STUDIES ON RESPONSES OF NON-TARGET PLANTS TO PESTICIDES: A REVIEW[1]

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ABSTRACT

Plant species that are unintentionally exposed to pesticides are treated as non-target plants Non-target plant effects are any direct or indirect effects that cause significant change in the survival, health or reproduction of non-target plant species or cause a change in plant community attributes. This review presents responses of several non-target plants to pesticide contamination. Non-target plants may have either one or a combination of responses to detoxify a pesticide. Development of detoxification mechanism depends on three factors: (1) concentration and type of pesticide present, (2) environmental conditions, and (3) plant characteristics. Comparison of metabolic pathways, detoxification mechanisms, and tolerance of various plants to pesticides may help us design vegetative treatment systems to decontaminate soil and water contaminated with pesticides.

1. INTRODUCTION

Pesticides cause regular or sporadic damage to established vegetation within the vicinity of agricultural lands. The off-target fate is particularly important when herbicides are involved. In many parts of the United States, herbicide drift-related problems have occurred for many years and, in some cases, have developed into complex social as well as biological and economic issues. Established vegetation bordering the target area is often non-target for the intended use of the pesticides. Increased impacts to non-target vegetation may result from increased herbicide usage for weed control in crops, roadsides, railroads, and industrial sites, and because of drift and run-off from non-point

sources. Botanical diversity in field buffer vegetation has declined considerably in recent decades in the U.K. (Bunce et al. 1994). Some of these changes may be attributable to pesticide spray-drifts and drift losses (Marrs et al., 1989).

During mid 20th century, the dominant organic pesticides were broad-spectrum, persistent in the environment, and bio-accumulative (e.g., DDT). In response to serious unintended ecological effects of such pesticide use and growing environmental concern, research and development programs focused on designing pesticides with greater specificity, shorter life-span in the environment, and less bio-accumulative potential. Prior to registration of a pesticide, a test is often conducted for environmental impacts on non-target aquatic and terrestrial plants and animals. It includes only a few species and often is not conducted on whole plants for long duration. Sensitivity to pesticides may differ by several orders of magnitude among species. It is possible that habitat alteration in aquatic/terrestrial plants through adverse effects of pesticides can affect non-target animals more than direct toxic effects. It is estimated that for every plant species that becomes extinct, 10-30 other non-plant organisms may also become extinct (Ware, 1993).

In this review we focus on the effect of pesticides on non-target plants with an emphasis on herbicides. The following observations support this approach: (1) field evidence shows unintended herbicide effects on non-target plants over wide geographic and taxonomic ranges (Kleijn and Snoeijing, 1997), (2) experimental evidence corroborates the role of herbicides (Marrs et al., 1993), (3) the herbicides implicated in non-target incidents are in widespread use (Marrs et a., 1991), and (4) many pesticides are persistent

in the environment and are toxic to plants, animals, and/or humans (Freemark and Boutin, 1995). This paper reviews responses of non-target plants to pesticides. Since there are already reviews on pesticide biotransformation in plants for target plants (Hall et al., 2001; Roberts, 2000) the emphasis in this review is on responses of non-target plants to pesticides.

2. NON-TARGET PLANTS

We define non-target plants as plant species that are unintentionally exposed to pesticide. Non-target plant effects are any direct or indirect effects that cause a significant change in the survival, health or reproduction of non-target plant species or cause a change in plant community attributes (such as species richness). Non-target plant effects include a range of symptoms, including vegetative growth changes, plant death, altered reproductive capability that can generally result in reduced fitness, and detrimental economic or ecological impacts. Altered susceptibility to disease of either the target or non-target plants may also be one of the unintended effects of herbicides (Altman, 1993).

Agricultural crops may be subjected to non-target effects by a variety of unintended exposure mechanisms, including accidents, local spray-drift from adjacent crop fields, aerial drift, surface water, groundwater, wind, and soil transport, and carryover in the soil from previous crops in crop rotation. Vapor derived from volatilization of residue from previous spraying events combined with rainfall can also contribute to non-target exposure (Marrs et al., 1989, 1991, and 1993; Obrigawitch, et al., 1998).

Native plant communities are often subjected to non-target exposure to pesticides and often manifest subtle but significant responses. Competitive interactions among species may be altered, thereby changing species dominance, richness, and distribution, which can lead to further repercussions in the ecosystem (Kleijn and Snoeijing, 1997). Table 1 lists numerous reports that document unintended effects of herbicides. The reports include documentation of unintended effects for a wide range of agricultural field crops, some trees, and a much less extensive coverage of native plant species (Obrigawitch et al., 1998). These results are for a single class of herbicides, the sulfonylureas. Comparable effects may be expected for other classes of compound on several non-target plants; but the effects are not well documented.

3. RESPONSE OF NON-TARGET PLANTS TO PESTICIDES

Certain selective herbicides are either metabolized differently or, more commonly, at different rates, in susceptible and tolerant species. Selective metabolism by susceptible and tolerant genotypes can aid development of technologies to remediate contaminated sites. Comparison of metabolic pathways, detoxification mechanisms, and tolerance of various plants may help us design treatment systems. Table 2 lists various possible responses of non-target plants to pesticides. Non-target species may have one, or a combination of responses to ultimately detoxify an agrochemical. If the plants do not develop a positive detoxification mechanism, they will ultimately die or have other unfavorable effects such as stunted growth. Development of a detoxification mechanism depends on three factors: (1) concentration of the pesticide present, (2) environmental conditions, and (3) plant characteristics.

3.1. Tolerance and establishment

The major basis for the tolerance of crops to most herbicides is differential rates and routes of herbicide metabolism. In many plants the selection pressure of pesticide pollution led to the natural evolutionary development of tolerant plant genotypes in response to a wide range of air- and soil-borne agrochemical pollutants. Many plant species are known to either possess or lack the genetic variability for tolerance of agrochemicals. For non-target plant species, there is sparse literature available about precise quantitative limits of tolerance, the actual dosage at which an agrochemical is toxic and the point beyond which no further adaptation can be achieved by a particular species. Desirable species of trees, shrubs, and herbaceous grasses and forbs, especially those sensitive to pesticide treatment, may be eliminated or greatly reduced. Reduced species richness has been cited as a consequence of herbicide treatment (Tomkins and Grant, 1977). Differential susceptibility to the nonselective herbicides can allow the establishment of resistant species and the elimination of susceptible ones along with their competitive effects, altering the species composition drastically (Dickinson et al., 1991).

Non-target plants growing on pesticide-polluted environments may experience local extinction. Desirable species of trees and herbaceous grasses and shrubs, especially those sensitive to pesticides, may be eliminated or greatly reduced (Boutin et al., 2000; Fink, 1972; Tomkins and Grant, 1997). Tolerant species and tolerant biotypes of herbaceous plants are favored by natural selection in pesticide-contaminated environments, due to their ability to survive or else to competitively exclude non-tolerant plants. In many herbaceous plants, the selection pressure of pollution has led to the natural evolutionary

development of tolerant plant genotypes in response to wide range of herbicides. Sensitive species exhibit marked effects on the vegetative growth and reproductive performance when exposed to pesticides. The seedling stage is the most sensitive period for most species, although surviving plants exposed to pesticides at later stages may show considerable effects on reproduction. Many annual or colonizing plants show rapid selection for tolerance. However, the scenario for trees and other long-lived perennials is different. They may respond to agrochemical pollution the same way they would respond to other temporary or fluctuating stresses such as drought.

3.2.Mutation

Exposure of plants to pesticides leads to the biochemical responses described below in section 3.3. However, in certain cases mutagenic activation of pesticides in plants can occur. The development of tolerance in plants can be due to proliferation of tolerant mutants or variants, which become favored by natural selection. Tolerant genotypes either may be produced randomly or induced by pesticide/plant interactions. Selection of tolerant plant genotypes can occur within a single generation or multiple generations. In some cases, tolerance to pesticides is probably maintained at low frequency within otherwise susceptible populations. After several generations of selection by exposure to the pesticide, the frequency of pesticide tolerant plants increases (Sandermann, 1992).

