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Chemical Ecology in Wheat Plant–Pest Interactions. How the Use of Modern Techniques and a Multidisciplinary Approach Can Throw New Light on a Well-known Phenomenon: Allelopathy

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A new holistic approach on research into allelopathy was launched in the FATEALLCHEM project. The project was financed by the European Commission in the 5th Framework Programme and involved agronomists, biologists, analytical chemists, organic chemists, environmental chemists, ecotoxicologists, and modelers. Benzoxazinones from wheat (*Triticum aestivum* L.) were the main group of compounds studied in the project. The project showed that future assessments of an extensive use of allelopathic crops must include the development of validated analytical methods, considerations of relevant concentrations, studies on soil transformation, ecotoxicological studies on individual compounds and mixtures, evaluation on human and mammal toxicity, and joint effect studies on weeds, insects, and pathogens. The project results clearly showed the relevance of optimizing the exploitation of cereal benzoxazinones. Crop rotation is a very traditional practice, which was given less importance for decades but is now regaining its importance in agricultural practice as a means of controlling weed seed banks and soilborne diseases and pests. When using cereals as catch crops and green manure, the allelopathic properties of the cereals could now be much more extensively exploited, choosing varieties with optimal production of benzoxazinones and optimizing the time of sowing in relation to the formation of bioactive metabolites.

KEYWORDS: Allelochemicals; benzoxazinones; hydroxamic acids; lactams; benzoxazolinones; phenoxazinones; secondary metabolites; LC-MSMSs; NMR; molecular modeling; synthesis; toxicology; assessment; fate

INTRODUCTION

The use of synthetic pesticides in Europe is high [300 000 metric tons in 1999 according to most recent Eurostat statistics (*1*)]. A large effort is still made to reduce pesticide use and thus diminish the environmental impact of agriculture and at the same time reduce the level of pesticide residues present in food. A program monitoring pesticide residues in food in 1999, consisting of 4 million individual determinations, showed an increase in the residue levels of synthetic pesticide residues in European crops (*2*). European consumers strongly demand a development toward reduced pesticide residue levels in food; 54% of the European population consider the absence of pesticides as an indicator for food safety (*3*, *4*). Alternative methods for controlling weeds, insects, pathogens, and other pests have therefore come into focus during the past decades.

Innate plant defense mechanisms were first described in the literature more than 2000 years ago. In the year 300 B.C., Theophrastus, a disciple of Aristotle, reported an example of the inhibitory effect of pigweed on alfalfa. The Roman Senator Plinius Secundo (who died in 79 A.D.) said in his *Naturalis Historia* that it was amazing how limited the growth of other plants was below a walnut tree because of the toxic agents coming from the leaves of the walnut tree (*5*). In 1937, Molisch constructed the word allelopathy from the Greek words “allelo” and “pathy”, “allelo” meaning “mutual” and “pathy” meaning “suffering” (*5*).

The possible exploitation of allelopathy for protecting agricultural plants against weeds, insects, pathogens, and other pests is one of several alternatives to the use of synthetic pesticides. Allelopathy can be exploited either (a) by cultivating crops with allelopathic properties, (b) intercropping of allelopathic plants together with the crop, (c) using allelopathic plants as green manure, and/or (d) using isolated allelochemicals as pesticides (*6*).

Earlier research into allelopathy focused mainly on field observations or laboratory studies with whole plant extracts. Thus, correlations between the application of a plant extract and a reduced growth of other plants could often be shown—but the causal relationship was not proven. The fact that the effect of allelochemicals in plant extracts could be different from

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the effects in the soil environment, due to binding and metabolic transformation, was seldom considered.

Chemical ecology is the study of the chemicals involved in the interactions of living organisms. With the application of modern chemistry techniques [liquid chromatography with tandem mass spectrometry detection (LC-MSMS), nuclear magnetic resonance (NMR)], molecular modeling, and advanced organic synthesis, allelopathy research can now be performed much more profoundly, as was done in this FATEALLCHEM project.

