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Ministry of Environment and Energy  
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# **Ecological Effects of Allelopathic Plants – a Review**

*NERI Technical Report, No. 315*



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# **Ecological Effects of Allelopathic Plants – a Review**

*NERI Technical Report, No. 315  
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Abstract: In this report actual literature concerning allelopathy has been reviewed. The objective of the report has been to discuss the potential of allelopathy in relation to genetically modification of crops and on this background to discuss how allelopathic crops may interfere with the environment through spread of GM-plants or transgenes outside agricultural areas. The last chapter discuss GM-allelopathic plants in relation to the ecological risk assessment.

Keywords: GMP; ecological risk assessment; allelopathy; ecological effects

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## **Preface**

This report is based on a literature review on allelopathy from an ecological impact point of view carried out in 1999. The study was initiated because recently published research results have suggested that the allelopathic activity of agricultural crops can be improved by genetic engineering.

The report describes allelopathy of selected crops and also summarises available information concerning the genetic studies on allelopathy in these crops. It discusses the ecological effects of allelopathic plants in natural ecosystems and factors of importance for the effects of these plants are pointed out. Finally the report presents suggestions for an ecological risk assessment of crops with an enhanced release of allelochemicals.

The report has been thoroughly reviewed and commented by Jan G. Højland from the National Forest and Nature Agency, Gösta Kjellsson, Christian Kjær and Helle Ravn from the National Environmental Research Institute.





# 1 Background

## *History*

The phenomenon of allelopathy, where a plant species chemically interferes with the germination, growth or development of other plant species has been known for over 2000 years. Statements as early as 300 years BC points to the phenomenon that many crop plants, including chick pea (*Cicer arietinum*) and barley (*Hordeum vulgare*), inhibit the growth of weeds and crop plants other than barley (Rice 1984).

## *The term allelopathy*

The term allelopathy, was introduced by Molisch in 1937, and is derived from the Greek words allelon 'of each other' and pathos 'to suffer' and mean the injurious effect of one upon the other (c.f. Rizvi et al. 1992). However, the term is today generally accepted to cover both inhibitory and stimulatory effects of one plant on another plant (Rice 1984). Some use the term in a wider sense, for instance entomologists, who include the effects of secondary compounds on plant-insect interactions. In 1996 The International Allelopathy Society defined allelopathy as follows: "*Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects*" (Torres et al. 1996).

## *Definition*

In the following, the term is used in accordance with Rice (1984), but effects of the chemical compounds involved in plant-plant interactions and the effects of allelopathic plants are discussed in a broader perspective than strictly related to the plant-plant interactions.

## *Allelochemicals*

Chemicals released from plants and imposing allelopathic influences are termed allelochemicals or allelochemicals. Most allelochemicals are classified as secondary metabolites and are produced as offshoots of the primary metabolic pathways of the plant. Often, their functioning in the plant is unknown, but some allelochemicals are known also to have structural functions (e.g. as intermediates of lignification) or to play a role in the general defence against herbivores and plant pathogens (e.g. Einhellig 1995, Corcuera 1993, Niemeyer 1988).

## *Multifunctional compounds*

Allelochemicals can be present in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flowers. Allelochemicals are released into the environment by root exudation, leaching from aboveground parts, and volatilisation and/or by decomposition of plant material (Rice 1984).

When susceptible plants are exposed to allelochemicals, germination, growth and development may be affected. The most frequent reported gross morphological effects on plants are inhibited or retarded seed germination, effects on coleoptile elongation and on radicle, shoot and root development.

## 1.1 Prospects for the application of allelopathy to farming

Allelopathic interactions between plants have been studied in both managed and natural ecosystems. In agricultural systems allelopathy can be part of the interference between crops and between crops and weeds and may therefore affect the economical outcome of the plant production. Both crop and weed species with allelopathic activity are known (e.g. Inderjit & Dakshini 1998, Inderjit & Foy 1999, Putnam & Weston 1985, Weston 1996).

Recently, several papers have suggested that allelopathy holds great prospects for finding alternative strategies for weed management. Thereby, the reliance on traditional herbicides in crop production can be reduced (An et al. 1998, Inderjit & Keating 1999, Macias 1995, Macias et al. 1997, Macias et al. 1998b, Olofsdotter 1998a, 1999, Wu et al. 1999). Today, the allelopathic activity of some crops, for example rye, is to some extent used in weed management (Weston 1996, Olofsdotter 1998b).

### *Enhanced allelopathic activity*

The search for genes involved in the production of allelopathic compounds in crops has begun, see chapter 3. This widens the opportunity for improving the allelopathic activity of crops through traditional breeding strategies or by genetic engineering. Biotechnological transfer of allelopathic traits between species has been suggested as a possibility and this could for example be from wild or cultivated plants into commercial crop cultivars (Chou 1999, Macias 1995). So far, a genetically modified plant with enhanced allelopathic activity has not been marketed.

### *Natural herbicides*

Another research area within allelopathy is the search and development of new herbicides through the isolation, identification and synthesis of active compounds from allelopathic plants (e.g. Duke 1998, Macias et al. 1997, Macias et al. 1998a, 1998b). These compounds are often referred to as 'natural herbicides' see section 2.3.

### *Reduced pollution?*

From the agronomic point of view, the research in allelopathy provides perspectives of a reduced reliance on traditional herbicides if weed control can be achieved by the release of allelochemicals from the crop. Also, in cropping systems where herbicides are not used, for example in organic farming, crop cultivars with enhanced allelopathic activity could be part of the weed management strategy.

Weed control mediated by allelopathy - either as natural herbicides or through the release of allelopathic compounds from a living crop cultivar or from plant residues - is often assumed to be advantageous for the environment compared to traditional herbicides. Due to their origin from natural sources, some authors suggest that the allelopathic compounds will be biodegradable and less polluting than traditional herbicides (e.g. Macias et al. 1998a, 1998b, Narwal et al. 1998). However, other authors emphasise that even though most compounds derived from natural sources appear to have short half-lives compared to synthetic pesticides, some of these products also

have toxicologically undesirable target effects (Duke et al. 1997). The need of ecotoxicological studies to unveil the consequences of growing allelopathic cultivars on large scale has also been stressed (e.g. Olofsdotter 1999).

With the possibility for development of genetically modified crops with enhanced allelopathic effect, the ecological consequences of the growth of such crops must be considered. This includes the possible spread of allelopathic plants to other ecosystems than the agricultural and spread of allelopathic traits to other plants.

## **1.2 Report objectives**

The intention with this report is to discuss potential ecological effects of allelopathic plants with focus on crop species. Therefore, background information of specific relevance for the ecological risk assessment of future genetically modified plants with allelopathic traits is provided.

Based on a literature study, the report intends to describe the challenges of demonstrating allelopathy and presents known effects of allelopathic plants in cultivated and non-cultivated ecosystems. In this context, environmental conditions of importance for the effect of allelopathic plants will be pointed out.

The allelopathic activity of some important agricultural non-GM-crops (not genetically modified) will be described to illustrate central aspects of weed control mediated by allelopathic crops.

Finally, the report presents suggestions for ecological risk assessment of allelopathic crops.



## 2 Demonstration of allelopathic activity

It has recently been said, that no area of plant science has provoked as much controversy as the study of allelopathy (Romeo & Weidenhamer 1998). Generally, the difficulties of separating the chemical interference (allelopathy) from other interference mechanisms have hindered the acceptance of many of the methods suggested to demonstrate allelopathic activity. Therefore, the validity of many test results within the field of allelopathy has been much debated.

Knowledge about the challenges related to the demonstration of allelopathy, as an ecological significant mechanism, is important in the assessment of ecological effects of allelopathic plants. This could for example be relevant if crop species with allelopathic traits are spread to other ecosystems or if the allelopathic traits are spread to other plant species e.g. by hybridisation.

### 2.1 Indications of allelopathy

Investigations of allelopathic activity have often been initialised by field observations mainly related to changes in agricultural, horticultural or silvicultural productivity or to changes in vegetation patterns in natural habitats.

#### *Autotoxicity*

Problems of growing the same crop in succeeding years because of poor establishment and stunted growth has lead to investigations of possible causes, including allelopathy. Allelopathy occurring among individuals of the same species is termed autotoxicity. Autotoxicity is known for example in *Medicago sativa* (alfalfa), *Trifolium* spp. (clovers) and *Asparagus officinalis* (asparagus) (e.g. Miller 1996, Chung & Miller 1995, Young 1986).

#### *Residue effect*

Inhibitory effects on germination and establishments of crops caused by residues of either crops or weeds have lead to investigation of the release of toxic compounds from such residues. For example, the allelopathic interference of both living plant and of plant residues of the highly aggressive weed *Elytrigia repens*, quackgrass, has been strongly indicated (Weston & Putnam 1985). Residues from several crop species have been examined for their potential to reduce weed germination (e.g. Creamer et al. 1996, Moyer & Huang 1997).

#### *Hazardous weeds*

In cases where the success of a plant, typically a weed, can not be explained by the competitive ability, allelopathy has been suspected to play a role. Investigations of such observations have established or strongly indicated an allelopathic activity of weeds, e.g. *Avena fatua* (wild oat), *E. repens* (quackgrass), *Cirsium arvense* (Canada thistle) and *Stellaria media* (common chickweed) (Putnam & Weston 1986, Seigler 1996, Inderjit & Dakshini 1998).

Reduced weed problems within a crop may indicate that the seed germination or development of weedy species is inhibited by the release of allelochemicals from the crop. This has for example been reported in cultivated fields of some *Brassica* species, where no herbicides were applied (Weston 1996). Also in fields of cultivated sunflower, the weed biomass was equally reduced in plots with or without herbicide treatments (Leather 1983).

Reduced weed densities following the growth of some crops has been observed and has naturally stimulated the research in allelopathy (e.g. Narwal et al. 1998).

#### *Halo zone and 'fairy rings'*

The observation of a weed-free zone around some up-land rice cultivars in a germplasm collection growing in a weed infested field has initiated an extensive research programme with the aim of finding allelopathic rice cultivars for weed control. Weed free zones (80-90% weed control) with a radius of up to 20 cm has been observed (Dilday 1994).

"Fairy rings" has also been observed both in fields with wild and cultivated sunflower (*Helianthus rigidus* and *H. annuus*, respectively). These rings are characterised by a decrease in the number of plants, and inflorescences as well as smaller size of individual plants in the middle of the ring (Rice 1984).

Distinct zones with sparse or without vegetation has been observed around some shrubs in chaparrals (Rice 1984, Williamson 1990) and under a number of trees (reviewed by Kohli 1998). This includes the observation of the inhibition of adjoining plants by *Juglans nigra* (black walnut) back in 1881 by Stickney & Hoy (Rice 1984).

#### *Replanting and reforestry problems*

Allelopathy has been investigated as an explanation of the difficulties of replanting fruit trees in orchards - for example apple (*Malus* spp.), citrus (*Citrus* spp.) and peach (*Prunus persica*) (Rice 1984, Putnam & Weston 1986).

The role of allelopathy in the interaction between forest trees and their understory species is also of current interests. For example, inadequate natural regeneration and reduced growth of planted seedlings has been attributed to the release of allelochemicals by herbaceous vegetation. Especially ericaceous shrubs have been investigated for their effect on seed germination, rooting ability and seedling growth of conifers (e.g. Mallik 1998, Pellisier & Souto 1999, Zackrisson & Nilsson 1992) also see chapter 4.

#### *Pure stands*

An example frequently referred to, is the formation of pure stands of *Brassica nigra* (black mustard), after invading annual grasslands of coastal California. In these pure stands of *B. nigra*, other plant species could not successfully invade (Bell & Muller 1973).

#### *Minor changes*

In other cases the effect of allelopathic activity may not be observed immediately if the development of visual symptoms is slow (Putnam & Tang 1986). Interactions may be caused by marginal but persistent presence of allelochemicals. This can result in changes in floristic

diversity and in changes in the distribution patterns of some plant species within a community (e.g. Chaves & Escudero 1997, Gentle & Duggin 1997). A reduction in the number of the plant species sensitive to allelochemicals might not be noticed at short term.

## 2.2 Challenges related to the demonstration of allelopathy

No commonly agreed well-defined methods exist for verification of allelopathic activity. Protocols have been suggested and attempted to verify that allelopathy is operating. These protocols are typically physiologically based (e.g. Rice 1984, Putnam & Tang 1986, Wallstedt et al. 1997). Such an approach implies that the release of allelochemicals must be demonstrated and the symptoms or suspected effects must be recreated in other plants by applying allelochemical(s) at the same concentrations and rates as those found under natural conditions when allelopathic plants are present.

Due to the complexity of allelopathic interactions (see below) the validity of these protocols based on a plant physiological approach has been questioned (e.g. Williamson 1990, Einhellig 1996, Inderjit & Del Moral 1997). The essence of these discussions both regarding some of the practical difficulties and the overall consideration about demonstrating allelopathic activity is outlined below.

