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Natural products for biocontrol: Review of their fate in the environment and impacts on biodiversity

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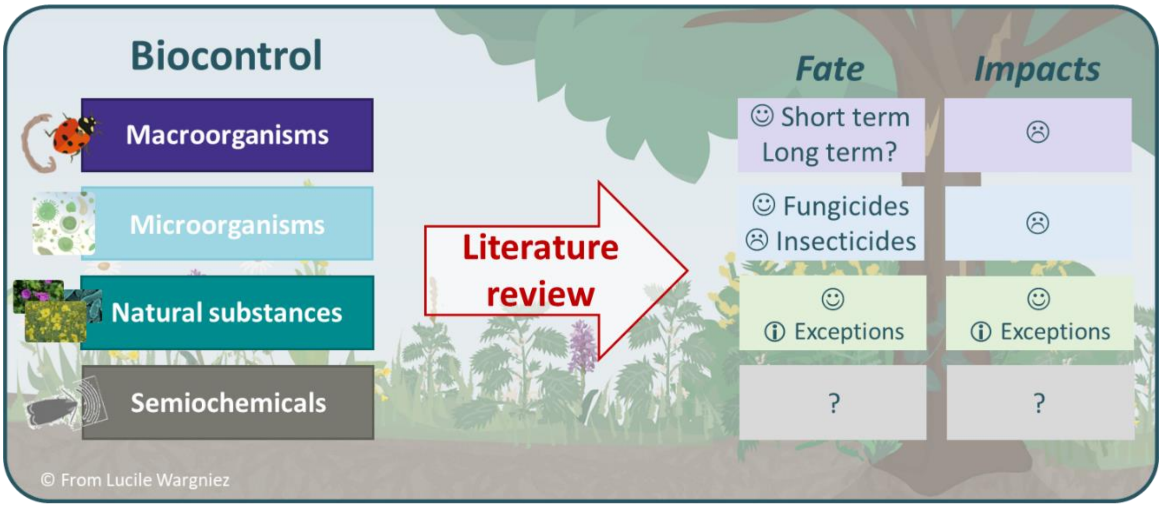
Abstract

Biocontrol solutions (macroorganisms, microorganisms, natural substances, semiochemicals) are presented as potential alternatives to conventional plant protection products (PPPs) because they are supposed to have lower impacts on ecosystems and human health. However, to ensure the sustainability of biocontrol solutions, it is necessary to document the unintended effects of their use. Thus, the objectives of this work were to review (1) the available biocontrol solutions and their regulation, (2) the contamination of the environment (soil, water, air) by biocontrol solutions, (3) the fate of biocontrol solutions in the environment, (4) their ecotoxicological impacts on biodiversity, and (5) the impacts of biocontrol solutions compared to those of conventional PPPs. Very few studies concern the presence of biocontrol solutions in the environment, their fate, and their impacts on biodiversity. The most important number of results were found for the organisms that have been used the longest, and most often from the angle of their interactions with other biocontrol agents. However, the use of living organisms (micro and macroorganisms) in biocontrol brings a specific dimension compared to conventional PPPs because they can survive, multiply, move and colonize other environments. The questioning of regulation stems from this specific dimension of the use of living organisms. Concerning natural substances, the few existing results indicate that while most of them have low ecotoxicity, others have a toxicity equivalent to or greater than that of the conventional PPPs. There are almost no result regarding semiochemicals. Knowledge of the unintended effects of biocontrol solutions has proved to be very incomplete. Research remains necessary to ensure their sustainability.

Graphical abstract



Keywords Biopesticides · Bioprotection · Biological control · Plant protection products · Contamination · Unintended effects · Ecotoxicology · Collective scientific assessment

Introduction

The European Directive 2009/128/EC (2009) establishing a framework for Community action to achieve a sustainable use of pesticides promotes the use of non-chemical methods of plant protection. In France, the Law for the Future of Agriculture, Food and Forestry states that “The State [...] supports professional actors in the development of biocontrol solutions [...]” (French Republic 2014). In addition, the recently implemented French National Strategy for Biocontrol Deployment (Ministry of Agriculture and Food and Ministry of Ecological Transition 2020) aims at implementing a series of measures (research, experiments, industrial innovation, field deployment) to consolidate the current dynamics to promote the design and use of biocontrol solutions as alternatives to conventional plant protection products (PPPs).

The “biocontrol” term appeared in a parliamentary report to the French Prime Minister in 2011 (Herth 2011). This French term should not be confused with the English “biocontrol” term, which is the use of beneficial insects (predators, parasitoids) or pathogens (bacteria, fungi, viruses) to control pests, weeds or plant pathogens, and which represents only a part of the French biocontrol (Eilenberg et al. 2001). The French biocontrol (referred as “biocontrol” in the manuscript) corresponds to a set of crop protection methods which has been defined by the French Rural and Maritime Fishing Code (FRMFC) - Article L-253-6 (French Republic 2023) as “agents and products using natural mechanisms as part of integrated pest management”. Biocontrol solutions are classified into four categories: (1) macroorganisms (insects, nematodes or mites that may be indigenous or exotic); (2) microorganisms (viruses, bacteria, oomycetes or fungi); (3) natural active substances (referred as natural substances) of plant, animal, microbial or mineral origin, either extracted from natural sources or synthesized identically; (4) semiochemicals such as pheromones and kairomones (mainly synthetic) (Table SI1). Therefore, biocontrol corresponds more closely to the broader English term "bioprotection", which includes biological control.

Biocontrol should also not be confused with organic farming, which is a production system that uses cultivation and breeding practices that respect natural balances and which is covered by Regulation (EU) No 2018/848 (2018). Thus, organic farming excludes the use of synthetic chemicals, of herbicides and of genetically modified organisms (GMOs), and limits the use of inputs (IFOAM 2022). Consequently, some substances of biocontrol which are not extracted from natural sources but synthesized identically are prohibited in organic farming (e.g., 6-benzyladenine, abamectin, gibberellic acid or phosphonates). On the contrary, organic farming allows the use of certain PPPs of mineral origin such as copper which is not listed as biocontrol solution especially because of its ecotoxicity to aquatic organisms (DGAL 2022; PPDB 2023) (Table SI1), and preparations based on natural substances that are listed in the European Commission implementing regulation regulation (EU) 2021/1165 (European Commission 2021) but that may not be listed as biocontrol products in France (for example azadirachtin).

Biocontrol has experienced an unprecedented boom in France over the past few years, representing 12% of the French PPP market in 2020, expecting 30% in 2030 (IBMA 2021). Indeed, the societal pressure coupled with the various regulations and restrictions concerning conventional PPPs has been an important lever to promote the use of biocontrol solutions. These solutions are presented as potential alternatives to conventional PPPs because they are supposed to have lower impacts on ecosystems and human health (Amichot et al. 2018; Boulogne et al. 2012; Mamy and Barriuso 2022; Robin and Marchand 2019). However, to ensure the sustainability of biocontrol solutions and the continuity of their development, it is necessary to document the unintended effects of their use to determine if biocontrol solutions are safe for the environment and biodiversity, and to compare their unintended effects with those of conventional PPPs. Recently, the three French Ministries responsible for the Environment, for Agriculture and for Research commissioned INRAE (French national research institute for agriculture, food and the environment) and the Ifremer (French national research institute for ocean science) to perform a collective scientific assessment (CSA) focused on the impacts of PPPs on biodiversity and ecosystem services (Mamy et al. 2022; Pesce et al. 2021; Pesce et al. 2024). Within this framework, to inform about the sustainability of biocontrol solutions, the objectives of this work were to review (1) the available biocontrol solutions and their regulation, (2) the contamination of the environment (soil, water, air) by biocontrol solutions, (3) the fate of biocontrol solutions in the environment, (4) their ecotoxicological impacts on biodiversity, and (5) the impacts of biocontrol solutions compared to those of conventional PPPs.

Bibliographic corpus

Construction of the queries and definition of the keywords

To review the literature on biocontrol solutions, some queries and related keywords were defined (Table SI2). The literature search was then conducted on the Web of Science™, from 2000 to 2020.

The first query (Q1) focused on biocontrol with fairly non-specific terms (Table SI2). The objective was to retrieve papers that were directly related to biocontrol, i.e., claimed as such by the authors through keywords or terms in the abstract.

The second query had two parts: one on microorganisms, natural substances and semiochemicals (Q2-1), the other on macroorganisms (Q2-2) (Table SI2). The Q2-1 query was based on the list published by the French Office of Inputs and Biocontrol of the French General Directorate of Food (DGAL 2022). The Q2-2 query was built on the list published in the Official Journal of the lists of "non-indigenous macroorganisms useful to plants, particularly in the context of biological control, exempted from requesting authorization to enter a territory and to be introduced into the environment" (French Republic 2015), on the list of requests for the introduction of macroorganisms of ANSES (French Agency for Food, Environmental and Occupational Health and Safety) (ANSES 2021), and on the list of indigenous macroorganisms used in augmentation (Robin and Marchand 2020).

The corpus of papers was then built by combining these queries (Q1 and (Q2-1 or Q2-2)). It was completed by various documents, papers and books known to the authors and which were not present in the screened database (Web of Science™).

Bibliographic corpus

The "Biocontrol" query Q1 collected 46,701 papers, the "Microorganisms, natural substances and semiochemicals" (Q2-1) and "Macroorganisms" (Q2-2) queries provided 228,605 and 6,914 papers, respectively. Combinations of the queries significantly reduced the number of papers: Q1 and Q2-1 collected 3,678 papers, while Q1 and Q2-1 collected 1,885 papers. Thus, the total number of items retrieved was 5,563. This total was modified by eliminating papers which were unusable or outside the selection criteria to finally reach 5,064 in December 2020. The corpus was completed by several documents taken into account a posteriori, until 2022.

The first selection criterion of papers was the reading of the titles, to eliminate papers describing the improvement of the production or use of a biocontrol solution (e.g., a new strain more easily handled, a multiplication method, etc.), papers describing methods for the physical or chemical characterization of natural or mineral extracts, and papers testing their efficacy under laboratory conditions. From this selection, a read of the abstract or of the content of the paper was performed. This step allowed to define which papers to retain for further analysis. In cases where the number of papers retained remained large (e.g., insecticides, see below), the papers were grouped according to similarity criteria and only the most representative papers of each group were examined in greater depth. A total of 4,662 papers was finally retained and analyzed in detail.

These 4,662 papers were distributed according to the use of the biocontrol solution: acaricide, bactericide, herbicide, fungicide, insecticide, molluscicide, or nematicide. The following distribution was obtained: 2,928 papers on insecticides, 1,292 on fungicides, 174 on acaricides, 123 on nematicides, 105 on bactericides, 20 on herbicides, and 20 on molluscicides.

It was interesting to note that a discrepancy appeared between the number of publications related to molluscicides and the French sale volumes. Indeed, molluscicides represent very few papers (0.47%) while they represent 26% of biocontrol product sales (IBMA 2021).

It has to be underlined that the use of cover crops for the management of weeds, which could limit the development of weed species through competition mechanisms (light and water preemption, mineral elements absorption) or through allelopathy mechanisms (emission of inhibiting substances), was not included in this review. The use of this biocontrol solution strongly depends on agricultural decision rules (sowing density, choice of plant species, destruction methods; Fernando and Shrestha 2023) which were outside the scope of this work.

At the end, a total of 487 papers were cited in the main report of the CSA (Mamy et al. 2022; Pesce et al. 2024). As this review is a summary of this report, only selected papers are cited here.

Available biocontrol solutions and their regulation

Biocontrol solutions aim at protecting crops by using the mechanisms that govern the interaction among species within agrosystems. Thus, biocontrol is based on managing the balance of pest populations rather than on eradicating them.

At the French national level, the Ministry for Agriculture and Food Sovereignty publishes a list gathering the authorized substances and biocontrol products which is updated and published every month (DGAL 2022). This list does not include macroorganisms but include insect traps combining pheromones, food attractants or conventional insecticides (e. g. deltamethrin) in a closed container.

The list considered in this work records 726 biocontrol products: 504 containing natural substances, 122 containing microorganisms, 86 containing semiochemicals, and 14 insect traps (Fig. 1) (DGAL 2022). While the 86 semiochemicals-based products and the 14 insect traps aim at limiting the populations of insects, the biocontrol products have various uses: acaricide, bactericide, fungicide, plant growth regulator, herbicide, insecticide, molluscicide, nematicide, repellent or protection against frost damage, and sometimes multiple actions (Fig. 2; Table SI1). Though the number of biocontrol solutions has increased significantly over the last 20 years, insecticides and fungicides remain the most numerous registered solutions. On the contrary, biocontrol solutions to control weeds, mites, nematodes and terrestrial mollusks remain very limited (Fig. 1 and 2; Table SI1).