3.3 Uptake, transport, and metabolism

Water and wind can transport pesticides for long distances. During this transport, pesticides could be altered physically, chemically, and/or biologically. Pesticides carried

by wind can be deposited onto terrestrial or aquatic vegetation. Pesticides may be transformed or biodegraded because of sunlight, microbial action, or plant enzymes. Water-transported pesticides might enter plants and be carried upward in the transpiration stream by vegetation during transpiration. Volatile pesticides may then enter the atmosphere from the large surface area of leaves and stems.

The metabolic fate of pesticides in or on a plant is a complex process starting with potential modification at reactive sites mostly found in leaves and roots. Plants take up pesticides mainly through leaf surfaces, fruits, and roots. Once within the plant the pesticide taken up can be distributed within the plant either from cell to cell or via the plant vascular system. The degree and manner in which a pesticide is taken up and distributed within the plant is dependent on the physical and chemical properties of the pesticide. A pesticide taken up by roots from the soil can take, alternatively or simultaneously, two pathways to reach xylem vessels where it is moved to the top of the plant with the transpiration stream in the xylem: (1) the apoplastic pathway; (2) the symplastic route. The symplastic system is the living plant tissue bounded by the plasmalemma and connected via plasmodesmata. It is a reactive environment that places chemicals in proximity to enzymes and other reactants. Movement within the conductive portion of the symplast (phloem) occurs by mass flow and diffusion. The apoplastic system includes all the nonliving portions of the plant. Cell walls and xylem elements form a water-permeable continuum through which both short- and long-distance solute transport occurs by mass flow and diffusion. Diffusive movement plays the primary role in short-distance transport. Some chemicals appear to be restricted to either the

apoplastic or symplastic pathways, while others, termed ambimobile, move in both domains. It is the balance between the distribution of pesticides in the apoplastic-symplastic compartments that determines the overall transport pattern. Generally, less-lipophilic pesticides take the apoplastic pathway and more-lipophilic pesticides tend to take the symplastic route. Movement to centers of metabolic activity such as the tips of roots and shoots occurs via active transport in the phloem (Boersma et al., 1988).

In general, plants have mechanisms inside the plant for the degradation or sequestration of most commercial pesticides, even though such chemicals are foreign to plants. Most often, these biotransformation mechanisms are independent of, and have no relation to, the mode of action and physiological lesions involved in the pesticidal activity; instead, they relate to the functional or reactive groups or linkages in the compound which are susceptible to enzymatic or chemical attack (Casida and Lykken, 1969). Plant metabolism can be divided into three phases, transformation (Phase I), conjugation (Phase II), and storage (Phase III) processes. The initial metabolic reactions, also known as Phase I reactions, are primarily catalyzed by cytochrome P450 enzymes by the generation of functional groups in the pesticide structure using different transformation mechanisms (Mougin et al., 2001). The generated functional groups such as hydroxyls serve as a 'handle' for subsequent conjugation and storage, catalyzed by Phase II and III enzymes. The types of transformations of pesticides that occur in plants include oxidation, hydroxylation, epoxidation, hydrolysis, reduction, N-dealkylation, O-dealkylation, desulfuration, dehalogenation, dehydrohalogenation, and dehydrogenation (Casida and Lykken, 1969).

Hoagland and Zablotowicz (2001) have reviewed the role of plant hydrolytic enzymes in pesticide hydrolysis reactions. Esterases, lipases, and proteases are involved in pesticide detoxification and degradation. Amide hydrolysis by amidases is important in the detoxification of alachlor and other pesticides with amide bonds. Monooxygenase enzymes are important in the transformation of nitroaromatic pesticides such as trifluralin (2, 6-dinitro-N, N-dipropyl-4-(trifluoromethylbenzenamine)). Zablotowicz et al. (2001) have reviewed the transformations of nitroaromatic pesticides in plants.

Phase II and III reactions involve the conjugation of the pesticide molecule or its metabolites with natural plant constituents. Phase II processes give rise to the formation of soluble conjugates (mainly with sugars and amino acids). The major plant products involved in conjugation are glucose, glutathione, malonic acid, and amino acids. Glutathione conjugation is an important Phase II transformation in plants (Hatzios, 2001). In the presence of the enzyme glutathione S-transferase, glutathione can react with a range of substrates including epoxides, aryl and alkyl halides, and other electrophilic compounds. Soluble conjugates can be stored in vacuoles during Phase II reactions (Korte et al., 2000; Sandermann, 1992). Cole and Edwards (2001) summarize research on several agrochemicals metabolized in plants by glutathione conjugation.

Conjugation to insoluble structures in the plant, for example co-polymerization with lignin, is referred to as a Phase III reaction. This results in the formation of non-extractable or bound residue. Significant pesticide incorporation into bound residues has

been reported by Sandermann et al. (2001) in a review of this phenomenon. It is a natural regulatory mechanism by which the plant decreases water solubility of toxic pesticides, thereby reducing their reactivity and toxicity. It also reduces mobility of pesticides in the plant symplast and allows their removal to the vacuole, apoplast, or plant matrix. Three major pathways have been detected for Phase III reactions in plants, export into cell vacuoles, export into the extracellular space, and deposition into lignin or other cell wall components (Roberts, 2000; Sandermann, 1992). Several enzymes have been detected in plants that were active against pesticides or other xenobiotics (Table 3). The levels of detoxifying enzymes in the plant depend on plant type and age.

An important unresolved issue is whether or the extent that bound residues in plants are bioavailable. Limited studies in which radiolabeled plant material was fed to animals indicate that most was not bioavailable for the particular compounds tested (see McCutcheon and Schnoor, 2003 for further discussion). There appears to be no published information for likely non-target plant species.

A large number of metabolites sometimes form because the degradative attack frequently takes place at more than one site on the chemical molecule, either simultaneously or sequentially. Not all these chemical changes are enzymatically mediated; some result from reaction with normal plant constituents by nonenzymatic mechanisms and others arise from photochemical mechanisms. The pathways of metabolism depend on the balance of enzymes of various capabilities, present in different plant species, and the chemical reactions that are possible. The rate of metabolic attack on a pesticide by plants

varies with the species type, the time of residence in or on the plants, and the degree of entry into the plant. With some major exceptions, microorganisms, insects, plants, and mammals metabolize foreign organic compounds by the same major pathways (Hall et al., 2001). The processes in plants are, in most cases, slower than in animals, if similar mechanisms are involved; most animals have better degrading, circulating, and excreting systems than plants. Plants tend to store pesticides and their metabolic products for longer periods. Pesticide metabolism generally results in detoxification but, at times, the transformation products have equal if not greater toxicity than the respective parent compounds; this factor sometimes contributes to selective toxicity.

The literature pertaining to plant metabolism of pesticides in target species is very large (Hall et al., 2001; Korte et al., 2000; Roberts, 2000; Sandermann, 1992). Since this review is of necessity brief, it reflects a survey of the literature that deals with metabolism or biotransformation of major agrochemicals in non-target plants, with emphasis on biochemical mechanisms and agents involved and on their importance to the subject of plant-based remediation of pesticide contaminated environments. A number of general and specific reviews exist on the subject of plant metabolism of agricultural chemicals (Roberts, 2000). The following sub-sections provide information on metabolism in various non-target species. We grouped non-target species into three categories, keeping in mind the design of treatment systems, namely trees, aquatic species, and field and horticultural crops. In an effort to make a complete survey of non-target species, several databases were used. SciFinder Scholar, Agricola, BIOSIS were searched with common and specific plant names linked to a wide range of pesticides.

References obtained were read whenever accessible and citations in these papers were examined to identify earlier works on the same or different species with the same or different pesticides. For plants not traditionally used as agricultural or forestry crops, and not weeds, there are not many papers documenting responses to pesticides.