### *Identification and isolation of allelochemicals*

The identification of allelochemicals involved in allelopathy is essential if a physiologically based protocol is to be followed. The active compound or compounds must be isolated in an amount adequate for identification and for further characterisation in bioassays.

The allelochemical or allelochemicals will not be released from the plant in isolation. Screening of fractions of plant extracts or leachates for their effects on seed germination of various plant species are frequently used to identify phytotoxic compounds (e.g. Macias 1995, Macias et al. 1998). In this process, the selection of extraction source and extracting agent must be carefully selected if ecological relevant data are to be obtained. To obtain ecologically relevant data, the use of organic solvents is not recommended and the isolation and identification of allelochemicals from the environment is by some researchers considered to be most significant in establishing allelopathy (Inderjit & Dakshini 1995).

### *Mixture effect*

The identification of an active phytotoxic compound from a suspected allelopathic plant does not establish that this is the only compound involved in allelopathy. The release of allelochemicals of different chemical classes from allelopathic plant species has been documented including tannins, cyanogenic glycosides, several flavonoids and phenolic acids such as ferulic, p-coumaric, syringic, vanillic, and p-hydroxybenzoic acids (c.f. Einhellig 1995a, 1995b). For example, both simple phenolic acids and cyclic hydroxamic acids with allelopathic effect are released from the living intact roots of *Elytrigia repens* (Friebe et al. 1995, Friebe et al. 1996). Einhellig (1995a)

states that an allelopathic inhibition under natural conditions is the result of the combined effect of several compounds.

*Mixed allelochemicals have greater effect than single compound*

Several laboratory experiments indicate that mixture solutions of allelochemicals have greater effect than the same concentrations of the compounds used separately (e.g. Blum et al. 1999, Einhellig 1995b, Chaves & Escudo 1997). Furthermore, these experiments have indicated that mixtures of some allelochemicals, e.g. phenolic acids and other organic compounds such as carbohydrates and amino acids can possess allelopathic activity even though concentrations of individual compounds are significantly below their inhibitory levels (Blum et al. 1993, Blum 1996).

In summary, laboratory experiments have indicated that several allelopathic compounds may be released from a plant and that these may act together to cause an allelopathic effect. Furthermore, the presence of compounds such as carbohydrates and other organic molecules may play important roles for the effects of allelochemicals. On this basis it is stressed that the interpretation of results of identification and testing of individual compounds in relation to the demonstration of allelopathy must be done with caution.

*Release rates of allelochemicals*

Allelochemicals are released and added to the soil over a time period and also continually removed and/or immobilised from the soil solution by plant uptake, adsorption to soil particles, and degradation by microorganisms (Cheng 1995). The estimation of the actual release rate of allelochemicals from living plants may be difficult. Allelopathic compounds released from different plant parts can either be released continuously, within specific periods (e.g. specific developmental stages) and/or in pulses when triggered by external factors as for example precipitation (e.g. Zackrisson & Nilsson 1992, Yoshida et al. 1993). For example, young barley plants release allelochemicals from roots and leaves when exposed to water (Lui & Lovett 1993, Yoshida et al. 1993 -see also chapter 3).

*Concentration of allelochemicals in soil*

The concentration of an allelochemical released at a given time can only be regarded as a snapshot of the present situation and measurements over longer periods of time must be carried out to establish the release rate of allelochemicals from plants. Certainly, one-time applications of compounds will not simulate continuous release of allelochemicals by plants under natural conditions.

Low environmental concentrations of allelochemicals at a given point of time is not necessarily an argument against their allelopathic role (Blum 1996, Weidenhamer 1996) or an evidence of their activity at very low concentrations. The toxicity of allelochemicals has been suggested to be a function of the static availability at a given point in time and of the dynamic availability based on the total amount of chemicals moving in and out of the system over a period of time (c.f. Weidenhamer 1996).

*Many factors interact with allelochemicals in soil*

The allelopathic effects may not solely depend on the concentration of allelochemicals in the soil solution. Laboratory experiments have shown that mixtures of phenolic acids and other organic compounds



can cause inhibitory effects even though the concentrations of individual compounds are below their inhibitory levels (e.g. Blum 1996). For phenolic acids, the magnitude of the allelopathic effects due to a given concentration of allelochemicals in soil is also influenced by factors such as soil pH, organic matter content, nutrient and moisture content (Blum et al. 1993, Blum 1995). The effectiveness of a given concentration of an allelochemical in inhibiting seedling growth can be influenced by the availability of other carbon sources to soil microorganisms (Blum et al. 1993, 1996). When a more readily available carbon source is present, the microbial utilisation of allelochemicals can be decreased, which increases the concentration of allelochemicals available for uptake by plant roots.

The concentration of allelochemicals in a medium will also depend on the density and age of the allelopathic plant. In arable areas the cultivation pattern can also have an influence (Inderjit 1996, Inderjit & Dakshini 1994, Inderjit et al. 1996).

In summary, the effect on an allelochemical in soil may not be directly related to the actual concentration of the allelochemical in soil. This means that the application of allelochemicals in a concentration corresponding to a concentration previously measured in the soil, in order to demonstrate allelopathy, will not necessarily result in an allelopathic effects.

#### *Transformation products from allelochemicals*

After release of allelochemicals to soil, transformation can take place due to biogeochemical active processes. The result can be the formation of more or less phytotoxic compounds. The transformation of compounds may cause practical problems for the identification and characterisation of allelochemicals. For example, the amount of a test compound can also be considerably reduced e.g. by volatilisation. When an alleged allelochemical was mixed in soil, 99 % was lost to volatilisation in 10-12 hours when hexane was used as solvent because the compound was poorly soluble in water. From the growing plant, the allelochemical is probably released more slowly and the residence time correspondingly longer (Choesin & Boerner 1991). Some compounds are relatively easily transformed whereas others, e.g. some alkaloids, may have longer persistence in soil due to their anti-microbial activity (e.g. Wink et al. 1998). A study by Inderjit et al. (1997) showed that allelopathic compounds have different recovery in soil.

Transformation products have been shown to be important allelochemicals and may even intensify the activity of those already present. For example, maize, wheat and rye release biologically active aglucones, DIMBOA (2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and DIBOA, which are degraded spontaneously to the corresponding benzoxazolinones MBOA (6-methoxy-benzoxazolin-2-one) and the desmethoxy derivate BOA. These compounds are also allelopathic. Additional phytotoxic compounds may be formed in the presence of microorganisms (e.g. Barnes & Putnam 1986, 1987, Nair et al. 1990, Niemeyer 1988, Pérez 1990 -see also chapter 3). Consequently, attempts to identify allelochemicals

responsible for an observed effect must consider biotic and abiotic transformation of released compounds.

*Interaction with biotic and abiotic factors*

Biotic and abiotic factors can influence both the production of allelochemicals by the donor species (the species from which the allelochemicals originate) and modify the effect of an allelochemical on the receiver plant. The influence of factors such as light, nutrient availability, water availability, pesticide treatment and disease can affect the amount of allelochemicals in a plant (e.g. Inderjit & Del Moral 1997, Reigosa et al. 1999). Even though the production of allelochemicals in a plant can increase in response to stress, it is not clear whether a corresponding release of allelochemicals to the environment also occur (Einhellig 1996, Inderjit & Del Moral 1997). In general the sensitivity of target plants to allelochemicals is affected by stress and typically it is increased (Einhellig 1996, Reigosa et al. 1999).

On the basis of several examples discussed by Einhellig (1996) and Inderjit & Del Moral (1997) the authors conclude that allelopathy and stresses interact under natural conditions. This implies that the result of an experiment designed to investigate allelopathic activity will be strongly influenced by the test conditions. Under laboratory conditions, which is typically less stressful than field conditions, the allelopathic effect might be reduced (Romeo & Weidenhamer 1998).

*Choice of effect parameter for demonstration of allelopathy*

The choice of measurement parameter for the demonstration of allelopathy must also be considered. In several bioassays, seed germination and seedling development is measured after the exposure to alleged allelochemicals because seed and seedlings development is generally considered to be the most susceptible stages (e.g. Leather & Einhellig 1986, Putnam & Tang 1986, Inderjit & Olofsdotter 1998). Unless plants are in contact with allelochemicals at their sensitive stages allelopathic effects will not be observed. This should always be taken into account in the design of experiments aimed at demonstration of allelopathy.

If major morphological changes are not apparent within the experimental period after the plants have been exposed to allelochemicals, the effects may be overlooked. Sometimes seed germination is not inhibited but the process may be delayed, cotyledon and root size diminished or radicle or seedling development abnormal e.g. in form of twisted growth or in form of adverse effects on their metabolism (e.g. Chaves & Escudo 1997, El-Khatib 1998, Lui & Lovett 1993). The effect on population size may be apparent only after a relatively long period of time when some of the seedlings in a population are inhibited.

## **2.3 Approaches in allelopathic research**

*Holistic approaches*

It is illustrated above that the protocols based on plant physiology may not be suitable to demonstrate allelopathy due to the complexity of the phenomena. This is in agreement with recent suggestions by Inderjit & Keating (1999) and Romeo & Weidenhamer (1998)

emphasising that holistic approaches are required to demonstrate allelopathy as an ecological significant mechanism. Careful consideration must be given to autecology, habitat, and life cycle pattern of allelopathic plants and the afflicted species. This implies that field observations must be carefully evaluated, qualitatively and quantitatively, to optimise the experimental design and that bioassays in laboratory, greenhouse or fields should preferably be conducted with plants that naturally occur together. Essentially, the role of biotic and abiotic environmental factors and the possible involvement of several allelochemicals must be taken into account.

#### *Various purposes of allelopathic research*

The debates about methods within allelopathic research have mainly focused on the demonstration of allelopathy as an ecological significant mechanism. Yet, several other purposes have been included in allelopathic research. In agricultural research there is an awareness of allelopathy as a tool in weed management, the research can therefore be different. For instance to distinguish cultivars with strong allelopathic properties from less allelopathic ones, evaluate species sensitivity, to identify the developmental stage that release allelochemicals and validate allelopathic strength under various conditions (Olofsdotter & Inderjit 1998, see also chapter 3).

In the search for potential herbicides derived from plants, the purpose is to demonstrate allelopathic activity of an isolated compound and to determine the activity range of the resultant allelochemical herbicide with respect to necessary dose and target weeds (e.g. Macias 1995, Macias et al. 1997, 1998). In such experiments, the effect on selected sensitive species such as lettuce and tomato may be important in the process, but the obtained effects must not be confused with the demonstration of allelopathy as an important ecological mechanism.

To demonstrate whether allelopathy offers the most reasonable explanation of an observed pattern, a series of experiments must typically be carried out and may include both laboratory and field tests. The design of each experiment will depend on the actual/precise purpose of the investigation and on the characteristics of the donor and afflicted plants and on habitat. Some of the approaches and factors affecting the sensitivity of the tests used as part of the allelopathic research are described below.

#### *Choice of bioassay material and bioassay conditions*

The bioassay conditions influence the effect concentration and thereby the results of the bioassay. For example in seed germination tests, test species, light conditions, osmotic potential and interactions between these factors strongly influence the result (Haugland & Brandsaeter 1996). Also, solution volumes and seed number can influence the result of seed germination bioassays (Weidenhamer et al. 1987). Factors such as seed size, seed dormancy and the length of the after-ripening period to which the seed has been subjected can influence on the concentration of allelopathic compound necessary to produce an effect on seed germination (Pérez 1990). The natural variation in seed germination may also in some cases pose some challenges to the design of experiments due to a low and inconsistent germination of relevant test species (e.g. Olofsdotter 1999).

Seeds, from species such as lettuce, tomato and cress, which germinate readily, are often used in various germination tests. Such test species can be practical and useful for fractionation, isolation, and purification of the most important allelopathic compounds (e.g. Macias 1995, Macias et al. 1998). However, to be able to relate the results to natural conditions species actual involved in the system must be evaluated.

#### *Density-dependent tests*

The density dependent test, as suggested by Weidenhamer et al. 1989, Thijs et al. 1994, implicates that the density of the donor species is kept constant while the density of the receiver species is increased. As phytotoxic effects are assumed to be density-dependent, maximum size of receiver plant will occur at an intermediate density, with reduced size at both low density (the result of phytotoxicity) and at high density (due to intense resource competition).

Density-dependent tests has both been carried out as a Petri-dish radicle elongation assays and as greenhouse and field experiments with whole plants (e.g. Choesin & Boerner 1991, Gentle & Duggin 1997, Thijs et al 1994, Weidenhamer et al. 1989).

#### *Competition experiments*

Competition experiments have been used to compare the competitive ability of genotypes with alleged difference in allelopathic activity toward a target species (Choesin & Boerner 1991, Malinowski et al. 1999). An enhanced release of the allelochemical would expectedly result in a better competitive ability of this genotype towards a target species compared to the genotype with a lower release.

The performance of the two genotypes must be analysed both as absolute yield and as relative yield total, where the relative yield of a genotype in a mixture is the ratio between its yield in that mixture and its yield in a pure stand.