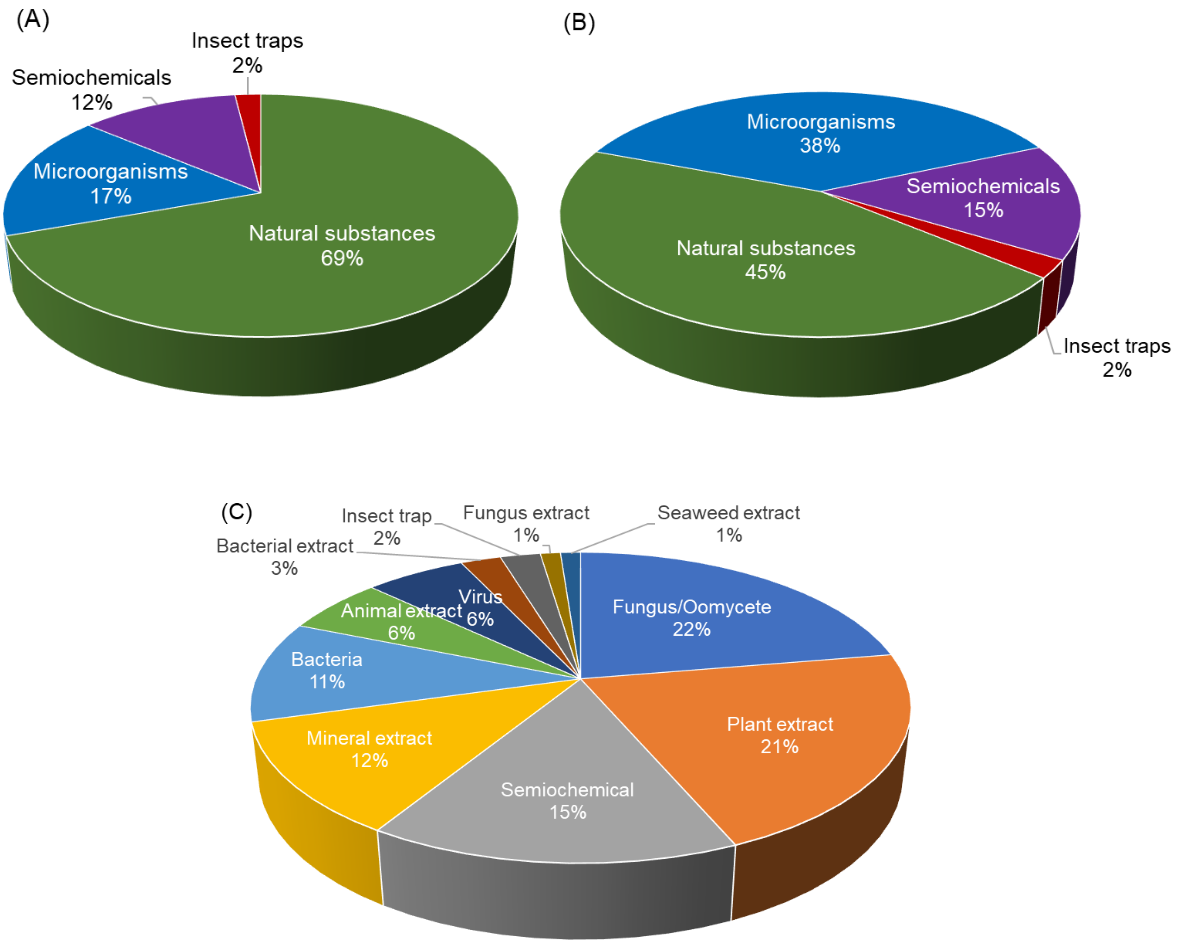


Fig. 1 (**A**) Distribution (in %) of the 726 biocontrol products with marketing authorization in the four categories of biocontrol (DGAL 2022), (**B**) Distribution of the 85 approved active ingredients in the four categories of biocontrol (DGAL 2022), **(C)** Details of the distribution of the 85 active ingredients

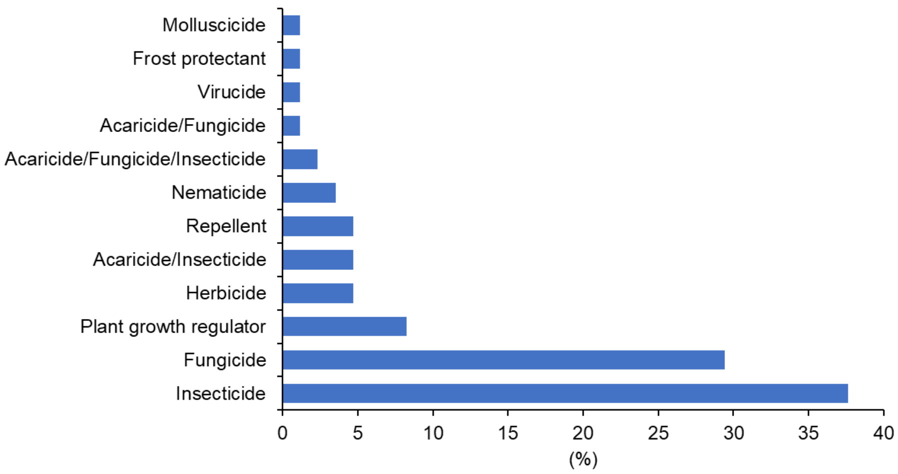


Fig. 2 Distribution of the natural substances and microorganism species according to their uses

Sulfur is the most widely used biocontrol product (15000 t sold), together with phosphonates (1500 t sold) (BNV-D 2021). A recent meta-analysis on "biological control" or "biocontrol" showed that the overall use of microorganisms remains limited because of their specificity as they usually control only one pest, and because they have limited efficacy to control the targeted pest (Hernandez-Rosas et al. 2020).

Macroorganisms

Insecticides

The majority of macroorganisms used for biocontrol are arthropods (insects, mites) used against other arthropods (insects and mites) and nematodes (Table SI1). These organisms are part of the crop protection agents (natural enemies or auxiliaries).

Arthropods. Beneficiary arthropods are used for predation or parasitism (Tables 1 & SI1). For predation, depending on the auxiliary species, the larvae or adults hunt and consume prey to ensure their development or reproduction. This predation is often not very specific: even if the predator has preferences, it generally consumes what it finds in the crop to be protected. Thus, the efficacy of biocontrol can be compromised, and biodiversity may be reduced by the impact of predation on non-target communities. Parasitism requires adults capable of reproduction. This method is based on the use of parasitoids that lay their eggs in (endoparasitism) or on (ectoparasitism) the host. After hatching, the parasitoid larvae will develop by feeding on the host. Depending on the parasitoid species, the parasitoid will lay its eggs in the host eggs, in the larvae or in the adults. Unlike predation, parasitism is often specific: oviposition will only take place if compatible insects are detected by the parasitoid, the compatibility being established at the species or even genus level. Interference or competition between parasitoids sharing the same hosts are events that can compromise the success of parasitism-based biocontrol.

Three methods of using auxiliary arthropods are used:

(1) Introduction/acclimatization of auxiliaries of the pest to be controlled: an invasive pest and its auxiliary from the same territory are considered. Past experience has shown that it is essential to ensure that the introduced auxiliary arthropods are not or do not become a threat to the environment in which they are introduced.

(2) Augmentation is also based on the use of auxiliaries of the pest, but they are endemic to the area being treated. As for introduction/acclimatization, it is necessary to mass-rear the biocontrol agents, which can be a significant hurdle to overcome because a substitute host or prey have to be found that must also be

mass-reared. Once the beneficials are reared, they need to be released in the areas to be treated (fields, greenhouses). Depending on the biological, physiological and/or morphological characteristics of the beneficials and pests, the releases will be repetitive or punctual via capsules or diffusers distributed over the area to be treated or released by an aerial vector. Thus, the releases will be inundative (implementation of large quantities of beneficials with an expected rapid control of the pest) or inoculative (less beneficials released with an expected reproduction in situ for a long-term control of the pest).

(3) Conservation consists of encouraging the presence of beneficial insects by manipulating the environment of the crops or the crops themselves (e.g., planting hedges, grassed strips, installing nest boxes for chickadees, etc.).

Table 1 Arthropods used in France for biocontrol of crop pests (adapted from Fauvergue et al. 2020)

Orders	Taxons used in France (examples)	Use	Main target
Dermaptera	*Forficula auricularia*	Predator	Aphids
Thysanoptera	*Franklinothrips*	Predator	Thrips
Hemiptera	*Orius, Macrolophus*	Predator	Thrips, Whiteflies
Neuroptera	*Chrysoperla*	Predator	Aphids
Coleoptera	*Coccinella*, *Harmonia*, *Radiola*	Predator	Aphids
Diptera	*Aphidoletes*, *Episyrphus*	Predator	Aphids
Hymenoptera	*Aphidius*, *Encarsia*	Parasitoid	Aphids, Whiteflies
Acari (subclass)	*Amblyseius*, *Neoseiulus*, *Phytoseiulus*	Predator	Thrips, Whiteflies, Acari

Nematodes. Two different families of nematodes (Heterorhabditidae and Steinernematidae) are used for biocontrol (Table 2). They are entomopathogenic and have similar lifestyles, mutualism with bacteria: Photorhabdus for Heterorhabditidae, and Xenorhabdus for Steinernematidae. Only the infective juvenile stage lives freely in the environment and is contaminating for insects, the other developmental stages take place in an insect. After entering the insect, the nematodes release their bacteria which release a series of toxins that neutralize the insect's immune response and kill it. The nematode feeds on the remains of the insect and enables its complete reproductive cycle. When the cadaver finishes disintegrating, there is a massive release into the environment of infective juveniles capable of attacking another insect. Nematodes search for their future prey in two different ways: ambush or active search (Grewal et al. 1994) however the range of insect species that can be attacked by nematodes is rather limited. It should be noted that a nematode of the Rhabditidae (*Phasmarhabditis hermaphrodita*) family has molluscicide properties and is used as such. This nematode also has a mutualistic bacterium, *Moraxella osloensis,* and its mode of reproduction is qualitatively identical to that of entomopathogenic nematodes. However, mass production of these nematodes is somewhat problematic. It can be done in vitro on an artificial medium (nematodes are multiplied in parallel with bacteria and then the two are combined) or in vivo using easily produced surrogate hosts. In all cases (nematodes, parasitoids and predators), there is a risk of reduced efficacy.

Table 2 Entomopathogenic nematodes used for biocontrol of agricultural pests (adapted from Tofangsazi et al. 2018). Although the mode of action of these nematodes is similar, they belong to distinct families of the order Rhabditida: Steinernematidae and Heterorhabditidae.

Species	Targets
Steinernema glaseri	White grubs (beetles, especially the Japanese beetle, *Popillia* sp.), banana root borers
Steinernema kraussei	Black vine weevil, *Otiorhynchus sulcatus*
Steinernema carpocapsae	Turf pests: Bugs, cutworms, armyworms, sod webworms, cereal bugs, tipulas Orchard, ornamental and vegetable pests: Banana moth, codling moth, cranberry rootworm, dogwood moth and other moth species, black vine weevil, peach moth, shore flies *(Scatella* spp.*)* Red palm weevil *Rhynchophorus ferrugineus*, palmivorous butterfly *Paysandisia archon*
Steinernema feltiae	Mushroom flies (*Bradysia* spp.), shore flies, western flower thrips, leaf miners
Steinernema scapterisci	Mole crickets (*Scapteriscus* spp.)
Steinernema riobrave	Citrus root weevil (*Diaprepes* spp.), mole crickets
Heterorhabditis bacteriophora	White grubs (beetles), cutworms, black vine weevil, flea beetles, maize rootworms, citrus root weevil, strawberry root weevil
Heterorhabditis megidis	Weevils
Heterorhabditis indica	Mushroom flies, root scales, grubs
Heterorhabditis marelatus	White grubs (beetles), cutworms, black vine weevil
Heterorhabditis zealandica	Beetle larvae

Herbicides

The use of macroorganisms to control the invasive development of plant species has been achieved several times over the last few centuries. The success of the management of *Opuntia stricta* invasion in Australia in the 1920s was made possible by the introduction of a *Cactoblastis cactorum* insect whose larvae consumed the plant and released entire territories (Zimmermann et al. 2004). However, the use of herbivore predators should be based on an overall assessment of the presence of this new species in terms of positive effects (efficacy in plant regulation) and negative effects (effects on the ecosystem) as for *Ctenopharyngodon idella* (Fedorenko and Fraser 1978). Research experiments are still required to use the synergistic potential effect of agricultural practices and macroorganims to regulate or to control weed populations (Foley et al. 2023).

Ambrosia artemisiifolia L. (common ragweed) is an invasive Asteraceae responsible for severe pollen allergy in areas of high densities of the plant. Its occurrence in Europe in contrasting open habitats (cultivated plots, rural environments, roads, river banks) made it difficult to develop classical control methods, and research were rapidly carried out on the potential of biological control (Reznik 1991). However, it was by accident that a biological control agent (*Ophraella communa*) was identified in Europe in 2013 (Müller-Schärer et al. 2014). Arriving probably via the airport of Milan (Italy), the proliferation of this small beetle (3 to 6 mm) which originates from North America like *A. artemisiifolia*, allowed to observe a very high level of predation of *A. artemisiifolia* plants to the extent of decreasing by more than five times the quantity of pollen in the air (Bonini et al. 2016). Studies under controlled conditions and modelling approaches confirmed the ability of *O. communa* to predate *A. artemisiifolia* in Europe (Augustinus et al. 2020). The predation of this beetle is all the more effective as the three larval stages and the adult stage contribute to the defoliation of the plant. The phenomenon is then amplified by the number of generations, which is three to four in Europe and six to seven in China, where large scale releases of beetles were successfully used to limit the negative allergenic effects of Ambrosia on local human populations (Zhou et al. 2014). *O. communa* mainly predates *A. artemisiifolia* and only rarely seems to consume other plant species, and it does not seem that the insect can significantly attack cultivated sunflower (*Helianthus annuus* L.; Augustinus et al. 2020), which is a major concern with regard to the introduction of this insect. Current work focuses on a better understanding of the plant-insect relationship (effect of genetic structuring of the two species, annual temperature, climate change) to promote predation intensity (Chen et al. 2018a; Sun et al. 2020). *O. communa* has now dispersed into Italy, Switzerland, Slovenia, Croatia, and recently in France (Müller-Schärer et al. 2014; Observatory of Species of Concern for Human Health 2023; Zandigiacomo et al. 2020).

For some years, in plots managed under conservation agriculture or in vineyards, the use of weed-control flocks (mainly sheep) has been tested to control cover crops and weed species before sowing the next crop (MacLaren et al. 2019). These strategies, developed by farmers on experimental sites, have not yet been validated from an agricultural and economic point of view. However, this reintroduction of herds during the fallow period is interesting for its potential efficacy and social impact. More specifically, for experiments on the management of common ragweed, flocks of sheep have been used with some success on the banks of French rivers (Drôme), areas where the use of PPPs is prohibited (Faton 2008). In general, the use of herds could be a weed regulation solution in agricultural or in peri-urban situations.

In field crops under conservation agriculture, the control of certain weeds can also be ensured by granivorous animals: small mammals, birds, and especially insects (carabidae; Honek et al. 2003; Bohan et al. 2011).

Finally, landscape management can be a lever to favor the action of beneficials (Davis and Liebman 2003; Petit et al. 2017).

