

# 1 Molecular Ecotoxicology: From Man-Made Pollutants to Multiple Environmental Stresses

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## 1.1 Overview

All life forms depend on plants as primary producers of food and feed and of substrates for decomposition. In the mid-1960s, it was realized that the productivity and biodiversity of whole ecosystems, including their vegetation, were threatened by man-made pollutants. Later on, other natural and man-made influences, such as climatic extremes, pathogens, herbivores or air pollution, were also studied as environmental stressors. The existence of highly effective plant acclimation and stress defense mechanisms is demonstrated by the occurrence of plants on extreme environmental sites and by the resistance of plants to most pathogens. Plants can also detoxify man-made chemicals, as shown by the selective action of many herbicides that kill weeds without inhibiting the growth of crop plants.

The defense mechanisms of plants are generally of a multipurpose nature. For example, the enzyme families detoxifying man-made chemicals can also protect against toxic metabolic by-products and natural toxins. Plant substances protecting against UV-B can also be antioxidants and antibiotics. Plant genes involved in pathogen defense can also be important for defense against air pollutants, and genes of normal protein assembly may be involved in protection against elevated temperature. Such cross-protective mechanisms are in many cases still tentative, but the recently completed plant genome projects and current array methods, as well as genetic overexpression or knock-out, are likely to identify the signal chains and molecular response programs involved. The purpose of this book is to provide an introduction into the newly developing research field of molecular ecotoxicology and to present a number of case studies.

## 1.2 Definitions of Ecotoxicology

Ecotoxicology is defined here as the science of the occurrence, transformation, bioavailability or exposure and the mode of action of physical, chemical and biological environmental stress factors. Ecotoxic effects need to be determined for individual plant species, for populations and biocoenoses and, finally, for whole ecosystems.

In the early 1960s, scientists first became aware of the detrimental effects of man-made pollutants (Farb 1965). The famous book, *Silent Spring*, by Rachel Carson (1962) had made a vast public aware of the possible toxic side effects that chlorinated insecticides and herbicides exerted on non-target organisms in the environment, the emphasis being on bird populations. The widespread use of insecticides had led to the emergence of resistant insect populations (Carson 1962; Farb 1965). Later on, pathogens resistant to fungicides appeared in agricultural ecosystems, and the spontaneous herbicide resistance of weeds also became a widespread problem (LeBaron and Gressel 1982). In the case of atrazine, it could be demonstrated that a point mutation in the chloroplast *psb A* gene led to an amino-acid change that made the chloroplast target protein insensitive to atrazine (Hirschberg and McIntosh 1983). By this mechanism, the empirical observation of herbicide resistance was explained at the molecular level. Later research clarified more mechanisms of herbicide resistance and its spread in weed populations (Powles and Holtum 1994). These various results document an early thorough case study of molecular ecotoxicology of plants, molecular mechanisms being successfully scaled up to the ecosystem level. Fungal subspecies with spontaneous fungicide resistance and insect subspecies with spontaneous insecticide resistance have also increased dramatically. These unintended developments can now be useful for the ecological assessment of transgenic plants with pesticidal traits (see Chapter 8).

Carson described many examples of undesirable ecotoxic side effects of pesticides, but the term “ecotoxicology” was used only subsequently when large-scale scientific research and legislative efforts were initiated. For example, the USA established the Toxic Substances Control Act (1976), and Germany banned DDT in 1972 and established a Chemicals Law in 1980. Many test procedures were developed to determine the ecotoxic character of anthropogenic pollutants. Bioaccumulation and biodegradation processes became understood, and today predicted environmental concentrations and predicted no-effect levels of chemicals are known at least for short-term tests and defined test species (including algae and higher plants).

Concerning plants, the modes of action and of selectivity of herbicides have been studied in particular detail. The target proteins and their genes often are unique for plants, such as in photosynthesis and branched-chain or aromatic amino acid biosynthesis. The absence of these targets in humans

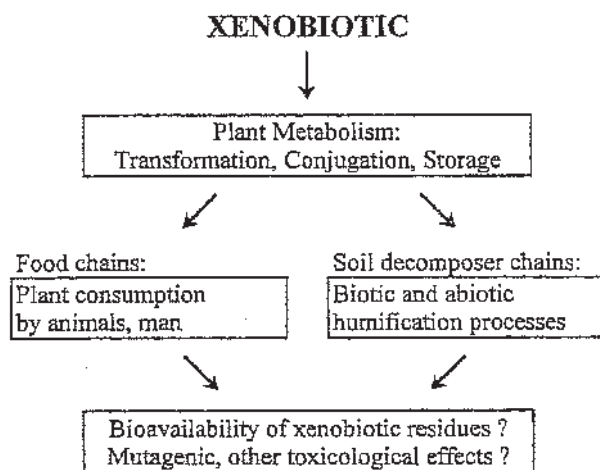


Fig. 1.1. Schematic summary of the toxicological potential of xenobiotic plant metabolites. (Sandermann 1994)

and animals could secure a low level of toxic side effects. Plants have been found to resemble animals in having similar metabolic enzymes of transformation, conjugation and compartmentalization. This similarity has led to the “green liver” concept and to the summary of ecotoxicological significance of plant pesticidal residues that is shown in Fig. 1.1. As in animals, plant metabolism can result in detoxification or bioactivation. Remarkably, similar enzyme classes were found to metabolize xenobiotics and natural substrates (Table 1.1; Sandermann 1992). These early observations have been confirmed and much extended by the detection of large homologous gene families in *Arabidopsis thaliana* (The *Arabidopsis* Genome Initiative 2000). This example already illustrates the enormous gain in information by present genome projects. Enzyme families, e.g., of cytochromes P450 and glucosyltransferases, are responsible for xenobiotic metabolism, but also for regulation of concentrations of free signalling molecules such as jasmonic and salicylic acids, auxins and cytokinins. Mutagenic effects of environmental stressors have recently been visualized using the recombination of a split and inactive  $\beta$ -glucuronidase marker gene to yield active enzyme producing a blue color. In this way, toxic effects of realistic UV-B (Ries et al. 2000) and heavy metal ions (Kovalchuk et al. 2001) were demonstrated in a dose-dependent manner.

