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OPINION

Research directions in plant protection chemistry

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Abstract – This Opinion paper briefly summarizes the views of the authors on the directions of research in the area of plant protection chemistry. We believe these directions need to focus on (1) the discovery of new pesticide active ingredients, and (2) the protection of human health and the environment. Research revenues are discussed thematically in topics of target site identification, pesticide discovery, environmental aspects, as well as keeping track with the international trends. The most fundamental approach, target site identification, covers both computer-aided molecular design and research on biochemical mechanisms. The discovery of various classes of pesticides is reviewed including classes that hold promise to date, as well as up-to-date methods of innovation, e.g. utilization of plant metabolomics in identification of novel target sites of biological activity. Environmental and ecological aspects represent a component of increasing importance in pesticide development by emphasizing the need to improve methods of environmental analysis and assess ecotoxicological side-effects, but also set new directions for future research. Last, but not least, pesticide chemistry and biochemistry constitute an integral part in the assessment of related fields of plant protection, e.g. agricultural biotechnology, therefore, issues of pesticide chemistry related to the development and cultivation of genetically modified crops are also discussed.

Keywords – research directions, plant protection chemistry, pesticides, pesticide resistance, environment, human health

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Contents

Introduction

Recently, an Editorial paper was published in this journal that discussed the history and the possible future of chemical plant protection (Komives, 2016). The paper emphasized the necessity of the development of new pesticide active ingredients but, in its conclusion, was rather ambiguous with regards to the importance of pesticides in plant protection in the foreseeable future. Still, we believe there is plenty of research to be carried out in the field of pesticide chemistry and related life sciences. Here we attempt to provide a list of the research tasks that we consider most promising for current research and development. Please note that the tasks listed below are tightly interdependent and their itemization is very personal. We are aware that the list is also incomplete: we would be glad to receive suggested modifications (with additions or deletions) from pesticide chemists and biochemists.

With the introduction of new research, development and innovation (RDI) methodologies in the early nineties, strategies in plant protection underwent a gradual

transformation, and as a result, the development of all biologically active substances both new pharmaceuticals (human or veterinary) and new pesticides became more difficult, more expensive and less successful (Komives, 2016). The main driver of this process has been our expanding knowledge on the unintended environmental, ecological and toxicity effects (both human toxicity and ecotoxicity) of biologically active substances developed previously. In turn, a currently developed substance, to be eligible for authorization as an active ingredient, has to comply with all toxicology requirements identified and codified to date. The response by both the pharmaceutical and pesticide industries has been seen in business economy and RDI strategies as well, including a strong capital concentration manifested in giant company mergers, and the introduction of RDI tools of increased capacity, mostly based on combinatorial chemistry and high throughput screening. It also has to be mentioned that the increasing strictness and cost of registration of new plant protection products not only reshaped research in pesticide chemistry, but also substantially contributed to the progress of agricultural biotechnology: instead of developing new pesticide active ingredients, plant protection technology innovators turned to development of genetically modified (GM) plants (crops) that produce or tolerate existing pesticide substances (so-called first generation GM crops intended for plant protection purposes) (Székács, 2017).

The above-mentioned trends in business and RDI strategies caused consequences in academic research as well, by narrowing the potential for small academic facilities in research of original lead compounds. Ironically, the shift in the RDI methodology not only limited original research, but also increased the appreciation of fundamental research and the human intellect leading to original ideas. This latter trend leaves substantial room for academic research as well. Therefore, we discuss potential areas that we consider promising in plant protection chemistry with the hope and encouragement that academic or other governmental and low capital capacity RDI facilities still hold currency in this sector.

Areas in need of further research

1. Target site identification

Identification of new target sites seems to be a very difficult task: conspicuously few new target sites (Komives, 2016), have been discovered during the last decades even though all "omics" methods have been used in the RDI process. An example of novel target site discovery is the ryanodine receptor, with flubendiamide, rynaxypyr and cyazypyr as successful insecticide active ingredients acting, although not as ryanodine analogs, on this receptor (Sattele et al., 2008). Yet, the number of such new target sites identified is rather limited. This has not been differently in pesticide discovery before: novel target sites were recognized and characterized as new substances were found to exert novel types of biological activities. Even though it would be desirable from an environmental-ecological aspect, it has been rare in pesticide discovery, maybe with the exception of biorational compounds (see 2.1. Natural compounds, below) that active substances were *ab ovo* designed to new sites of action.