Regulation of macroorganisms

Contrary to microorganisms, natural substances and semiochemicals, macroorganisms are not covered by the European Regulation (EC) No 1107/2009 (2009) (Fig. 3).

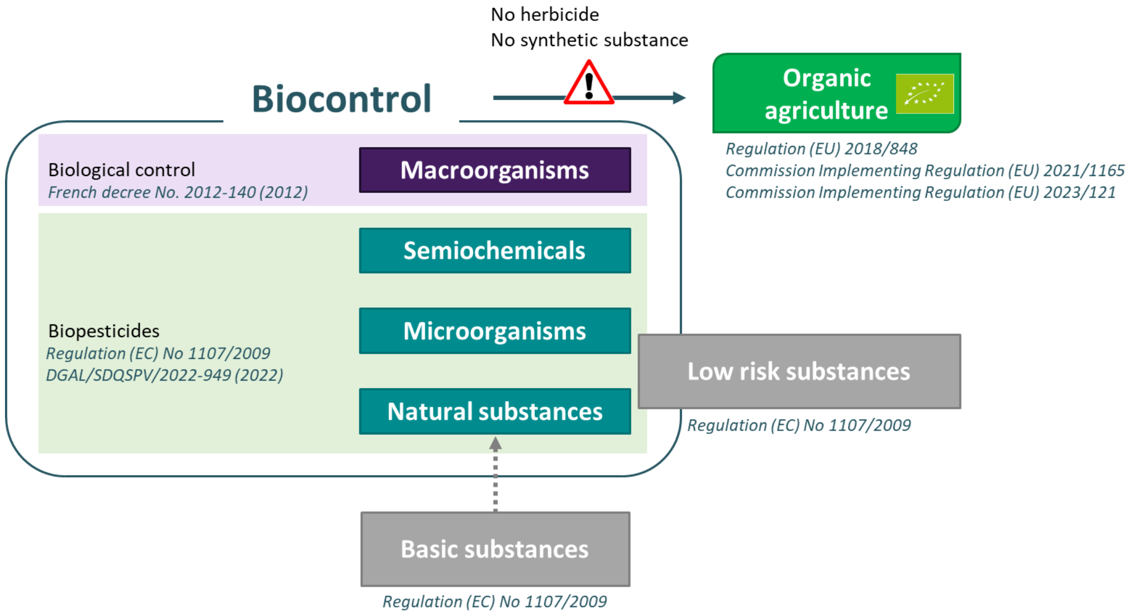


Fig. 3 The different categories of biocontrol solutions, their positioning in the European and French regulations, and their implication in organic agriculture

The introduction of non-native macroorganisms (not installed on the French territories) may present specific risks for the environment (e.g., invasive species). Therefore, since 2012, macroorganisms have been subject to the French Decree No. 2012-140 of 30 January 2012 (French Republic 2012a; 2012b; 2023) on the conditions for authorizing the entry into the territory and introduction into the environment of non-indigenous macroorganisms useful to plants, particularly in the context of biological control (Fig. 3). However, non-indigenous macroorganisms that have been introduced for several years, before the date of entry into force of the decree, and that do not present a particular risk, are exempted from an application for authorization of entry or introduction into the national territory.

In total, 448 macroorganisms have been declared, corresponding to 125 indigenous and non-indigenous species (Table SI1). The list is regularly updated by ANSES (2021).

Microorganisms

Insecticides

A range of *Bacillus thuringiensis* strains are known as insecticides and listed as biocontrol solutions (Table SI1). Discovered in Japan in dead insects and formally identified in Germany at the beginning of the 20th century, *B. thuringiensis* has been first exploited as bioinsecticide in France since 1930. The mode of action of *B. thuringiensis* can be summarized as follows: after being ingested by the insects, the spores germinate in their intestine and release Cry enthomopathogenic toxins forming holes in the intestine and causing the death of the insects (Bravo et al. 2007; Bravo et al. 2011; de Almeida Melo et al. 2016). The bacteria can multiply in the insect cadaver and then sporulate when nutrients are no longer available. The various *B. thuringiensis* strains are differing by the range of toxins they are able to produce and which define the range of species against which this strain will be toxic (Table 3). In France, *B. thuringiensis* var. *kurstaki* producing Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa and Cry2Ab toxins, and *B. thuringiensis* var. *aizawai* producing Cry1Aa, Cry1Ab, Cry1Ba, Cry1Ca and Cry1Da toxins have an agreement as biocontrol agent to fight against lepidoptera insects (DGAL 2022).

Table 3 Toxicity of some Cry toxins of *B. thuringiensis* towards different orders of insects (X: insect order comprising species targeted by the toxin; +: insect order comprising at least one sensitive species to the toxin)

Toxin	Lepidoptera	Diptera	Coleoptera	Hemiptera	Hymenoptera
Cry1Ab	X			+	
Cry1Ac	X	+		+	
Cry2Aa	X	+		+	
Cry3Aa			X	+	+
Cry4Aa		X		+	

Other Bacillus strains have been found for their insecticidal activity (Table SI1): as an example, a strain of *B. subtilis* (Abs3b strain) has been identified for its insecticidal activity on *Bactrocera olea* (Mostakim et al. 2012). Interestingly, as with *B. thuringiensis*, a chitinase activity has been identified as important for the insecticidal function of *B. subtilis* (Chandrasekaran et al. 2014). Some surfactant-like compounds (surfactin isomers: iso-C14 [Leu7], iso-C14 [Val7] and anteiso-C15 [Leu7]) have also shown insecticidal activity on aphids by *B. subtilis* (Yang et al. 2017). In addition, a strain of *B. amyloliquefasciens* (G1) showed insecticidal action on aphids by means of a surfactin (Yun et al. 2013). Finally, a strain of another bacterial species, *Serratia marcescens*, produces an enzyme that degrades the wax present on the cuticle of certain insects. This gives it a proven insecticidal action against the mealy bug *Maconellicoccus hirsutus* (Salunkhe et al. 2013).

The two species *Metarhizium anisopliae* and *Beauveria bassiana* are the most commonly used fungal strains as bioinsecticides (Table SI1). Several laboratory tests describe other potentially interesting strains or species of fungi but they are not effective in the field. Better consideration and knowledge of the ecological and physiological parameters of this species in its environment are needed (Lacey et al. 2015).

Fungicides

Bacteria. Three Bacillus genera have anti-fungal activities, *B. amyloliquefaciens*, *B. subtilis* and *B. pumilus*, and have been approved to control many fungal and bacterial diseases in vineyards, orchards, and arable crops (Table SI1) (E-Phy 2023). They mainly act by direct antagonism, due to secreted lipopeptides or volatile compounds (VOCs) which inhibit mycelial growth and/or spore germination of pathogens, but they also act as stimulators of plant defenses (Chowdhury et al. 2015; EFSA et al. 2021a; Islam et al. 2016). For example, *B. pumilus* physically limits fungal spore germination, damages the cellular integrity of fungal cells, competes for nutrients, and can induce systemic resistance (EFSA 2013b). Other bacteria, like *Pseudomonas chlororaphis*, exerts antibiosis action via the production of antifungal compounds (e.g., phenazine, pyrrolnitrin, lipopeptides) (Huang et al. 2018), but they are also able to stimulate plant defenses and even to promote plant growth (Ganeshan and Kumar 2005). The Streptomyces (formerly *Streptomyces griseoviridis*) actinobacteria has antifungal or antibacterial properties (Lee et al. 2018), with modes of action similar to those of *B. amyloliquefaciens*: spatial and nutritional competition, production of antifungal products, cell lysis followed by hyperparasitism, biostimulation of plant growth (Table SI1).

Fungi and oomycetes. The review of mycofungicides by Thambugala et al. (2020) describes 300 antagonistic fungi, with the Trichoderma genus reportedly having the greatest potential. However, there are limiting factors for the development of mycopesticides, such as lower than expected efficacy or environmental sensitivity of propagules (Zaki et al. 2020), and they are often less used than bacterial solutions. Most fungal solutions act as mycoparasitic, antagonistic or fungicidal fungi, i.e., *Coniothyrium minitans*, *Clonostachys rosea*, Trichoderma (*Trichoderma asperellum*, *Trichoderma atroviride*, *Trichoderma harzanium*), *Aureobasidium pullulans*, and *Ampelomyces quisqualis* (Table SI1). As for bacteria, several modes of action coexist, ranging from competition for nutrients to production of anti-fungal molecules, stimulation of plant defenses, and hyperparasitism (EFSA 2013a). For example, the Trichoderma genus can compete with a pest, inactivate pathogen infection processes by producing hydrolytic enzymes (chitinases, proteases) and antibiotics, stimulate plant defenses, or promote the solubilization of inorganic nutrients in the plant (EFSA 2012a; EFSA 2013e; Trivedi et al. 2016). Antagonistic yeasts (*Candida oleophila* and *Metschnikowia fructicola*) are also used as mycofungicides, whose mode of action is largely due to competition for nutrients (EFSA 2012b; Spadaro et al. 2013). A single oomycete, *Pythium oligandrum* (registered as oospores), controls pathogens predominately by mycoparasitism and via the production of antimicrobial compounds. In addition, like many microorganisms, it also enhances plant defenses and induces systemic acquired resistance (Table SI1) (Benhamou et al. 2012).

Bactericides

Biocontrol products with bactericidal properties are based on different modes of action including bactericidal, bacteriostatic and antagonistic effect (Table SI1). The bacterium *B. amyloliquefaciens* strain Ar10 exhibits glycolipid-mediated antagonistic properties to *Pectinobacterium carotovorum* that causes potato soft rot (Azaiez et al. 2018). *B. amyloliquefaciens* strain KC-1, an endophytic bacterium, was shown to be effective in controlling the development of *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), which causes Chinese cabbage soft rot, in vitro and in vivo (Cui et al. 2019). The *B. amyloliquefaciens* strain P41, isolated from olive phylloplane, was shown in vitro and in planta to be effective in controlling *Pseudomonas savastanoi* pv. *savastanoi* causing gall of olive through the production of VOCs, siderophores, and lytic enzymes (Mina et al. 2020). Similarly, greenhouse experiments have shown an efficacy of 47-78% of PGPR (Plant Growth Promoting Rhizobacteria) bacterial strains (*Serratia* strain J2, *Pseudomonas fluorescens* strain J3, *Bacillus* strain BB11) in controlling the development of *Ralstonia solanacearum* which causes soft rot of tomato (Guo et al. 2004). The PGPR bacterium *Pseudomonas syringae* strain Cit7 is effective in controlling the development of *P. syringae* pv. *tomato* and eliminating tomato speckles by stimulating the plant defense mechanisms (Ji et al. 2006). In combination with copper hydroxide (which is not a biocontrol solution), *B. subtilis* strain QST 713 is more effective than conventional chemical treatments combining mancozeb and copper in controlling the development of spots on tomatoes caused by *Xanthomonas euvesicatoria* and *Xanthomonas perforans* (Roberts et al. 2008). A strain isolated from the tomato rhizosphere, *B. amyloliquefaciens* strain SQRT3, is not only able to form a biofilm on tomato roots, to produce siderophores and proteases to suppress *Ralstonia solanacearum* but also to induce tomato defense mechanisms via the jasmonic acid signalling pathway indicating its interest for biocontrol (Li et al. 2017). Similarly, *Bacillus* strain B014, an endophytic strain isolated from healthy *Anthurium* tissue, controls the development of *Xanthomonas axonopodis* pv. *dieffenbachiae* by activating enzymes involved in plant defense mechanisms such as phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase (Li et al. 2012).

Pseudomonas sp. 23S, producing siderophores, acetic indole and hydrogen cyanide, is able to control *Clavibacter michiganesis* subsp. *michiganesis* responsible for bacterial canker of tomato by inducing a systemic resistance response via the salicylic acid pathway (Takishita et al. 2018). The combination of the application of PGPR bacteria (*B. pumilus* strain INR7) and a chemical inducer (benzothiadazole) was shown to be effective in inducing plant defense mechanisms to control *Xanthomonas axonopodis* in tobacco and pepper (Yi et al. 2013). Halotolerant isolates of *B. amyloliquefaciens* capable of producing siderophores are used to control *Acidovorax oryzae* infecting rice crops (Masum et al. 2018). Filtrates from *B. amyloliquefaciens* strain K5-3 and strain PPB6 produced damage to the cell membrane of *Acidovorax oryzae* leading to a decrease in its abundance, mobility, and ability to form biofilms. In addition, yeast strains (*Pichia anomala* and *Candida oleophila*) are 27-60% effective in antagonising the development of the parasitic complex responsible for banana root rot (Lassois et al. 2008).

Nematicides

Only few studies have been found on the use of microorganisms for their nematocidal activity. Bacillus species, such as *B. firmus* (Table SI1), is used against nematodes of the genus Meloidogyne. This bacterium is an antagonist nematode capable of degrading and colonizing Meloidogyne eggs. It is also able to induce systemic resistance in plants; however, this effect varies depending on the host plant. Some bacterial isolates are active over a wide temperature range with an optimum at 35°C (Ghahremani et al. 2020). The most commonly used fungal nematocidal agent is *Paecilomyces lilacinus* which attacks nematode eggs (Anastasiadis et al. 2008; Mukhtar et al. 2013). In addition, it was proposed to use entomopathogenic fungi or bacteria for their nematocidal activity (Muniz et al. 2020; Iqbal et al. 2018; Kiewnick and Sikora 2004; Mukhtar et al. 2013; Temitope et al. 2020).

Acaricides

Some works on the search for entomopathogenic fungi (*B. bassiana*, *Metharizium anisopliae*, *Acremonium hansfordii*) effective against *Tetranychus* species (Bugeme et al. 2014; Shang et al. 2018; Wekesa et al. 2005) showed that strains had low efficacy. In combination with thymol, *B. bassiana* or *M. anisopliae* have an increased acaricide efficacy against the varroa mite (Sinia and Guzman-Novoa 2018).

Natural substances

Insecticides, acaricides

Several natural substances of various origins have insecticide/acaricide properties: abamectin and spinosad which are from bacterial origin, diatomaceous earth, aluminium silicate and paraffin oil from mineral origins; fatty acids, maltodextrin, orange oil, pyrethrins, rapeseed oil, and terpenoid blend which are extracted from plants (Table SI1). These substances were developed to eliminate a wide range of species of harmful insects such as caterpillars, flies, snout moths, soil pests, thrips, etc. (E-Phy 2023).