The ratio between the donor species and the target species can be varied in competition experiments (Choesin & Boerner 1991). The effect of density on the allelopathic effects is thereby considered.

#### *Toxicity assessment of soil samples.*

Experiments have been set up to determine if allelochemicals are present in soil samples in active concentrations, so associated plant species are influenced. Soil samples can be collected from the rhizosphere of the alleged allelopathic plant and seeds of test species can thereafter be placed in that soil to germinate. Germination percentage, speed of germination and plant development can then be compared to controls. Soils samples from adjacent fields or from sites in the same fields where the alleged allelopathic plant is not present can be used as controls (El-Khatib 1998, Inderjit et al. 1996).

#### *Amendments plus fertilisation*

Amendment of plant material to soil to test the allelopathic effect has often been carried out. However, the enhanced concentration of organic material may result in enhanced microbial activity, which may result in depletion of some nutrients. Thereby the effect caused by allelopathic toxicity can not be separated from the effect of microbial activity. To avoid that any growth response after the addition of plant material are caused by nitrogen and phosphorus

depletion in the soil due to enhanced microbial activity and not caused by allelopathic toxicity (see Michelsen et al 1995), fertilisers has been added in some experiments (Inderjit & Foy 1999). In some cases or as part of the set-up, sterilisation of such soils for experimental purposes could also be considered.

*Recovery of allelochemicals from soils*

The recovery of some allelopathic compounds (phenolics) has been compared between soils infested with a suspected allelopathic plant and non-infested soils. The quantitative increase in the allelopathic pool of soil owing to an allelopathic plant has been determined. The phytotoxicity of the soils can then be compared (Inderjit & Dakshini 1998). Such an experimental design can demonstrate whether or not a plant has the potential of releasing allelopathic compounds into the rhizosphere and to affect the growth of other plant species. Still, as previously discussed, the importance of allelopathic interactions in ecosystems can neither be determined by the actual nor by net changes in the concentration of allelochemicals in soil.

*Detoxification of allelochemicals*

Activated carbon has been used to detoxify allelochemicals - either directly on the soil surface, incorporated into the soil, with plant extracts or in hydroponic culture (Nilsson 1994, Asao et al. 1998, El-Khatib 1998, Inderjit & Foy 1999). Anticipating that the activated carbon totally absorbs all the allelochemicals and does not influence other factors of significance, the effect of allelochemicals can be estimated by comparison to controls without activated carbon.

## **2.4 Conclusive remarks**

The demonstration of allelopathy as an ecological significant mechanism comprises several challenges. Especially, the interactions with abiotic and biotic factors are considered to play an important role in the expression of allelopathy. A holistic approach where the experimental designs are adapted to the species and the ecosystems under investigation has been recommended in recent years.



## 3 Research in allelopathic crops

Numerous crops have been investigated more or less thoroughly for allelopathic activity towards weeds or other crops. A suppressive effect on weed, possibly mediated by the release of allelochemicals has been reported for a wide range of temperate and tropic crops. These include alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), clovers (*Trifolium* spp., *Melilotus* spp.) oats (*Avena sativa*) pearl millet (*Pennisetum glaucum*), rice (*Oryza sativa*) rye (*Secale cereale*), sorghums (*Sorghum* spp.), sunflower (*Helianthus annuus*), sweet potato (*Ipomoea batatas*) and wheat (*Triticum aestivum*) (e.g. Dilday et al. 1994, Narwal 1996, Narwal et al. 1998, Miller 1996, Weston 1996).

In this chapter, some of the essential findings regarding the allelopathic activity of the important agricultural crops, rye, barley, wheat, oats and rice are summarised. These crops share in common that their allelopathic activity has been examined in more recent research programmes within crop-weed allelopathy. This includes available information about identified allelochemicals, specificity of allelochemicals, the developmental stages of the plant where allelochemicals are produced and released. Finally it is referred whether the genes coding for the production or release of some of the allelochemicals has been identified.

### 3.1 Use of allelopathic crops

Allelopathic crops can be used to control weeds by:

- 1) Use of crop cultivars with allelopathic properties
- 2) Application of residues and straw of allelopathic crops as mulches
- 3) Use of an allelopathic crop in a rotational sequence where the allelopathic crop can function as a smother crop or where residues are left to interfere with the weed population of the next crop

For further reading see for example An et al. (1998), Barnes & Putnam (1986), Narwal et al. (1998), Weston (1996).

Furthermore, suggestions for the use of allelopathy in weed control also include the application of allelochemicals or modified allelochemicals as herbicides (e.g. Macias 1995).

So far, an extended use of the allelopathic properties of crop species has mainly been considered a promising supplement to other weed management strategies (An et al. 1998, Inderjit & Olofsdotter 1998, Krishnan et al. 1998, Moyer & Huang 1997, Olofsdotter 1998a, 1999).

#### 3.1.1 Designs of allelopathic crops

Improved season-long weed suppression by allelopathy has been suggested to be obtained by manipulating germplasm resources to

enhance the production of allelochemicals or by regulating the release rate of allelochemicals in both living plants and their residues (Weston 1996). Some environmental and agricultural factors that influence the request to allelopathic crops and the possibility of improving their allelopathic properties are described below.

*Cultivation practice*

The demands to an allelopathic crop depend on the cultivation practices of the crop (e.g. time of sowing, tillage practices). In production systems with no-till or conservation tillage that leave nearly all crop residues on the soil surface, the release of allelochemicals from both the growing plants and during residue decomposition could be advantageous. Clearly, the release of allelochemicals from the living plant would be the goal in cases where almost no residues are left after harvest. The interest in determining the allelopathic and physical effects of crop residues on weed seed germination, and on the establishment of the following crop, has been stimulated by the recent trend in some regions towards no- or minimum-tillage direct seeding cropping systems (Miller 1996, Moyer & Huang 1997).

*Competitive ability*

The use of a combination of allelopathy and a strong competitive ability in the crop has been suggested as a beneficial combination for weed management in many crops (Wang & Olofsdotter 1996). This is considered to be especially important in early stages of plant growth because competitive hierarchies often form during early stages of plant development. Therefore, a maximal allelopathic effect of seedlings and young plants would be advantageous in crops that later can form a dense canopy. In crops with an open canopy structure, a season-long allelopathic effect would be an advantageous character. Another alternative in such crops, is the use of herbicides early in the season and the allelopathic effect later in the season for weed control (Leather 1987).

*Specificity*

Allelopathic effect against a broad spectrum of weeds has been proposed as a valuable character of an allelopathic crop and the possibility of inserting resistance genes towards one or several weeds as part of a breeding strategy of a crop has been mentioned (Olofsdotter et al. 1997).

*Sensitive stage of target plants*

Many plant species are most susceptible to allelochemicals in the seed seedling stage. This means, that, as weeds grow they are less likely to be affected by allelochemicals released in their rhizosphere. To obtain a direct allelopathic effect, the ideal allelopathic cultivar must therefore release allelochemicals in bioactive concentrations before the target weeds grow to old. Knowledge about both the critical developmental stage where the crop starts releasing allelochemicals and the critical sensitive stage of the target weeds is therefore essential (Inderjit & Olofsdotter 1998).

*Genetic/genotype variation*

The amount of allelochemicals present in a plant is often found to exhibit considerable variation between genotypes and between cultivars (section 3.2). In barley and rye it has been shown that the concentration within the leaves and roots, respectively, does not correlate with the actual release (section 3.2). This illustrates that the



allelopathic activity of a crop can not directly be related to the content of allelochemicals within the plant.

#### *Screening /selection of crop varieties*

The variation in allelopathic potential between genotypes can be used in the search for crop cultivars with enhanced allelopathic properties. So far relatively few attempts have been made to enhance the weed suppressive properties of crop plants. Differences in allelopathic potential between genotypes has been investigated among accessions (genetical different lines or strains of a species) of barley, cucumber (*Cucumis sativus*), oats, soybean (*Glycine max*), sunflower, sorghum (*Sorghum bicolor*), rice and wheat (e.g. Copaja 1999, Dilday et al. 1994, Narwal 1996, Miller 1996, Yoshida et al. 1993, Wu et al. 1998).

Screening programmes with the aim of identifying cultivars with enhanced allelopathic activity has during the last decade been carried out for rice and has more recently been started for wheat and barley (Dilday 1994, Olofsdotter 1999, Wu et al. 1999).

#### *Genetically modified plants (GMP's) with enhanced allelopathic activity*

Genetic modification of crop plants to improve their allelopathic properties and enhancement of their weed-suppressing ability has been suggested as a possibility. A regulation of the biosynthesis and the release rate to enhance the release of allelochemicals or to prolong the period of release of allelochemicals has been suggested (Weston 1996, Wu et al. 1999). Use of biotechnological transfer of allelopathic traits between cultivars of the same species or between species has also been proposed (Chou 1999, Macias 1995, Macias et al. 1998, Rice 1984). Wu et al. (1999) supposed, that the use of advances in plant biotechnology, such as RFLP (restriction fragment length polymorphism) markers, will increase the efficiency in unveiling the inheritance of allelopathic traits. On the other hand, it has been stated, by Wu et al. (1999), that even though genetic manipulation seems promising, it might be more feasible to select for crop cultivars with improved allelopathic properties using conventional breeding methods, because of the strict regulation and public concern about transgenic crops.

## **3.2 Allelopathic activity of selected crops**

### **3.2.1 Rye (*Secale cereale*)**

The allelopathic activity of rye has mainly been investigated in relation to the weed suppressive ability when used as green manure or as cover crop. The release of allelochemicals via root exudates has also been documented (e.g. Barnes & Putnam 1986, 1987, Creamer et al. 1996, Hoffman et al. 1996). Especially, in the US, rye is grown as winter annual cover crop and efficiently reduces soil erosion and nutrient loss. Rye grows well on marginal soil, produces an extensive root system and a dense canopy and competes effectively with weed species for light, moisture and nutrients.

Especially due to the massive production of biomass, rye has the potential to influence the growth of succeeding plant species through the release of allelochemicals from the residue (Barnes et al. 1985).

Still, the weed suppression effect of mulch can be due to a combination of physical factors and a release of chemicals from the degrading plant material.

### 3.2.1.1 Allelochemicals identified for rye

Several compounds with phytotoxic effect have been isolated from rye (Barnes et al. 1985, Barnes & Putnam 1987 and references in Narwal 1996), but their role and significance under field conditions are not clearly understood.

#### *Phenolic acids*

The phenolic acids beta-phenyl-lactic acid (PLA) and beta-hydroxybutyric acid (BHA) have been identified as allelochemicals in water extracts of rye residues (references in Narwal 1996).

#### *Hydroxamic acids*

The two hydroxamic acids DIBOA (2,4-dihydroxy-1,4(2H)-benzoxazin-3-one) and BOA (2(3H)-benzoxazolinone) has also been isolated from root and shoot tissue of rye and were found to be toxic to many weed species (Barnes & Putnam 1986, 1987). Rye root exudates containing hydroxamates also inhibited the development of a wild oat, *Avena fatua*, in a seed germination test (Pérez & Ormemeño-Núñez 1991).

DIBOA exists in the plant as the glucoside DIBOA-glc that readily can hydrolyse to DIBOA when the tissue is destroyed. In water, DIBOA decomposes immediately to BOA (Niemeyer 1988).

#### *Transformation compounds*

Additional phytotoxic compounds may be formed by microbial transformation of compounds from rye residues. AZOB (2,2'-oxo-1,1'-azobenzene), an azoperoxide, has been isolated from non-sterilised soil after addition of BOA or DIBOA. AZOB was found to be more toxic to seedling growth than BOA or DIBOA in seedling bioassays (Nair et al. 1990).

The three compounds DIBOA, BOA and the transformation product AZOB have been evaluated singly and in mixtures for activity against various crop species and one weed in a seedling bioassay. Significant synergistic activity was observed and especially when AZOB was present (Chase et al. 1991). This result indicates that the allelopathic compounds released from the plant can act together with its transformation product, resulting in increased toxicity (Chase et al. 1991).

### 3.2.1.2 Release of rye allelochemicals

Allelochemicals does not seem to be released from the shoots of living rye plants in bioactive concentrations. When rye plants of different ages were misted, the leachates had no effect on the germination of plant species, known to be sensitive to rye root leachates (Barnes et al. 1985).

The release of hydroxamic acids from rye cultivars during the period between emergence and first leaf stage has been reported (Pérez & Ormemeño-Núñez 1991). DIBOA was found in root exudates of some cultivars of rye by using continuous root exudates trapping system. In the same experiment, the amount of DIBOA exuded by rye plants

was not proportional to the content of the compound in the roots, which indicate that exudation of DIBOA is an active process (Pérez & Ormemeño-Núñez 1991). In the cultivar found to exude the highest amount of DIBOA, the concentration was 25.0  $\mu\text{mol kg}^{-1}$  fw.