1.1. Molecular modeling

Computerized molecular modeling studies provide insight on relationships between chemical structure and biological activity. Far progressed from traditional quantitative structure-activity relationship studies and molecular modeling by molecular mechanics, quantum mechanics and molecular dynamics, computer-aided molecular design (CAMD) is capable to consider biochemical target enzymes, receptors or binding proteins, also in interaction with thousands of molecules (e.g., solvation), and allows molecular docking, pharmacophore modeling and mapping, comparative molecular field and similarity analyses, or even virtual screening. And although the particular strength of CAMD remains to be optimization on the basis of already identified lead compounds (Bordás et al., 2003; Delaney et al., 2006), it offers some utility in *de novo* molecular design of biologically active agents, and thus, in some cases in lead compound generation. Lately, it has also been extensively applied in absorption, distribution, metabolism, excretion and toxicity prediction (Benfenati, 2016), and toxicity prediction power is hoped to be extended among various classes of chemicals, pharmaceuticals, pesticides and cosmetics (Alves et al., 2017). CAMD helps identify most promising chemical structure from a number of analogous derivatives, but modeling of interactions with proteins and lipids may also lead to the discovery of new structures; therefore, it plays an important role in pesticide discovery programs. With regards to identification of new target sites, molecular modeling that uses 3D structures of proteins and docking 3D models of designed pesticide candidate molecules are the most promising.

1.2. Mode of action investigations

Mode of action-based pesticide design faces a dilemma between specificity to target pests and the development of pest resistance. To avoid unintended adverse side-effects, physiological modes of action rather specific to the target pests have been preferred lately, since the ecological and toxicological side-effects are being heavily considered in pesticide registration. However, the more specific the mode of action of a biological agent, acting by a single biochemical mechanism on a target-specific site of action, the larger is, in principle, the likelihood of mutations within the test population that render the mutant individuals resistant to the given agent, and the selection of the resistant subpopulation (Georghiou and Saito. 1983; Clark and Yamaguchi. 2001; Darvas and Székács, 2006).

Resistance of weeds, insects and disease-causing microorganisms against pesticides is a major concern for growers. Approximately 85 different modes of action of resistance of weeds, fungi or insects to pesticides have been reported (Aliferis and Jabaji, 2011). Knowledge of the mechanism of action of pesticide active ingredients is an important tool in resistance management (Clark and Yamaguchi, 2001). Therefore, the determination of the (bio)chemical causes of resistance will greatly improve the efficacy of pest control.

Cross-resistance is of particular concern, when related substances or close structural analogs, acting by the same mechanisms, are applied simultaneously. Under such conditions, resistance gained to a given substance can provide tolerance to related substances as well. As an example: cross-resistance in the case of microbial Cry toxins has been found to develop more readily if individual Cry toxins are applied alone (in the form of bioinsecticides or single genetic event insect resistant GM crops), than if related toxins act in combination (Székács and Darvas, 2012a). The biochemical background of the development of resistance or cross-resistance is facilitated by knowledge gained in receptor research.

As mentioned above, pesticide active ingredients with extensively broad spectrum of activity are not favored from an ecological aspect, while compounds with narrow spectrum of activity are prone to the development of resistance against them. Therefore, there is a need for products that can control several pests with a single application. In addition, development of resistance against such combinations of active ingredients (preferably exerting their effects by different modes of action) is hoped to be significantly less probable. As a result, design and preparation of new, original combinations of active ingredients remains an important task.