RESULTS AND DISCUSSION

FATEALLCHEM Project. In the FATEALLCHEM project (Fate and Toxicity of Allelochemicals in Relation to Environment and Consumer), the chemical compounds of interest were the benzoxazinone derivatives present in wheat (*Triticum aestivum*), rye (*Secale cereale*), and maize (*Zea mays*). If alternative strategies for suppressing weeds, insects, pathogens, and other pests are exploited extensively, these strategies cannot automatically be considered harmless to the environment and the consumer. Thus, the overall objective of the FATEALLCHEM project was to perform an environmental and human risk assessment of exploiting the allelopathic properties of wheat in modern farming and to develop a framework for future assessments of allelopathic crops. The following 17 papers in this issue of the *Journal of Agricultural and Food Chemistry* present a number of important findings from the FATEALLCHEM project. The project was organized in the following Work-packages, which also give the order in which the papers are presented: WP1, isolation and synthesis; WP2, development of analytical methods and determination of levels of benzoxazinones; WP3, target effects; WP4, degradation in soil; WP5, nontarget effects; WP6, molecular modeling.

Analysis of Benzoxazinones. During 20 years of research in benzoxazinone derivatives, little attention has been devoted to the importance of doing a thorough validation of the analytical methods and performing the analyses with LC-MSMS equipment. The development of analytical methods in LC-MS and LC-MSMS for the secondary metabolites in the wheat plant or soil matrix was found to be complex, due to the instability of the compounds in standard solutions, difficulties in choosing an efficient extraction procedure that does not destroy the compounds, and severe ion suppression effects (*7*−*11*). It is strongly recommended that future studies on allelochemicals should be done with mass spectrometry detection instead of HPLC-UV methods and that validation of the linearity, accuracy, reproducibility, repeatability, and specificity should be done at a level that follows the general recommendations for chemical analysis of xenobiotics as given by EURACHEM (*12*), the U.S. Environmental Protection Agency (EPA), or the U.S. Food and Drug Administration (FDA). Fast and reliable highly sensitive methods for benzoxazinone derivatives in the FATEALLCHEM project and the first intercalibration study ever on the analytical procedures for analyzing benzoxazinones in plant material were published (*13*). The application of the methods on wheat varieties grown under different conditions revealed a genetic difference between varieties in the inherent content of natural defense compounds as well as environmental effects on the production by the plants (*14*−*16*).

Degradation in Soil. Benzoxazinones and benzoxazolinones from wheat were shown to degrade very rapidly in soil with DT_{50} values of ≤1−2 days (*17*−*19*). The formation of degradation products depended on the initial concentration of the benzoxazinones in soil or test media, and a complex pattern of transformation products was formed (*20*−*24*). Schemes with the structure and names of the metabolites are shown in Etzerodt et al. (*24*). Future studies on the effects of allelochemicals should definitely consider the relevant concentration levels, if natural systems are to be simulated, and should include metabolic profiling of the soil environment when effects on weeds or soilborne diseases are investigated. The transformation products showed more pronounced biological activity than the parent compounds from wheat. The transformation product 2-aminophenoxazin-3-one (APO) had significantly higher suppressive target effects on both weeds and fungi than the parent compound (*25*−*28*). A number of hydroxylated, acetylated, and methoxylated APO-related transformation products in soil are currently being characterized. The importance of the microbial transformation of allelochemicals in soil is clear: The conclusions in earlier published studies, in which the effect of wheat benzoxazinones on weeds or soilborne diseases has been studied without any focus on the transformation that occurred during the time of study, must now be revised. In research on soil health and agricultural sustainability these results should be considered as well. Chemical soil quality is determined not only by the content of nutrients, salt concentration, soil texture, etc., but also by the presence of biologically active chemical compounds.