The allelopathic effect of rye has mainly been considered in relation to the use as green manure in the field. On the basis of estimated concentrations of DIBOA, and the relatively small amount of BOA, in the shoots, the amount of DIBOA and BOA possibly released in the field has been estimated to 13.5 - 16  $\text{kg ha}^{-1}$  (Barnes & Putnam 1987, Mwaja et al. 1995). Compared to results from bioassays, Barnes & Putnam (1987) concluded that data suggests that under field conditions, a sufficient quantity of residues would be present to affect the growth of weedy species, if their seeds are placed close to the residues.

### **3.2.1.3 Effects of environmental conditions on the production of rye allelochemicals**

The toxicity of rye and the concentration of some allelochemicals can be influenced by environmental conditions. The concentrations of BOA and DIBOA in rye shoots are influenced by nutrient availability and were highest when rye was grown under low or moderate nutrient availability compared to high availability (Mwaja et al. 1995). In the studies by Mwaja et al. (1995), the higher concentration of hydroxamic acids in shoots were found to correlate with an enhanced toxicity of rye residues. Also, the iron status of the plant significantly affects the release of hydroxamates from rye seedlings. When rye seedlings were grown in a nutrient solution containing iron, the secretion of hydroxamates increased considerably (Pethó 1992a).

The release of hydroxamic acids from roots of rye seedlings can probably be affected by biotic stresses. It has been shown that the release of hydroxamic acids through root exudates is affected by defoliation of rye seedlings. The result of repeated defoliation of rye seedlings, was an increase in the allocation of hydroxamic acids to roots and root exudates (Collantes et al. 1999). It was suggested that increase in the exudation of hydroxamic acids, could possibly lead to an advantage in the acquisition of resources for the regeneration of lost biomass if it affects neighbouring plants of other species negatively (Collantes et al. 1999).

### **3.2.1.4 Effect and specificity of rye allelochemicals**

#### *Visible effects*

Characteristically of allelopathic interference, it has been reported that some of the species emerging through the rye residue were chlorotic and stunted, although nutrient supplies were optimal. The apical root meristem of lettuce have been reported to become discoloured with a subsequent inhibition of root growth when seeds germinated close to rye residues (Barnes & Putnam 1986, 1987).

#### *Species affected*

Rye interferes with the growth of numerous plants. Several experiments have demonstrated strong species dependent response to rye allelochemicals, exudates and residues. Overall, various dicotyledons were found to be more sensitive than monocotyledons

to the rye allelochemicals, DIBOA, BOA, PLA and HBA when effects on seed germination and seedling development were tested (Barnes & Putnam 1987).

Among the species inhibited by rye allelochemicals, the following cultivated and weedy species have been mentioned: *Echinochloa crus-galli*, *Lactuca sativa*, *Lepidum sativum*, *Panicum miliaceum* and *Lycopersicon esculentum* (Barnes & Putnam 1986, 1987, Hoffmann et al. 1996, Mwaja et al. 1995). Hydroxamic acids from rye suppressed the growth of wild oat *Avena fatua* (Friebe et al. 1996, Pérez & Ormemeño-Núñez 1993), whereas *Avena sativa* showed high tolerance to hydroxamic acids (Friebe et al. 1996).

A stimulating effect of rye on some species, including *Vicia villosa* and *Bromus secalinus* has also been reported by Hoffman et al. (1996).

In a field plot with a rye cultivar exuding hydroxamic acid, the total biomass of the mixed population of the following species was reduced: *Veronica persica*, *Lamium amplexicaule*, *Chenopodium album*, *Polygonum aviculare* and *Bilderdykia convulvulus* (Pérez & Ormemeño-Núñez 1993).

#### *Microbial degradation*

The tolerance to BOA of certain plant species can possibly be due to species dependent microbial metabolism of the allelochemical. Investigating the degradation by root-colonising bacteria, no microbial metabolism was found with roots of *Triticum aestivum* and *Secale cereale* whereas microbial metabolism was found with roots of *Avena sativa* and *Vicia faba* (Friebe et al. 1996). The phytotoxic influence of BOA on *Avena sativa* increased when the microbial degradation of this allelochemical was prevented. In conclusion, the inhibitory influence by BOA can be significantly reduced by root-related microbial degradation.

#### **3.2.1.5 Weed suppressive ability**

#### *Rye residues*

Most work concerning allelopathic effects of rye has been carried out using residues. Rye residues have been employed as mulches or cover crops in no-tillage cropping systems to suppress certain weed species (Barnes & Putnam 1986).

In contrast, results obtained by Creamer et al. (1996) by leaching rye of its water soluble allelopathic compounds and using it as an inert material, indicated that the physical suppression of rye was responsible for the reduced emergence of two weedy species, eastern black night shade (*Solanum ptycanthum*) and yellow foxtail (*Setaria glauca*). Even though the emergence of one of the weeds was further reduced when unleached (allelopathic) rye material was used compared to leached material (not allelopathic) the reduction was not statistically significant. However, it is possible that other species not included in the tests may be affected.

#### *Root exudates from living plants*

The interference of rye with other plants growing simultaneously has also been examined. For example, a field study by Pérez & Ormemeño-Núñez (1993) indicates that living rye can reduce the weed population by allelopathy. In the experiment, a rye cultivar

exuding hydroxamic acids through its roots reduced the weed biomass by up to 83 % compared to a wheat cultivar that also produces hydroxamic acids, but appears unable to exude them. When compared to forage oats (*Avena strigosa*) with high competitive performance and no production of hydroxamic acid, the weed biomass was reduced by up to 76 % by the hydroxamic acid exuding rye (Pérez & Ormemeño-Núñez 1993). These results indicate that it is not the physical performance of rye and not the content of hydroxamic acids within the plant, but probably the release of hydroxamic acids, which accounts for the weed inhibiting effect.

### 3.2.1.6 Genetics of rye allelopathy

Information about the location of genes coding for the production or release of allelochemicals in rye has not yet been published.

### 3.2.2 Wheat (*Triticum aestivum*).

The allelopathic effect of wheat has mainly been studied in relation to its use as green manure/straw. Wheat residues suppress weeds due to the physical effect and to the production of allelochemicals. The release of allelochemicals from living wheat plants has also been documented (Pethó 1992a).

#### 3.2.2.1 Wheat allelochemicals

#### *Phenolic acids*

Phytotoxic phenolic acids and simple acids have been identified in wheat residues (references in Narwal 1996). The content of total phenolics has been measured in water extracts of dried residues of 38 different wheat cultivars. The allelopathic activity of the extracts was evaluated for effects against *Lolium rigidum* by an laboratory seed germination bioassay. The allelopathic effect was positively correlated with the total phenolic content in the tissue of the wheat cultivars (Wu et al 1998).

#### *Hydroxamic acids*

Hydroxamic acids have also been identified in shoot and root tissue of wheat. The most abundant of these acids in wheat tissues is DIMBOA. When the content of hydroxamic acids was examined in wheat seedling during 7 days of germination, DIBOA was also found in roots and leaves of the three cultivars examined. Hydroxamic acids were not detected within the seeds (Copaja et al. 1999).

High concentrations, up to 6 mmol/kg fw, of hydroxamic acids have been recorded in roots of some wheat cultivars and has been suggested to be valuable in the allelopathic control of weeds (Copaja et al. 1999). However, it was not confirmed that the content of allelochemicals in the roots corresponds to the actual release.

Furthermore, the decomposition product MBOA has been examined for its phytotoxic effect, see section “activity and specificity of wheat allelochemicals”. DIMBOA decomposed to MBOA after uptake in seed of *Avena sativa* within a period of 48 hours.

#### 3.2.2.2 Release of wheat allelochemicals

It has been documented that DIBOA and DIMBOA accumulate in the roots and in the leaves of wheat during germination (e.g. Copaja et

al. 1999). However in hydroponic culture, these compounds were not found to be released from living roots in the period between emergence and the first leaf stage when three cultivars were examined (Pérez & Ormeño-Nuñez 1991). However, Pethó (1992a), found both DIBOA and DIMBOA in the secretions from the roots of 10 and 14 days old wheat plants (one cultivar tested) with DIBOA present in larger amount. These contradictory observations can either be due methodological differences or to differences between the cultivars examined.

Similarly to the observations from rye, hydroxamic acids seem to occur as aglucones in wheat root exudates while in root extracts they occur as glucosides, suggesting that transformation takes place before release (Niemeyer & Pérez 1995, Pérez & Ormeño-Nuñez 1991, Pethó 1992a).

Apparently hydroxamic acids are not released via xylem exudates or in guttation drops of wheat plants (Niemeyer 1988).

Experiments with three different wheat genotypes indicate that the concentration of hydroxamic acids decreases in all parts of the plant at later stages of germination, although the total amount of hydroxamic acids remains stable, indicating a growth dilution effect (Copaja et al. 1999). How this change in concentration affects the amount of hydroxamic acids released from roots have apparently not been examined.

### **3.2.2.3 Activity and specificity of wheat allelochemicals**

DIMBOA and its decomposition product MBOA have been tested for their effect on wild oat, *Avena fatua*, and both compounds inhibits root growth and seed germination (Pérez 1990).

The decomposition product, MBOA, inhibited the seed germination of *A. fatua* more than DIMBOA when tested at concentrations between 0 and 8 mM (Pérez 1990). This was suggested to be due to the documented and significant faster and more extensive uptake of MBOA than of DIMBOA by the tested seeds (Pérez 1990). When comparing the uptake of the two compounds the transformation of DIMBOA to MBOA in the plant should also be taken into account.

#### *Species affected*

Pethó (1992b) found that a relatively high concentration of DIMBOA had only a low toxic effect on the germination of the grasses *Zea mays*, *Hordeum districhon*, *Triticum aestivum*, *Secale sereale* and *Sorghum spp.* The germination of the dicotyledons *Amaranthus caudatus* and *Lepidum sativum* was significantly inhibited even at low concentrations of DIMBOA. Two lilaceous species were rather similar in sensitivity to the dicotyledonous species.

Based on experiments performed under dark conditions, Pérez (1990) concluded that it seems likely that phytotoxicity of hydroxamic acids is related to interference with the normal activity of auxin.

#### **3.2.2.4 Effect of environmental conditions on the production of wheat allelochemicals**

Temperature influences the concentration of hydroxamic acids in wheat seedlings. However, the significant increase in concentration of hydroxamic acids in seedlings of one wheat cultivar when grown at higher temperatures was suggested to be fully explained by the increase in plant growth rate (Gianoli & Niemeyer 1997).

The iron status of the plant significantly affects the DIMBOA-glucoside content in the roots and the amount of DIMBOA released from the roots. When wheat seedlings were grown in a nutrient solution containing iron, the secretion of hydroxamates decreased (Pethó 1992a). In contrast, the secretion of hydroxamates from rye roots increased considerably when rye seedlings were grown under the same experimental conditions (Pethó 1992a).

Some wheat cultivars showed an increased concentration of hydroxamic acids after a short-term infestation by aphids, whereas in other wheat cultivars the hydroxamic acid concentration was unaffected (Gianoli & Niemeyer 1998). The level of hydroxamic acids seems to be constitutive in some wheat cultivars and inducible in other cultivars.

#### **3.2.2.5 Genetics of wheat allelopathy**

Attempts have been made to locate the chromosomal position of genes conferring the accumulation of hydroxamic acids in wheat. The control of hydroxamic acid accumulation in wheat seems to be multigenic involving several chromosomes. Chromosomes of group 4 and 5B are apparently involved in the accumulation of hydroxamic acids (Niemeyer & Jerez 1997).

The location of genes involved in the accumulation of hydroxamic acids was explored in relation to the breeding of wheat for higher levels of hydroxamic acids in order to develop wheat cultivars resistant to aphids (Niemeyer & Jerez 1997).

#### **3.2.3 Barley (*Hordeum vulgare* /*Hordeum* spp.)**

Barley is known as a “smother” crop. This effect has both been attributed to the competitive ability for nutrients and water and to the direct effect of allelochemicals released from barley. Also the residues of barley have been associated with phytotoxicity (Overland 1966, Lovett & Houlton 1995).

##### **3.2.3.1 Barley allelochemicals**

###### *Phenolic acids*

Phytotoxic phenolic compounds, including ferulic, vanillic and p-hydroxybenzoic acids, have been identified in cold water extract of barley straw and in methanol extracts of living barley roots (Börner 1960).

###### *Alkaloids*

The two alkaloids, gramine (N,N-dimethyl-3-amino-methylindole) and hordenine (N,N-dimethyltyramine) have been confirmed to play an important role in the phytotoxic ability of barley (Lovett & Houlton 1995, Overland 1966)

Hydroxamic acids are absent in cultivated barley, but DIBOA has been found in wild *Hordeum* species by Barria et al. 1992 (Gianoli & Niemeyer 1998). Hence, the production of DIBOA by cultivated barley could possibly be achieved by transferring genetic material from wild barley species (Gianoli & Niemeyer 1998).

### **3.2.3.2 Release of allelochemicals from barley**

The release of alkaloids has been documented both from roots and from leaves of living plants (e.g. Liu & Lovett 1993, Yoshida et al. 1993).