3.3.1. Trees

Phenoxy herbicides

The tolerance of trees to several pesticides has been studied in relation to artificial regeneration of forests of important hardwood tree species. The feasibility of successful establishment of hardwood plantations has been enhanced by the use of various chemical methods to control undesirable competing vegetation. Some herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), developed as agricultural chemicals, have been extended to kill tropical trees, unwanted woody species, and shrubs. Thus, some trees have been investigated as target species and others as non-target species. Few studies have been conducted to determine the translocation mechanisms and detoxification mechanisms of herbicides in woody plants. Studies are even more limited on woody plants that have been exposed to herbicides as non-target plants. For implementation of riparian buffer strips, woody species are often included. It is important to determine if they can prove useful in detoxifying runoff of various pesticides to prevent their entry to waterbodies.

Hamner and Tukey (1946) studied the effects of 2,4-D on juniper (*Juniperis communis* L.), elm (*Ulmus americana* L.), poplar (*Populus nigra* L. var. *Italica* Du Roi), spruce

(*Picea canadensis* Mill.), willow (*Salix nigra* Marsh.), plum (*Prunus domestica* L.), pear (*Pyrus communis* L.), pine (*Pinus strobus* L.), and other woody plants. A concentration of 1400 ppm of 2,4-D was applied to the foliage of elm, juniper, and other "woody weeds". Plants were treated during early spring and late spring. The leaves of all treated plants except juniper started to bend, discolor, and wilt within a few days during warm weather. Two additional sprays at 4000 ppm were applied to the juniper plants but again without apparent effect. Salves and powder of the same material at 20% concentration were applied on cuts made in the stems of peach, poplar, pine, spruce, elm, and willow. Treated plants responded generally by typical curvatures of new shoot growth within two days after treatment but varied otherwise in the degree and type of response. Leaves at the top of 40-foot poplar trees showed downward bending of the petioles within two days. Progressive browning and drying followed, and within a month the leaves at the tops of the trees were dead, and the leaves on the lower parts of the trees were becoming twisted, chlorotic, and brown. In the case of peach trees, flowers and leaves were distorted and became brown and dry, limbs and branches exuded gum from many discolored areas and the trees were completely dead within 60 days after application. In the case of pine, the new terminal growth became severely twisted; the young cones became pendant, the bark split, and gum exuded from patches on the branches and trunks, which had a water-soaked appearance. In general, young trees were more responsive than older ones, and the elm tree was the most responsive of all plants tested. Its long system of uninterrupted vessels perhaps aids in the rapid transport of the material throughout the plant.

Pallas (1963) simulated field application of triethylamine salt of 2,4-D or 2,4,5-T on red maple (*Acer rubrum* L.), yellow poplar (*Liriodendron tulipifera* L.), sweetgum (*Liquidambar stryraciflua* L.), and white ash (*Fraxinus americana* L.) to ascertain if these test species were resistant to the herbicides. All plants used in the study were at least two years old and actively growing in the greenhouse. The formulations consisted of an aqueous solution of the triethylamine salt of 2,4-D containing 500 ppm acid equivalent or the triethylamine salt of 2,4,5-T, 580 ppm acid equivalent and the chemicals were applied to foliage. In general, 2,4,5-T was never absorbed or translocated as efficiently as 2,4-D. Red maple leaves absorbed the greatest quantity of 2,4-D after 4 days followed by yellow poplar, sweetgum, and white ash. The results suggested continued absorption of 2,4-D and 2,4,5-T as indicated by increased concentrations of the parent compounds in the plant tissues. Poor absorption of 2,4,5-T by the leaves of white ash may account for its physical resistance. It is highly variable and modified by environment and growth stage. This study showed that absorption and translocation of 2,4-D and 2,4,5-T were not limiting and no unknown metabolites were found indicating a metabolic shunt. In this study, there was no evidence that decarboxylation of the herbicides occurred as a detoxification mechanism. This study provided some basis for selectivity of the herbicides in woody plants, although much remains to be clarified.

Sundaram (1965) studied the relative mobility of ^{14}C-labelled 2,4,5-T in the trunks of four different tree species native to West Africa. The chemical was applied at the region of basal bark. Two susceptible (*Piptadeniastrum africanum*, *Celtis mildbraedii*) and two tolerant (*Xylopia quintasii*, *Ricinodendron heudelotii*) species were used in the

investigation. All the trees could grow up to at least 100 ft and were found commonly in West African deciduous and evergreen forests. Tree samples were taken after 45, 75, and 150 days of application. There was a consistent pattern of absorption and retention of 2,4,5-T by the phloem of sensitive trees and downward movement of the chemical was marked, but in the resistant trees, the chemical was distributed upward to a greater extent in the transpiration stream.

Absorption of chemicals such as amitrole and 2,4,5-T after bark application was considerably superior to that after foliar application in red maple (*Acer rubrum* L.) and white ash (*Fraxinus americana* L.) (Leonard et al., 1966). When applied to leaves, herbicide may either migrate in the apoplast following penetration of the cuticle or passage through stomata or other openings; such transport proceeds toward the margins of laminas. A portion of the herbicide may be absorbed into living cells and then be translocated in the symplast (Leonard et al., 1966).

Norris and Freed (1966a, b, and c) studied absorption and translocation of several phenoxyalkyl acid and chlorophenoxyalkyl acid herbicides in bigleaf maple (*Acer macrophyllum* Pursh.). In an experiment in which the leaves were treated with 2,4-D or 2,4,5-T as the acid, triethanolamine salt or 2-ethylhexyl ester formulations, it was found that absorption increased with decreasing polarity of the chemical (Norris and Freed, 1966a). In general, an inverse relationship between the polarity of the compound and its degree of absorption exists. Also, inherent toxicity of the compound to plant transport mechanisms, the degree to which it is accumulated by non-transport tissues in the

vascular system of the plant, and the possible influence of water solubility of the translocated compound are factors which might interact to produce differential translocation. However, the relative importance of each of these factors is not yet known. Norris and Freed (1966b) concluded that phenoxy herbicides were poorly translocated by bigleaf maple. They suggested that those herbicides might be toxic to phloem transport tissues, which could be a limiting factor in translocation. In the later study (Norris and Freed, 1966c), they tested 4-(2,4-dichlorophenoxy) butyric acid (2,4-DB), which is supposed to have superior translocation characteristics (Norris and Freed, 1966c). Intact maple seedlings were treated with 2,4-DB and after 3 days, the plants were harvested, sectioned, and extracted. The results showed that 2,4-DB was oxidized to 2,4-D in several plant parts. While α-oxidation is not unknown in plants, β-oxidation probably resulted in this case. Roots of maple seedlings were found to have a greater ability to convert 2,4-DB to 2,4-D than stem phloem. This agrees with a previous study by Norris and Freed (1966b) that 2,4-D was not translocated in maple seedlings. They concluded that the rate of transformation from 2,4-DB to 2,4-D was relatively low in the leaves and stems (Norris and Freed, 1966c).

Triazine herbicides

Dhillion et al. (1968) investigated the uptake, distribution, accumulation, and degradation of simazine, a triazine herbicide, within young red pine (*Pinus resinosa* Ait.) seedlings. Simazine readily entered red pine seedling roots. The movement from roots to needles was rapid and occurred within 24 hours. Simazine accumulation in roots reached a maximum after 72 hr of incubation in 5 and 10 ppm simazine treatments, and then

declined slightly. This decline in concentration could be attributed in part to the redistribution of simazine into stems and needles. It is also possible that the roots were either absorbing less herbicide or metabolizing it faster than the rate of uptake. For the plant, simazine degradation was much less than its absorption. The translocation of simazine from roots to stem and needles did not appear to be influenced by changes in uptake of simazine by roots. Continuously increasing amounts of simazine were found in stem and needles with increasing incubation time. This could be expected since simazine is largely a xylem-mobile compound in several plants. Continuous accumulation of simazine in needles of red pine seedlings could injure young red pine seedlings. Degradation experiments of simazine in pine seedlings have shown the presence of three unknown metabolites. In wheat, a plant species very sensitive to simazine, one of the metabolites was found to be hydroxysimazine (Hamilton, 1964). Resistant species such as maize degrade simazine to hydroxysimazine nonenzymatically. A compound named benzoxazinone was isolated from maize extracts, and appears to be responsible for apparent catalytic degradation of simazine. It is possible that red pine seedlings contain very little benzoxazinone. This was evident from the fact that the amount of simazine degraded by red pine seedlings was small and there was no hydroxysimazine (Dhillion et al., 1968).