2. Pesticide discovery

2.1. Natural compounds

Natural compounds represent an almost endless source of useful products, such as surfactants, drugs, cosmetics, etc. Many of them were characterized with a potential to be developed as active ingredients of plant protection chemicals (Beck et al., 2013). For example, a fungal metabolite strobilurin A (Figure 1) was the starting point for a highly potent group a fungicides (azoxystrobin, trifloxystrobin, kresoxim methyl, etc.), the plant growth hormone indoleacetic acid (Figure 1) was the template of phenoxyacid herbicides (2,4-D, MCPA, MCPP, etc.), modeling natural insect hormones led to potent insect growth regulators (novaluron, pyriproxyfen), and elucidation of the chemical structure of annelid marine toxin nereistoxin (Figure 1) led to the discovery of new insecticides (e.g., thiocyclam, cartap, bensultap or thiosultap) (Hammock et al., 1989; Casida and Quistad, 1998; Horowitz et al., 2009; Darvas and Székács, 2006). This area of 'biorational' substances holds promise not only in the utilization of new biological modes of action or attack at some already identified target site (e.g. cartap/nereistoxin are anticholinergics), but also often offer improved ecotoxicity features. Nonetheless, the biorational origin of a given compound is promising in tailoring biological activities, but is not a guarantee against unintended side-effects, as has been seen for some of the above-mentioned and other classes of pesticide substances.

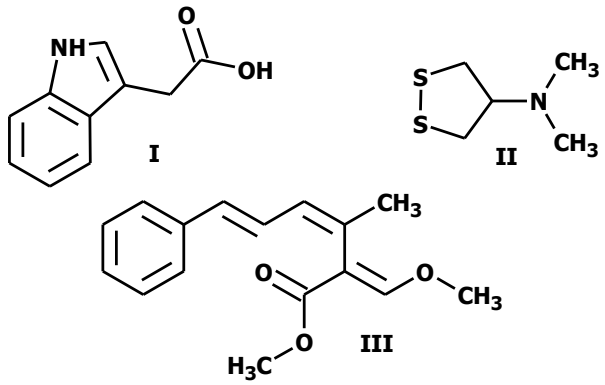

Figure 1. Chemical structures of indoleacetic acid (I), nereistoxin (II), and strobilurin A (III)

A particular group among natural compounds is represented by semiochemicals, chemical compounds produced by plants and animals as a means of communication. These natural substances modulate communication among members of the same species (e.g., insect attracting members or the same species or alarm them about danger; insect pheromones) and among different species (e.g., plants and insects or plants and other plants; allelochemicals) (Petroski et al., 2015). Due to their special biochemical mode of action and physiological role, certain groups of these natural substances are discussed separately below.

Allelochemicals may act among different plant species: the weed suppressive activity of certain plant allelochemicals (e.g. in sorghum) have been described. Also, chemicals play important roles in the communication between plants and microorganisms, and ultimately this communication determines whether the interaction will be symbiotic or a pathogenic. Identification of these signal transmitting molecules may lead to new plant protection agents.

Isolation and elucidation of chemical structure of active substances from plant extracts can also serve as a basis of substance development in plant protection chemistry. Knowledge gained here can possibly be utilized directly in the form of natural substances or indirectly as lead compounds for pesticide development, similarly as both directions represented in botanical pesticides (azadirachtin from neem, avermectin from a soil actinomycete, Cry and Vip toxins from *Bacillus thuringiensis* strains, etc.).

2.2. Chemical communication of insects

Many insects use chemicals as a means of communication, which can also serve as a basis of use in plant protection. Sex pheromones are a good example: after elucidation of their chemical structure they are synthesized in the laboratory in order to lure or to confuse male insect pests that are searching for a mate (Schulz, 2004, 2005). Although pheromones cannot be classified as pesticides, derivatives of natural sex pheromones represent a direction of biorational utilization. Thus, pheromone analogs gained utility in integrated pest management with varying success, in aerial saturation technologies, and in monitoring the dispersal of beneficial insects used in biological crop protection, and are considered particularly applicable in pest population forecasting and subsequent crop damage prevention (Chandler et al., 2011; Arora et al., 2016). Nonetheless, sex pheromones and attractants are not directly involved for control purposes in plant protection practice. There is no applied technology in this respect.

A severe limitation of super-selective approaches to pest modulation, such as the use of pheromones, is that a control method devised against a single pest cannot be practically effective when the damage is caused by pest communities. This is a severe constraint in plant protection, as the vast majority of crop losses are attributed to guilds of pest populations.