Abamectin (produced by fermentation of *Streptomyces avermitilis*) and spinosad (produced by bacterial fermentation of *Saccharopolyspora spinosa*) are neurotoxic which raise the question of their compatibility with the insect natural enemies (Table SI1) (Williams et al. 2003). Diatomaceous earth, which consists mainly of silicon dioxide, interfers with physiological processes by destroying the natural water barrier, the waxy layer of the cuticle, and hence disrupting the functioning of the water preservation mechanism (Table SI1) (BPDB 2023; ECHA 2016). Aluminium silicate (kaolin) is an insect repellent due to the film formed on the surface of the plants and creating a physical barrier (Table SI1) (BPDB 2023). Pyrethrins are neurotoxic to insects, stabilizing the opened form of the sodium channel in axon membranes (Table SI1) (BPDB 2023). Oils are used as contact insecticides: by forming an impermeable film on the surface of the plant, they isolate the insect and its eggs by suffocating them. Maltodextrin acts like oils by plugging the respiratory orifices of insects and engulfing them (Table SI1) (Siegwart and Lavoir 2020). Fatty acids act by contact, having a burn-down effect (Table SI1) (BPDB 2023).

Fungicides

The natural substances used as fungicides are from plant (fatty acids, eugenol, geraniol, clove oil, orange oil, thymol) or mineral (potassium hydrogen carbonate, disodium phosphonate, potassium phosphonates, sulfur) origins (Table SI1) (E-Phy 2023).

The modes of action of these substances are not well known. Fatty acids act by contact while eugenol prohibits the growth of both Gram-positive and Gram-negative bacteria and fungi (Table SI1) (BPDB 2023). Potassium hydrogen carbonate causes the collapse of hyphal walls and shrinkage of fungal conidia, and potassium phosphonates have direct toxicity to plant pathogens reducing populations, but also promote of plant natural defenses (Table SI1) (BPDB 2023). Sulfur is a non-systemic, protective fungicide with contact and vapor action inhibiting respiration. It is a non-specific thiol reactant which also acts as a multi-site fungicide (EFSA 2008).

Essential oils (clove, thymol, eugenol, geraniol, orange) often show a good efficacy in laboratories but, in field experiments, it decreases drastically because these substances are very volatile, and their persistence on the crop is low. To improve their efficacy, they should be encapsulated (Milicevic et al. 2022).

Herbicides

Due to the lack of workers to weed the cultivated fields, natural substances were used at the end of the 19th century as herbicides to increase the efficacy of weed management (Table 4). Iron sulfate and sea salt allowed the first experiments to be carried out to apply an herbicide molecule to control weeds in cultivated fields (Chauvel et al. 2022). After the Second World War, the development of synthetic molecules, which were cheaper and more effective, virtually eliminated the use of natural substances. Nevertheless, in the current context, herbicidal biocontrol solutions are presented as an alternative to conventional active substances whose negative effects on the environment have been demonstrated, and also as a potential solution for managing weed species that have selected resistance genes.

Table 4 Natural substances for herbicide uses: number of commercial products in France, use in agricultural and non-agricultural areas, dose and target

Active substance	Number of commercial products in France (ACTA 2022)	Agricultural areas	Non-agricultural areas	Dose	Target
Acetic acid*	5	Yes	Yes	From 250 to 1 000 L/ha	Plant
Capric acid* (+ caprylic acid)	3	No	Yes	1 000 L/ha	Plant, bryophyte
Caprylic acid*	1	Yes	Yes	80 L/ha	Plant, bryophyte
Sodium chloride	-	No	Yes	10-100g/stump	*Baccharis halimifolia*
Iron sulfate**	6	Yes	Yes	From 150 to 280 kg /ha	Bryophyte
Pelargonic acid*	20	Yes	Yes	16 to 166 L/ha	Plant, Bryophyte-seaweed, lichen
Vinegar*, ***	-	Yes	Yes	100 L/ha	Plant

* Plant origin; ** Mineral origin; *** Only ion medicinal aromatic and perfume

The natural substances currently used (Table 4, Table SI1) are partly fatty acids (capric acid, caprylic acid, pelargonic acid) and acetic acid which have a non-selective action on weeds (EFSA 2013i; EFSA 2013h). They are also used to limit the development of bryophytes in urban areas. The efficacy of pelargonic acid, the first herbicide natural active substance to be marketed in France, appears to be higher for the management of eudicotyledons (seedlings) than for the management of monocotyledons (Travlos et al. 2020) but the experimental conditions seem to strongly influence the efficacy of the compound. Sodium chloride (NaCl) was approved in March 2021 at the European level (BPDB 2023). Its use is currently limited to the destruction of the stump of the invasive species (*Baccharis halimifolia* L.) in coastal areas by spot application of pure salt in holes drilled in tree stumps, and on the ground in the direct vicinity of the stumps (10-100 g/treated stump; pure salt). Studies are being carried out on the use of seawater (alone or in combination with synthetic molecules) for the management of turfgrass (Uddin et al. 2011). At currently registered doses, iron sulfate has limited efficacy for the management of bryophytes (ACTA 2022). Although many potential herbicide molecules are being studied today, few natural solutions that are viable from economic and agricultural points of view are currently available to farmers, despite the very strong pressure to withdraw synthetic molecules.

Allelopathy is a population regulation mechanism that is often mentioned in ecology and agronomy. Allelopathy consists of the production by a given plant species of one or more chemical substances that can limit the germination and growth of other plant species that are spatially close to it (Rice 1984). Although many works on crop-weed relationships are entirely devoted to allelopathy (Cheema et al. 2013; Rice 1984), there is very little scientific data to confirm that this biological regulation is effective in cultivated environments. Proposed as alternatives to conventional herbicides, allelopathic compounds from plants could be a potential source of new herbicide molecules. Literature reviews indicated that about 200 molecules were identified as potentially having an allelopathic effect under controlled and semi-controlled conditions (Aslam et al. 2017; Jabran and Farooq 2013). Several species belonging to the Asteraceae, Brassicaceae, Poaceae, and Polygonaceae families were investigated for their allelopathic potential in managing weed communities (Delabays et al. 2009; Jabran et al. 2015) but efficient applications in the field seem to be very limited at the moment. As allelopathic substances released into the environment can be leached, bound and immobilized by soil organic matter, or degraded by microbial communities (Zeng 2014), there are few concrete achievements of the agricultural use of these molecules. A review carried out on the allelopathic potential of cultivated varieties showed, from 523 papers published from 1956 to 2020, the relevance of an allelopathic effect was demonstrated only in seven cases (Mahe et al. 2022). Although many studies have been carried out over the past ten years, further work is still needed to understand the functioning of these molecules, which seem to have a broad spectrum of action (what synergies between allelopathic molecules?). The fate of these new molecules in soil remains to be determined as well as the identification of their modes of action in the plant (Macias et al. 2019) before considering their real use in the field.

Other uses

Five biocontrol substances are currently used as plant growth regulators: 6-benzyladenine, gibberellic acid, indolbutyric acid, gibberellins and spearmint oil (Table SI1). Auxins (indolbutyric acid) and gibberellins (e.g., gibberellic acid), with their numerous actions on cell divisions and elongation, are the main molecules used (Santner et al. 2009), especially for vegetable crops, vineyards, orchards, and ornamental crops. In recent years, an increase of more than 15% in sales has been observed (Robin and Marchand 2019).

Among the available plant elicitors (Table SI1), laminarin, a polyoside extracted from brown seaweed, is approved against various pathogens, including many fungi (Poveda and Diez-Mendez 2022; Siegwart and Lavoir 2020). The COS-OGA active substance consists of a complex of chitosan fragments (chitooligosaccharides, COS), which are compounds found in crustacean exoskeletons, that are associated with pectin fragments (oligogalacturonides, OGA) originating from plant cell walls (van Aubel et al. 2014). Although the COS-OGA elicitor is not directly toxic to pathogens, it is detected by the plant, which then switches on signaling cascades that result in defense reactions against potential invaders. It has been demonstrated that the COS-OGA complex triggers signal transduction through the salicylic acid (SA) pathway (de Miccolis Angelini et al. 2019; van Aubel et al. 2014). Finally, cell wall derivatives from *Saccharomyces cerevisisae*, cerevisane, also acts as an elicitor of plant defenses and, by modulating the gene expression, it can also be effective against oomycetes (de Miccolis Aneglini et al. 2019).

Repellents are mainly substances of animal origin: blood meal, sheep fat, fish oil, but there are also quartz sand and aluminium silicate (Table SI1).

Only one molluscicide (ferric phosphate) and one nematicide (garlic extract) are approved as biocontrol solutions (Table SI1). Finally, heptamaloxylglucan (natural component of dicotyledone plant walls) is approved to protect crops against frost damage (Table SI1).

Semiochemicals

Semiochemicals are molecules used either to trap, disorient or repel pests or to attract predators or parasitoids of these pests. The molecules used to trap or disorient pests are pheromones: they are normally emitted by females to attract males very efficiently for reproduction, and they are usually very species-specific. In crop bioprotection, pheromones are used either at low doses or high doses. At low doses, the objective is to attract males into traps from which they will be physically unable to leave or in which they will be poisoned by insecticides. At high doses, the atmosphere will be saturated, making the female undetectable to the male. In the latter case, this is called sexual confusion. The molecules that repel pests or attract their predators or parasitoids are kairomones. They can be emitted by the pest itself or by the attacked plant. In the context of biocontrol and given their very low production by the emitting organisms, pheromones and kairomones are not extracted but synthetized in identical form.

Regulation of microorganisms, natural substances and semiochemicals

Microorganisms, natural substances and semiochemicals are covered by the European Regulation (EC) No 1107/2009 (2009) (Fig. 3). Among these biocontrol solutions, some are considered as “Low risk active substances” (e.g., cerevisane, COS-OGA, ferric phosphate, *Pepino mosaic* virus, etc.) (Table SI1) and have to be specifically approved according to the Articles 22 and 47 of the Regulation (EC) No 1107/2009 (2009); while others are considered as “Basic substances” (e.g., garlic extract, beer, vinegar) (Table SI1) needing to be approved according to the Article 23 of the Regulation (EC) No 1107/2009 (2009) (Fig. 3). After obtaining the approval, biocontrol solutions are listed in the Annex II of the European Regulation (EC) No. 889/2008 (2008).

The French regulations that apply to biocontrol solutions (Article L.253-6 of the FRMFC; French Republic 2023) are specific and aim at facilitating their placing on the market. They benefit from a reduced tax for approval and authorization applications, a reduced evaluation period, and various exemptions (Article R.253-11 of the FRMFC; French Republic 2023). For example, they are exempted from the prohibition of discounts, rebates and refunds, and from certain sales conditions applied to other PPPs (Articles L.253-5.1- of the FRMFC; French Republic 2023). Approval as PPP is not compulsory for use as a service when the product does not carry any danger mention (Article L.254-1 of the FRMFC; French Republic 2023). Some advertising, prohibited for conventional PPPs, is authorized for biocontrol (Article D.253-43-2 of the FRMFC; French Republic 2023). The use of biocontrol solutions is exempted from the obligation to implement measures to protect people near inhabited areas or areas used for recreational purposes (Article L.253-8 II of the FRMFC; French Republic 2023). The biocontrol solutions of the DGAL list (DGAL 2022) can be sold and used by public persons and for green spaces, forests, roads or public walks (Article L.253-7 of the FRMFC; French Republic 2023). They are also exempted from actions aiming at reducing the use of PPPs and from PPP saving certificates (Articles L.254-10 to L254-10-9 of the FRMFC; French Republic 2023).

Contamination of the environment by biocontrol solutions

The macroorganisms, microorganisms, natural substances and semiochemicals used for biocontrol are still very rarely monitored in the environment after their application. As some of them are naturally present (fatty acids, potassium hydrogen carbonate, aluminium silicate, sulfur, etc.), it is difficult to distinguish, in the soil, water and air, the fraction coming from the biocontrol solutions from the one that is present at the origin, especially since the quantities added may be negligible (E-Phy 2023). In addition, some compounds have a chemical nature that is not compatible with analytical monitoring (sheep fat, fish oil, etc.). It is also difficult to determine, for example, the quantities of semiochemicals brought by treatments. Thus, the few results presented below concern exogenous biocontrol substances that can be measured in the environment.

Soil and water contamination

There is almost no data on the contamination of soil and aquatic environments, freshwater or marine ones, by biocontrol solutions. However, knowledge of their fate in soils, water and sediments can provide some information: the more persistent and/or mobile a compound is, the more likely it is to lead to the contamination of the environment (soil, water, sediment, plant).

A recent review on the behavior of natural substances in soils showed that most of them were not very persistent (degradation half-life DT50 < 60 days), except abamectin, paraffin oil, spinosad and phosphonates (Mamy and Barriuso 2022). On the other hand, some substances have a high mobility (in particular acetic acid: adsorption coefficient normalized to soil carbon organic content Koc = 0 L/kg), while others will be almost immobile in the soil (oils, pyrethrins: Koc > 30,000 L/kg) (Mamy and Barriuso 2022) (more details are given in the "Fate of biocontrol solutions in the environment" section). Consequently, most of the natural substances should lead to a low risk of contamination of soil and water, but data are needed.

The environmental fate of *B. thuringiensis*-derived proteins has been the subject of two recent reviews (Brühl et al. 2020; Liu et al. 2021), which indicate, among other things, that these toxins would be biologically active even after adsorption to soil, particularly clays where they are highly retained and less rapidly degraded than their free form, and that they can be immobilized in sediments or sequestered in algae for several years. In leaf litter from a mosquito breeding area in the French Rhône-Alpes region treated with *B. thuringiensis* var. *israelensis*, extensive environmental contamination and toxin production were observed several months after application (Cry4Aa and Cry4Ba) (Tetreau et al. 2012).