Nontarget Effects. Toxicological evaluations of allelochemicals have generally not been considered. In fact, often the argument for exploiting natural substances as an alternative to synthetic pesticides is that these compounds are harmless because they are natural and that they are expected to degrade rapidly. However, an extensive exploitation of allelopathic properties of plants must include an evaluation of nontarget effects. In the FATEALLCHEM project a toxicological evaluation of benzoxazinone allelochemicals and their soil metabolites was performed for the first time. The ecotoxicological studies showed that the soil transformation products suppressing weeds and pathogenic fungi also were the most toxic to beneficial organisms [carabid beetle larvae (*Poecilus cupreus*), collembola (*Folsomia candida*), and selected aquatic organisms] (*29*−*32*). Comparisons were made with a structure-related herbicidal compound, benazolin (4-chloro-2-oxobenzothiazolin-3-ylacetic acid) and its formulated product Cresopur (4-chloro-2-oxobenzothiazolin-3-ylacetic acid), and with two standard reference synthetic pesticides. However, the adverse effects of the most active metabolites did not exceed the negative effects of these substances (*29*, *30*, *32*). Molecular modeling was applied to the benzoxazinone derivatives and their soil metabolites, and the results were compared with the empirical results of the toxicological evaluations. Quantitative structure−activity relationship (QSAR) and QSAR with comparative molecular field analysis (CoMFA) studies showed that in silico approaches could be usefully applied to predict the toxicity of benzoxazinones to *Folsomia candida* and *Daphnia magna* (*33*, *34*).

Target Effects. This project focused mainly on the benzoxazinone derivatives from wheat, rye, and maize and their soil metabolites. However, other groups of secondary metabolites, such as phenolic acids and polyphenols with defense properties, have also been identified in these crop species. Joint action studies on selected weed species showed that binary mixtures of benzoxazinones generally followed an additive dose model, as did binary mixtures of phenolic acids. However, binary and tertiary mixtures of benzoxazinone derivatives and phenolic acids responded primarily antagonistically (*35*). Thus, the assumption by some that synergistic effects of otherwise weakly active allelochemicals exist could not be supported. Significant differences in the capacity of different wheat varieties for suppressing weeds were seen after incorporation of wheat in the soil (*28*), and significant differences between varieties were observed with regard to the inhibition of the intrinsic rate of increase of aphids living on wheat plants (*36*). These differences, however, could not be correlated with the concentrations of benzoxazinone derivatives.

Future Prospects and Research Needs. The project results clearly showed the relevance of optimizing the exploitation of cereal benzoxazinones. Crop rotation is a very traditional practice, which was given less importance for decades but is now regaining its importance in agricultural practice as a means of controlling weed seed banks and soilborne diseases and pests. Also, cover crops and green manure are used more extensively in modern sustainable agriculture. By growing cereals as a catch crop or for green manure, the allelopathic properties of the cereals could now be much more extensively exploited, choosing varieties with maximum production of benzoxazinones and optimizing the time of sowing in relation to the formation of bioactive metabolites in soil. However, concurrent research should be performed on all of the significant groups of allelochemicals in a crop. Research into allelopathy must be performed with single compounds as well as combined plant extracts. However, studies with plant extracts must include chemical identification and quantification of the active chemical substances. Thus, the development of basic organic chemistry methods for isolation and/or chemical synthesis of the investigated substances is indispensable, as was done in the FATEALLCHEM project (*19*, *22*, *24*, *37*).

The mode of action of allelochemicals is another important future aspect of allelopathy research (*38*). One of the benzoxazinone derivatives, 2-benzoxazolinone, seems to possess multiple modes of action, affecting simultaneously many different physiological pathways (*39*, *40*).

A framework for future assessments of allelopathic crops must include the development of validated analytical methods, considerations of relevant concentrations, soil transformation studies, ecotoxicological studies on individual compounds and mixtures, evaluation of human and mammal toxicity, and efficacy studies on weeds, insects, pathogens, and other pests. Combining the results of such studies with knowledge on the biosynthetic pathways of allelochemicals and studies on gene expression can result in substantial gains for sustainable agriculture.

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