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ECOSYSTEMS AND ENVIRONMENT

Environment, Natural or Anthropogenic Pressures

Biocontrol of Plant Disease

Recent Advances and Prospects in Plant Protection

Coordinated by
Claire Prigent-Combaret
Bernard Dumas

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Introduction

Promoting Biocontrol and Sustainable Crop Protection Strategies, a Major Challenge for the 21st Century

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During the 20th century, in response to the need to significantly increase production, agriculture resorted to the massive use of chemical inputs (fertilizers and pesticides). This made it possible to provide crops with all the essential nutrients needed for their growth (nitrogen fertilizers, phosphorus, etc.) while effectively protecting them from diseases and pests (fungicides, insecticides, herbicides). Although these products have been extremely effective and have resulted in a significant increase in crop yield, their large-scale use has led, in some cases, to the degradation of soil quality and has had a dramatic impact on natural flora and fauna (a reduction in biodiversity, the appearance of resistant individuals, etc.). Today, the increased availability of better-quality food has made it possible to achieve food safety and has also provided customers with wide access to a healthy diverse diet. These beneficial effects have, however, come at the cost of adverse health effects associated with the use of certain inputs with a hazardous toxicological profile. As a result, the way in which we develop and use agricultural inputs has seen a major reorientation following a shift in policies to drastically reduce agricultural inputs originating from synthetic chemistry (including chemical fertilizers and phytosanitary products), which we have been systematically implementing for the last several years to focus on developing alternative solutions with a lower environmental impact. The main goal of these policies is the sustainable production of quality foods that are safe for both the environment and the consumer.

Twenty-first century agriculture must therefore face the added constraint of reducing environmental impacts in addition to ensuring adequate production capacity to maintain the viability of its economic model. There are enormous economic challenges to be addressed for developing a sustainable agriculture that also respects the environment. Today, consumers are increasingly aware of the need to produce healthy food albeit with a low environmental impact. To this end, they are increasingly turning to products from alternative systems; these may be products with zero residue specifications from organic farming, or local production sold via short supply chains. This calls into question our historical production model with respect to its choice of crop species, inputs used and marketing channels.

To address these new challenges, a significant research and development effort is needed to optimize and integrate new agronomic methods so that we can circumvent the massive use of non-natural products originating from so-called “conventional” synthetic chemistry. Such research involves setting up new farming practices (tillage, crop rotation, etc.) and the genetic selection of varieties that guarantee yield and improve resistance to stress and which are in symbiosis with soil microbial communities, while at the same time developing new inputs with a low environmental impact. In this context, the implementation of effective solutions as an alternative to “conventional” treatments will be decisive for the competitiveness of our current agricultural model.

One approach that is currently being developed is through the use of natural compounds to combat weeds, diseases and pathogens (biocontrol) and to optimize nutrition and plant development (biostimulant). For several years now, the application of these two types of products has seen a sharp increase, particularly in the context of organic farming. Manufacturers in this sector have set themselves the ambitious goal of capturing 30% of the crop protection market by 2030, which is in line with national public policies, notably in the European Union (using the strategy “Farm to Fork”, which aims for a 50% reduction in plant protection products and 25% conversion to organic farming by 2030 – https://ec.europa.eu/food/farm2fork_en). The major obstacle to achieving these ambitious objectives, however, remains identifying new active substances or living organisms of agronomic interest which are more environmentally friendly. Moreover, in order to exploit the potential of these active substances and provide solutions to ensure optimal crop protection while guaranteeing better yield, we also need to understand their mode of action in the complex environment that constitutes our agricultural system (Figure I.1).