Gramine is not present in seeds or roots of barley, but is a constituent of barley leaves. In the leaves, gramine is located in mesophyll parenchyma and in epidermis. Both the surface gramine and some inner gramine can be released by rain (artificial rain, 20 min of treatment) (Hanson et al. 1981, Yoshida et al. 1993). The content of gramine in barley shoots reaches a maximum during the first two weeks of growth of barley seedlings, decreasing afterwards (Hanson et al. 1983, Lovett et al. 1994) to near zero for some genotypes (c.f. Moharramipour et al. 1999). The effect on the release of gramine does not seem to have been investigated.

The gramine content in wild barley (*H. spontaneum*) was considerable higher both in the leaves and on their surface than it was in four cultivated accessions 15 days after germination. For the five accessions of barley, there was no correlation between the amount of gramine within the leaves and the amount of gramine on the surface of the leaves (Yoshida et al. 1993). The higher content of gramine on the surface would probably allow a higher release of gramine from the leaves of wild barley compared to the cultivated accessions with a lower amount of surface gramine when in touch with rain.

Hordenine is not found in seeds of barley, but appears in the roots from the first day of germination and can be released from roots of barley for up to 60 days in a hydroponic system. From one barley line, the maximum release of hordenine,  $2 \mu\text{g plant}^{-1} \text{day}^{-1}$  was observed after 36 days and then declined (Liu & Lovett 1993).

### **3.2.3.3 Effects and specificity of barley allelochemicals**

A synergistic interaction between gramine and hordenine was apparent in a bioassay when these allelochemicals were tested for their effect on the seed germination of white mustard, *Sinapis alba*, with concentrations ranging between 0 and 50 ppm of each compound. In addition, the equimolar combination depressed seed germination more than a combination of different concentrations. In the combination of gramine and hordenine, the synergistic effects of equal concentrations were higher than unequal concentrations (Liu & Lovett 1993).

The effects of gramine and hordenine on the ultrastructure of root tip cells of *Sinapis alba* includes increases in both size and number of vacuoles. Even though no significant changes in gross morphology could be observed on the radicle of *S. alba* after seedlings were treated with 22 ppm gramine, changes on the ultrastructural level



were observed by electron microscopy. In the cells, a substantial response was observed, typically as an increase in the number and size of vacuoles. Also, many lipids appeared when root tips were treated with gramine and hordenine and may indicate the disruption of food metabolism. Therefore, when measuring a secondary effect, no observed effect can not be interpreted as a true non-effect, but as a no difference in a secondary indicator (Lui & Lovett 1993). Based on these and previous results, Liu & Lovett (1993) argued that although allelochemicals present are insufficient to cause death of the seedlings, the metabolism may be adversely affected. Depending on the concentrations of allelochemicals, a reduction of the speed of germination, of radicle length and of the health and vigour of radicle tips may be caused by barley allelochemicals.

#### *Species affected*

Both gramine and hordenine has been reported to inhibit the growth of various plants including; *Capsella bursa-pastoris* (shepherd's purse), *Nicotiana tabaccum* (tobacco), *Sinapsis alba*, *Stellaria media* (common chickweed), whereas *Triticum aestivum* was not inhibited (Liu & Lovett 1993, Overland 1966). Allelopathic compounds released from residues of barley apparently inhibit the emergence of *Setaria glauca* (yellow foxtail) whereas *Solanum ptycanthum* (eastern black nightshade) was apparently only affected by the physical suppression of barley straws (Creamer et al. 1996).

#### **3.2.3.4 Effects of environmental conditions on the production of barley allelochemicals**

Temperature and availability of nitrates tend to increase gramine content in barley (Corcuera 1993, Hanson et al. 1983). Also, when barley lines were grown under increased light intensity and increased temperature at outdoor conditions compared to a growth chamber, gramine production was enhanced up to 100% for some barley lines (Lovett et al. 1994).

In contrast, the production of hordenine was higher when the plants were grown under the cooler conditions. The balance of hordenine and gramine production changes in response to environmental changes (Lovett et al 1994). In conclusion, the production of allelochemicals by barley is influenced by environmental conditions e.g. stress.

#### **3.2.3.5 Genetics of barley allelopathy**

A gramine synthesis gene has been detected on chromosome 5 in barley (Yoshida et al. 1997). The genetics behind the gramine content in adult barley plants has recently been studied by crossing wild and cultivated barley lines with high and low gramine content respectively (Moharramipour et al. 1999). The ratio of progeny containing gramine to progeny without or with a low content of gramine suggested that one or two genes control the synthesis of gramine. The objective of the study of the inheritance of gramine in adult barley was to find a combination of the two properties, gramine provided aphid resistance in the leaves, and a gramine content in barley that does not depress the performance of ruminants (Moharramipour et al. 1999).

In contrast to gramine production, hordenine production does not seem to be under strong genetic control but responds much more to changes in the environment (Lovett et al. 1994).

No information about the genetics of the release of barley allelochemicals was found.

### 3.2.4 Oats (*Avena spp*)

*Scopoletin*

The allelopathic activity of oats has not been extensively studied even though 3000 accessions of *Avena spp.* have been screened for capacity to exude scopoletin (6-methoxy-7-hydroxy coumarin), a chemical identified as phytotoxic towards several plant species (Fay & Duke 1977). Four accessions apparently exuded up to three times as much scopoletin as a standard oat cultivar. When one of the accessions were grown in sand culture with wild mustard *Brassica kaber*, the growth of the mustard was significantly less than when it was grown with an accession that exuded a lower amount of scopoletin. However, in a soil system there was no correlation between scopoletin content of the plant and magnitude of interference with *B. kaber*. Even though the phytotoxic symptoms observed was similar to those caused by synthetic chemicals, the authors stressed that it can not be assumed that the phytotoxic effect exhibited was due to scopoletin alone (Fay & Duke 1977).

### 3.2.5 Rice (*Oryza sativa*)

In recent years, extensive screening programmes have been carried out in the search for rice accessions with an enhanced allelopathic activity. More than 12000 rice accessions have been evaluated for allelopathic activity against at least one weed (Olofsdotter 1998a and references herein). The results are summarised below.

#### 3.2.5.1 Rice allelochemicals

*Phenolic acids*

The phenolic acids 3-hydroxybenzoic acid, 4-hydroxy-benzoic acid, 4-hydroxyhydrocinnamic acid, 3,4-dihydroxyhydrocinnamic acid and 4-hydroxyphenylacetic acid have been identified in higher concentrations in water from allelopathic rice cultivars compared to water from a non-allelopathic cultivar (c.f. Olofsdotter et al. 1997). These phenolic acids have been described as allelochemicals in many plant species and Olofsdotter et al. (1997) suggests that the allelochemicals in rice should be more specific due to the very selective mode of action seen in field testing of allelopathic cultivars.

#### 3.2.5.2 Selectivity in allelopathic activity among rice accessions

The allelopathic activity of rice accessions is highly selective. In field tests, 412 rice accessions out of 12000 were allelopathic against *Hetherantera limosa* and 145 out of 5000 were allelopathic against purple ammannia when the allelopathic activity was evaluated as a weed free zone around rice plants. Sixteen rice accessions inhibited both weed species (c.f. Olofsdotter et al. 1997).

In another field experiment, 1000 accessions were screened for allelopathic activity against the two weedy species, *Echinochloa crus-galli* and *Cyperus difformis*. Of these 45 accessions showed allelopathic

activity against one of the weeds and five accessions inhibited both species (c.f. Olofsdotter et al. 1997). These experiments showing selectivity in weed control among accessions of rice, indicate that several chemical compounds, with selective mode of action against particular weeds are involved in rice allelopathy (Olofsdotter et al. 1997).

### **3.2.5.3 Genetics of rice allelopathy**

Preliminary genetic studies have indicated that allelopathy in rice is quantitatively inherited and has no major genes (Olofsdotter et al. 1997 and references herein).

A research programme aiming at identification of the genes responsible for allelopathy in rice has been initiated (Olofsdotter 1998 b).

### **3.2.5.4 Physiological costs?**

So far, no genetic correlation between yield and allelopathy seem to exist in rice. Physiological cost in form of yield reduction due to allelopathy has not been recognised, but further experiments are needed to clarify whether allelopathy is energy costly for rice plants or not (Olofsdotter et al. 1997).

## **3.3 Conclusive remarks**

Several researchers have suggested improvement of allelopathic properties of crop cultivars by traditional breeding or by genetic manipulation. So far, no crop with strong allelopathic activity has apparently been developed, but research in this area is receiving attention. The inheritance of allelopathic traits has not yet been fully unveiled for the crops described above, but may be under current investigation. At least for barley and wheat, some of the genes involved in the production of allelochemicals have been located. In rice, the search for the genes involved in rice allelopathy has started.

Apart from genotype, the production and/or release of allelochemicals from crops, e.g. rye, wheat, barley and rice, typically depend on the developmental stage of the plant and/or on external factors such as temperature, nutrient status and herbivory.

Especially the hydroxamic acids, DIMBOA, DIBOA and the degradation products MBOA and BOA, have been examined for allelopathic effects against weeds. Among the agricultural species discussed here, hydroxamic acids have been documented to be released from living roots of cultivated wheat and rye and have also been found in wild barley. Production of hydroxamic acids in cultivated barley has been suggested by transferring genetic material from wild barley species. Still, it is not concluded that these allelochemicals would be among the ones that should be selected to obtain cultivars with higher allelopathic activity.

Regarding the phenolic acids also frequently investigated as crop allelochemicals, no information was found on the inheritance of phenolic acids with allelopathic effect. However, some progress has

been made on genetic studies of these compounds in tobacco, *Nicotiana tabaccum*, and French bean, *Phaseolus vulgaris*, but these investigations were carried out in relation to insect resistance (Wu et al. 1999 and references herein).

Even though the allelopathic potential of one class of compounds may be demonstrated, the involvement of other compounds should not automatically be ruled out (Inderjit & Keating 1999). Factors such as the amount of compound detected, its biological activity, laboratory facilities and expertise may influence which compounds researchers are focusing on. Therefore, the improvement of the allelopathic properties of a crop, either by traditional breeding or by genetic manipulation, may not necessarily involve an enhanced or prolonged release of the compounds identified as allelochemicals in crops at present.

Both laboratory and field experiments have indicated a selective response in different plant species to crop allelochemicals. At least two studies indicated that dicotyledons were more sensitive than monocotyledons to allelochemicals released from grasses. Also, members of the same tribe can differ in sensitivity to an allelochemical as demonstrated for the response of *Avena sativa* and *Avena fatua* to rye allelochemicals.

## 4 Ecological effects of allelopathic plants

From an agronomic point of view, allelopathic weed management seems immediately advantageous as an alternative or a supplement to other weed management practices in crop production. Reduced reliance on traditional herbicides via the use of allelopathy has frequently been mentioned as environmentally favourable (e.g. Macias 1995, Narwal et al. 1998).

Nevertheless, apart from the effect on target weeds, it must be considered whether allelochemicals affect non-target organisms and whether the allelopathic plant itself has adverse effects in the cultivated field or in natural environments. Before a genetically modified crop with allelopathic traits can be released, essential questions must be answered such as: Are non-target organisms affected? Will the change in allelopathic activity lead to changes in the invasive and competitive ability of the plant? Can the allelopathic trait spread to other species by gene flow? Will there be long-term effects associated with long-term use either as a result of accumulation of allelochemicals or accumulation of effects through a long-term influence.

In this chapter we present examples of ecological effects of selected allelochemicals and discuss the role of allelopathy for establishment and invasion of non-native species in natural and semi-natural ecosystems. Characteristics of allelopathic plants and of the plant habitat that may influence the allelopathic expression are also included.

### 4.1 Effects of allelochemicals

The previous chapter described the allelopathic activity of some agricultural crops and allelopathic effects on several weedy species or crop plants. Several of the allelochemicals identified as phytotoxic compounds are also involved in insect resistance. This indicates that some allelopathic interactions are part of a general plant defence.

#### 4.1.1 Selectivity of allelochemicals

The visible effects of allelopathy frequently observed as inhibited or delayed seed germination or reduced seedling growth, are secondary expressions of primary effects on metabolic processes.

Analogous to the marketed herbicides, the diversity of structure among allelochemicals suggests that they have no common mode of action. The primary molecular target whereby an allelochemical interferes with physiology and growth is, with a few exceptions, unknown. Similarities within certain groups exists and will probably be further exploited as the functioning of allelochemicals on

*Sites influenced by allelochemicals*

molecular level is a fast growing research area (Einhellig 1996). Exact information about the allelochemical, or allelochemicals, responsible for the allelopathic effects must be sought when assessing the effects of plants with enhanced allelopathic traits (see chapter 5). For reviews about classes of allelopathic compounds, the following are suggested: Apple 1993, Einhellig 1995a 1995b, Gallet & Pellissier 1997, Inderjit & Keating 1999, Putnam 1985, Rice 1984, Wink et al. 1998).