2.3. Chemical communication between plants and micro-organisms

Chemicals play important roles in the communication between plants and microorganisms as well (Blande and Glinwood, 2016). Ultimately, this communication determines whether the interaction will be symbiotic or pathogenic type (Lareen et al., 2016). Plant–virus or plant–microorganism interactions may be transmitted by insect vector, and both direct (plant–virus or plant–microorganism) and indirect (plant–insect–virus or plant–insect–microorganism) interactions, eventually mediated by small volatile organic compounds, are produced constitutively or induced by the plant pathogen. Identification of these signal transmitting molecules may lead to new plant protection agents.

2.4. Plant metabolomics

Plant metabolomics as a distinct area of genetic research emerged from phytochemistry, focusing on the chemical endeavor of plants, and currently both fields continue to co-exist. The techniques are new, but the goal remains the same: identification and characterization of plant metabolic routes and metabolites (Dixon et al., 2006; Aliferis and Jabaji, 2011). The information obtained by highly complex metabolomic investigations is used to characterize the influence of water supply, temperature, nutrients, salts, heavy metals, and abiotic and biotic stresses on plant metabolism. Metabolomic studies contribute to the improvement of the quality of crops as well as to the identification of chemicals important in pest and disease resistance of plants. Metabolomic analysis facilitates pesticide research in the discovery of bioactive compounds with described or novel modes of action in interspecific and intraspecific interactions (Weckwerth, 2003), and has been mainly developed for the investigation of the mode of action of phytotoxic, antifungal and antimicrobial compounds, and to a lesser extent for that of insecticides. It can also help assessment of their ecotoxicological and toxicological risks, the prediction of their effects on non-target organisms, to combat pest resistance, as well as the evaluation of risks related to genetically modified crops. Interestingly, metabolomics has been utilized in pesticide residue analysis as well (Sugitate et al., 2015). Although at present there still exist numerous technical bottlenecks in metabolite analysis techniques, regarding both chemical structure identification and data analysis, the approach will certainly play a major role in pesticide research and development. It can help the isolation of specific metabolites (Komives, 2017) from plants and microorganisms that can be useful as pesticide candidates as well as resistance-inducing natural substances.

2.5. Pesticide application technologies

Typically, only a small percentage of the pesticides utilized in agriculture reach their target site (Darvas and Székács, 2006). New methods of formulation (e.g. nano-technology) and application could increase this percentage significantly, in addition to reducing the pollution of the environment.

From this aspect, chemical crop protection technologies have undergone a major conceptual change during the last decades. Before the seventies, slow environmental decomposition of an active ingredient used to be considered an advantage by providing long-lasting effects. However, as persistence has been identified as a factor exerting extensive chemical pressure on natural habitats and ecosystems and, mainly triggered by the publication of “Silent Spring” (Carson, 1962), determination of the environmental fate of pesticide active ingredient received particular emphasis in their assessment, and currently substances susceptible to rapid environmental decomposition are preferred.

This development direction aims to minimize pesticide release, to improve technology economy, but primarily to reduce chemical pressure on the environment (see in detail below). Moreover, such technology is required due to the ongoing shrinkage in the range of active ingredient availability.

3. Environmental aspects

3.1. Analytical chemistry

In the absence of new pesticidal active ingredients it is more probable that some of the older active ingredients will accumulate in the environment to a harmful level (Komives, 2016). This trend is particularly seen in the

example of glyphosate, currently the world most used herbicide (Dill et al., 2010; Székács and Darvas, 2012b; Benbrook, 2016), alone representing globally a stable 11.8% of the overall pesticide market and 12.5% of the market of synthetic pesticides, propelled by increasing adoption of GM crops. A rather unfavorable trend for environmental and public health is that with possible limitations on the use of current pesticides, particularly herbicides due to their extensive use in combination with so-called herbicide tolerant GM crops, "old" and somewhat obsoleted active ingredients are being re-introduced along with GM crops tolerant to them. Examples include bromoxinyl or 2,4-D, the former classified as possible human carcinogen (Group C) by EPA (EPA, 1998) and the latter as possibly carcinogenic to humans (Group 2B) by IARC (Loomis et al., 2015). The IARC classification of glyphosate, probably carcinogenic to humans (Group 2A), is even worse than of 2,4-D (IARC 2015), even though EFSA attributed no inadmissible risk (EFSA 2015) to this hazard. In contrast, no incriminatory data have been released so far about other alternative herbicide active ingredients glufosinate or isoxaflutole.