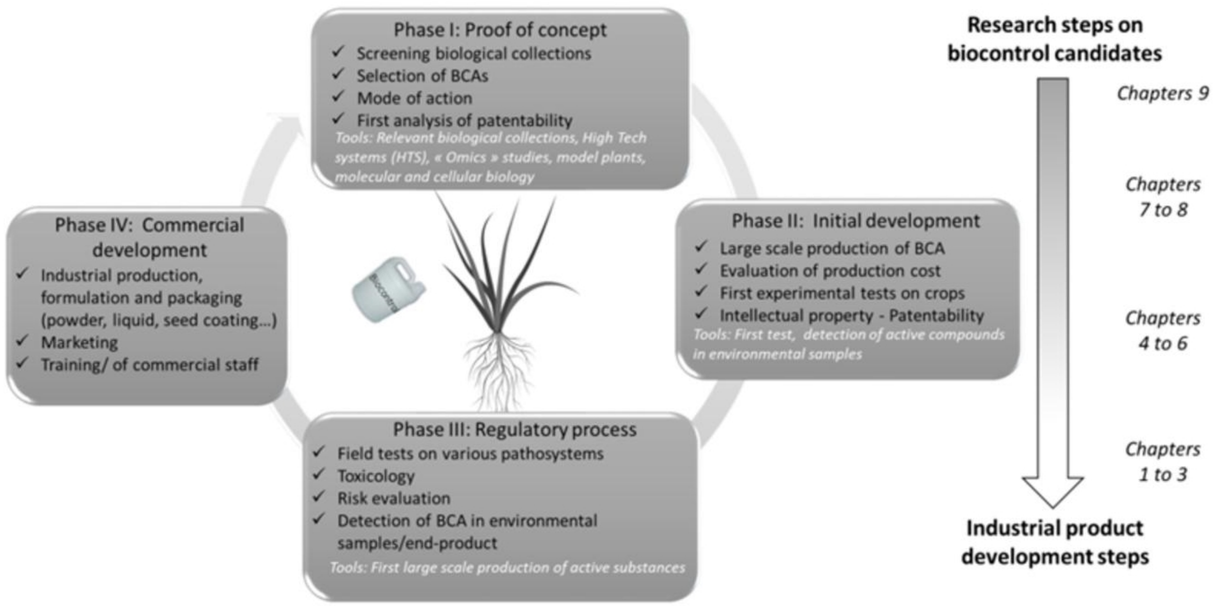

Figure I.1 *Necessary steps in the development of biocontrol agents (BCAs)*

The objective of this book is to gather works written by leading scientists in their fields in the form of chapters to illustrate the multifaceted aspects of the research devoted to finding new environmentally compatible solutions to protect plants against diseases, while maintaining crop yields. This book also addresses the important question of the current regulatory process needed to launch plant production products on the market (Figure I.1). We have chosen articles from research works presenting new advances on plant disease management through innovative strategies. Since an exhaustive panorama of biocontrol strategies is out of the scope of this book, certain topics such as the mechanisms involved in the protection of plants against insects by indirect action (e.g. the use of pheromones and kairomones and other natural defense stimulators) will not be covered in this book. However, the ability of biocontrol agents to protect plants against bacterial, fungal or oomycete pathogens or diseases triggered by insects or nematodes directly (e.g. by producing antimicrobial peptides, using the quorum quenching strategy with microorganisms, using plant or agro-industrial by-products as biopesticides, etc.) or indirectly (e.g. by increasing plant defense signaling pathways (via induced systemic resistance (ISR)), or by stimulating plant growth and development) will be described. We also want to address new strategies such as the development of phage-based biocontrol and those in which preventing pathogen-induced dysbiosis of plant microbiota is considered to be key to ensuring the overall health of the plant.

The different phases in the development of BCAs are identical to those when developing chemicals. The first phase is to identify candidates by screening biological collections that usually involve collaborative work between academic labs and industrial partners. At this stage, it is crucial to analyze the patentability of the selected candidates. The second phase concerns the initial development of BCAs, including the analysis of their activity on target crops and the evaluation of their industrial production feasibility. The knowledge acquired during phase I and II is essential for the subsequent development of the products. Phases I and II are intimately linked and only a few candidates are selected for phase III. Phase III (regulatory process), done on the main candidates, is probably the one that is the most expensive and time consuming. This phase involves toxicological analyses and several years of field tests to demonstrate the efficacy of the product. Finally, phase IV includes the final steps to launch the product on the market. Together, these phases need about 10 years and an investment of several hundreds of millions of euros to be completed. Even if the investment is mainly in phase III, regulations defined by authorities influence the decision to select a certain BCA (phase I) and to continue with its initial development (phase II). The positioning of the chapters in relation to these different stages is indicated on the right side of the figure.

A crucial step in introducing a plant protection product onto the market is the associated regulatory process, which directly impacts the investment needed to launch the product. The way in which this regulatory process is defined also influences the search for new active compounds in that those that have the greatest chance to be homologated will be preferred (Figure I.1). For these reasons, the first part of this book focuses on issues related to commercial biocontrol compounds. First, a description of the rules and regulations for the commercialization of biocontrol products is given by Robin et al. (Chapter 1). This chapter starts by giving definitions of the term "biocontrol" and also of other denominations used in the context of plant protection products (PPP) as required by the European regulation (e.g. "BioControl Agents", "Active Substances", "Biorationals", etc.) even though the word "biocontrol" is not used as such. This chapter also discusses the problematic issues in these regulations regarding the use of biocontrol substances and the slow and long path to gaining the approval of biocontrol products in the PPP regulation. Limitations in the French and European regulations on so-called "biocontrol" products and the different possible suggestions to reform these regulations are also discussed.