The following sites or processes are known targets for allelochemicals: cell division, production of plant hormones and their balance, membrane stability and permeability, germination of pollen, mineral uptake, movement of stomata, pigment synthesis, photosynthesis, respiration, amino acid syntheses, nitrogen fixation, specific enzyme activities and conduction tissue (after Rizvi et al. 1992 and Wink et al. 1998).

Some of the reports are based only on results of *in vitro* bioassays. It remains to be answered whether the processes are also affected *in vivo* and how many processes are inhibited in addition (Rizvi et al. 1992, Wink et al. 1998).

#### *Allelochemicals as multipurpose defence compounds*

Several allelochemicals have been shown to possess a broad activity spectrum. *In vitro* experiments with more than 70 alkaloids indicate that most alkaloids are toxic or inhibitory to more than one group of organisms including plant seedlings, bacteria, insects and mammals (Wink et al. 1998). The authors conclude that alkaloids can be considered as “multipurpose” defence substances due to their wide activity range. (An example is gramine (see chapter 3) which is toxic to mammals, insects, bacteria, plant pathogenic fungi and plants. The broad biological activity of gramine could be explained by its effects on energy metabolism as it inhibits photo-phosphorylation, Pi-ATP exchange reaction, proton gradient and enhances electron transport in thylakoid membranes (Corcuera et al. 1993). Further, it was suggested, that compounds that affect one, or several, basic molecular targets are more likely to affect a wide range of organisms than compounds that affect targets specific to one organism (Wink et al. 1998).

#### *Phenolic compounds*

Similarly, Gallet & Pellissier (1997) argue that the understanding of the role of phenolic compounds produced by a plant with allelopathic activity, on the establishment of other plant species, requires an ecosystem approach. This is because the phenolic compounds are also likely to have biological toxicity towards other organisms such as microflora and soil animals.

#### *Terpenoids*

The ecological roles of terpenoids have been reviewed by Langheim (1994). Terpenoids are in contrast to phenolic and alkaloids, not commonly identified as allelochemicals in temperate agricultural crops (chapter 3), but occur abundantly in particularly conifers, composites, mints and euphorbias (after Langheim 1994). The review illustrated that terpenoids produced by a plant may contribute to: 1) seed germination inhibition 2) defence against generalist and specialist herbivores, 3) defence against insect vectored fungi and

pathogenic fungi, 4) attraction of pollinators and 5) inhibition of soil bacteria. Numerous individual mono-terpenoids have been demonstrated to have multiple effects (Langheim 1994).

Although multiple effects may appear to be broad spectrum, the effect may be quite specific in terms of dosage levels on different populations of organisms in different communities. This way, the dosage factor can create a degree of specificity (Langheim 1994).

#### *Effects on non-target plants*

The allelochemicals released from a crop may affect non target species, both in fields and in natural habitats if the allelopathic plant is spread. In most agricultural research experiments, the effects of allelochemicals or allelopathic plants has been tested on weedy species or cultivated plants that may be affected by the allelopathic crops due to cultivation practices (e.g. rotational practices) or on species that are valuable as test species in seed germination tests due to their synchronic germination (e.g. Barnes & Putnam 1986, 1987, Macias 1995, Moyer & Huang 1997, Olofsdotter 1999). As concluded in chapter 3, the results of both laboratory and field experiments has indicated a selective response of different plant species to allelochemicals. Despite some studies have indicated that dicotyledons were more sensitive than monocotyledons to allelochemicals released from grasses, the response of plant species to allelochemicals cannot be predicted until the exact mode of action of the allelochemicals is known. Seed characteristics such as seed size and seed coat permeability may influence the uptake and effects of allelochemicals in seeds. In seed germination assays it was shown that species with small seeds were more inhibited than larger seeded species at a given concentration of allelochemicals (Barnes & Putnam 1987, Pérez 1990, Chase et al. 1991).

#### *Effects on other organisms - some examples*

The hydroxamic acids are often mentioned and examined as allelochemicals released from intact plants of cereal crops such as wheat and rye (chapter 3). They are present in leaves, stems and roots of cereal plants. Their potential in the control of pest and diseases of the crops has often been discussed (e.g. Niemeyer & Perez 1995). The role of hydroxamic acids in crop resistance to aphids, fungi and bacteria is well documented. For instance, constitutive levels of hydroxamic acids have been shown to deter aphid feeding and decrease aphid survival and reproduction (e.g. Niemeyer 1988, Niemeyer & Perez 1995 and references therein).

In wheat and barley seedlings, inverse correlation have been found between concentrations of hydroxamic acids or gramine, respectively, and population growth rate of two aphids species, *Metopolophium dirhodum* and *Schizaphis graminum*, on the plants (Corcuera et al. 1992). Even though the performance of these aphid species on the wheat plants was negatively affected by hydroxamic acid, the wheat seedlings with the higher content of hydroxamic acids were more damaged by aphids. This was explained by the feeding behaviour of the aphids as at least *S. graminum* probed for a longer time and thereby made more damage on the seedlings with higher contents of hydroxamic acids (Corcuera et al. 1992). Hence the plant content of compounds that can be released as allelochemicals

and also function as insect deterring agents can both be advantageous and disadvantageous for the success of the plant.

Gramine is also considered to be an “anti-quality factor” in barley as gramine may be toxic to mammals (Hanson et al. 1981). It has been reported to be toxic to sheep, cattle, meadow voles, insects, pathogenic fungi and bacteria (Corcuera et al. 1992). The mode of action has been described earlier in this section.

#### *Effects on microbial symbioses*

Allelochemicals may affect plant species through inhibition of their microbial symbionts such as mycorrhiza and nitrogen fixing bacteria. Under natural conditions, seedlings of black spruce (*Picea mariana*) growing close to the allelopathic shrub, *Kalmia angustifolia*, had significantly lower rate of mycorrhizal infection than seedlings further away (Yamaski et al. 1998). The presence of *K. angustifolia* was found to reduce the growth of specific parts of the seedling, e.g. foliage, twigs and stem, as well as foliage concentrations of nitrogen and phosphorus. *Kalmia angustifolia* extensively invades many *P. mariana* plantations in central New Foundland and at many of these sites an increase in *K. angustifolia* cover is associated with chlorosis and decreased height growth of *P. mariana*.

Previous laboratory experiments also indicated that extracts of *K. angustifolia* affect the growth of certain ectomycorrhizal fungi associated with *P. mariana*. It seems possible that *K. angustifolia* specifically affects the uptake of nutrients by *P. mariana* by affecting the activity of mycorrhizal fungi (Yamaski et al. 1998). However, the importance of this factor compared to the ability of *K. angustifolia* to out-compete spruce seedlings by nutrient competition, allelochemicals or by increasing the occurrence of the pseudo-mycorrhizal fungus *Phialocephala dimorphospora* on spruce roots could not be ruled out under natural conditions (Yamaski et al. 1998).

Laboratory and greenhouse experiments have also shown that aqueous extracts of crowberry *Empetrum hermaphroditum* reduced the spread of mycorrhizal infection in seedlings of Scots pine, *Pinus sylvestris* (Nilsson et al. 1993). The N uptake by *P. sylvestris* seedlings was reduced when extract of *E. hermaphroditum* was added. The negative effect on N uptake was particularly evident on seedlings infested with mycorrhiza. Apparently, the extract of *E. hermaphroditum* reduced the ability of mycorrhiza to take up N (Nilsson et al. 1993). Under field conditions extracts and exudates of *E. hermaphroditum* affect seed germination and seedling development of *P. sylvestris* negatively (Zackrisson & Nilsson 1992).

Compounds released from both living and herbicide-killed plant material of *Elytrigia repens* apparently inhibit the symbiosis between *Rhizobium* and legume species (Weston & Putnam 1985, Putnam & Weston 1986). The nodulation and development of legume species was inhibited by both living and herbicide (glyphosate)- killed *E. repens*. Laboratory studies indicated that compounds from *E. repens* are particularly inhibitory to root hair formation, which subsequently prevents the infection by *Rhizobium*. Compounds that inhibited root hair formation did not inhibit the growth of several species of



*Rhizobium*. This means that the morphological changes induced by allelochemicals can reduce nodulation in legume species.

Decomposing leaves of nodding thistle, *Carduus nutans* affect the symbiotic nitrogen fixation negatively of white clover, *Trifolium repens*, in pastures dominated by white clover and perennial ryegrass, *Lolium perenne* (Wardle et al. 1998 and references herein).

#### 4.1.2 Effects on soil properties and nutrient availability

Apart from the direct toxic effect on other plants, some allelochemicals are supposed to influence the availability of nutrients in the soil.

#### Changes in chemical characteristics of the soil

It has been hypothesised that allelopathic plants in addition to qualitative and quantitative changes in the soil content of allelochemicals also may cause changes in soil chemical characteristics (Inderjit 1998). In one study, the presence of *Pluchea lanceolata*, an aggressive evergreen asteracean weed, apparently influence certain soil properties. In addition to the higher phenolic content of soils in the vicinity of *P. lanceolata* compared to soils between 10 and 40 m away, pH, electrical conductivity, potassium (K<sup>+</sup>) and soluble chloride (Cl) were influenced in the soil in contact with *P. lanceolata*. However, it was not established that the observed nutrient alterations resulted from phenolics excreted from *P. lanceolata* (Inderjit 1998). As the *P. lanceolata*-infested soils had significant negative effects on seedling growth of various crop plants compared to non-infested soils, it is possible that the effect of allelopathic plants can be due to the allelochemicals in the soil and/or to altered soil nutrients. Generally, phenolic acids are considered to have important influence on nutrient cycling in terrestrial ecosystems. Phenolic monomers and phenolic acids can form complexes with nutrients and thereby influence the nutrient availability and nutrients turn over in soil (Apple 1993, Kuiters 1991).

In conclusion it is possible that some allelochemicals, at least phenolic acids, in addition to their direct effects on other plants may also interfere via altered nutrient availability.

#### Nitrification

Investigations by Rice & Pancholy (cf Rice 1984) have indicated that phenolic compounds can inhibit the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> through toxicity towards nitrifiers (Rice 1984). These results have been much discussed by Bremner & McCarty (1993) who found no inhibitory effects using pure phenolic compounds on soil and reported that phenolics and terpenoids enhanced the immobilisation of NH<sub>4</sub><sup>+</sup> by soil organisms rather than the inhibition of nitrifying bacteria.

## 4.2 Effects of allelopathic plants in natural ecosystems

One of the important objectives within agricultural allelopathy research is to be able to affect the species composition in the field in a

way that favours the growth of the crop or succeeding crops (see chapter 3). An important question is whether a cultivar with enhanced allelopathic activity would also be more likely to invade and/or establish in natural habitats. These questions are discussed below with examples from natural habitats.

#### 4.2.1 Effects on population and community structure

##### *Reduced species richness*

In Spanish scrublands, the floristic diversity, richness and evenness of herbs found beneath the Mediterranean scrub Crimson spot rockrose (*Cistus ladanifer*) is significantly lower than in adjacent plots without this plant. The allelopathic activity of *C. ladanifer* is thought to play an important role as leaf exudates of this plant inhibit the germination of seeds of species that are absent from - but found growing adjacent to *C. ladanifer* scrublands. The distribution of these species are apparently limited by the allelopathic action of *C. ladanifer* (Chaves & Escudero 1997). Also some species growing close to *C. ladanifer* are affected by the exudates by delayed seed germination and reduced seedling growth. On the basis of these results, it is suggested that the allelopathic activity of a plant may reduce both number and population size of other species by reducing their competitive ability (Chaves & Escudero 1997). In interpreting the results, it must be considered that the exudates were extracted in ethanol and that the seed germination tests were carried out only under laboratory conditions and without osmotic controls.

##### *Changes in genotypic variation in afflicted plant populations*

Allelopathic plants may induce genetic changes within associated plant populations. The release of allelochemicals from *Ailanthus altissima*, tree-of-heaven, seems to be responsible for altering the genetic pool of susceptible neighbouring plant species. Both close (<1 m) and distant populations (> 10 m) of *Tridens flavus* are inhibited by *Ailanthus* toxins. But, the distal population includes a class of highly susceptible individuals not present in the proximal population. It is suggested that the genotypes sensitive to the allelochemicals have been removed from the gene pool of the proximal population by selection (Lawrence et al. 1991).

#### 4.2.2 Invasion and dominance

Allelopathy has been discussed as one of several factors affecting the ability of a plant to invade and establish in a new ecosystem. A few examples demonstrating the importance of allelopathy for successful invasion are presented below.

##### *Aggressive colonizers*

The release of allelochemicals from plants known as aggressive colonisers e.g. *Elytrigia repens* (quackgrass) and *Vulpia myuros* (silvergrass), have lead some to suggest that allelopathy is involved in successful invasions (e.g. Friebe et al. 1995, An et al. 1997). However, the actual importance of the release of allelochemicals by these coloniser plants has rarely been demonstrated under natural conditions. For some aggressive coloniser species previously reported to be allelopathic, further investigations have not confirmed that release of allelochemicals was essential for their ability to establish in new habitats.