Lund-Hoie (1969) studied uptake, distribution, and fate of simazine in 4-year old seedlings of Norway spruce (*Picea abies* L. Karst.). Simazine was found to be readily absorbed and distributed in the spruce seedlings. There was a positive correlation between the amount of simazine absorbed and the growth of the plants. This indicates an

active mechanism of simazine uptake by Norway spruce. Simazine taken up by spruce was fixed in roots and stems. Fixed simazine was degraded by roots and stems to hydroxysimazine and two other unknown metabolites. Since no simazine was observed in spruce needles, this indicates a very rapid rate of metabolism in the roots and stems. However, there was a very slow degradation of metabolites to CO_2 as one of the final products. Three possible reasons may account for tolerance of spruce to simazine. Firstly, simazine is very insoluble in water and could be strongly sorbed to soil. Secondly, the root system of spruce may present a barrier to the uptake of simazine due to it is lipophilicity. Thirdly, the resistance of spruce may be correlated with metabolic inactivation or detoxification of simazine. In this study, simazine was absorbed by the roots and hydroxysimazine, the degradation product, was translocated to the needles, with a direct relationship between the amount of simazine absorbed and the transpiration intensity. As expected, seedlings at apparent dormancy translocated less simazine compared to seedlings at intense internode elongation (Lund-Hoie, 1969). An important conclusion is that in northern latitudes, spruce may serve as a useful species to assist clean up of simazine.

Erickson and Lee (1989) have reviewed the pathways for degradation of simazine and atrazine as shown in Figures 1 and 2 and Table 4. The pathways appear to be the same for degradation in soil and in plants; however, the rate of the hydrolysis process to form hydroxyatrazine in soil is influenced by nucleophilic compounds that are present in soil. Within plants, other compounds appear to be important as described below for simazine.

The main pathway for simazine metabolism in tolerant species such as maize is found to be a hydrolysis reaction by which a non-toxic hydroxysimazine is formed (see Figure 1). This hydrolysis of simazine (CEET) to hydroxysimazine (EEOT) is considered to be non-enzymatic, and may be catalyzed by the plant constituent benzoxazinone or glucoside. The glucose derivative of benzoxazinone was extracted from all parts of spruce seedlings and is probably responsible for the hydrolysis of simazine (Lund-Hoie, 1969). These findings related to metabolism of simazine by spruce differ very much from those reported by Dhillon et al (1968) for simazine degradation by red pine seedlings. In the red pine, no benzoxazinone could be detected and the amount of simazine degraded was found be negligible (Dhillion et al., 1968). Hence red pine would be a poor species to use in buffer strips or for remediation at heavily contaminated spill sites.

A study was conducted to investigate factors such as uptake and degradation of simazine by black walnut (*Juglans nigra* L.) and yellow poplar (*Liriodendron tulipifera* L.) trees (Wichman and Byrnes, 1975). One-year old and three-month old seedlings of walnut and poplar grown in a growth chamber were used to determine the degree of tolerance for simazine. Seedlings were fed with ^{14}C-simazine for three days and then removed and placed in distilled water for 15 min to remove simazine adsorbed onto root surfaces. At the end of 3, 6, and 9 days of growth in silica sand, seedlings were harvested and divided into leaves, stems, and roots. Simazine and its degradation products were extracted from leaves, stems, and roots. Concentrations of simazine and monodealkylated simazine (CEAT in Figure 1) were found in various tissues of one-year old black walnut and yellow poplar seedlings. The concentrations were higher in yellow poplar leaves than in

black walnut leaves. For both species, simazine levels in leaves decreased from day 3 to day 9. Almost all simazine had disappeared from black walnut leaves and had decreased by 50% in yellow poplar leaves by day 9 due to degradation of the herbicide. CEAT and other degradation products were found in leaves of both species. Gysin and Knusli (1960) reported that monodealkylated simazine is phytotoxic and an effective inhibitor of the Hill reaction in isolated chloroplasts. Since simazine is a photosynthetic inhibitor and its degradation product, CEAT is also an inhibitor, tolerance of a plant should be inversely related to the concentration of phytotoxic compounds in the leaves of the plant. Consequently, it shows that black walnut was more tolerant than yellow poplar to simazine. Walnut is a high value species that might profitably be used in riparian buffer strips.

Wichman and Byrnes (1975) examined concentrations of simazine and CEAT in black walnut and yellow poplar roots and stems. For all plant tissues combined, roots, stems, and leaves, 1.9% of simazine absorbed by black walnut seedlings remained as the undegraded simazine on day 9. For yellow poplar, 7.3% remained in the undegraded form on day 9. This indicated that both species degraded simazine rapidly but that black walnut was more efficient. For both phytotoxic compounds, simazine and CEAT, 12% remained in black walnut and 29% remained in yellow poplar on day 9. It was concluded that differential uptake, translocation, and degradation were factors which contributed to lower levels of toxic compounds in black walnut leaves and as a result greater tolerance of black walnut for simazine (Wichman and Byrnes, 1975).

The results of Wichman and Byrnes (1975) showed that both black walnut and yellow poplar degraded simazine rapidly and have a considerable degree of simazine tolerance. Four other degradation products were identified. The first compound, 2-chloro-4, 6-(diamino)-s-triazine (CAAT in Figure 1), is the product of N-dealkylation of CEAT, and is considered nontoxic to plants (Gysin and Knusli, 1960). This metabolite was identified in black walnut leaves, stems, and roots on days 3 and 6, but not in roots on day 9. It was found in leaves and stems of yellow poplar on all three days, but in roots on day 3 only. The second compound, identified as hydroxysimazine (EEOT), was found in the roots of yellow poplar and not found in any other plant tissue. Two other unidentified degradation products were found in both black walnut and yellow poplar. It was believed that they represent the products of further hydroxylation or conjugation reactions. From this study a major pathway of simazine degradation was found to be N-dealkylation in both species (see Figure 1).

Akinyemiju et al. (1983) reported differential tolerance to simazine among poplar clones (simazine-intolerant clone, *Populus maximowiczii* × *Populus trichocarpa* cv. Kingston; tolerant clone, *Populus* × *euramericana* cv. I-45/51). The pattern of simazine translocation, accumulation, and metabolism in simazine-tolerant and –intolerant poplar clones in a controlled environment was evaluated. Simazine was absorbed from sand by the roots and translocated acropetally to the stem and leaves of both clones. Partitioning of absorbed simazine in the two clones was similar. The presence of CEAT (Figure 1), a less toxic simazine metabolite, was detected in shoots and roots of the tolerant poplar clone after 12 h and it increased steadily through one week after simazine application. In

contrast, no trace of the metabolite was found in the susceptible poplar clone. The data from this study indicate that metabolism of absorbed simazine to a relatively less toxic form plays a significant role in the observed difference in simazine tolerance among poplar clones. Although the CEAT (Figure 1) does not completely detoxify the parent compound, the accumulation of this metabolite has been reported to account for intermediate triazine resistance in pea plants (Shimabukuro et al., 1966). Transformation of CEAT to non-toxic 2-hydroxy-4-amino-6-ethylamino-s-triazine (EAOT in Figure 1) or conjugate formation by tolerant poplar clones is possible but not a prerequisite for the moderate simazine tolerance shown by some clones (Akinyemiju et al., 1983).