Air contamination

Among the substances used for biocontrol, only pyrethrins were searched by some French accredited air quality monitoring associations (AASQA) in 2011 and in 2016, but they were not detected (PhytAtmo Database 2023). In 2019, because of its physico-chemical properties, abamectin was to be studied in the framework of the French national pesticide exploratory campaign in air (CNEP) (ANSES 2020) but the monitoring was impossible due to problems with the compound trapping efficiency. In the United States, measurements of pheromone concentrations have been made in treated plots (forest, cotton crop) (Koch et al. 2009; Thorpe et al. 2007) but no result on a larger contamination of the atmosphere due to pheromones used in agriculture have been published. In a very local study, Koch et al. (2009), observing some persistence of compounds (a few hours) in fields after removal of pheromone delivery systems, attributed these concentrations to either canopy release or persistence of the product within the canopy air.

Fate of biocontrol solutions in the environment

Fate of macroorganisms in the environment

The fate of a macroorganism in the environment is greatly influenced by its ability to move to find a prey or a host which is crucial for the success of crop protection. The relationships between movement and success of bioprotection are demonstrated for example with syrphid predators of the rosy apple aphid (Dib et al. 2017). This ability to move can be problematic when the crop to be protected is in close proximity to a sink crop, which can distract the predator from its objective (Madeira et al. 2014). The movement of predators and their prey has been the focus of many modelling studies (Briggs and Hoopes 2004). As for predators, the movement of parasitoids is the subject of much work, and the ability to move can be an important parameter in their successful use (Stacconi et al. 2018). The dispersal ability of a parasitoid also influences its persistence in the environment (Kuske et al. 2003).

To facilitate the persistence of a macroorganism in the environment, so its efficacy, it is possible to consider feeding to help its establishment after a release. However, this action has contrasting effects depending on the predator/prey pair considered. For example, supplying pollen can reduce thrips predation by *Orius laevigatus* (Hemiptera) and *Neoseiulus cucumeris* (mite), whereas supplying *T. viride* has no effect (Skirvin et al. 2007). Other works give more disparate results, still focusing on thrips predation by mites: an addition of pollen increased predation by *Amblyseius swirskii,* but had no effect on the efficacy of *Euseius ovalis* (Ghasemzadeh et al. 2017). In addition, the supply of pollen reduced the protection of plants by two mites (*N. cucumeris* and *A. swirskii*) against a thrips (Delisle et al. 2015). Thus, it seems difficult to draw generalizations concerning the feeding of predators, and a thorough knowledge of their ecology is necessary to try to control their maintenance in the environment.

Bank plants can be seen as a variant of the feeding concept. Plants are placed in the vicinity of the crops to be protected which will host herbivores which will be consumed by the predators if their preferred prey (the pests) run out on the crops. This strategy has also been applied to parasitoids and its efficacy in different agricultural systems has been discussed in a review (Frank 2010). It may reduce the number of predator (or parasitoid) releases, but problems may arise with maintaining bank plants.

The ability of macroorganisms to move is also used as such in bioprotection, so-called entomovectoring. For example, predatory mites are used as vectors to infect their prey, the thrips *Frankliniella occidentalis*, with the entomopathogenic fungus *B. bassiana* (Lin et al. 2017), as this fungus is not very offensive to mites. As for a mite species, entomovection is also considered using *Harmonia axyridis* and *Chrysoperla carnea* as vectors of *B. bassiana* for biocontrol of the aphid *Myzus persicae* (Zhu and Kim 2012).

While favoring the persistence of a macroorganism will increase its efficacy in bioprotection, this persistence may also be the source of potential problems: change of prey/host range, competition with endemic species, etc. For example, at the scale of several countries (France, Italy, Serbia, etc.) and over several years, *Lysiphlebus testaceipes* has demonstrated its migration capabilities (Mitrovic et al. 2013). This was also observed for *Torymus sinensis* in Spain from France (Nieves-Aldrey et al. 2019) or in Slovenia from Italy (Kos et al. 2021). One predator is now unambiguously considered invasive: *H. axyridis* (Lombaert et al. 2014). It has significant migration capacity with typical flight of 18 km long, but flights of up to 120 km have been recorded indicating a high capacity for long-distance dispersal (Jeffries et al. 2013). In addition, *H. axyridis* reproduction happens early and extends over a larger period than endogenous insects, both criteria favoring this invasive character (Tayeh et al. 2015). The problems posed by *H. axyridis* are sufficiently important to raise the question of its control. Thus, several strategies have been tested using the fungus *B. bassiana* (Roy et al. 2008), the parasitoid *Dinocampus coccinella* (Berkvens et al. 2010; Dindo et al. 2016) or the predator *Podisus maculiventris* (De Clercq et al. 2003) without a satisfactory solution being found. Contrary to *H. axyridis*, the flight distance of *Trichogramma ostriniae* is small and was estimated to be 16 m on average, with a maximum < 45 m (Chapman et al. 2009).

Global climate change has also motivated one overview which provides further insight by considering this change in relation to insect phenology and the possible consequences (Damien and Tougeron 2019). It seems likely to the authors that species with close links (host/parasitoid or prey/specialized predator) should retain some synchronicity. Consequently, it can be expected that global warming will have an impact on the environmental fate of predators/parasitoids released for crop bioprotection.

Fate of microorganisms in the environment

Like macroorganisms, microorganisms are able to grow and disperse after their application to the crop. This makes it difficult to predict their dynamics after their application, and until now only a few studies address this point (Köhl et al. 2019). Nonetheless microorganisms used for biocontrol are entering in competition with indigenous soil microbiota and are supposed to rapidly disappear after their application. However, the number of works monitoring the dissipation of microorganisms introduced for biocontrol is low.

Studying the impact of *B. amyloliquefaciens* strain FZB42 on the native rhizosphere community by metagenome sequencing, Kröber et al. (2014) showed that it remained in the rhizosphere for the five weeks of the field trial.

According to Zeng et al. (2012), populations of *C. minitans, Trichoderma* and *Streptomyces* species are stable throughout the season, and maintaining high populations of biological control agents is key to effective sclerotinia control. However, the population of *C. minitans* has been gradually decreasing during the season and this trend may continue to decrease during the following winter (Zeng et al. 2012). *C. minitans* sprayed on oilseed rape survives on flower petals for five days suggesting that the fungus can protect petals from colonisation by *S. sclerotiorum* ascospores and thus reduce sclerotinia diseases on this crop (Yang et al. 2007).

Several studies on the persistence of Trichoderma are available. It has been shown that *T. asperellum* populations in soil (per gram of soil) do not change significantly over time up to 12 weeks (Widmer and Shishkoff 2017). The persistence of Trichoderma, followed at three temperature regimes, increased during the first few days of incubation, and decreased over the 253-day experiment until it reached the limit of detection (Weaver et al. 2005). A study with *T. atroviride* in vineyard revealed dispersion in the soil surface for 18 weeks (Longa et al. 2009). However, when inoculated at high concentration, populations declined after two years and reached the level of the indigenous population. An application of *B. amyloliquefaciens* in an orchard displayed a stability of propagules over 21 days, and then dropped drastically after 120 days (Vilanova et al. 2018).

As indicated in the "Contamination of the environment by biocontrol solutions" section, there are few results on the fate of *B. thuringiensis* in the environment. *B. thuringiensis* are biologically active even after adsorption to soil, and they can be immobilized in sediments or sequestered in algae for several years (Brühl et al. 2020; Liu et al. 2021). It is noteworthy that the persistence of *B. thuringiensis* is influenced by the commercial formulation and the nature of the soil where *B. thuringiensis* is applied (Paul et al. 2017). *B. thuringiensis* var. *kurstaki* can persist for 28 months in an oak forest environment. The Cry toxins can be just as persistent but the insecticidal properties are drastically reduced from 14 months. Still in oak forest, but in a different terroir, *B. thuringiensis* var. *kurstaki* was found 88 months after spraying, the temporal limit of the study (Vettori et al. 2003). *B. thuringiensis* var. *israelensis* can also be found for several months in the environment while retaining its toxicity, and can even be found in areas where it has not been used. In leaf litter from a mosquito breeding area in the French Rhône-Alpes region treated with *B. thuringiensis* var. *israelensis*, widespread contamination of the environment and production of toxins (Cry4Aa and Cry4Ba) were observed several months after the application (Tetreau et al. 2012).

Fate of natural substances in the environment

Mamy and Barriuso (2022) recently reviewed the fate of natural substances in the environment, and especially in the soil which occupies a central position in the regulation of the fate of PPPs. Some data were already presented in the “Contamination of the environment by biocontrol solutions” section above: natural substances tend to be less persistent than conventional PPPs, and the variability of their mobility was found to be similar to that of conventional PPPs (Mamy and Barriuso 2022). It has to be underlined that for many natural substances, no DT50 or Koc value could be found (Mamy and Barriuso 2022).

In soils, the persistence of abamectin (mixture of B1a and B1b avermectin) is generally low (DT50 < 2 days) but its degradation leads to the formation of many transformation products that can be significantly more persistent (Bai and Ogbourne 2016; EFSA et al. 2020a). Its mobility is low (Freundlich adsorption coefficient normalized to soil carbon organic content Kfoc = 6631) (Bai and Ogbourne 2016; BPDB 2023; Dionisio and Rath 2016; EFSA et al. 2020a), so it is unlikely to be found in groundwater, but it could be present in surface water. In water-sediment systems, DT50 range from 20 to 91 days (EFSA et al. 2020a). Paraffin oil (mixture of C17-C31 alkanes) is persistent in soils however it can be degraded by microorganisms (EFSA 2009; Pozdnyakova et al. 2008; Spini et al. 2018). It appears to have low mobility (Koc = 462,000 L/kg; BPDB 2023) but results are scarce. Paraffin oil dissipates rapidly in water to adsorb on sediments (EFSA 2009). The persistence of spinosad in soil in the field is highly variable (0.3 d < DT50 < 104 days), increasing with soil pH and as soil moisture decreases (Adak and Mukherjee 2016; EFSA et al. 2018; Huan et al. 2015; Sharma et al. 2007; Thompson et al. 2002; Williams et al. 2003). During degradation, spinosad forms transformation products that may be more persistent than the active substance (EFSA et al. 2018). This insecticide is otherwise highly adsorbed in soils (Koc = 34,600 L/kg; BPDB 2023) which induces a low risk of groundwater contamination (EFSA et al. 2018; Mottes et al. 2017). Nevertheless, it is likely to be found in surface water but data are lacking while spinosad is persistent in water-sediment systems (DT50 > 78 days) (EFSA et al. 2018). In the soil, the DT50 of disodium phosphonate is up to 281 days (EFSA 2013c) and that of potassium phosphonates up to 196 days (EFSA 2012d). Their mobility ranges from medium (Koc = 454 L/kg for potassium phosphonates; BPDB 2023) to low (Kfoc = 952 for disodium phosphonate; PPDB 2023). The renewal assessment reports do not contain data characterizing their behavior in aquatic environments (EFSA 2012d; EFSA 2013c). The degradation of pyrethrins needs to consider the evaluation of the degradation kinetics of its six major components: pyrethrin I and II, cinerin I and II, and jasmoline I and II (Angioni et al. 2005; Feng et al. 2018). In general, pyrethrins are not persistent in the environment, with laboratory DT50 lower than three days (EFSA 2013d).

For biocontrol, deltamethrin is only approved in insect traps, so it is not likely to be in contact with the environment (in particular soil and water). However, it must be stressed that existing results show that this substance is persistent (22 days < DT50 < 231 days) in soils and is very strongly adsorbed (Koc = 1.024 10^7 L/kg; PPDB 2023). On the contrary, its degradation is very rapid in water-sediment systems (European Commission 2017).

Except some basic substances (*Salix alba*, *Equisetum arvense*) or some complex mixtures without maximum residue levels (MRLs) requirement (cerevisane, aqueous extract of *Lupinus albus*), no natural complex extract is actually approved in Europe as biocontrol solutions and, to the best of our knowledge, no environmental fate studies are available for these complex. One of the potential reasons is the limitation of classic methodologies as it is difficult to identify and track in environmental matrices derived products from such complex mixtures.

Environmental untargeted meta-metabolomic was recently considered to offer a novel "universal" tool for assessing the environmental fate and impact of commercial formulations and in-course-of-development of biocontrol solutions (Ghosson et al. 2022). This metabolomic approach, introduced by Patil et al. (2016), was called "Environmental Metabolic Footprinting" (EMF). The EMF integrates extraction, detection and analysis of the xenometabolome of an applied PPP, and the endometabolome of the environmental matrix. The xenometabolome includes the active substance, the adjuvants and the co-formulants of the commercial formulation, and the transformation products derived from the active substance. The endometabolome consists of metabolites produced by microbiome living in the studied environmental matrix. The xenometabolome and the endometabolome will then constitute the meta-metabolome that will be the target of the extraction, the chemical analyses, and the data processing (Ghosson et al. 2022). The study of the kinetics of EMFs allows the definition of two new proxies: the resilience time and the dissipation time. The resilience time is reached when the statistical multivariate comparative analysis (principal component analysis-PCA or orthogonal partial least squares discriminant analysis-OPLS-DA) of the metabolic footprints clearly shows no difference between the treated and untreated matrix (Ghosson et al. 2022; Patil et al. 2016; Salvia et al. 2018). The resilience time provides more information than the DT50 as it describes various phenomena such as the formation of transformation products and the effect on biodiversity. The EMF approach was used to evaluate the impact of natural β-triketone herbicides in soil (Patil et al. 2016). It was also useful to study the impact of commercial solutions of *B. thuringiensis* var.

israelensis on sediment (Salvia et al. 2018). Recently, the EMF approach has been adapted to fruit matrices and to target only the xenometabolome to study the fate of complex biocontrol solutions and the dissipation of their residues in treated crops. In this adaptation, the EMF approach was able to exclusively target the dissipation of biocontrol treatment residues (xenometabolome). It was also able to determine the "dissipation interval" which corresponds to the time needed to have no difference between the residue profiles of the treated sample and the profile of the control samples (Ramos et al. 2022). The EMF approach could play a very important role in the coming years, as more and more biocontrol products are developed.

Semiochemicals

To the best of our knowledge, there was no result on the fate of semiochemicals in the environment. The "Contamination of the environment by biocontrol solutions" section summarized the few papers that were found in this literature review.