### *Lantana camara*

The importance of allelopathy has been examined for the ability of the exotic, invasive, woody weed *Lantana camara*, to invade, establish and form dominant components within certain susceptible ecosystems including various types of Australian forests (Gentle & Duggin 1997). In an experiment by Gentle & Duggin (1997) it was attempted to distinguish between suppressed seedling growth caused by possible phytotoxins and density dependent resource competition respectively. The results provided evidence that *L. camara* is capable of interrupting the regeneration processes by decreasing germination, reducing early growth rates, and reducing survival of two indigenous species by allelopathy. These changes are expected to lead to disruption of community development because *L. camara* can also aggressively compete with indigenous seedlings.

### *Release of allelochemicals from decomposing leaves*

The release of allelochemicals from decomposing leaves of nodding thistle, *Carduus nutans*, has been reported to be involved in the establishment of this species in pastures of *Lolium perenne* and *Trifolium repens* in New Zealand. Initially, bare patches in the pasture allowed seed of *C. nutans* to germinate. The allelochemicals suppressed *T. repens* and thereby provided new bare patches where seeds of *C. nutans* can germinate. Bioassays have indicated that the decomposing material of *C. nutans* is allelopathic and that the nature of this allelopathy is highly selective. Leguminous species appeared to be considerably more sensitive than grass species. The suppression of *T. repens* seemed to be caused by a reduction in *T. repens* nodulation whereby the symbiotic nitrogen fixation became adversely affected (Wardle et al. 1998 and references herein). It was also suggested that the decline in soil nitrogen input may benefit the subsequent *C. nutans* cohorts as this species tolerate low nitrogen conditions better than most forage species. This example illustrates that the allelopathic activity of a plant species may contribute to an increased population size and perhaps also to changed ecosystem functioning.

### *Pyrrolizidine alkaloids*

The concentration of pyrrolizidine alkaloids in roots of the grass tall fescue, *Festuca arundinacea*, is suggested to influence its competitive ability through an allelopathic effect on neighbouring species. In a study, the concentration of pyrrolizidine alkaloids was higher in roots tall fescue infected with endophytes compared to non-infected tall fescue plants (Malinowski et al. 1999). When it was grown in monoculture, endophyte infection reduced the dry matter of roots by 16 %. Competition experiments suggested a negative interaction between red clover and infected tall fescue, where the endophyte infection resulted in an increased concentration of pyrrolizidine alkaloids in the roots (Malinowski et al. 1999). The actual release of the pyrrolizidine alkaloids was not measured in the experiment. From previous studies this compound is known to influence the germination and seedling growth of some plant species (c.f. Malinowski et al. 1999). The experiment suggests that allelochemicals may be important for the ability of a species to increase its dominance at least at low species diversity.

### *Bunia orientalis*

It has been investigated whether allelopathic effects might promote the rapid spread of *Bunia orientalis* (a perennial *Brassicaceae*) in

Central Europe (Dietz et al. 1996). Leaf extracts and root exudates were found to significantly inhibit seedling growth of two sensitive agricultural species, e.g. lettuce and barley, whereas two species naturally co-occurring with *B. orientalis* showed only weak response to root exudates. When leaf litter leachates and decaying leaves of *B. orientalis* were added to soil in concentrations corresponding to those expected under natural conditions, seed germination of four species were only slightly reduced compared to leaf litter of decaying leaves of a species mixture. On the basis of these results, Dietz et al. (1996) concluded that even though allelopathic effects can be obtained in seed germination tests, the release of allelochemicals under natural conditions will not be a substantial factor in the rapid establishment of *B. orientalis* (Dietz et al. 1996).

#### *Brassica species*

Choesin & Börner (1991) investigated whether the aggressive colonisation and persistence of *Brassica* species in plant communities could be attributed to the release of allelochemicals. In replacement series experiments, the competitive ability of a wild type and a mutant type of *Brassica napus* were tested. The wild type released more of a suspected allelochemical, allyl glucosinolate, than the mutant type. The two *Brassica* genotypes suppressed the growth of the test species, *Medicago sativa*, equally well and were equal competitors when tested in the intraspecific replacement experiment. This result led Choesin & Börner (1991) to conclude, that *B. napus* showed no indications of being allelopathic and that the future studies of aggressive colonisations of *Brassica* species should focus on other mechanisms than allelopathy. However, it must be considered that other species may be more susceptible to the release of allyl glucosinolate from *B. napus* than *M. sativa* or that other compounds produced in equal amounts were responsible for the observed suppression.

#### *Elytrigia repens*

The grass *Elytrigia repens*, has often been reported to be allelopathic and the release of several phenolic compounds and hydroxamic acids from dead plant material and from intact roots has been described earlier in this chapter. However, when studying the competitive interaction between the two grasses *E. repens* and *Puccinellia distans*, it was concluded that the allelopathic activity of *E. repens* does not seem to affect the biomass production of *P. distans*. Furthermore, it was questioned whether *E. repens* was truly allelopathic (Beyschlag et al. 1996). This was based on experiments where three different allelochemicals were added to monocultures of *P. distans* grown in hydroculture pots. The concentration and selection of allelochemicals was made on the basis on references in the literature. The biomass of *P. distans* was not significantly affected by the three allelochemicals tested. Again, before final conclusions can be drawn, the experimental conditions must be evaluated. It should be considered that the allelochemicals, were added individually and a possible mixture effect could not be detected in this procedure (see also chapter 2). In addition, other allelochemicals than the ones tested may be involved - for example, the release of hydroxamic acids, DIBOA and DIMBOA, from intact roots of *E. repens* has also been reported (Friebe et al. 1995).

Allelopathy is supposed to play a role in the inhibition of several coniferous species by rapid growing ericaceous shrubs (Mallik 1998). In cool, temperate climate, rapid vegetative growth and release of allelochemicals from *Calluna*, *Empetrum*, *Kalmia* and *Gaultheria* result in failure of conifer regeneration after disturbances, e.g. Zackrisson & Nilsson (1992), Mallik (1998) see also section 4.5.

### 4.3 Ecological characteristics influencing the allelopathic expression

The allelopathic effect is largely determined by the biological active concentration of allelopathic compounds in the soil (Rice 1984). This is often influenced by biological and chemical characteristics of the soil such as nutrients, microorganisms, organic matter and pH. Also, the timing between the release of allelochemicals and the sensitive stage of the susceptible species is important (see chapter 2 and 3). Other characteristics of the allelopathic plant itself may be important for its ability through competition to influence plant community structure.

#### *Life cycle of the plant*

Perennial plant species are likely to release and add allelochemicals to the soil over more than one season. This is probably the reason why most reports on the importance of allelopathy for the ability of a plant to invade or dominate in an ecosystem has involved perennials, including *Empetrum hermaphroditum*, *Kalmia angustifolia*, and *Lantana camara* (Zackrisson & Nilsson 1992, Gentle & Duggin 1997, Mallik 1998).

For annual species, the allelopathic activity has been suggested to be part of their success as weeds. Experiments with weedy species indicate that the life cycle pattern is an important factor for the allelopathic interference between weeds and crops (Inderjit & Dakshini 1995, 1996, 1998). The allelopathic effect of the perennial weed, *Pluchea lanceolata*, towards several crop species has been established under field conditions, see also section 4.1.2 (Inderjit & Dakshini 1996, Inderjit et al. 1996). Also, the polycarpic annual weed, *Stellaria media*, has been shown to interfere allelopathically with wheat. The growth pattern of *S. media* with several life cycles completed within one life span of most crop species maintains the pool of allelochemicals in the crop environment. The simultaneous presence of several age-class plants in the same population may also be important because the critical life-stage for release of allelochemicals is more or less continuously represented (Inderjit & Dakshini 1998).

In another experiment, chemical interference of the monocarpic weed, *Polypogon monspeliensis* with the crops (radish and cluster bean) during the growth season could be ruled out. However, interference was observed with the following crop, probably due to a high content of phenolic compounds in the straw of *Polypogon monspeliensis* (Inderjit & Dakshini 1995).

It has been suggested, that an uptake of allelochemicals by insensitive species can detoxify the soil (Thijs et al. 1994 - see below). Thereby, the presence of insensitive species reduces the effect on sensitive species. Hence, not only the species diversity, but also the relative proportion between sensitive and insensitive species could be important for the expression of allelopathy.

#### *Species diversity*

It has been suggested that an effective and consistent allelopathic inhibition of one species by another is more likely to occur in species poor communities than in species rich. In a community with high plant species diversity, it is less likely that one species would reach sufficient dominance for its allelochemicals to dominate the soil biochemistry (Wardle et al. 1996). The above arguments are based on a comparison of the results of two experimental series by Stowe (1979) and by Wardle et al. (1996). In the experiments by Stowe (1979), bioassays did not correlate with field observations at sites with a high diversity of plant species. In contrast, Wardle et al. (1996) detected a statistical relationship between bioassays and field experiments when the field sites consisted of pure swards of each species tested.

Furthermore, due to the large variation in the rate of uptake and sensitivity to allelochemicals between different species (e.g. Thijs et al. 1994, Pérez 1990) the effect on some susceptible species is likely to be reduced by the uptake of allelochemicals by other species.

These results indicate that allelopathic plants are more likely to become established and/or dominate in communities of low species diversity. This would particularly be the case if the phytotoxic effects were directed towards a broad spectrum of species, of course dependent upon the nature and diversity of the receiving ecosystem. Severe effects would also be expected in the case where an invasive species is phytotoxic to a crop.

#### *Plant density*

The relative density between donor and receiver species has been suggested to be an important factor in the degree of expression of allelopathy. This has been suggested as a method to distinguish between allelopathy and resource competition (see chapter 2). Survival and growth of susceptible species should be positively correlated with the density of the non-sensitive neighbouring species due to a dilution of the effect of allelochemicals (Weidenhamer et al. 1989, Thijs et al. 1994).

The principle of density-dependent-effect has been used under field conditions, by Gentle & Duggin (1997) to verify that allelopathy is an important factor for the ability of *Lantana camara* to invade different forest ecosystems. Both the density of the allelopathic plant and the density of the susceptible species have been documented to influence the degree of allelopathic inhibition. In field studies, seedling growth of two susceptible species was negatively related to increasing density of *Lantana camara*. When the densities of the two susceptible species were increased, the average seedling biomass for both species increased (Gentle & Duggin 1997).

In cultivated fields, the relative density between weed(s) and an allelopathic crop (e.g. rye or barley, or future crop varieties developed through traditional or biotechnological breeding) is most probably different from the relative density between the susceptible plants and the allelopathic crop in cases where the allelopathic crop is spread to other ecosystems. Therefore, results from the cultivated system may not be valuable as an indication of how a crop with allelopathic traits will affect susceptible plants in other ecosystems.

When discussing the influence of density and species diversity on allelopathic expression, the physical distance between allelopathic plants and afflicted species should be considered.

### *Co-adaptation*

Newman (1978) has tested the hypothesis that predicts that plant species are more tolerant to allelochemicals produced by neighbouring species on the basis of literature data. It is acknowledged that there is evidence that on a strictly local scale, tolerance to allelochemicals allow a plant species to grow near or under the allelopathic plant (Newman 1978). However, no clear differences in response to allelochemicals of local plants between native, introduced and alien species in the examples studied. These results suggest that long coexistence does not result in species becoming more tolerant to each other's allelochemicals. Thus, the author concludes, that there is not often specific selection for tolerance of allelopathy. It was suggested that tolerance to allelochemicals from other plants depend upon characters such as rooting depth, cuticle thickness, cell membrane properties and the relative importance of alternative metabolic pathways, which are subject to other selection pressures (Newman 1978).

It has been shown that individuals of neighbouring plant species previously unexposed to the allelochemicals, released from the tree *Ailanthus altissima*, were more susceptible to the allelochemicals than individuals previously exposed. Also, populations previously exposed to the allelopathic influence of *A. altissima* were more tolerant to *Ailanthus* toxins than previously unexposed plant population. The experimental data suggested that the seeds of individuals previously exposed to *Ailanthus* were better suited for growth in *Ailanthus*-contaminated environments (Lawrence et al. 1991). This result indicates that on the local level, populations may adapt to the allelochemicals.

Discussing the importance of co-adaptation, it must again be stressed that the effect of allelochemicals and allelopathic plants should not be looked at isolated from microbial activities and physical factors of the soil (e.g. Rice 1984, Inderjit et al. 1996). Inderjit et al. (1996) also showed that differences in agricultural practices at individual sites possibly affected the quantitative and qualitative availability of phenolics in soil. As the allelopathic activity of a species can vary in soils from different sites, the effect of an allelopathic plant can be different when a plant species (or cultivar) is introduced to a new area.

### *Allelopathic hierarchy?*

An interesting aspect is that some plants have allelopathic effect against plants, which themselves have been reported to be allelopathic (e.g. Wardle et al. 1996). This raises the question of whether an allelopathic hierarchy exists. A hypothesis relating to whether species that are more allelopathic also are those that are less sensitive to allelopathic effects of other species has been tested by comparing the results of various bioassays. No relationship was detected between the response and the effect of ten grassland forage species, more or less allelopathic, towards the allelopathic weed *Carduus nutans*. This indicates that the effects of allelochemicals and response to allelochemicals are unrelated and that there is little evidence for an allelopathic hierarchy (Wardle et al. 1996).