Chapter 2 by Guibert et al. gives an overview of the various biocontrol products used in horticulture. Horticulture is a vast agronomic sector involving the cultivation of fruits, vegetables and ornamental plants. It is a major market for biocontrol products and targets both fresh and, in some cases, perishable products to improve shelf life. In the case of edible products, plant protection strategies should scrupulously avoid contamination by toxic substances and efforts should also be dedicated to preserving the gustative quality of the food in addition to conserving their nutritional value. In this sector, consumers are strongly concerned about the safety aspects of food products, as confirmed by the continuously increasing demand for organic farm produce. Although biocontrol has been integrated into horticultural practices for more than 40 years, the reduced or banned application of phytopharmaceutical products (e.g. glyphosate) has motivated cultivators to make fundamental changes and has also challenged many entrenched agricultural practices. The increase in the use of biocontrol methods and biostimulants, which is now under way, is largely driven by the greater awareness in society of the negative impact of pesticides, as described in Chapter 2. The authors describe the different trade-offs that must be considered in horticulture and the difficulties that farmers could encounter: the trade-off between plant growth and plant protection against disease as well as economic trade-offs linked to eco-innovations (increased costs, productivity gains, critical importance of certification and labeling on the marketplace, etc.). Further studies on the safety and environmental sustainability of biocontrol products are still needed before their deployment on the large scale as an alternative to PPP.

Chapter 3 by Ghosson et al. discusses this issue in detail. While biocontrol products are generally considered to be safer for the environment than chemical products, they are, by definition, biologically active products, and it is therefore necessary to analyze their environmental fate following their deployment. This aspect is related to regulatory rules that apply to biocontrol products, which are largely inspired by the existing rules for chemicals. However, a natural product is much more complex than its chemical alternative which is generally composed of a nearly pure molecule with formulants and adjuvant, and whose degradation products can be traced. Plant and microbial extracts contain thousands of metabolites, and it is almost impossible to predict the diversity of compounds produced by a living microorganism. As noticed by Ghosson et al. a way to circumvent these difficulties could be to apply “omics” or “meta-omics” strategies, notably genomics and metabolomics, to get a wider overview of a given product in the “omic” environment.

The second part of this book focuses on the development of biocontrol products based on active natural compounds obtained from plants (also known as botanicals) and microorganisms. Chapter 4 by Ntalli and Caboni presents a survey of the literature from the last 10 years on plant-based products whose ability to control root-knot nematodes has been assessed in vitro or in vivo, including in fields. Nematodes belonging to the *Meloidogyne* genus are major agricultural pests, and developing effective biocontrol strategies to combat root-knot nematodes is currently the subject of much research; significant advances have been made in recent times. Nevertheless, molecular targets within the nematode on which botanicals interact have only rarely been identified. In this context, Ntalli and Caboni describe few recent works which have investigated the mode of action of plant secondary metabolites on root-knot nematodes.

Agro-industrial wastes and by-products are inexpensive and abundant sources of bioactive molecules, notably those from the agroforestry sector or other industries that process plant materials. These products contain antioxidants, antimicrobials, insecticides and nematicides. With a special emphasis on nematicide activities, Chapter 5 by Andres and González Coloma presents the activities of agro-industrial wastes, which have been the subject of intense study in recent times. Such wastes include biochar produced from the pyrolysis of wood and other plant materials, by-products from the production of essential oils (hydrolates or hydrosols), and wastes from olive oil production. Through these examples, Andres and González Coloma suggest that agro-industrial wastes constitute an almost inexhaustible source of biopesticides, but more research is needed to improve their efficiency and reduce the potential phytotoxicity of these products.

We end the second part with Chapter 6 by Montesinos et al. which focuses on the development of novel plant disease protection strategies based on the use of antimicrobial peptides and defense elicitor peptides. Plants, animals and microorganisms synthesize a wide diversity of peptides that have antimicrobial properties, or are able to trigger the immune response of the plant. Several peptides have already shown their activity against plant pathogens in vitro or in planta under greenhouse conditions, but reports on their efficiency in fields are still scarce. Such peptides could be produced naturally or synthetically via heterologous expression systems implemented in plants or microorganisms. However, there are several challenges, including their facile synthesis in a cost-effective manner, improving their delivery/formulation and stability, all of which determine their efficiency in plant disease protection. A clear regulatory framework for their application as biocontrol products is also needed.