Determination of novel routes of distribution of pesticide residues in plant/commodity and environmental matrices is also of high concern. Wide residue distribution in biological matrices is of particular concern for systemic pesticide active ingredients, as seen in the occurrence of neonicotinoid residues in the guttation liquids of crops (van der Sluijs et al., 2015; Mörtl et al., 2016, 2017).

As increased release of these re-introduced herbicides and other pesticidal compounds, and their consequent occurrence in environmental and biological matrices is anticipated, development of innovative, specific, and sensitive analytical methods (for example, immunoanalysis, biosensorics) for the determination of pesticides and their degradation products (e.g. DBHA from bromoxynil, TCDD from 2,4-D or AMPA from glyphosate) in the environment is of key importance. Such studies will provide data on the fate (movement, (bio)chemical transformation, and distribution) of pesticides in the environment (active ingredients and formulating agents, e.g. glyphosate and polyethoxylated tallow amine) thereby contributing to our knowledge on their persistence as well as on their effects on the environment and human health. Based on the data obtained a pesticide database can be constructed on their (a) cytotoxicity/genotoxicity/carcinogenicity, (b) endocrine disrupting effects, c) teratogenicity and (d) immunomodulant effects, and the combined information can be utilized in the assessment of the risks to human health and the environment.

Pesticide residue analysis in environmental matrices is a routine task in quality control in environmental, food and health safety. Pesticide residues in main environmental matrices such as surface water, sediment, and soil represent a permanent problem that requires continuous monitoring and occasionally, pollutant removal or purification (see below). Uninterrupted periodic monitoring of pesticide residues e.g., in surface waters (Rathore and Nollet, 2012; Eurostat, 2013; Székács et al., 2015; Knauer, 2016) not only generates valuable data-sets on annual and seasonal variations of contamination that can be correlated to technology characteristics or policy aspects, but also provides information on the long term tendencies of chemical pressure on the environment, as well as possible correlation between pesticide usage and residues and climate change. In this context, the development of new plant protection products and technologies based on them, and utilization of existing ones requires a close connection between pesticide chemistry research and water science.

3.2. Managing resistance of pests against pesticides

Combinations of pesticides are very useful in this respect. It is wise to prepare in advance for the period of patent termination: in your design experiment with compounds developed by different companies. Research may include the a) determination of (bio)chemical causes, b) generation of resistant plants (crops) by molecular biological techniques, and c) occurrence of resistant plants (weeds) as an unwanted side-effect of the use of herbicide tolerant crops (Tabashnik, 1989).

3.3. Remediation of pesticide-polluted sites

Continuous use of certain pesticides may result in polluted environment, and some of these contaminants may be persistent. Chemical, biochemical, and biological methods need to be developed to decontaminate agricultural soils and groundwater (Komives and Gullner, 2006).

3.4. Chemicals important in the protection of GM crops

The development of first generation GM plants, namely insect resistant and herbicide tolerant GM crops, follows a strategy somewhat opposing the concept of the development of novel pesticide substances. New pesticide candidate compounds are being designed, screened and developed with the intention to replace or complement previous active ingredients as the latter become obsolete. First generation GM plants, in contrast, emerged with an opposing concept by either producing a transgenic insecticide protein or being tolerant via their genetic modification to existing herbicide active ingredients. One way or another, these crops are directly related to pesticide application: insect resistant GM plants can be considered a unique form of pesticide preparation, "formulated" in the biological matrix of the host plant, while herbicide tolerant GM crops actually rely on herbicide agrochemicals applied on them. Therefore, these GM crops do not widen the range of pesticide compounds to be applied, but rather shrink it to the particular ones involved in the given genetic modification (e.g. Cry or Vip toxins in the case of insect resistant GM plants or glyphosate in the case of

herbicide tolerant GM crops). Therefore, increased chemical pressure on the environment has to be carefully assessed. Moreover, risks associated with GM technology must be controlled by preliminary assessment of pesticides that may have a possible use in the protection of genetically modified plants as the re-introduction of "old" substances is a potential source of risks (as discussed above). A clear example to pesticide-based risk assessment as applies for herbicide tolerant GM plants is the case of GM crops tolerant to bromoxynil. Although the US EPA registered bromoxynil-tolerant GM crops, it did not currently authorize the use of bromoxynil on them (ICAC, 1998) due to its earlier classification as a possible human carcinogen (EPA, 1998), rendering the cultivation of these GM crops useless.