Impacts of biocontrol solutions on biodiversity

Impacts of macroorganisms on biodiversity

The assessment of the unintended effects of non-indigenous macroorganisms is difficult because it has to consider host specificity, and the establishment of the potential host range of a generalist in a new area (Loomans 2021). Some authors suggest building qualitative food webs containing information on feeding relationships and abundance measures which may be useful for illustrating the connections between species and thus identifying the species at risk of indirect effects from the release of a macroorganism (Todd et al. 2021). This network model would allow for a better assessment of post-release or pre-release risks in new regions (Todd et al. 2021).

Predators

A difficulty often encountered with predators is that they are able to feed on species other than those they are released against. Predators can thus affect the biodiversity of an area in several ways: their feeding habits, their ability to move, and their ability to reproduce. These last two characteristics, by going beyond the norms of the species considered, can lead to its classification as an invasive species, as was done with *H. axyridis*. This species can consume other predators (intraguild predation) without being detrimental to the efficacy of biocontrol (Gardiner and Landis 2007). *H. axyridis*, in addition to intraguild predation, may also show a definite inclination towards cannibalism if natural prey (aphids) becomes scarce (Rondoni et al. 2012) or in populations that have become invasive compared to natural populations of *H. axyridis* (Tayeh et al. 2014). These aspects of the biology of *H. axyridis* have recently been reviewed (Rondoni et al. 2021).

Moreover, this disturbance can be complex as it depends on the season and on the habitat. Indeed, one study shows that *H. axyridis* alters the balance of local ladybird species in lime trees but not in pine trees or nettles (Brown and Roy 2018). This impact on species balance is different depending on the species considered: *H. axyridis* can negatively affect the demography of another coleopteran predator (*Coccinella septempunctata*) but not those of a dipteran (*Aphidoletes aphidimyza*) or neuropteran (*Chrysopidae*) predators (Brown 2003). A review of the impact of *H. axyridis* on local populations shows all these nuances (Li et al. 2021).

H. axyridis is not the only macroorganism to cause environmental problems. Releases of mass-reared individuals of *Macrolophus pygmaeus* led to “hybridization” between the released and native individuals (Streito et al. 2017). The “hybridization” term is maybe too strong as individuals are of the same species thus genetic mixing between farmed and “wild” individuals should not be a problem unless the farmed individuals carry genetic traits that weaken the population (reduced fecundity, susceptibility to disease, etc.).

Parasitoids

Parasitoids can interact with each other, particularly in the case of superparasitism when a host is parasitized by several individuals of the same or different species. Thus, in the Hawaiian archipelago, the joint use of two Hymenopteran parasitoids, *Fopius arisanus* and *Diachasmimorpha tryoni*, against *Ceratitis capitata* had an effect that was difficult to predict a priori. Indeed, as *F. arisanus* parasitizes the eggs and *D. tryoni* the larvae, it turned out that the larvae of *F. arisanus* having developed before those of *D. tryoni* were able to kill the latter. *F. arisanus* had thus supplanted *D. tryoni* in parasitism of *C. capitata*. *D. tryoni* had changed host and had started to parasitize two non-target insects (*Eutreta xanthochaeta* and *Procecidochares utlis*) which were themselves introduced for crop protection (Wang and Messing 2003). Similarly, *Trissolcus basalis* and *Trichopoda pilipes* parasitoids, which were introduced to control *Nezara viridula* (green bug), attacked *Coleotichus blackburniae*, an endemic non-target species. Host switching of these parasitoids were demonstrated to depend on the climate (altitude variation) and on the density of *C. blackburniae* populations (Johnson et al. 2005). In Europe, Ferracini et al. (2015) showed that the parasitoid *Torymus sinensis* (Hymenoptera), used against the chestnut sawfly *Dryocosmus kuriphilus*, had a broader ecological host range than previously reported, and that it was attracted by non-target hosts other than *D.*

kuriphilus. It has also been observed that the release of parasitoids can lead to hybridization phenomena between neighbouring species, for example *T. sinensis* and *T. beneficus* (Yara 2014).

Interactions between predators and parasitoids

Interactions between predators and parasitoids can be neutral, positive or negative. If negative interference exists, it can be monodirectional (the predator influences the parasitoid or vice versa) or bidirectional. An example of the latter is the pairing of the parasitoid *Leptomastix dactylopii* and the predator *Cryptolaemus montrouzieri* used against the citrus mealybug *Planococcus citri*. The predator will consume parasitized mealybugs as long as they are consumable (after a certain period of parasitism, the mummy hardens). The parasitoid will be less active on the mealybug if the predator is present (Chong and Oetting 2007). These bidirectional interactions are staggered in time. A one-way interaction involves the predator *H. axyridis* and the parasitoid *Tamarixia radiata*. Traces of semiochemicals from the predator on the surface of a leaf alter the host-seeking behaviour of the parasitoid (Nakashima et al. 2004; Shrestha and Stelinski 2019). Conversely, the predator *Nesidiocoris tenuis* will become cannibalistic or herbivore and neglect its prey (*Tuta absoluta*) if the parasitoid *Trichogramma brassicae* is present (Mirhosseini et al. 2019). A final example demonstrates the absence of negative interaction (as long as the prey is present): in the woolly apple aphid (*Eriosoma lanigerum*)/parasitoid (*Aphelinus mali*)/predator (forficula, hoverfly, ladybirds, spiders) system, the concomitant presence of both types of biocontrol agents always led to an increase in aphid control efficacy compared to observations made with each agent alone (Gontijo et al. 2015).

The wealth and diversity of the literature confirm the great complexity of the ways in which macroorganisms interact with each other or with organisms already present in the environment. These interactions can be direct (predation, parasitism, hybridization) or indirect (competition for resources), sometimes linked to unexpected phenomena such as changes in hosts or prey.

Among the outputs of the EU ERBIC (Evaluating Environmental Risks of Biological Control Introductions into Europe) project, which lasted four years from 1998 to 2002, two publications proposed a scheme for organizing experiments (mainly in the laboratory) to determine the potential ecological risks associated with predators or parasitoids, and a procedure for assessing the environmental risk of such releases in the field (van Lenteren et al. 2003; van Lenteren et al. 2006). For example, *Hippodamia convergens*, *H. axyridis* and *T. brassicae* species have been labelled with high risk indices.

A literature review of macroorganism release campaigns and their impacts is worth quoting here (Louda et al. 2003). The findings highlight some of the problems associated with the use of macroorganisms: (1) species phylogenetically related to the pest are most likely to be attacked; (2) host-specificity testing defines physiological host range, but not ecological range; (3) prediction of ecological consequences requires population data; (4) level of impact varied, often in relation to environmental conditions; (5) information on magnitude of non-target impact is sparse; (6) attack on rare native species can accelerate their decline; (7) non-target effects can be indirect; (8) macroorganisms disperse from agroecosystems; (9) whole assemblages of species can be perturbed; and (10) no evidence on adaptation is available in these cases.

Impacts of microorganisms on biodiversity

Insecticides and bactericides

As mentioned above, *B. thuringiensis* was shown to be persistent over long-period of time in various environments causing, on the one hand, the appearance of resistances to *B. thuringiensis* (Tilquin et al. 2008) and, on the other hand, ecotoxicological impacts on non-target organisms. Indeed, *B. thuringiensis* var. *kurstaki* was demonstrated to affect both soil bacterial and fungal communities, as well as arbuscular mycorrhizal colonization of plant roots (Ferreira et al. 2003). In addition, the ingestion of *B. thuringiensis* var. *kurstaki* by insect larvae (Drosophilidae) led to the slowdown in their development (Babin et al. 2020). Larval mortality was observed at the highest dose applied (annual application dose x1000). Further analyses showed that the slowdown of the development of insect larvae in response to the ingestion of *B. thuringiensis* var. *kurstaki* was due to changes in gut physiology, to the volume of food intake, to the composition of the gut microbiota, and to the quality of the diet (Nawrot-Esposito et al. 2020). In larval amphibians, *B. thuringiensis* var. *kurstaki* did not induce mortality at agricultural application doses but only at the highest application dose tested (application dose x650) (Weeks and Parris 2020). Furthermore, *B. thuringiensis* var. *kurstaki* had no effect on soil arthropods (Beck et al. 2004) and on spiders (Bajwa and Aliniazee 2001). *B. thuringiensis* Cry1Ah did not affect the survival, longevity, pollen consumption, and physiology of honeybee workers (*Apis mellifera* and *Apis cerana*) (Dai et al. 2012). Harwood et al. (2006) studied the transfer of Cry1Ab-*B. thuringiensis* endotoxin along the maize-slug-carabid food chain: they showed that, despite the uptake of *B. thuringiensis* endotoxins by the slug *Deroceras laeve*, no *B. thuringiensis* endotoxin was detected in the carabid beetles *Scarites subterraneus*. *B. thuringiensis* can also modify food webs by reducing arthropod food resources for birds. This has been documented for house martins (*Delichon urbicum*) in the French region of Camargue, where treatments to control mosquito populations with *B. thuringiensis* var. *israelensis* reduced the number of prey for these birds. As a result, the average number of offspring per nest fell from 3.2 to 2.3 (Poulin et al. 2010). A similar observation was made for *B. thuringiensis* var. *kurstaki* applied to control the gypsy moth, an important prey of the vermivorous warbler (*Helmitheros vermivorus*): the reduction in the number of preys decreased the number of young fledged per nest (Awkerman et al. 2011).

Until now, there are only a very limited number of papers evaluating the effect of microbial active ingredients with antimicrobial properties on indigenous microbial communities or on living organisms in the environment. The behaviour of the bacteriophagous nematode *Cephalobus brevicauda*, which is attracted to Gram-negative bacteria, was not affected by different biocontrol products containing *B. thuringiensis*, *B. pumilis* or *B. subtilis* as active ingredient (Salinas et al. 2007). The survival of *B. amyloliquefaciens* strains inoculated to suppress *Ralstonia solanacearum* in the rhizosphere of tomato plants showed that their abundance remained high over a period of five weeks (>10^7 cfu/g soil) allowing the control of *R. solanacearum* compared to the non-inoculated control, and that they were able to develop inside the plant and promote its growth (Tan et al. 2013). The effect of *B. amyloliquefaciens* strain ZM9 on the suppression of tobacco wilt-causing *R. solanacearum* and on the rhizosphere microbial community of this plant was evaluated by a 16S rRNA amplicon sequencing approach (Wu et al. 2016). In samples treated with ZM9, the abundance of OTUs (Operational Taxonomic Units) affiliated with *R. solanacearum* was lower than in untreated samples. The tobacco rhizosphere microbial community dominated by OTUs affiliated to proteobacteria, acidobacteria, bacteroidetes, gematimonadetes, and actinobacteria was affected by treatment with *B. amyloliquefaciens* strain ZM9 in the early stages of tobacco development but the composition of the rhizosphere bacterial community was resilient by the end of tobacco cultivation. In the early stages, three groups of OTUs, affiliated to *Sphingosinicella*, *Gemmatimonas* and *Gp1*, negatively correlated to the abundance of *R. solanacarum,* were identified in samples treated with *B. amyloliquefaciens* strain ZM9, which also showed a higher abundance of OTUs affiliated to bacterial genera known for their PGPR properties (Wu et al. 2016).

Unfortunately, most of these studies focused on the impact caused by the microorganisms on indigenous soil microbiota without evaluating the impact on ecological functions supported by soil microbiota, and on other soil meso- and macro-fauna. Additional efforts are required to monitor the ecotoxicological impact of active ingredients of microbial origin on in-soil living organisms.

Fungicides

Thanks to their ability to produce a wide range of molecules (phytohormones, antibiotics, hydrolytic enzymes, plant elicitors, etc.), microorganisms can affect microbial communities and plant growth. Thus, the effects of microorganisms on the physico-chemical properties of the soil, and a modification of the functions of microbial communities have been demonstrated. This is notably the case of *C. rosea*, applied at high doses, which modulates bacterial populations by favoring proteobacteria, firmicutes and actinobacteria, and by reducing acidobacteria, without reducing protists (Fournier et al. 2020). Ravnskov et al. (2006) reported that *C. rosea* increased overall bacterial biomass (especially Gram-positive bacteria) but limited protozoa, suggesting that these populations were either sensitive to toxins produced by *C. rosea*. The fungus *Phlebiopsis gigantea* reduces the bacterial richness of early decaying spruce strains with, as expected for other microorganisms, an attenuation of the negative effects on microbial diversity over time (Sun et al. 2013). As the assessments of the impacts on biodiversity may not reveal significant impacts on ecological functions, detailed analyses of microbial communities are needed to sensitively assess the impact of pest management practices on the soil ecosystem (Fournier et al. 2020; Rillig et al. 2019).

An assessment of the effects of *T. atroviride* on soil microbial communities revealed that, while microbial diversity was slightly altered in short term (3 days), in longer term (9 months) the fungal and bacterial communities were identical to those observed in uninoculated soils (Cordier and Alabouvette 2009). Similar findings were found in vineyards in Italy: *T. atroviride* had no major long-term impact, and thus environmental conditions had more effect than the fungus (Savazzini et al. 2009). In contrast, a study with *T. harzianum* strain T-22 indicated that it altered the communities of microorganisms in the rhizosphere of carrot by increasing the population size of rhizobacteria, including *Bacillus* species and *Pseudomonas* species, and reducing the size of the fungal population in the rhizosphere (Patkowska et al. 2020). For *B. amyloliquefaciens*, a transient or negligible effect on rhizosphere or soil microbial populations has been shown (Kröber et al. 2014). If changes in microbial community structure were sometimes observed (in crops grown in hydroponics in controlled conditions in greenhouse), the initial structure of microbial community was restored after 40 days (Wan et al. 2018). However, *B. amyloliquefaciens* can induce a decrease in fungal abundance and diversity, enhance soil urease, catalase and phosphatase activities, and decrease cellulase activity (Tian et al. 2018) with an increase in bacterial/fungus ratios (Chen et al. 2018b). Further greenhouse studies with *B. subtilis* showed no significant effect on the rhizosphere microbiota in sandy and loamy soils, but some effect in clay soil (Li et al. 2016). Moreover, some Bacillus devoted up to 8% of their genetic material to the synthesis of antimicrobial compounds (lytic enzymes, antibiotics, lipopeptides, polyketides), capable of triggering plant defense mechanisms (Cawoy et al. 2014; Cawoy et al. 2015; Chen et al. 2009). Few studies describe the effects of Bacillus on microbial diversity. Evidence of changes in the bacterial microflora after the introduction of *Pseudomonas* or the oomycete *P. oligandrum* led to the same conclusions: transient changes and no sustainable impact on the bacterial communities (Schreiter et al. 2018; Vallance et al.