Paterson and Schnoor (1992) found that atrazine and alachlor degraded more rapidly in a riparian zone soil under hybrid poplars (*Populus deltoides* $\times$ *nigra* cv. "Imperial Carolina") than in soils where herbicides were applied to row crops. In a recent study, Burken and Schnoor (1996) applied atrazine, another triazine herbicide, to the same hybrid poplars grown in silica sand. They showed that 91% of atrazine could be assimilated in less than 10 days yielding less toxic metabolites (Burken and Schnoor, 1997). The evapotranspiration rate of poplar trees has a significant influence on contaminant uptake. The formation of bound residues from atrazine is considered to be a detoxification mechanism. Burken and Schnoor (1997) proposed atrazine fate mechanism in poplar tissues, with major degradation steps involving hydroxylation, dealkylation, and oxidation. Twelve organic contaminants including atrazine were later studied for uptake by hybrid poplar trees (Burken and Schnoor, 1998). Translocation and subsequent transpiration of volatile organic compounds from the leaves to the atmosphere

was a significant pathway for several organic compounds. However, for atrazine, volatilization from leaves was found to be negligible (Burken and Schnoor, 1997).

Other Pesticides

The herbicide bentazon was degraded within black willow (*Salix sp.*) trees, as indicated by bentazon loss during a nursery study and the identification of metabolites within the tree (Conger and Portier, 1997). Schnabel and White (2001) investigated the phytoremediation potential of mycorrhizal systems for the remediation of soils contaminated with aldrin, an organochlorine pesticide. Feltleaf willow (*Salix alaxensis*) and balsam poplar (*Populus balsamifera*) were grown in soil contaminated with 0.8 mg of radiolabeled-aldrin/kg of soil. Most of the radiolabeled material in roots was found to be extractable dieldrin, an epoxide of aldrin. Aldrin was not detected in any vegetative matrix. Dieldrin constituted less than 1% of the radiolabel in the willow leaf material and dieldrin was not detected in the poplar leaves, although poplars took up approximately the same amount of radiolabel as the willows.

Komives et al. (2003) have examined the ability of a poplar (*Populus nigra* L.) to detoxify some of the chloroacetanilide herbicides. Leaves of the plants were exposed to seven herbicides through the cut petiole. Leaves tolerated all seven tested herbicides and all treatments induced increased levels of glutathione-S-transferase. On the basis of these results, it was reported that such trees are being used for phytoremediation in Hungary.

Woody shrubs, including small trees, have been tested for their tolerance to two commonly used pre-emergent herbicides in the ornamental horticulture industry (Baz and Fernandez, 2002). The rationale is that these non-target species may be useful to control run-off from containerized plant production, which frequently leads to significant run-off levels of herbicide resulting in undesirable impact on surrounding water bodies. Isoxaben at the levels of exposure tested here (4 mg/L for 9 d) was toxic to Virginia sweetspire (*Itea virginica*), white willow (*Salix alba*) and black pussywillow (*S. gracilistyla*), but the first two species were able to tolerate oryzalin, a dinitroaniline herbicide, with little effect. See discussion below (section 3.3.3) for additional trees, shrubs and landscape plants, exposed to single treatments of the preemergent herbicides at rates used in commercial production.

3.3.2 Aquatic Plants

Triazine herbicides

Wilson et al (2000) used radiolabelled simazine to characterize the interaction within two ornamentals, sweet flag (*Acorus gramenius*) and pickerel weed (*Pontederia cordata*). Both are emergent wetland plants. The radioactivity in the exposure solution with either of the plants decreased with time. Nearly all of the radioactivity removed from the exposure solution was detected in the plants. Dissected plant tissues of *A. gramenius* revealed significant accumulations of radioactivity primarily in the leaves and to a lesser extent in the roots. No accumulation was seen in the rhizomes, indicating that they serve primarily as a pathway for further transport. Analysis of dissected plant tissues of *P. cordata* showed accumulation of radioactivity in all plant parts. However, the majority

of the radioactivity accumulated in the leaf petioles and blades after more days of exposure. In this study, plant-incorporated simazine was acropetally distributed from the roots primarily into the leaves. Simazine uptake was directly related to water uptake with the transpiration stream.

Simazine was primarily accumulated in the leaves of parrot feather (*Myriophyllum aquaticum*) and canna (*Canna hybrida* L.) when those plants were grown in simazine-contaminated water (Knuteson et al., 2002). They concluded that four-week old parrot feather and canna were more tolerant of simazine than two-week old plants. The tissue burden with simazine, normalized for plant biomass, was less for the four-week old plants. The authors attributed simazine biodegradation to dealkylation of simazine. They suggested that simazine could be metabolized either to CEAT or to EEOT followed by storage of end products in the vacuoles. However, they also concluded that the main form of detoxification was through conjugation to glutathione using glutathione-S-transferases. In general, the foliage of plants has the highest levels of glutathione and glutathione-S-transferases. Hatton et al (1996) had demonstrated that the levels of glutathione in maize plants declined after 30 days of growth. However, in the work of Knuteson et al. (2002), plant tolerance for simazine increased with plant age and size. Both parrot feather and canna were more vulnerable to simazine shortly after emergence (Knuteson et al., 2002).

Other pesticides

Herbicide phytoremediation with semi aquatic plants has been examined in the horticulture industry (Fernandez et al., 1999). Three species including *Canna generalis* (canna), *Pontaderia cordata* (pickerel weed), and hybrid Iris were cultured in the presence of 5 mg/L isoxaben or oryzalin for 9 days. These levels were chosen to represent actual reported levels in runoff water from nursery operations. Isoxaben was toxic to all three species, while pickerel weed was the most susceptible to oryzalin. Functional parameters of the plants including growth and photosynthesis (chlorophyll fluorescence) were monitored and indicated that isoxaben was directly affecting photosynthesis, whereas the impact of oryzalin was elsewhere. Oryzalin is a mitosis inhibitor and may have worked primarily by damage to root function. All three species could be effective in phytoremediation of oryzalin but none proved satisfactory for isoxaben at the levels tested. The variability of response of these semi aquatic plants, and woody shrubs referred to above, indicates that appropriate selection of plants useful for remediation of some herbicides, such as isoxaben, may be difficult and may depend on empirical selection procedures. See section 3.3.3 below for a listing of plants that tolerate single dosing of isoxaben as normally applied in commercial production. Some of these may prove effective for phytoremediation of incidental over spray or run-off.

The uptake and metabolism of organophosphorous (OP) pesticides was investigated using aquatic plants such as parrot feather (*Myriophyllum aquaticum*), duckweed (*Spirodela oligorrhiza*), and elodea (*Elodea canadensis*) (Gao et al., 2000a). The decay kinetics of

those pesticides from the aqueous medium followed first-order kinetics. However, rate and extent of decay depended on both the physiochemical properties of the pesticide compounds and the nature of the plant species (Gao et al., 2000a). This study indicated that OP pesticides could be taken up by aquatic plants and subsequently transformed with the subsequent plant-mediated transformation attributed to enzymatic reactions.

Another uptake study was conducted on the organochlorine (OC) pesticide, dichloro-diphenyl-trichloro-ethane (DDT), using parrot feather, duckweed, and elodea (Gao et al. 2000b). The decay profile of DDT from the aqueous culture medium followed first-order kinetics for all three plants. Almost all of the DDT was removed from the medium in a 6-day period, and most of it accumulated in or was transformed by those plants. The authors concluded that plant-mediated metabolism/degradation of DDT in all three aquatic plants may be at least partly attributed to dehalogenation and dehydrochlorination reactions. Those reactions appeared to involve a general plant dehalogenase. In addition, even more DDT was apparently irreversibly bound to the plant tissue (Gao et al., 2000b; Garrison et al., 2000).

Water hyacinth (*Eichornia crassipes*) is a rapidly growing tropical species, which has been tested for use in phytoremediation of several pesticides (Xia et al., 2002a,b). The presence of water hyacinth, at a level of about 1 g.f.w./25 mL treatment solution greatly stimulated the disappearance of ethion, dicofol and cyhalothrin from solution concentrations of 1 mg/L over a 10-day treatment. For methyl parathion, a potent insecticide, the presence of water hyacinth increased disappearance from 40 to 99% in a

10-day exposure, beginning with 10 mg/L. In all instances there was a microbial, as well as a plant contribution to the degradation process, but in each case the majority of the effect was attributable to plants.