3.5. Interactions of pesticides and other chemicals

Two main aspects lacking in current toxicology and ecotoxicology are possible effects of substances upon long term exposure at sub-acute dosages and combined toxicity in parallel exposure to numerous toxicants. As classical toxicology is fundamentally built on the assessment of dose-dependence of effects of single toxicants upon singular or non-continuous exposure, the resulting acute or even chronic toxicity data do not properly describe the consequences of long term (much less lifelong) exposures. Similarly, only targeted surveys can reveal how individual toxicants act in combination with each other. Thus, a serious concern regarding the toxicological consequences of pesticide residues is related to the combined effects by compounds of agricultural (or other) origin. It has been evidenced that toxicity of given pesticide residues not only adds up upon co-exposure, but may show synergistic features in interaction.

Synergy is often targeted to amplify pesticide main effects, e.g. as in the case of pyrethroids applied in combination with pesticide metabolism inhibitor piperonyl butoxide. Modified pesticide metabolism is also utilized by the use of pesticide safeners or antidotes (Komives, 1992), e.g. when tolerance of a given crop towards a herbicide compound is achieved by its enhanced metabolic decomposition.

Synergistic toxicity has been demonstrated between insecticide active ingredient chlorpyrifos and cadmium exposure (He et al., 2015; Budai et al., 2015), and the ecotoxicity of insecticides used in fog spray is enhanced and far surpassed by propellant oils (kerosene-type oils, fuel oils, diesel oils, etc.). Even more alarming are the findings that toxic effects exerted by formulated pesticides often immensely exceed those of the corresponding active ingredients, e.g. in the case of 9 formulated pesticides (3 major herbicides, 3 insecticides and 3 fungicides) and their active ingredients (glyphosate, isoproturon, fluroxypyr, pirimicarb, imidacloprid, acetamiprid, tebuconazole, epoxiconazole and prochloraz) (Mesnage et al., 2014) or for neonicotinoid formulations (Takács et al., 2017). Moreover, glyphosate has been shown to undergo biological dissipation by algal biofilms alone and in its formulated preparation (Klátyik et al., 2017b). These results indicate a common misconception in pesticide chemistry and toxicology, namely that additives used in pesticides are "inert". In contrast, in their adverse effects they may interact with the active ingredients in the exerted unintended side-effects. This interaction has been extensively demonstrated for the herbicide active ingredient glyphosate and its adjuvant polyethoxylated tallow amine (Székács and Darvas, 2012b; Székács et al., 2014; Defarge et al., 2016; Engdahl, 2017) that led the European Commission to recommend a ban on this chemical from glyphosate-based products in 2017 (EC, 2016). These finding may necessitate new authorization regulations for surfactants used in formulated veterinary drugs and plant protection products (Klátyik et al., 2017a).

3.6. Interactions of chemical and biological pesticides

There is an increasing number of biopesticides used in crop protection. Since they are seldom applied in combination, little is known on their possible interactions with traditional pesticides. Their joint effects may be harmful but can also be beneficial (Sharon et al., 1992): a complex, but certainly interesting area of study.

4. Continuous tracking of international trends

Finally, we add an often neglected, but equally important point. A continuous collection of data on the most recent advances in chemical plant protection (chemistry, formulation, and application technologies) will help the timely prediction of a technological shift.

Conclusions

Further research is necessary to develop new, more efficient, and safer pesticides. In addition, new analytical methods are needed to assess the environmental effects of pesticides and technologies to remediate pesticides-polluted sites.

Note

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Public interest statement
Wide scale use of pesticides led to substantial benefits in food security of billions of people, but at the same time resulted in environmental pollution and destabilized food safety. This paper lists narrower and wider areas of research that may lead to safer and more efficient use of these chemicals.

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