The third and last part of this book addresses the question of how biocontrol products may impact pathogen microbial development, notably by exploiting microbial signaling and microbe–microbe interactions. Biocontrol of plant pathogens via quorum quenching is discussed in Chapter 7 by Faure and Latour. Quorum quenching (QQ) refers to any of the mechanisms involved in the degradation of quorum-sensing (QS) molecules, whose concentrations increase with the proliferation of QS-emitting populations and thus act as signals. These signal molecules, when reaching a concentration threshold, can bind and activate transcriptional regulators to control the expression of QS-regulated genes, especially those involved in the biosynthesis of virulence factors, in several bacterial pathogens. Quorum quenchers can alter either of the two main steps in QS signaling pathways, namely the QS synthesis or the accumulation and interaction of QS signals with their regulators. Accordingly, there are two main types of quorum quenchers: QS inhibitors and QS-degrading enzymes having QQ activities and bacterial populations sharing this property that could be used as biocontrol agents. These agents target virulence factors of plant pathogens, but do not inhibit their population growth. QQ activity can be stimulated by using treatments that stimulate the proliferation of externally introduced or native QQ populations. Thus, QQ is an emerging novel biocontrol trait that is widely distributed in microbial soil communities. The efficiency of QQ treatments in combination with antibiosis approaches is currently under evaluation in field assays, and promising results are expected from these new biocontrol strategies.

Another strategy to counteract pathogenic development is by using bacteriophages. Bacteriophages (or phages) are an essential and often underestimated components of plant microbiota, notably in the rhizosphere. Due to their pathogenicity towards phytopathogenic bacteria and their specificity, phages are potentially attractive biocontrol agents. As described in Chapter 8 by Clavijo-Coppens et al. a number of phages targeting major phytopathogenic bacteria belonging to various genera (*Acidovorax*, *Burkholderia*, *Erwinia*, etc.) have been characterized and some of them have already been shown, at least in experiments under controlled conditions, to successfully control bacterial diseases on agronomically relevant plants. However, these encouraging results should be taken with caution in the face of technical challenges, which could hamper the use of phage preparations in agriculture. The cost of large-scale preparation of phage-based products has to be compatible with the overall cost of the commercial end-product in order to be acceptable to farmers, and progress also needs to be made in the formulation of phage-based products, notably regarding phage viability. An attractive approach, as for other biocontrol substances, could be coating seeds with a phage preparation, which could be an elegant way to protect plants while avoiding subsequent treatments in the field. Finally, the authors discuss the regulatory challenges faced by industrial actors who are willing to commercialize these new products. Despite the great potential of this strategy, there is little doubt that a deeper understanding of the role of phages in the plant ecosystem will be necessary to encourage the development of phage-based products.

Up to now, the development of biocontrol products has been mainly viewed as a replacement of chemicals by natural products exhibiting antimicrobial activities, hoping that these compounds are less harmful to the environment because of their natural origin. However, plant diseases can also be tackled by optimizing the functioning of the plant microbiota, which increasingly is being recognized as an essential component of plant health. Plant phyllosphere and rhizosphere host a myriad of microorganisms collectively designated as the microbiota. The microbiota is essential for plant life and fitness: it is a key contributor to plant nutrition and its resistance to biotic and abiotic stresses. In the last decade, metabarcoding investigations have pointed out the diversity of plant microbiota (rhizosphere, phyllosphere, spermosphere) in various plant species growing in contrasted environmental conditions. Recent results have shown that the composition of microbiota is in part, determined by the plant, notably with respect to the composition of plant exudates, and can be tuned to respond to new environmental conditions, and that certain microbial species can play a tremendous role in altering the biological function of the microbiota. In agricultural systems, chemical inputs which have been massively used for close to a century, has caused soil depletion and has had a dramatic impact on the diversity and functioning of plant microbiota. Thus, a major challenge of modern agriculture is to reconcile the highly reduced use of chemicals while maintaining high yields in agricultural environments that is today also strongly impacted by urbanization and climate change. One solution could come from the use of microbial formulations to help plants get nutrients and combat diseases while allowing the soil to recover its properties. However, the use of active microbial strains in agricultural systems has thus far not been optimized. Chapter 9 by Yu et al. addresses this essential issue and, on the basis of the latest progress made in this field, shows that new strategies could be proposed in the near future to optimize plant microbiota.