The metabolism of halogenated organic compounds, including pesticides, through the corresponding oxidative and reductive transformation products identified in the aquatic plant rhizosphere, stems, and leaves suggested that more than one pathway, requiring different enzymes, may be involved in phytotransformation reactions (Nzengung and Jeffers, 2001). Four mechanisms were found to be important in the removal of the halogenated organic compounds from water by aquatic plants (parrot feather and elodea), namely (1) rapid sequestration by partitioning to the lipophilic plant cuticles; (2) phytoreduction to less halogenated metabolites; (3) phytooxidation; and (4) assimilation into plant tissues as nonphytotoxic products, presumably produced by covalent binding with the plant tissues.

3.3.3. Field and Horticultural Crops/Grasses

Insecticides

Field crops grown in insecticide treated soils may contain measurable amounts of insecticides in the top portion of the plant. Translocation of chlorinated hydrocarbon insecticides in food crops has been studied extensively (Lichtenstein, 1959, 1960; Lichtenstein and Schulz, 1960, 1965; Lichtenstein et al., 1965). Most such studies were conducted with root crops (Harris and Sans, 1967). A few studies were conducted with cucumbers (*Cucumis sativus*) (Lichtenstein, 1960), lettuce (*Lactuca Sativa*) (Lichtenstein,

1960), alfalfa (*Medicago sativa* L.) (King et al., 1966; Zins et al., 1991), canola (*Brassica napus* L.) (Dupont and Khan, 1993), and soybeans (*Glycine max* L.) (Bruce and Decker, 1966). Generally, terrestrial plants do not accumulate highly non-polar chlorinated hydrocarbons; however, hexachlorocyclohexane (HCH), is known to be taken up and translocated by plants while DDT is mainly found associated with roots (Lichtenstein and Schulz, 1960). Minimal translocation of insecticides was detected in oat plants grown in soil with bound residues of DDT and HCH as compared to organophosphorus and carbamate compounds (Fuhremann and Lichtenstein, 1978). Most of the DDT and HCH residues accumulated by the oat plants were found bound to the plant tissues. The plant-bound residues of parathion in oats were 5% in greens and 18% in roots when oats were grown in freshly treated soil or soil with bound residues (Fuhremann and Lichtenstein, 1978). Thus, a significant fraction of the DDT and HCH is transformed by the plants.

Soybeans and cotton (*Gossypium hirsutum* L.) were grown in soils contaminated with 0.5 ppm each of ^{14}C-DDT, -dieldrin, -endrin, and –heptachlor. The top portions of the plants were harvested periodically to assay for insecticide (Nash et al., 1970). At maturity, seed and cotton lint were also assayed. Total ^{14}C-residue concentrations in soybeans for all four insecticides increased linearly during active growth stages, whereas ^{14}C-residues in cotton were found highest at the first harvest. No parent DDT or heptachlor was detected in any of the soybean or cotton plant samples. Heptachlor epoxide was present in soybeans, however there was no heptachlor epoxide found in cotton (Nash et al., 1970). This shows that the transformation of heptachlor and possibly other organochlorine pesticides by soybeans would be via epoxidation.

The effects of DDT on the germination and growth of various plants were studied by Mitra and Raghu (1989). Of the species tested, oil-rich seeds of plants, such as peanut (*Arachis hypogaea*) and mustard (*Brassica juncea*), were more prone to DDT induced inhibition of germination and subsequent plant growth than cereals, pulses and fiber crops, like rice (*Oryza sativa*), barley (*Hordeum vulgare*), mung bean (*Vigna radiata*), pigeon pea (*Cajanus cajan*), and cotton (*Gossypium hirsutum*). It is suggested that lipids of the plant cell solubilize and disperse DDT in the cytoplasm, which, in turn, affects normal metabolism within the cell (Mitra and Raghu, 1989). While the growth of peanut and mustard plants was significantly inhibited at 100 ppm of DDT, cotton plants were not affected, although cotton could be classified as an oilseed crop because of high oil content. Based on their study, barley was chosen as representative plant with high tolerance and peanut as one of the low tolerant plants to DDT. Soybean was considered of medium tolerance to DDT. Barley showed no effect even at 1000 ppm of DDT in soil.

Rapid uptake of organochlorine and organophosphate pesticides into plants containing high fatty acid contents is a very common phenomenon. Residues of aldrin and heptachlor in seeds were reported to be directly related to the oil content of the seeds (Nash et al., 1970). High uptake of DDT by tobacco plants was also correlated to the high fatty acid content of this plant (Rosa and Cheng, 1973). The binding of DDT to the lipid fraction of the cell membrane might affect membrane permeability and transport in root cells. This, in turn, may affect the uptake and translocation of essential plant nutrients from soil to plants, resulting in inhibition of plant growth.

Verma and Pillai (1991) studied bioavailability of soil-bound residues of DDT and HCH to green gram (*Cicer arietinum*), maize (*Zea mays*), and dryland rice (*Oryza sativa*). Uniformly labeled ^{14}C-DDT and ^{14}C-HCH were used to form bound residues in sandy loam soil. The results clearly indicated that gram, maize, and rice plants accumulate soil-bound residues of DDT and HCH. The majority of the residues assimilated by the plants were found to be associated with the roots while the shoots had minimal residues, suggesting low translocation of DDT and HCH in the plants. This was attributed to the low water solubility of the insecticides. Generally, binding of DDT was more in the shoots and that of HCH in the roots (Verma and Pillai, 1991). They also showed that 8-11% of the bound residues of DDT and HCH in soil were converted to extractable forms in the soil after planting. This conversion may be carried out by microbial activity associated with the plants. From their study, it is apparent that soil-bound residues of DDT and HCH are susceptible to environmental interactions when present in the root zone. However, the toxicological consequences of translocated residues may be minimal, as the concentrations of residues accumulated are much less as compared to comparable insecticide residues when available in labile form (Verma and Pillai, 1991).

Kiflom et al. (1999) studied the extent to which DDT may be absorbed and translocated from contaminated soils into cowpea (*Vigna sp*.) plant tissues. Substantial absorption and accumulation of residues was observed for the cowpeas grown in DDT contaminated soils. Within a period of 90 days, DDT residues in the cowpea plants ranged from 1 to 8 mg/kg, while that in soils ranged from 90 to 32.0 mg/kg (Kiflom et al., 1999). White

(2000) conducted a series of field experiments to determine the efficacy of plant-assisted remediation of weathered (> 30 years) *p,p'*-DDE ((2,2-bis(p-chlorophenyl)1,1-dichloroethylene)) residues in agricultural soil. Alfalfa, ryegrass (*Lolium multiflorum*), and pole bean (*Phaseolus vulgaris*) were planted in soil containing 185 to 230 ng/g of *p,p'*-DDE. There were statistically significant decreases in the concentration of *p,p'*-DDE in soils with alfalfa and ryegrass. No measurable concentration of *p,p'*-DDE was found in the shoots of any of the plants (White, 2000). The role of organic acids in plant uptake of *p,p'*-DDE was examined in a separate study (White, 2003). Addition of low molecular weight organic acids caused partial dissolution of the soil structure by chelating inorganic structural ions, resulting in increased bioavailability of persistent organic pollutants for plant uptake (White, 2003). Field experiments were conducted to assess bioavailability of weathered *p,p'*-DDE in soil to plants in the *Cucurbita* (squash and pumpkin) and *Cucumis* (cucumber and melon) genera (White, 2003). Significant variability existed in the uptake of *p,p'*-DDE between plants of different genera. For all plants, the highest concentration of *p,p'*-DDE was in the roots. However, this concentration comprised less than 2% of the total plant biomass (White, 2003).

What we can conclude from studies such as these is that many species are likely to take up enough of the pesticide that it may pose a significant risk to organisms consuming the plants. DDT and HCH are well known to bioaccumulate strongly in animal species. Because plants seem able to convert only a portion of DDT and HCH to bound and less toxic forms, application of plants for phytoremediation of these materials will require considerable caution. While DDT is banned in the U. S. it is still in use in other parts of

the world and safe means of remediation are still being sought. For other highly chlorinated hydrocarbons such as biphenyls, plants have shown some success (Davis et al., 2002; McCutcheon and Schnoor, 2003).