2012). Although the introduction of microorganisms used as biocontrol active ingredient can affect microbial and fungal communities, this tends to be more or less transient with a return to the normal balance over time.

Regarding the effects of fungicide microorganisms on macroorganisms and beneficial organisms, the literature generally showed limited or no effect. For example, *B. amyloliquefaciens* had no effect on earthworms (Lagerlöf et al. 2015). But, on the contrary, *B. thuringiensis* can have negative effects on caterpillars of some butterfly species: the density of *Gelechia ribesella* and *Euhyponomeutoides gracilariella* were reduced by 60% and 23%, respectively, in the leaf-feeding guild on sprayed *Ribes cereum* plants compared to control plants (Boulton et al. 2002).

Many microorganisms can induce induced systemic resistance (ISR) in plants and indirectly have plant-mediated fungicidal action (Ownley et al. 2010) by producing various redox enzymes and *PR* proteins (Duke et al. 2017). They can also produce volatile (COVs) anti-pathogenic or plant-acting compounds, but it is not known how these COVs can impact non-target organisms (Asari et al. 2016). Like all PPPs, microorganism based-biocontrol solutions should be investigated specifically. For example, in 2016, EFSA published a scientific opinion on the risks for human health of *B. cereus* and *B. thuringiensis* in food products (EFSA Panel Biological Hazards BIOHAZ et al. 2016). They reported that the indirect effects of microorganisms (and macroorganisms) are difficult to assess, and until now remains poorly considered, because of the complexity of connections between species and the ecological community.

Finally, the increasing use of microorganisms as biocontrol solutions raises the question of the risks of microbial invasions in agriculture after mass use with potential effects on soils and ecosystem services (parasitism, promoting invasive plants, suppressive soil) (Jack et al. 2021).

Impacts of natural substances on biodiversity

Insecticides, acaricides

Abamectin was found to have significant toxicity on coccinellids (James 2003), and on several predators (*O. insidiosus*, *A. swirskii*) and parasitoids (*Eretmocerus eremicus*) (Gradish et al. 2011). It also has reproductive effects on earthworms and enchytreids (Bai and Ogbourne 2016; Diao et al. 2007; EFSA et al. 2020a; Jensen et al. 2007; Kolar et al. 2008; Lumaret et al. 2012), and a high toxicity to predatory mites (Fountain and Medd 2015). On the contrary, abamectin appeared to have low toxicity on terrestrial vertebrates, but effects are observed on pollinators and aquatic organisms (EFSA et al. 2020a).

The situation is qualitatively similar for spinosad, which is highly toxic to parasitoids, having many sublethal effects such as inability to develop into the adult stage and build a cocoon, and decrease in the reproductive abilities, offspring size and ability to forage for hosts (D'Avila et al. 2018; Williams et al. 2003). Spinosad is also toxic to *H. axyridis* (Galvan et al. 2006), *Drosophila* (Martelli et al. 2022), and *Daphnia* (Duchet et al. 2010). It has lethal effects on larvae and adults of wild social bees of the Melipona group (Botina et al. 2020). dos Santos Araújo et al. (2023) demonstrated that the ingestion of spinosad decreased survival and food consumption of *A. mellifera*, and that exposure of the bees to spinosad LC50 (Lethal Concentration for 50% of exposed organisms) reduced flight capacity, respiration rate, and superoxide dismutase activity. Spinosad causes a decrease in predatory activity of forficula (*Forficula auricularia*) (Malagnoux et al. 2015), alters their physiology and behavior, and reduces larval growth (Fountain and Harris 2015). It also induces a reduction in the abundance of some predatory ants (Pereira et al. 2010), and of many spider families in apple orchards (Marliac et al. 2016) but not in cabbage crops (Liu et al. 2013). Results have further shown that predatory mites can develop resistance due to prolonged exposure to spinosad (Duso et al. 2014; Fountain and Medd 2015). Transient effects on microorganisms and on some soil enzyme activities have been observed (Telesinski et al. 2015). Finally, spinosad can cause indirect effects on food webs resulting in reduced food resources (often representing 50% reduction in invertebrate abundances) for insectivorous terrestrial vertebrates (Poulin et al. 2010; Poulin and Lefebvre 2018).

Pyrethrins have low toxicity to terrestrial vertebrates, however, effects are observed on aquatic organisms, bees and earthworms (EFSA 2013d). They have no effect on thrips (Nikolova et al. 2015), but they cause a decrease in the abundance of many spider families (Marliac et al. 2016). Regarding aquatic vertebrates, exposure of bullfrogs (*Lithobates catesbeianus*) to pyrethrins resulted in an increase in leukocytes with the conventional formulation, but an increase in erythrocyte numbers and impaired cell division with the nanoscale formulation (Oliveira et al. 2019).

Paraffin oil has little effect on earthworms, but the effects depend on soil type (EFSA 2009; Erlacher et al. 2013). It has no effect on the abundance of many spider families (Bajwa and Aliniazee 2001) but it does cause a decrease in ladybug densities (Karagounis et al. 2006). Paraffin oil has moderate toxicity to fish and aquatic invertebrates (European Commission 2009a). Minor effects on soil microorganisms have been observed after application of paraffin oil (Bundy et al. 2004; Engelen et al. 1998).

Aluminium silicate is used as insecticide, but also as repellent. A study carried out on *Bombus terrestris* did not show any direct lethal effect of aluminium silicate, but this substance can induce a loss of water and thus reduce the survival of bumblebees at 28°C (Karise et al. 2016a). The use of biocontrol solution containing aluminium silicate and fungi (*C. rosea* or *B. bassiana*) led to an increase in cuticular water loss of *B. terrestris*, to a reduction in their survival, and to their mortality due to the presence of entomopathogenic spores of *B. bassiana* (Karise et al. 2016b). Aluminium silicate had no effect on ladybugs (Karagounis et al. 2006), but a decrease in community abundance and species richness, and a change in the structure of the communities of bugs, beetles, spiders has been observed (Marko et al. 2010).

According to EFSA (2013f), a high risk to bees cannot be excluded following the use of maltodextrin but, in general, the substance has a low ecotoxicity. For orange oil, a lack of data was evidenced, and in particular to characterize the risk to birds and mammals, including secondary poisoning, and the risk to aquatic organisms, including the chronic risk assessment and the potential for bioaccumulation (EFSA 2013g). Rapeseed oil has no critical of concern in ecotoxicology (low risk was identified for birds, mammals, earthworms, non-target terrestrial plants, soil microorganisms), but EFSA et al. (2022a) concluded of high risk for bees, non-target arthropods other than bees, and soil macroorganisms other than earthworms. Diatomaceous earth presents a low risk to birds, wild mammals, aquatic organisms, bees, non-target arthropods other than bees, earthworms, soil organisms, non-target terrestrial plants, and sewage treatment organisms (EFSA et al. 2020b). Some results showed that the ecotoxicity of terpenoid blend to non-target soil macroorganisms was low, but data are lacking to characterize the effects of this blend on aquatic organisms, bees, and non-target arthropods (EFSA 2014c).

Fungicides

Potassium hydrogen carbonate is a common substance in soils, it has low ecotoxicity (EFSA et al. 2021c). In a two-season field experiment, the efficacy and phytotoxicity of this substance were evaluated for the control of apple scab. Potassium hydrogen carbonate significantly reduced apple scab severity on the leaves and fruits of the three tested apple cultivars, and it did not affect the summer density of the beneficial phytophagous mite predator *Typhlodromus pyri* (Jamar et al. 2008).

Potassium or disodium phosphonates release phosphorous acid that can accumulate in different plant organs (fruits, buds) (Malusa and Tosi 2005). However, at the environmental concentration, an Australian study showed no effect of phosphonate treatments on vegetation structure, and the functionality of impacted areas would be maintained (Barrett and Rathbone 2018). On the contrary, Lambers et al. (2013) described an impact on biodiversity for plants adapted to phosphorus-poor soils and calls for finding alternatives to phosphonates. According to EFSA (EFSA 2012d; 2013c), potassium phosphonates have low ecotoxicity, while disodium phosphonate is moderately toxic to most environmental organisms (birds, earthworms, fish, aquatic invertebrates, sediment dwelling organisms), except for bees and mammals for which it has low ecotoxicity. Thus, phosphonates seem to be a controversial topic.

Overall, sulfur has low ecotoxicity (Carcamo et al. 1998; EFSA 2008). It is not toxic to earthworms (Carcamo et al. 1998; EFSA 2008), it stimulates soil enzyme activities (dehydrogenase, arylsulfatase; Ram et al. 2017), and carabid beetles were reported to be unaffected by sulfur (Carcamo et al. 1998). In addition, Jamar et al. (2008) demonstrated that wettable sulfur and lime sulfur had no effect on the summer density of *T. pyri*. On the contrary, sulfur impacts enchytreids (Ohtonen et al. 1992), it affects microorganisms as it induces a decrease in soil pH (Czerwonka et al. 2017), and it has a significant effect on the abundance of mycophagous beetles (Sutherland et al. 2010). Despite sulfur is used as a fungicide, it has a side effect that can be considered as an insecticidal action. Indeed, sulfur dust causes a decrease in the number of eggs and consequently in the numbers of *Lobesia botrana*, a lepidopteran pest of grapevine, but it has no effect on a predatory mite (Tacoli et al. 2020).

For eugenol, EFSA (2012e) concluded as a low risk for earthworms, honeybees, non-target arthropods, soil microorganisms, and terrestrial non-target plant. However, there were data gaps to consider the short-term and long-term risks to insectivorous birds, and the risk to aquatic organisms. For geraniol, risk assessment for birds, mammals and aquatic organisms could not be finalized (EFSA 2012f) while for thymol, a high risk was identified for aquatic organisms (EFSA 2012g). Data were missing to characterize the risk for birds and mammals.

Finally, no data were available to assess the ecotoxicity of clove oil (EFSA 2012h). It is reported that some essential oils which are not authorized in France (*Melaleuca* or *Artemisia*) have a toxic effect on aquatic invertebrates (*Daphnia*) and on unicellular green algae. It should be remembered that a substance such as rotenone, which was withdrawn in EU (European Commission 2008), is toxic to mammals, fish, and insects (Chaudhari et al. 2021).

Herbicides

Fatty acids are not free of toxicity even at doses considered as sublethal (EFSA et al. 2021b; Techer et al. 2015), and risks were identified for aquatic organisms, more specifically for aquatic invertebrates (capric, caprylic and pelargonic acids) (Table SI1). Toxicity to other organisms (earthworms, birds) was found to be very low but experiments are required to assess the ecotoxicological risk of these fatty acids towards aquatic organisms (EFSA et al. 2021b). Regarding acetic acid, a high risk was identified for mammals, honeybees, non-target arthropods, and aquatic organisms (EFSA 2013h). On the contrary, the risk to soil-dwelling organisms was found to be low (EFSA 2013h). Data gaps were identified for birds, non-target plants, and mammals for acute toxicity (EFSA

2013h). Iron sulfate, as for it, is moderately toxic to mammals, birds, fish and aquatic invertebrates, and has low toxicity to earthworms and bees (EFSA 2012c). Other fatty acids are being studied (for example: *Cuphea* species oils; Tisserat et al. 2012) to find new herbicide solutions with less negative impacts on the environment. Research into essential oils with herbicidal activity is currently underway, but has not yet produced convincing results due to the selectivity and toxicity of these molecules. The development of such herbicides would be an immediate alternative, provided that the environmental safety of these new molecules can be demonstrated.

Other uses

The number of data for natural substances used as molluscicides, nematicides, plant growth regulators, plant elicitors, repellents, and protection against frost damage (Table SI1) is low. Phosphonates (plant elicitor) and aluminium silicate (repellent) are also used as fungicides and insecticides, respectively, and has been discussed above.

Ferric phosphate is the only natural substance approved for molluscicide use. Overall, it has low toxicity to mammals and bees but, in contrast, it has some ecotoxicity to aquatic organisms (EFSA 2015). Contradictory results were observed for earthworms: EFSA (2015) and Langan and Shaw (2006) demonstrated that ferric phosphate was toxic while Edwards et al. (2009) observed no effect. Some studies showed that microorganisms were not able to solubilize phosphorus when it is in the form of ferric phosphate (Matos et al. 2017; Spagnoletti et al. 2017).

Only one substance is approved for nematicide use: garlic extract (Table SI1). In general, this substance has a low ecotoxicity but data are missing to assess its effects on aquatic organisms, bees, and non-target arthropods (EFSA et al. 2020c).

Looking at plant growth regulators (Table SI1), the data published at the regulatory level in the renewal assessment reports showed an overall low ecotoxicity of indolbutyric acid, 6-benzyladenine, gibberellic acid, and gibberellins but there were nevertheless some data gaps (such as for aquatic macrophytes) (EFSA 2010a; 2010b; 2012i; 2012j).

The cerevisane (EFSA 2014a) plant elicitor is of no concern for ecotoxicology. COS-OGA (EFSA 2014b) was also demonstrated to have low ecotoxicity but there is a data gap for aquatic organisms. Laminarin, which is a natural polysaccharide, has low toxicity to earthworms (EFSA et al. 2017), and has no effect on the activity of chitinase, which plays an important role in soil carbon and nitrogen cycles (Ueno and Miyashita 2000).