To conclude, these contributions together offer us a large panorama of the various strategies that are currently available or being developed to offer alternative environmentally safe products to combat plant diseases. They also point out the bottlenecks that hamper the introduction of these compounds into the market, notably the lengthy regulatory process that, at least in European countries, does not distinguish natural and chemical pesticides. However, the urgent need for alternative solutions to replace conventional products will undoubtedly support strong investment in this research field. Nevertheless, the road ahead is still long in facilitating regular use and improving the efficiency of biocontrol strategies. The transition from results acquired under greenhouse conditions to those acquired in fields over several consecutive years is still the most difficult step in the development of a biocontrol product. However, high magnitude and quality of research efforts on biocontrol strategies worldwide allows us to be optimistic about being able to provide farmers with novel nature-inspired biomimetics and efficient biocontrol solutions.

1
Regulatory Aspects of Biocontrol

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Within the term “biocontrol”, only “biological control” thus macroorganisms (typical concept), “BioControl Agents” (BCAs) encompassing the three pillars outlined by Plant Protection Products Regulation EC 1107/2009 (namely microorganisms, semiochemicals and natural substances of plant, animal, mineral and microbial origin) and macroorganisms (fourth pillar) are considered. Macroorganisms are currently unregulated, except in France. For those considered as Plant Protection active substances, an approval pathway through EU Regulations follows general rules although some of these may be waived, depending on the pillar. In fact, 216 BioControl active substances are approved at the EU level, representing around 48% of total active substances (38% in 2011). Globally, and in a fairly stable way since 2011, around 50% are natural substances, 1/3 are microorganisms and 20% are semiochemicals. However, this significant progression, taking into account that 19 BioControl active substances were removed during the same period of time, is hiding the fact that many BioControl candidate substances are not being approved due to the inability to evaluate these substances (especially natural substances), which has left dozens of applicant substances unapproved.

1.1. Regulatory definition of biocontrol

1.1.1. *Definitions of biocontrol*

Biocontrol can be considered as including only biological control and macroorganisms, which follows the typical concept in the English-speaking world, or alternatively all the BioControl Agents (BCAs) encompassing the three pillars: microorganisms, semiochemicals and natural substances (of plant, animal, mineral and microbial origin) and occasionally including macroorganisms (fourth pillar), following the rather French and European concept set out by the International Biocontrol Manufacturers' Association (IBMA) (Robin and Marchand 2019a). Sometimes this definition is extended to physical barriers (nets, monitoring traps, etc.), but this thinking has no specific regulations to date.

1.1.2. *Applicable regulations*

Biocontrol, within its BCA limits (BioControl Agents and Biocontrol products), has been under the control of the phytopharmaceutical regulation EC 1107/2009 (EC 2009b) at the European level, since 2011. Macroorganisms are not regulated in EU but when species are not endogenous, they are regulated in France (Robin and Marchand 2020).

1.1.2.1. *Denominations – wording*

Therefore, these products, and the substances which support them, even if they are not defined in such a way, are considered as "pesticides" sometimes with the alternate qualifiers of "biological pesticides", "biopesticides", "biorationals" or "BioControl agents" (BCA) in English.

1.1.2.2. *Denominations – glossary*

“Active substance” (a.s.) – substance from the three pillars regulated by Plant Protection Products Regulation EC 1107/2009 and listed in Regulation EC 540/2011.

“Product” – formulated approved active substance with market authorizations.

“Biocontrol product” – product with biocontrol active substance from the three pillars.

“Semiochemicals” – semiochemicals are defined by the European commission as substances or mixtures of substances emitted by plants, animals and other organisms that evoke a behavioral or physiological response in individuals of the same (= pheromones) or other (= allelochemicals) species. Natural-identical synthesized molecules are also included in this definition.

“Natural substances” – active substance of plant, mineral, animal or microbial origin, non-transformed or activated.

“Biological control” – generally dedicated to macroorganism uses.

“Biorationals” – generally considered as “natural substances” or of biological origin.

“Biocontrol” – may also be attributed to crop protection by living organisms: macroorganisms and microorganisms.

“BioControl Agents” or “BCAs” – commonly attributed to all biocontrol plant protection active substances.

1.2. Current issues and limitations

First, it has to be mentioned that the word “biocontrol” is absent in the Plant protection Products (PPP) regulation (EC 2009a). The notion of biocontrol is therefore external to the regulation that manages ¾ of the categories.