A comprehensive study was conducted of the uptake of weathered soil residues of chlordane, an organochlorine insecticide, and its translocation in the tissues of food crops under both greenhouse and field conditions (Mattina et al., 2000). Chlordane was detected in the edible root tissue of the three root crops examined; carrots (*Daucus carota*), beets (*Beta vulgaris*), and potatoes (*Solanum tuberosum*) and detected in the edible aerial tissue of spinach (*Spinacia oleracea*), lettuce, dandelion (*Taraxacum sp*.), and zucchini (*Cucurbita sp*.). It was not detected in edible aerial tissue of tomatoes (*Lycopersicon esculentum*), peppers (*Capsicum sp*.), and maize and only trace amounts were detected in bush beans and eggplants (*Solanum melongena*). Mattina et al. (2000) concluded that under field conditions, soil-to-plant transport dominated compared to the air-to-plant uptake route. Zucchini was found to bioaccumulate chlordane very efficiently in its edible fruits under controlled conditions (Mattina et al., 2000). Since there was no metabolite detected in this study, it seems that all those plants efficiently translocated chlordane via the transpiration stream and it bioaccumulated in the tissues. White (2003) studied cycling of chlordane in soil with zucchini. Zucchini was planted in a greenhouse in three bays containing chlordane-contaminated soil. Significant levels of chlordane were detected in the vegetation, the amount varying in different plant tissues from a maximum in roots to a minimum in fruit (White, 2003).

Emametin benzoate, an effective lepidopteran insecticide was not found to be accumulated in rotational crops, representing small grains (barley), root crops (carrots), and leafy vegetables (lettuce) (Chukwudebe et al., 1996).

Herbicides

While the largest application of herbicides and insecticides occurs for field crops and forages, there are significant uses of both in horticulture, particularly in the production phases for trees, shrubs and herbaceous plants that are to be distributed to home growers. Examples were cited in previous sections for plants tested for potential ability to assist phytoremediation of contaminated water. A number of tests have been done for pre-emergent herbicides to determine their suitability for use in containerized plant production. These may serve as useful indicators of the likely tolerance of comparable cultivars of the same species of plants, which might be applicable in phytoremediation. A few recent examples are described here. There is a broad, dispersed literature on tolerance, some of which is cited by the authors whose work is discussed here. Proceedings of regional weed control and weed science societies report such studies.

Bhandary et al. (1997) examined the response of shrubby species including Gardenia, Ilex (holly), and Rhododendron, and the monocots Pennisetum (fountaingrass) and Hemerocallis (daylily) when treated with two concentrations of oryzalin, oxyfluorfen, and isoxaben (1 and 10 mg/L). There were few effects. The high dosage of oryzalin and oxyfluorfen inhibited fountaingrass while the high level of oryzalin inhibited daylily. The high level of isoxaben inhibited root growth in the holly. The levels used for treatment

were equivalent to those reported to be encountered in irrigation run-off during nursery operation.

Neal and Senesac (1990) did extensive studies with isoxaben alone and in combination with oryzalin and trifluralin, and in comparison to simazine. Isoxaben was originally released for control of broadleaf weed in cereals. It is evidently resistant to phytodegradation and can accumulate to phytotoxic levels in woody plants, as indicated in section 3.3.1.

Diflufenican, a widely used preemergence or early postemergence carboxyamide-herbicide to control weeds in cereals in Europe, was not readily taken up in main (wheat) and rotational (maize) crops (Conte et al., 1998). Soybean and canola (*Brassica napus*) were the most successful plant species in the germination and survival tests conducted with soils contaminated with atrazine (Bruce and Decker, 1966). Dupont and Khan (1993) studied the fate of bound and extractable ^{14}C residues in resistant and susceptible varieties of canola plants treated with radiolabelled atrazine. The results from the study showed that both a resistant and a susceptible variety of canola metabolized atrazine and formed bound residues in almost equal proportions. It was concluded that bound residue formation does not contribute to the differential tolerance of the two-canola varieties (Dupont and Khan, 1993). However, in several plants, the tolerance mechanism for toxic agrochemicals would be by forming bound residues of toxic contaminants with plant tissues. The key issues are the toxicity and bioavailablity of such residues.

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Table 1. Non-target effects of herbicides [This table is mostly from Obrigawitch et al. 1998]

Non-target Plant	Exposure Mechanism	Effect(s)
Trees		
Douglas Fir (*Abies sp*.)	crop injury	stunted vegetative growth
Maple, red (*Acer rubrum*)	crop injury	no apparent effect
Pear (*Pyrus communis*)	Drift	no apparent effect
Peach (*Prunus persica*)	Drift	fruit size reduction
Pine (*Pinus taeda*)	Drift	no apparent effect
Field Crops/Grasses		
Alfalfa (*Medicago sativa*)	windblown soil	stunted vegetative growth, reduced seed yield and root weight, and reduced root nodulation
Barley (*Hordeum vulgare*)	crop injury	reduced seed yield
Buckwheat (*Fagopyrum esculentum* Moench)	crop injury	reduced seed size
Canola (*Brassica napus*)	Drift	delay in crop maturity and reduced seed yield
Corn (*Zea mays*)	drift, windblown soil	reduced seed yield
Cotton (*Gossypium hirsutum*)	Drift	flower number, fruit set, and boll size were unaffected
Red fescue (*Festuca* rubra L.)	Drift	seed yield, weight, and number decreased
Ryegrass (*Lolium perenne* L.)	Drift	seed yield, weight, and number decreased
Sorghum (*Sorghum vulgare*)	Drift	no apparent effect
Soybeans (*Glycine max*)	Drift	no apparent effect
Sunflowers (*Helianthus annuus*)	Drift	Reduced seed yield, visual injury, and reduced emergence
Wheat (*Triticum aestivum*)	crop injury	Visual injury and nutritional malfunction

Table 2. Response of non-target plants to pesticides.

Response
Complete death or stunted growth (Norris and Freed, 1966a,b, and c; Dhillion et al., 1968)
Tolerance (Hamner and Tukey, 1946; Pallas, 1963; Leonard et al., 1966)
Acclimation (Dickinson et al., 1991)
Detoxification (Lund-Hoie, 1969; Wichman and Byrnes, 1975; Burken and Schnoor, 1996; 1997)
Metabolism (Burken and Schnoor, 1996; 1997; 1998; Schnabel and White, 2001)
Mutation and resistance (Sandermann, 1992)
Phenotype alteration (Sandermann, 1992)
Root or shoot uptake (Wichman and Byrnes, 1975; Akinyemiju et al., 1983; Burken and Schnoor, 1996)
Translocation (Lund-Hoie, 1969; Gao et al., 2000a; 2000b; Wilson et al., 2000)

Table 3. Enzymes detected in plants responsible for detoxification of various pesticides (Hall et al., 2001).

Enzymes
Cytochrome 450
Peroxidases
Aryl Acylamidases
Esterases
Lipases
Proteases
Amidases
Oxygenases
Reductases

Table 4. Names and structures of s-triazine compounds shown in Figure 1 [This table is from Erickson and Lee, 1989]. See Figure 2 for general structure of s-triazine compounds.