Ecotoxicology was initially developed as a scientific discipline limited to studying the biological consequences of anthropogenic chemicals in the environment (e.g., Korte 1980). A broader definition has included any physical (heat, radiation), chemical or biological factor that creates a potential source of pollution (Ramade 1979). This extended definition still limited the scope of ecotoxicology to man-made pollutants, whereas the present definition also includes natural substances as well as physical parameters. Some of these stressors are listed in Table 1.2. In the typical situation of low-level exposure to man-made pollutants, their ecotoxic effects may be masked by those of nat-

**Table 1.1.** Plant enzyme classes for either xenobiotic or natural substrates. (Sandermann 1992)

Enzyme class	Xenobiotic substrates	Natural substrates
Cytochromes P450	4-Chloro- <i>N</i> -methyl-aniline	Cinnamic acid, pterocarpan
Glutathione <i>S</i> -transferases	Fluorodifen, alachlor, atrazine	Cinnamic acid
Carboxylesterases	Diethylhexylphthalate	Lipids, acetylcholine
<i>O</i> -Glucosyltransferases	Chlorinated phenols	Flavonoids, coniferyl alcohol
<i>O</i> -Malonyltransferases	$\beta$ -D-Glucosides of penta chloro-phenol and of 4-hydroxy-2,5-di-chloro-phenoxyacetic acid	$\beta$ -D-Glucosides of flavonoids and isoflavonoids
<i>N</i> -Glucosyltransferases	Chlorinated anilines, metribuzin	Nicotinic acid
<i>N</i> -Malonyltransferases	Chlorinated anilines	1-Aminocyclopropyl carboxylic acid, D-amino acids, anthranilic acid

**Table 1.2.** Selected environmental stress factors

Abiotic	Biotic
Air pollutants	Allelopathy
Allelochemicals	Herbivores
Fire	Microbial pathogens
Heavy metals	Viral pathogens
Salinity	Nematodes
Mechanical stress	Plant/plant competition
Nutrient deficiencies	Pollinators
Pesticides	Symbiont availability
Radiation	
Shading	
Temperature extremes	
Toxins	
Water deficit or excess	

ural stressors. In any case, plants are seen as targets as well as producers of environmental stress, both aspects being involved in determining plant fitness.

### 1.3 Environmental Stress

It has long been known that many stress and selection factors exist in the environment. Ernst Haeckel, in 1866, first used the term “ecology” in the sense of the “economy of nature” and the “science of all interactions between organisms and their environment.” The ability of organisms to adapt to their environmental conditions seemed important to withstand parasitism and to fight for survival (Haeckel 1911). Exposure to the natural environment thus was seen already to constitute stress. It is still a difficult problem to judge the significance of superimposed anthropogenic stress in ecosystems. The book, *Silent Spring*, (Carson 1962) presented the following example of natural ecotoxic stress. Roses in a park in a city in Holland suffered from heavy infestations by tiny nematode worms. Instead of chemical treatments, marigolds were planted among the roses. The marigolds released an excretion from their roots that killed the soil nematodes and allowed the roses to flourish. This story is based on the allelopathic principle first described by H. Molisch in 1937. Allelopathy has been defined as chemical competition between plants (Harborne 1993). Another natural ecotoxic principle, that of phytoalexins, was first described by Müller and Börger in 1941. Phytoalexins are plant compounds that are toxic for fungi. These initial and later discoveries were well summarized in Harborne's *Introduction to Ecological Chemistry* (1993).

Plants employ the two mentioned and many other chemical mechanisms to defend themselves against a broad range of biotic and abiotic stressors. The active principles of plants can be seen as “natural pesticides” that can be constitutive (Wittstock and Gershenzon 2002) or induced (Ebel 1986; Harborne 1993). As with man-made pesticides, there are natural herbicides (allelochemicals), fungicides (phytoalexins), antibiotics, insecticides, acaricides, etc., all of which can also exert non-target effects on plants as well as animals and humans. Natural pesticides can be acutely toxic (e.g., alkaloids), estrogenic (e.g., soybean isoflavonoids) or neurotoxic (e.g., hallucinogens). Natural plant compounds thus open a broad field of toxicology and ecotoxicology with basically the same questions as in the case of man-made chemicals. A greatly simplified sequence to analyze the stress exposure of plants is outlined in Fig. 1.2.

Many physical and environmental stressors act on plants as mimics of biotic elicitors, which were first characterized in the case of cell wall fractions of the plant-pathogenic oomycete *Phytophthora megasperma*. This elicitor was defined by its ability to induce phytoalexins in soybean plants and cell cultures. Subsequently, the elicitor was recognized also to induce many other