Regarding repellents, sheep fat (EFSA et al. 2022b), fish oil (EFSA et al. 2022c) and pepper (EFSA 2011), no critical ecotoxicology issues were identified. Blood meal is also used as a food additive and fertilizer thus, at the regulatory level, no data were provided to characterize its effects (EFSA et al. 2020d). Bonilla et al. (2012) and Cayuela et al. (2009) showed that blood meal stimulates microbial activity and has no effect on soil enzyme activities. The quartz sand repellent is largely composed of the mineral quartz, the major constituent of which is silicon dioxide. In the area of ecotoxicology, a low risk to all non-target organisms was concluded based on the low exposure in the environment and relevant food items for non-target organisms (EFSA et al. 2022d).

Heptamaloxyloglucan which is used against frost damage is a natural component of dicotyledone plant walls which is present in different food commodities of plant origin, among them apple juice, and dietary supplement. This substance has a low ecotoxicity (EFSA et al. 2022e).

Impacts of semiochemicals on biodiversity

Insect attractants are described for predators (Hesler 2016) as well as for pests for trapping or detection purposes (Toth et al. 2012; Royer 2019).

The literature highlights auxiliaries, such as the predator *O. laevigatus*, can induce the emission of kairomones/allomones by plants. *O. laevigatus* can have an occasional phytophagous behaviour leading to the plant emission of odors having a repulsive action for *Bemisia tabaci* and for *Franklinellia occidentalis*, and an attractive action for the parasitoid *Encarsia formosa* (Bouagga et al. 2018). The role of kairomones/allomones emitted by plants has been confirmed with *Arabidopsis thaliana* which produces an alarm pheromone for *M. persicae* resulting in aphid dispersal and attraction of the parasitoid *Diaeretiella rapae* (Beale et al. 2006).

The pheromones are long-chain hydrocarbons which disperse freely in the environment and thus reach many organisms. At the same time, similar molecules such as (Z)-5-tetradecen-1-ol may play a similar role in mice. Indeed, this molecule is present in the urine of males and attracts females (Gomez-Diaz et al. 2013). It is possible, given the similarities of the chemical structures, that there may be interference between the detection systems of these molecules among vertebrates and invertebrates. To the best of our knowledge, this area of research has not been explored.

Impacts of insect traps on biodiversity

Deltamethrin is the sole conventional insecticide authorized for biocontrol in France, and only in insect traps (DGAL 2022). Consequently, non-target organisms should not be exposed to deltamethrin. However, in the event of accidental exposure (destruction of the trap, consumption by predators of contaminated insects, poor layout of the trap allowing deltamethrin to be released or to be accessible to organisms not normally exposed, etc.), this insecticide may have ecotoxicological effects at local scale such as decrease in the abundance of terrestrial invertebrates (spiders, carabid beetles, staphylins, lacewings, ladybirds, ants, parasitoids, natural enemies) (Khans and Alhewairini 2019; Macfadyen and Zalucki 2012; Rodriguez et al. 2003) or aquatic invertebrates (McKnight et al. 2015). Small terrestrial vertebrates can also be affected (Ansari et al. 2008; Brander et al. 2016; Peveling et al. 2003) as well as fish (Brander et al. 2016).

Comparison of the impacts of biocontrol solutions with those of conventional PPPs

Very few studies compare the impacts of biocontrol solutions with those of conventional PPPs. Such a comparison is especially difficult to make with microorganisms or macroorganisms since treatments are very different in nature from treatments with conventional PPPs. Indeed, micro and macroorganisms can multiply upon their release in the crops, and their mode of action can be extremely complex. Moreover, their behavior in the environment is different in terms of spatial distribution or dispersion. Thus, comparing the effects of the action of living organisms and synthetic or natural molecules requires the consideration of very different approaches (characterization of their dispersion in the environment, their persistence and their ecotoxicity at different scales ranging from the individual to the communities) and the mobilization of scientific concepts from ecology (such as coalescence concept) and from evolution (such as intra- and interspecific competition) to understand the fate and impact of biocontrol organisms. While this has been analyzed for conventional PPPs, few biocontrol agents can boast such comprehensive analyses.

Some results were found comparing the ecotoxicity of natural substances and microorganisms to that of conventional PPPs but there was no result for macroorganisms, neither for semiochemicals.

Comparaison of the impacts of microorganisms with those of conventional PPPs having the same usages

In general, the literature shows that microorganisms have a lower ecotoxicological effect than conventional PPPs.

Regarding the fungicides, *B. amyloliquefaciens* has a lower effect on the rhizosphere microbial communities than the association of thiram and carbendazim conventional fungicides: phenotypic indices of culturable heterotrophic bacterial colonies obtained from the soybean rhizosphere were similar in the untreated control and following treatment with *B. amyloliquefaciens* while they decreased by 18% and increased by 23%, respectively, after fungicides application (Correa et al. 2009). Compared to the control (intensity of mycorrhization of 20.8%, abundance of mycorrhization of 5.2%), the reduction in the intensity and abundance of structures involved in the mycorrhizal symbiosis developed between soybean roots and arbuscular mycorrhizal fungi was lower in *B. amyloliquefaciens*-treated plants (14.5% and 1.9%) than in the fungicides-treated ones (8.5% and 0.97%) (Correa et al. 2009). On chrysanthemum crop, *B. subtilis* increased significantly soil urease (+17% on average) and acid phosphatase activities (+44.5%) compared to the control but had no effect on catalase activity, while dazomet decreased catalase (-48%) and urease (-22%) activities, but had no effect on acid phosphatase activity (Chen et al. 2018b). The effect caused by *B. subtilis* strain Tpb55 isolated from tobacco phyllosphera to control *Phytophthora parasitica* var. *nicotianae* on the rhizosphere bacterial community was compared with that caused by a mixture of two fungicides, metalaxyl and mancozeb (You et al. 2016). In response to treatment with *B. subtilis* strain Tpb55 or with the fungicide mixture, the abundance of the two dominant phyla (acidobacteria and proteobacteria) was altered compared to the untreated control, with a decrease in acidobacteria for the fungicide mixture (-7%) and an increase in proteobacteria (alpha and gamma proteobacteria) for both treatments (+11.5 and +12%, respectively).

Treatments with *B. subtilis*, *Burkholderia ambifaria*, *Trichoderma virens* or *T. harzianum* tended to favor *Pseudomonas* and *Trichoderma* communities in contrast to the conventional fungicide treatment consisting of a mixture of thiophanate-methyl, mancozeb and cymoxanil (Larkin 2016). The microorganisms had little effect on bacterial communities (as do conventional fungicides), and their effect on fungi was variable. These treatments also generally resulted in increased microbial activity (e.g., 12% increase following treatment with *B. subtilis*) and substrate use, unlike the conventional PPP mixture (6% decrease in microbial activity) (Larkin 2016). Similarly, *T. harzianum* and *P. oligandrum* have less marked effects on population density and community structure of soil oribatid mites than conventional fungicides (metalaxyl-M + copper or mancozeb) (Al-Assiuty et al. 2014). Recently, Fournier et al. (2020) evaluated the impact on microbial populations of two conventional fungicides, fosetyl-aluminium and propamocarb hydrochloride, and of a *C. rosea*-based biofungicide. The fungicides (conventional fungicide and biofungicide) had no effect on soil bacterial, fungal, and protist diversity. However, both types of fungicides decreased the complexity of the soil microbial network. In addition, they had contrasting impacts on the composition of microbial communities, and on the identity of key taxa: *C. rosea* impacted keystone taxa which structured the soil microbial network while the conventional fungicides modified biotic interactions favouring taxa which are less efficient at degrading organic compounds.

For insecticides, Duso et al. (2008) showed that exposure to *B. Bassiana* led to a low mortality rate of *Tetranychus urticae* (two-spotted spider mite) (33%), a major pest of agricultural systems, compared to imidacloprid (77%) and pymetrozine (66%) insecticides, but increased that of *P. persimilis* (43% for *B. Bassiana*, 34% for imidacloprid, and 35% for pymetrozine), a predatory mite that specializes on the *Tetranychus* species. *B. Bassiana* was found to strongly decrease egg hatchings (only 3.7% of egg hatching) of *T. urticae* compared to the two conventional insecticides (75% for imidacloprid, 98% for pymetrozine), while they all had no effect on those of *P. persimilis*. *B. thuringiensis* has a lower toxicity (decrease in mortality, increase in offspring production and reproductive capacity) for *O. laevigatus* than the metaflumizone conventional insecticide, and lower or similar toxicity than indoxacarb (Biondi et al. 2012).

Comparaison of the impacts of natural substances with those of conventional PPPs having the same usages

Abamectin and spinosad were found to be more toxic to the *O. laevigatus* predator than conventional insecticides (Biondi et al. 2012): mortality was 75% and 98% after exposure to spinosad and abamectin, respectively, while it was lower than 44% for metaflumizone and indoxacarb. Spinosad is 19 and 37 times more toxic (based on LC50) to a parasitoid (*Aphidius colemani*) than imidacloprid or lambda-cyhalothrin, respectively (D'Avila et al. 2018). It is also more toxic (reduction in the Ca^{2+} response mediated by Dα6 protein, negative effect on larval survival, vision loss, widespread brain vacuolation) to *Drosophila* than imidacloprid (Martelli et al. 2022). Conversely, spinosad had less effect than lambda-cyhalothrin on spider abundance and diversity, and the Shannon-Wiener Index and the hierarchical richness index were on average two times higher in the spinosad treated plots than in the lambda-cyhalothrin ones (Liu et al. 2013). Mortality of *H. axyridis* following exposure to spinosad was two (third instars) to ten (adults) times lower than following exposure to indoxacarb (Galvan et al., 2006). Lastly, spinosad was found to be less toxic (considering survival) to all stages of *H. axyridis* (eggs, first and third instars, pupae, adults) than chlorpyrifos, carbaryl, bifenthrin and lambda-cyhalothrin, and abundances of *H. axyridis* in soybean and sweet corn crops in spinosad treated plots were found to be higher than in conventional insecticides treated ones (Galvan et al. 2005).

Some effects of paraffin oil on soil microorganisms have been observed after application (change in species frequency distribution), however they were minor compared to those of metamitron and, especially to those of dinoterb (Engelen et al. 1998). Paraffin oil was also less toxic than bifenthrin to the lacewing *Chrysoperla rufilabris* (larvae) and to the ladybug *Rhyzobius lophanthae* (adults), with a mortality rate two to ten times lower, but it can lead to slightly higher mortality of both species than pyriproxyfen, spiromesifen, and spirotetramat (Quesada and Sadof 2020). Biondi et al. (2012) demonstrated that paraffin oil decreased the mortality of *O. laevigatus* (mortality rates ranging from 20 to 40%) compared to metaflumizone (40 to 80%) and indoxacarb (30 to 40%), that it tended to increase the offspring production (from 10 to <20 nymphs produced) compared to metaflumizone (< 15) but to decrease it compared to indoxacarb (15 to 17), and that paraffin oil decreased the reproductive capacity of *O. laevigatus* compared to metaflumizone and indoxacarb. Biondi et al. (2012) also showed that rapeseed oil decreased the mortality of *O. laevigatus* (mortality rates ranging from > 25 to 80%) compared to metaflumizone (35 to 80%) but increased the mortality compared to indoxacarb (30 to 40%); on the contrary rapeseed oil increased offspring production (from 10 to 22 nymphs produced) and reproductive capacity compared to metaflumizone (< 15) and indoxacarb (15 to 17).

Finally, pyrethrins were found to lead to higher mortality rate of *T. urticae* and *P. persimilis* (> 90% and 100%, respectively) than imidacloprid (77% and 34 %, respectively) and pymetrozine (66% and 35%, respectively), but hatching of *T. urticae* was higher in the presence of pyrethrins (98%) than in the presence of imidacloprid (75%). There was no difference in hatching of *P. persimilis* after exposure to pyrethrins, imidacloprid or pymetrozine. Oviposition rates of *T. urticae* were two times lower following treatments with pyrethrins than with imidacloprid (Duso et al. 2008).

Overall, biocontrol solutions seem to have lower ecotoxicity than conventional PPPs, but there are some exceptions, and the number of data is low highlighting the need of more research on this topic.

Conclusion

Overall, this review showed that little is known about the contamination of soil, water and air by biocontrol solutions (macroorganisms, microorganisms, natural substances, semiochemicals), their fate in the environment, and their impacts on biodiversity.

However, the existing works report that biocontrol solutions have an impact on the environment and biodiversity, and that there are a wide range of interactions between macroorganisms and their environment. While natural substances and semiochemicals could be considered as an alternative to conventional PPPs (as they are organic or inorganic substances), the application of living biocontrol organisms (macro and microorganisms) to protect crops has different consequences. Indeed, their interactions with the various components of the environment are complex since biocontrol organisms can survive, multiply, colonize, and move in different habitats where they can interact with indigenous organisms. As a result, the ecosystem resilience which can be observed when a conventional PPP is dissipated may not be observed if the biocontrol organism becomes resident in the environment where it was introduced to control a given pest or in another environment following its invasion. While it is legitimate to assess the efficacy of biocontrol, the assessment of the unintented effects and associated risks of biocontrol solutions for the environment is also critical: there are ways of improvement and it may connect to other regulations, including those governing the control of invasive species (European Union 2014).

The comparison of the impacts of biocontrol solutions on biodiversity with those of conventional PPPs demonstrated that microorganisms tend to have lower impacts than PPPs, and that while most of natural substances have low ecotoxicity, others have a toxicity equivalent to or greater than that of the conventional PPPs. However, the number of studies is low, and there is no data for macroorganisms and semiochemicals.

A great deal of research remains to be done to better understand and characterize the processes governing the fate and dispersal of biocontrole solutions in the environment as well as their ecotoxicological effects on environmental health. In addition to research conducted in laboratory, it would be relevant to rely on instrumented study sites and/or long-term monitoring, such as sites associated with the International Long Term Ecological Research (ILTER) network (Vanderbilt et al. 2015) on a global scale or on network such as the Biovigilance 500 ENI (non-intended effects) one (Andrade et al. 2021) at national scale.

The consideration of the unintended effects of biocontrol solutions will help to ensure their sustainability, and as an ultimate goal to avoid replicating the difficulties associated with the near widespread use of conventional PPPs. This could likely have implications for risk definitions and regulations associated with biocontrol.

Supplementary Information The online version contains supplementary material available at…

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