Names and abbreviations	Structure
Atrazine 2-chloro-4-ethylamino-6-isopropylamino-s-triazine [CIET]*	$R_2 = Cl$ $R_4 = C_2H_5N$ $R_6 = i\ C_3H_7NH$
Simazine 2-chloro-4,6-bis(-ethylamino)-s-triazine [CEET]	$R_2 = Cl$ $R_4 = C_2H_5NH$ $R_6 = C_2H_5NH$
Deethylatrazine 2-chloro-4-amino-6-isopropylamino-s-triazine [CIAT]	$R_2 = Cl$ $R_4 = NH_2$ $R_6 = i\ C_3H_7NH$
Deethylsimazine 2-chloro-4-amino-6-ethylamino-s-triazine [CEAT]	$R_2 = Cl$ $R_4 = NH_2$ $R_6 = C_2H_5NH$
2-chloro-4,6-diamino-s-triazine [CAAT]	$R_2 = Cl$ $R_4 = NH_2$ $R_6 = NH_2$
2-chloro-4-amino-6-hydroxy-s-triazine [CAOT]	$R_2 = Cl$ $R_4 = NH_2$ $R_6 = OH$

Names and abbreviations	Structure
2-chloro-4-amino-s-triazine [CAHT]	$R_2 = Cl$ $R_4 = NH_2$ $R_6 = H$
Hydroxyatrazine 2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine [IEOT]	$R_2 = OH$ $R_4 = C_2H_5NH$ $R_6 = i\ C_3H_7NH$
Hydroxysimazine 2-hydroxy-46-bis(ethylamino)-s-triazine [EEOT]	$R_2 = OH$ $R_4 = C_2H_5NH$ $R_6 = C_2H_5NH$
N-Ethylammeline 2-ethylamino-4-amino-6-hydroxy-s-triazine [EAOT]	$R_2 = C_2H_5NH$ $R_4 = NH_2$ $R_6 = OH$
N-Ethylammelide 2-ethylamino-4,6-dihydroxy-s-triazine [EOOT]	$R_2 = C_2H_5NH$ $R_4 = O$ $R_6 = OH$
Melamine 2,4,6-triamino-s-triazine [AAAT]	$R_2 = NH_2$ $R_4 = NH_2$ $R_6 = NH_2$

Names and abbreviations	Structure
Ammeline [AAOT]	$R_2 = NH_2$ $R_4 = NH_2$ $R_6 = OH$
Ammelide [AOOT]	$R_2 = NH_2$ $R_4 = OH$ $R_6 = OH$
Cyauric acid [OOOT]	$R_2 = OH$ $R_4 = OH$ $R_6 = OH$

*The four-letter code has one letter for each substituent [A = amino, C = chloro, E = ethlamino, I = isopropylamino, O = oxo (or hydroxy when written as the enol tautomer) and T for the s-triazine ring].

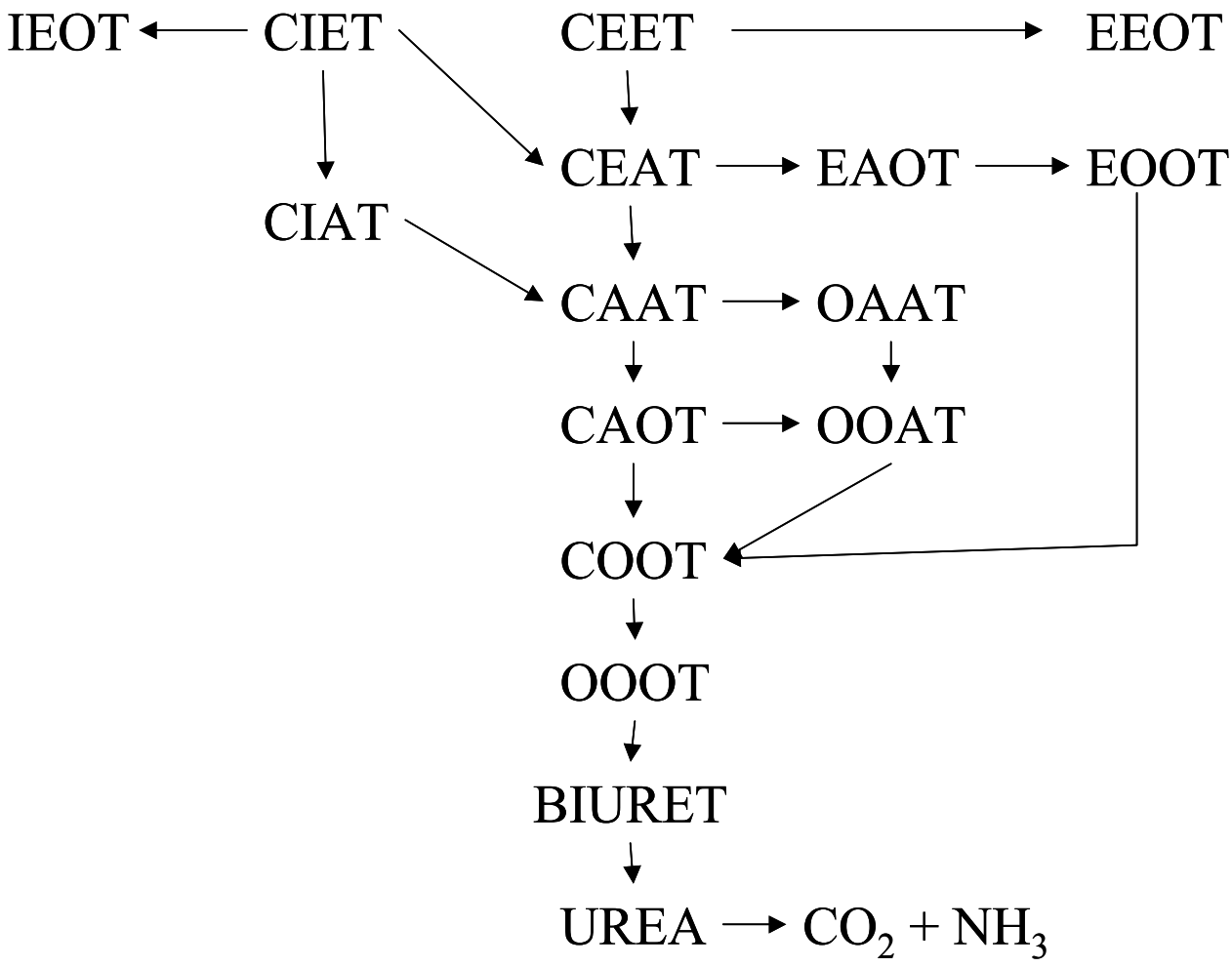

Figure 1. Degradation pathways for atrazine (CIET) and simazine (CEET) and related compounds. See Table 4 for nomenclature. [This figure is modified from Erickson and Lee (1989)].

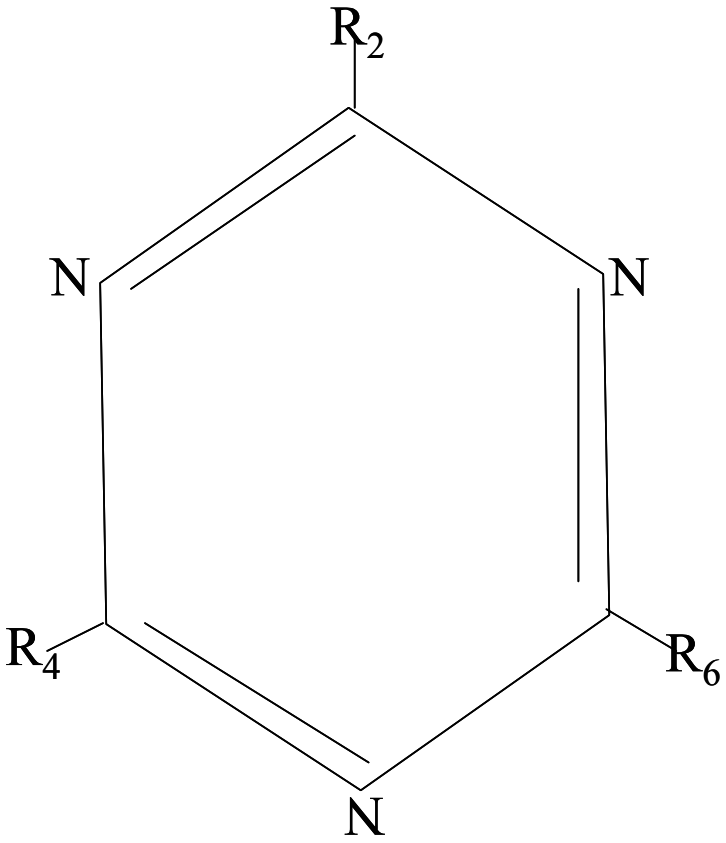

Figure 2. General structure of s-triazine herbicides and related degradation products. R_2, R_4, and R_6 are identified in Table 4 for each s-triazine considered in this manuscript.