It is possible that allelopathy in some instances may act as a stress factor on exposed susceptible species and thereby also affect the production of allelochemicals by the exposed species.

### *Sensitive ecosystems?*

It has been emphasised that allelopathy is particularly evident under conditions where allelochemicals can affect seeds or seedlings or accumulate in soil or plant litter. For instance, the slow metabolism of phenolic acids with phytotoxic properties under mor humus conditions may explain why species typically for mor sites are often found to have allelopathic activity (Kuiters 1991). Based on their observation of interaction between allelopathic effect and plant density Weidenhamer et al. (1989) suggested that the allelopathic effect might be intensified in natural communities where overall plant densities are lower for example because of harsh environmental conditions and other constraints. Communities such as the Florida scrub, the California coastal chaparral, dry tropical scrub communities were mentioned as examples (Weidenhamer et al. 1989). Similarly, Langheim (1994) argues that for terpenes, there appear to be interacting effects between allelopathy and some other conditions, such as drought, soil type and intraspecific competition, or intraspecific diversity effects.

Agricultural systems, where disturbances, crop rotation and addition of fertilisers are frequently occurring, may according to the descriptions above not be characterised as ecosystems especially sensitive to allelochemicals. Generally a high density of donor plants and a stressful environment may be important for the allelopathic effect.

## **4.4 Spread of allelopathic traits between species?**

Transfer of allelopathic traits from *Agropyron glaucum* to a cultivar of wheat, *Triticum aestivum* has been carried through hybridisation (Panchuk & Prutenskaya 1973). It was found that water extracts of residues of the hybrid were more toxic than extracts of wheat residues against seed germination of radish, *Raphanus sativa*, and against root growth of cress, *Lepidium sativum*. The first generation hybrids exhibited chiefly *A. glaucum* characteristics and manifested high inhibitory activity. This example illustrates that the transfer of



allelopathic traits between species is possible, but has not been reported from natural habitats.

## 4.5 Competition through allelopathic interference

### *Allelopathy and competition*

The difficulty of separating allelopathic interference from other kind of competitive interactions makes the importance of allelopathy in plant-plant interactions somewhat unclear. Hence, it is important to notice that allelopathy in many cases should be seen as part of the strategy of a species, rather than being the main determinant of interaction in natural ecosystems. Neither should it be seen as a species characteristic being in opposition to competition. In considering the competitive properties of a plant, allelopathy could be seen as part of an arms arsenal also including nutrient competition, water competition, light and space competition etc. In some situations allelopathy may be unimportant. However, in stressed situations allelopathy may be the factor that tips the outcome of competition to the advantage of the allelopathic species.

Weidenhamer (1996) argues that the difficulty in distinguishing between chemical interference and resource competition may be an obstacle for the use of allelopathy in weed management. However, the reality is that the allelopathic effect of some crops, for example rye, to some extent already is used for weed management (Weston 1996, Olofsdotter 1998b). Though Weidenhamer argues that full proof of allelopathy may never be attained, some investigations aiming at separating allelopathy from other factors of competition have been reported (e.g. Rice 1984; Weidenhamer et al. 1989; Nilsson 1994; Thijs et al. 1994). Two of these are presented below.

### *Helianthus annuus*

The striking distribution pattern of herbaceous species around common sunflower *Helianthus annuus* led Wilson and Rice (citation of W&R (1968) in Rice 1984) to believe that allelopathy could be the explanation. Through experiments and observations they showed that *Erigeron canadensis*, and *Rudbeckia serotina* were significantly inhibited near sunflowers, whereas other species were slightly inhibited or even stimulated. Growth experiments comparing soil from below the sunflower and nearby soil confirmed the observations, as did experiments with leaves or roots from sunflower added to soil. The toxin(s) responsible for the effect was/were not identified, neither were the mechanisms of production and release or uptake in the receiver plants.

### *Empetrum and Pinus*

In the Swedish boreal forest, crowberry, *Empetrum hermaphroditum*, forms extensive clones in postfire successions on acid soils. Regeneration of Scots pine in such crowberry-dominated areas has often failed. Explanations have focussed harsh climate, unfavourable soils and dense plant cover until it was suggested that chemical inhibition could be involved (Zackrisson and Nilsson 1992). In experiments it was shown that leaf exudates from *E. hermaphroditum* strongly inhibited seed germination of Scots pine both in field experiments and under controlled indoor conditions (Nilsson 1994). The toxin responsible for the inhibition was batatasin III, which in its

purified form strongly inhibited seed germination of Scots pine. Batatasin III was produced in secretory gland cells in the leaves of *Empetrum hermaphroditum* (Wallstedt et al. 1997; Nilsson et al 1998). Finally, it was shown that the chemical inhibition causing reduced seed germination, slow growth and high mortality of Scots pine could be reduced by adding activated carbon to the soil (Nilsson & Zackrisson 1992).

#### *Influence of allelopathy on succession*

The effect of *Empetrum hermaphroditum* on *Pinus sylvestris* regeneration slows down the succession towards coniferous forest, which otherwise would be the natural climax vegetation in boreal areas. Under natural conditions this effect would be prevented by forest fires, which occur frequently enough to prevent the extensive dominance of the slow growing crowberry shrub. However, anthropogenic influence has decreased the natural fire frequency as well as the affected area in order to protect the wood production, with the consequence that crowberry shrubs become dominant and thereby a problem for forest establishment (Zackrisson et al. 1997). Allelochemical inhibition of ectomycorrhiza forming fungi and other symbiotic fungi is another way to influence tree stages in succession that relies on symbiosis (Nilsson et al. 1993; Zackrisson et al. 1997). Control of the succession can also take place in a more indirect manner through allelopathic inhibition of nitrogen fixing microorganisms, e.g. *Rhizobium spp.* and many genera within the Cyanobacteriae. Inhibition of nitrification and ammonification is another way of controlling the amount of plant available nitrogen. Such inhibition can be advantageous to species tolerant to low nitrogen availability (Rice 1984). Such tolerance is typical for species belonging to early succession stages.

Another example of allelopathic plants having influence on succession has been hypothesized by Rice (1984) to explain why the first pioneer weed stage is so rapidly replaced by prairie threeawn *Aristida oligantha* in abandoned fields in Oklahoma and Kansas. Rice explained the observation with self-elimination due to production of toxins in the species belonging to the first pioneer stage. *Aristida oligantha*, being insensitive to these toxins, therefore could replace the pioneer weed stage much faster than would be the case if this community level autotoxicity was not present.

## **4.6 Conclusive remarks**

The direct chemical interference between an allelopathic plant and susceptible species may not be the only effect of an allelopathic plant. Apart from the direct phytotoxic effects of some of the allelochemicals so far identified in the selected temperate allelopathic crops described in chapter 3, effects on other organisms and on ecosystem processes, such as nutrient availability, have been demonstrated or debated in the literature. In conclusion, allelopathy may act directly on plants and other organisms and indirectly through alteration of soil properties, nutrient status and altered population and/or activity of harmful or beneficial organisms like

microorganisms, insects, nematodes etc. Therefore, allelopathic plants may have wide-ranging effects in ecosystems.

Plant populations may be affected in the vicinity of allelopathic species, both species diversity and genetic variation may be affected e.g. by development of resistance towards certain allelochemicals.

The importance of allelopathy in competition between species is not fully understood. At least in a few cases it has been shown that the release of allelochemicals is important for the dominance of the donor species, which apart from becoming dominant also is able to control or at least to some degree slow down the natural succession rate. The control can be by direct inhibition of the germination of woody species, inhibition of symbiosis or through control of available nitrogen by inhibition of nitrogen-fixing microorganisms. However, it is also possible that autotoxicity can speed up succession due to faster elimination of some succession stages.



## 5 Assessment of ecological effects of plants with allelopathic traits

The use of allelopathy in agriculture has been suggested as an additional tool in the weed management in several recent papers. Crop cultivars with enhanced allelopathic activity can possibly be obtained by selection among the existing cultivars, through traditional breeding methods or by genetic manipulation (chapter 3).

In the design of experiments to assess the ecological effects of allelopathic crops, the interaction between allelopathy and physiological, chemical and biological components of ecosystems must be considered.

### 5.1 Environmental risk assessment

#### *EEC directive*

Before a genetically modified allelopathic crop can be released within the EEC an environmental risk assessment is required (EU directive 90/220/EEC). This means that a more thorough assessment of the effects of a genetically modified crop with enhanced allelopathic activity is required compared to crop cultivars with a strong allelopathic activity obtained by selection or traditional breeding methods.

#### *Approval of allelopathic crops obtained through GM*

For a genetically modified allelopathic crop, the allelochemical, or allelochemicals, responsible for the target effect has to be identified. The trait(s) and characteristics, which have been introduced or modified must be described in applications for approval of genetically modified crops. Other obligatory information includes; Information on the sequences actually inserted/deleted:

- a) size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP;
- b) in case of deletion, size and function of the deleted region(s); copy number of the insert location(s) of the insert(s) in the plant cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination;"

In contrast, it will be possible through traditional cultivation programmes to select and grow crop cultivars with enhanced allelopathic activity toward one or more weedy species without identifying the allelochemicals responsible for the effect and without assessment of possible ecological effects.

#### *Guidelines*

A structured system has been developed to identify the information needed to perform a profound ecological risk assessment of genetically modified plants (Kjær et al. 1999). This information forms basis for the evaluation of the risk of invasion and establishment, risk

of effects on non-target organisms and risk of spread of the inserted trait to other plant species.

### *Effects from plant debris*

The effects of the dead plant material of a genetically modified crop on the vegetation are not considered in the approach by Kjær et al. (1999). For several allelopathic plants the release of allelochemicals from decaying plant material influence the germination and development of plant species in the vicinity. Therefore, this aspect must be included in the risk assessment of genetically modified plants with allelopathic traits.

The approach implies that a specific risk assessment should accompany a particular case of genetically modified higher plants (Kjær et al. 1999). This will also include each new case of genetically modified plants with allelopathic traits.

## **5.2 Reflections concerning experimental designs to assess the effects of allelopathic crops**

The validity of the methods so far used to demonstrate that allelopathy occurs under natural conditions has been questioned and debated (chapter 2 and 4). In risk assessment, in contrast to the demonstration of allelopathy as an ecological significant factor, the difference in allelopathic activity between genetically modified and non-modified crop cultivars provides material for comparative studies between cultivars originally similar with respect to morphological and physiological characters. Comparative studies may be a valuable tool in the assessment of the effects of an enhanced release of allelochemicals.

Even though, the allelochemicals responsible for the observed effects have been identified and comparative studies are possible, still the demonstration of some of the possible ecological effects of a plant with allelopathic traits might possess some challenges due to the interaction between allelopathy and biotic and abiotic factors.

### **5.2.1 Factors to be integrated in the experimental design to risk assessment of allelopathic crops**

Conclusions from the previous chapters of this report, suggests that the following factors must be integrated in the design of experiments to assess the effects of a plant with allelopathic traits:

- 1) Plant species differ considerably in sensitivity towards allelochemicals (chapter 2, 3, 4). Therefore, it must be critically evaluated how many species it is essential to test to be able to "predict" the selectivity of an allelochemical. The response of plant species in habitats whereto the allelopathic crop (or allelopathic trait) might spread must be considered.
- 2) The production and release of allelochemicals might be altered by various stresses and by environmental conditions (chapter 2 and 3).

- 3) Allelopathic effects are influenced by biotic and abiotic factors (chapter 2 and 3).
- 4) Some allelochemicals may influence nutrient availability in the soil (chapter 4).
- 5) Allelopathic plants often release several allelochemicals. These can have different mode of action - the effect might be antagonistic, additive or synergistic (chapter 2 and 3). Even though, the release of one allelochemical would be dominant from a genetically modified crop, this allelochemical may interact with other allelochemicals in the habitat.
- 6) Species density and species diversity may influence the allelopathic effect on sensitive species (chapter 4). The importance of the relative density between allelopathic plants and receiver plants must be carefully examined.
- 7) Agricultural ecosystems where allelopathic crops might be grown may not be the only habitats sensitive to the allelochemicals released (chapter 4). The effects of allelopathic agricultural species may be different in other ecosystems. This should also be considered in test procedures.

### *Monitoring*

The design of monitoring programmes accompanying the approval and release of GM-allelopathic crops and trees should be careful to include potential affected ecosystems due to spread of the allelopathic crop or modified genes to wild relatives through hybridisation.

### **5.3 Conclusive remarks**

If a crop with enhanced allelopathic traits is developed by genetic modification, the ecological risk assessment shall take the allelopathic properties into account. The effects of release of allelochemicals from plant debris on seed germination and plant development of other species will be special to allelopathic crops and trees and must be included to complete the risk assessment.





## 6 References

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