification of non-ionic organic pesticides for environmental distribution: a qsar approach. International Journal of Environmental Analytical Chemistry 84(1-3):65-74.

Parfitt RL, Whitton JS, Susarla S. 1995. Removal of DDT residues from soil by leaching with surfactants. Communications in Soil Science and Plant Analysis 26:2231-2241.

Patten LC, Glick BR. 2002. Role of Pseudomonas putida Indoleacetic Acid in Development of the Host Plant Root System. Applied and Environmental Microbiology 68(8):3795-3801.

Penrose DM, Glick BR. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiologia Plantarum 118:10-15.

Persello-Cartieaux F, Nussaume L, Robaglia C. 2003. Tales from the underground: Molecular plant-rhizobacteria interactions. Plant, Cell and Environment 26:189-199.

Pramanik MHR, Nagai M, Asao T, Matsui Y. 2000. Effects of temperature and photoperiod on phytotoxic root exudates of cucumber (Cucumis sativus) in hydroponic culture. Journal of Chemical Ecology 26:1953-1966.

Rajiv A, Niranjan A, Natesan M, Ashwani K. 2001. Metabolism of 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene by Alcaligenes Denitrificans. Biotechnology Letters 23:423-426.

Rajkumar B, Manonmani HK. 2002. Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by Serratia marcescens DT-1P. Process Biochemistry 38:49-56.

Richardson PT, Baker DA, Ho LC. 1982. The chemical composition of cucurbit vascular exudates. Journal of Experimental Botany 33:1239-1247.

Rovira AD. 1965. Interaction between plant roots and soil microorganisms. Annual Review of Microbiology 19:241-266.

Russel PF. 1955. Man's mastery of malaria. New York: Oxford University Press. 308 p.

Salt DE, Blaylock M, Kumar NPBA, Doshenkov V, Ensley BD, Chet C, Raskin I. 1995. Phytoremediation. A novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468-474.

Samuel T, Pillai MKK. 1989. The effect of temperature and solar radiation on volatilization, mineralization and degradation of 14C-DDT in soil. Environmental Pollution 57:63-77.

Saxena P, KrishnaRaj S, Dan TV, Perras MR, Vettakkorumakankav N. 1999. Phytoremediation of heavy metal contarninated and polluted soil. In: Prasad M, Hagemeyer J, editors. Heavy Meta1 Stress in Plants From Molecules to Ecosystem. Heidelberg: Springer-Verlag. p 305-329.

Schwitzguebel J-P. 2001. Hype or Hope: The Potential of Phytoremediation as an Emerging Green Technology. Remediation 11(4):63-78.

Singh J. 1962. Activities of the malaria institute of India during world war II. Indian Journal Malariol 16:504.

Singh OV, Jain RK. 2003. Phytoremedation of toxic aromatic pollutants from soil. Applied Microbiology and Biotechnology 63:128-135.

Suntio LR, Shiu WY, Mackay D, Seiber JN, Glotfelty D. 1988. Critical review of Henry's constants for pesticides. Reviews of Environmental Contamination & Toxicology 103:1-59.

Suresh B, Ravishankar GA. 2004. Phytoremediation-a novel and promising approach for

environmental clean-up. Critical Reviews in Biotechnology 24(2-3):97-124.

Suresh B, Sherkhane PD, Kale S, Eapen S, Ravishankar GA. 2005. Uptake and degradation of DDT by hairy root cultures of Cichorium intybus and Brassica juncea. Chemosphere 61(9):1288-1292.

UNEP. 2001. UNEP Protection of Human Health and the Environment from POPs. The Stockholm Convention. Sweden: United Nations Environment Programme.

USEPA. 1980. Ambient water quality criteria for DDTs. Office of water regulations and standards, U.S. Environmental Protection Agency.

USEPA. 1996. Method 3550B Ultrasonic extraction. U.S.Environmental Protection Agency. p 1-14.

USEPA. 2000. Introduction to Phytoremediation. National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency.

Villa DR, Carvalho DdEFG, Carbo L, Cunha MLF. 2006. Dissipation of DDT in a heavily contaminated soil in Mato Grosso. Chempsphere 64:549-554.

Waliszewski SM, Carvajal O, Infanzon MR, Trujillo P, Aguirre AA, Maxwell M. 2004. Levels of organochlorine pesticides in soils and rye plant tissues in a field study. Journal of Agriculture and Food Chemistry 52:7045-7050.

Walker T, Bais H, Grotewold E, Vivanco J. 2003. Root exudation and rhizosphere biology. Plant Physiology 132:44-51.

Wang X, White JC, Gent MPN, Iannucci-Berger W, Eitzer BD, Mattina MI. 2004. Phytoextraction of weathered p,p'-DDE by zucchini (Cucurbita pepo) and cucumber (Cucumis sativus) under different cultivation conditions. International Journal of

Phytoremediation 6:363-385.

White JC. 2000. Phytoremediation of weathered p,p'-DDE residues in soil. International Journal of Phytoremediation 2(2):133-144.

White JC. 2001. Plant-facilitated mobilization and translocation of weathered 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE) from an agricultural soil. Environmental Toxicology and Chemistry 20:2047-2052.

White JC, Kottler BD. 2002. Citrate-mediated increase in the uptake of weathered p,p'-DDE residues by plants. Environmental Toxicology and Chemistry 21:550-556.

White JC, Mattina MI, Lee W-Y, Eitzer BD, Iannucci-Berger W. 2003. Role of organic acids in enhancing the uptake of weathered p,p'-DDE by plants. Environmental Pollution 124:71-80.

Whitfield Aslund ML, Zeeb BA, Rutter A, Reimer KJ. 2007. In situ phytoextraction of polychlorinated biphenyl-(PCB) contaminated soil. Science of the Total Environment 374:1-12.

WHO. 1989. DDT and its derivatives-Environmental aspects. World Health Organization, the United Nations Environment Programme, the International Labour Organisation.

Worthing CR, Hance RJ. 1991. The pesticide manual: a world compendium. Farnham, Surrey, UK: British Crop Protection Council.