Moreover, the word “biocontrol” is also absent in the Sustainable Use of pesticides Directive (SUD) (EC 2009b), although integrated pest management is mainstream, and biocontrol is one form of this concept. Consecutively, the concept of biocontrol or the biocontrol qualification of the substances and the corresponding product is from an external point of view; consequently, there is no official specific pathway or wavers during approval or renewal.

1.2.1. *The 3 PPP pillars*

During our study on their evolution, we showed at the ITAB institute that “biocontrol” active substances (a.s.) can belong to all parts from A to E (Robin and Marchand 2019b), and all types (active substance, low-risk, basic or candidate for substitution) of PPP regulation (Robin and Marchand 2021a). The notion of biocontrol applied to these substances is therefore diluted, and these substances diluted in EC Regulation 540/2011 and its sub-parts without distinction (Robin and Marchand 2021b) although the concept and the word “biocontrol” was not written in the PPP regulation. Many of these natural substances with non-biocidal properties and modes of action (MoA) are of very low toxicological concern and therefore do not have maximum residue limits (MRLs) (Charon et al. 2019). However, even if some would want to generalize that belief, their toxicity is not always trivial.

1.2.2. *Fourth pillar*

Global biocontrol includes macroorganisms, which fall under neither PPP regulation nor any specific regulation at the European level. At the national level, only France regulates transfers of non-native macroorganisms from mainland France to overseas territories, including Corsica (Journal Officiel “Lois et Décrets” (JORF) 2022), and vice versa (Robin and Marchand 2020). It aims to avoid the potential problems of the propagation of non-native macroorganisms, which could become invasive species outside their indigenous territory. Use is granted when this risk is guaranteed to be null (inadequate adaptation to the European climate, for example), and global warming could affect certain authorizations in the long term. In addition, French regulations may be partially or completely taken up by Europe in the long term. This path is indeed currently followed by the EU with recent regulatory incentives (EU 2021a) paving the way for the regulation of macroorganisms.

1.2.3. *Limits with crop protection in Organic Agriculture*

First of all, the four pillars of biocontrol are acceptable in Organic Farming ruled by Regulation EU 2018/848 (EU 2018b), the question of GMOs or GMO-produced substances being regulated in a similar way to the previous regulation (EU 2008b). The current global restriction avoiding the use of herbicides (a de facto total ban) implies that weeds are managed mainly by physical methods (mechanically, thermically or electrically). The global question of crop protection is managed primarily by protection from natural predators, the choice of species and varieties, crop rotation, cultivation techniques, mechanical methods and physical and thermal processes. Questions on new herbicide techniques (electricity) are currently being assessed. The question of “transformed” natural substances is also at the heart of debates. Three out of four pillars are automatically authorized in Organic Production: macroorganisms, microorganisms and chemical mediators. The authorizations of natural substances (of plant, animal, mineral and microbial origin) are managed on an ad hoc and individual basis for each active substance and are subject to voluntary inclusion files in Annex I (protection of crops and foodstuffs and stored products by extension), deposited at the national level and managed by the DGAgri through EGTOP PPP examination mandates (EGTOP 2017). Most of these natural biocontrol substances are thus accepted (Marchand 2017), except herbicides and substances that regulate positive (auxins) or negative (herbicides) growth. Annex I is under deep discussion, with the creation of sub-parts that did not previously exist, such as natural substances obtained from microorganisms (currently spinosad, cerevisane, ABE IT 56), specific parts for low-risk substances (EU 2017b; Robin and Marchand 2021b, 2021c) and basic substances (Marchand 2015, 2017; Robin and Marchand 2019a; Marchand et al. 2021). In conclusion, almost all biocontrol substances and products of the four pillars are accepted in Organic Agriculture (EGTOP 2021).

1.2.4. *Limits of biocontrol: contentious substances!*

Natural substances, including some chemical mediators, are not free from toxicity, and many examples show this, such as rotenone. Aside the natural substances historically used for lethal purposes (Bacalexi and Katouzian-Safadi 2018), some natural substances have been withdrawn from approval (e.g. crude tall oil) (EU 2017a), or never got approved in PPP Regulation (e.g. rotenone, nicotine) (EC 2008a). Direct comparisons show that the differences between “synthetic” and “natural” substances are not as sharp and the conclusions are sometimes not as dichotomous as society may think (Smith and Perfetti 2020). A similar acceptance problem may be encountered with copper compounds that are candidates for the substitution (as for emamectin, for example (EU 2020; Robin and Marchand 2021b)).