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Can organic agriculture cope without copper for disease control?

Synthesis of the Collective Scientific Assessment Report

Didier Andrivon, Isabelle Savini, eds

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This book summarizes the collective scientific assessment report jointly requested in 2017 by the French Technical Institute for Organic Agriculture (ITAB) and the Sustainable Management of Crop Health (SMaCH) metaprogramme of INRAE. The contents of the full report and this abridged version are the sole responsibility of the authors. The overall report, source of this version, was created by the scientific experts without condition of preliminary approval by the sponsors or INRAE. The abridged version was validated by the authors of the report.

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Contacts:
Lead Scientist: Didier Andrivon – INRAE, Plant Health and Environment Division, IGEPP, Rennes.
didier.andrivon@inrae.fr
Project management, drafting and editorial coordination: Isabelle Savini – INRAE, Delegation for Collective Scientific Assessment, Foresight and Advanced Studies (DEPE).
isabelle.savini@inrae.fr
Publication Director: Bertrand Schmitt – INRAE, DEPE.

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Contents

Introduction

Significant uses of copper, subject to increasing levels of regulation

SINCE THE ADVENT OF BORDEAUX MIXTURE in the late 19th century, copper has been a major element in crop protection methods against a variety of fungal and bacterial diseases of plants, particularly in viticulture, fruit, and vegetable production. Copper is used in a range of "conventional" agricultural systems, in combination with other pesticides, but it plays a critical role in most organic agricultural systems (OA). It is currently the only active ingredient approved for use in OA that has both a strong biocidal effect and a wide spectrum of action.

While most uses of copper are justified by their biological effectiveness, they also generate ecotoxicological problems (proven risks for soil microbial populations, earthworms, some aquatic organisms, and beneficial microbes). The demonstrated environmental impacts of copper have led to regulatory restrictions on its use (e.g. a maximum allowable application rate per hectare per year), and even to its total prohibition as a pesticide in some European countries (such as the Netherlands or Denmark). This situation creates an uneven competitive landscape for organic growers across Europe.

Alternatives to the use of copper: considerable research prompting the need for a critical synthesis

THE INCREASED RESTRICTIONS ON THE AMOUNT of copper growers can apply, together with the looming threat of a total ban at the European level, presents a challenge for organic growers who cannot replace it by other synthetic pesticides. A recurrent demand thus exists for research on "alternatives" to copper. First articulated some twenty years ago, the need for viable alternatives to the use of copper continues to appear on recent lists of agricultural research priorities (for example, within the French framework programme for OA development *Ambition Bio*).

As a result, the question of "alternatives" to copper has been the focus of considerable research and R&D activity, including three major European research programs since the beginning of the 2000s alongside numerous other prominent, but more limited, research efforts in different parts of the world. Countless trials of alternative methods and products have been conducted, both by technical centers and by growers themselves, to evaluate the potential of different molecules and/or formulations. Other research has focused on elucidating the underlying biological mechanisms involved (in particular the elicitation of plant defenses, the ecology of disease organisms and of biocontrol agents, etc.).

While a significant number of scientific and technical references has thus been accumulated, practical adoption of these potential innovations remains limited. Relevant findings are scattered across a variety of sources, are often fragmentary in nature, and are not always readily accessible. No complete critical synthesis of this research has been published to date. Scientists and technicians alike lack access to a consolidated "state of the art" on the topic, one which offers a scientific evaluation of the efficacy and limitations of the various possible alternatives to copper. Such a review could assist in identifying research priorities and developing recommendations for the practical implementation of these alternatives.

Organization and intent of a Collective Scientific Expertise

Within this context, and in response to a series of meetings with relevant stakeholders, INRA's Internal Committee on Organic Agriculture (CIAB) suggested that a critical analysis of the full range of available and validated information on the subject should be undertaken. This suggestion was taken up by the French Technical Institute for Organic Agriculture (*Institut Technique de l'Agriculture Biologique* - ITAB) and by INRA's metaprogramme "Sustainable Management of Crop Health" (SMaCH), which jointly requested a Collective Scientific Assessment (*Expertise Scientifique Collective* – ESCo) – a multidisciplinary, critical review of all the relevant scientific and technical information – on the topic. This type of exercise is conducted at INRA by its Delegation for Collective Scientific Assessment, Foresight and Advanced Studies (*Délégation à l'Expertise scientifique collective, à la Prospective et aux Etudes* – DEPE), following clearly established rules and procedures (Box I.1). An ESCo consists in the critical analysis of the existing international scientific literature on a topic (with an emphasis on academic research) by group of scientific experts (researchers from public research and higher education institutions). While an ESCo is intended to provide clarification, it does not formulate specific recommendations.

The goal of the ESCo was to produce a summary of *published* information that could be used by stakeholders to guide their decisions with respect to research or R&D efforts seeking to favor the emergence of "zero copper" or "very low copper" disease management strategies, and applicable in organic agricultural systems. Its scope was to include:

- the range of possible technical solutions: treatments based on natural substances with biocidal effects and/or which act to stimulate natural plant defenses; the introduction of microbiological control agents; the use of disease-resistant crop varieties; and the management of crops and crop areas to prevent the spread of disease;
- the insertion of these individual solutions within integrated production/crop protection systems;
- constraints and necessary conditions to the diffusion and adoption of alternative methods.

The ESCo considered *a priori* all approved "uses" (combinations of *crop x pathogen*) for copper-based treatments, placing an emphasis on a small number of "major" uses (in terms of the economic importance of the crops involved). These uses for copper have received the most attention from researchers and are the focus of the largest number of published references.

The analysis focused on the case of OA, which is both the mode of production most dependent on copper and the context addressed by a large number of the available references. Nevertheless, the ESCo findings are relevant to all forms of agriculture seeking to reduce the use of synthetic pesticides.

Status and organization of this document

The present document is a synopsis of the full report (in French) produced by the expert group, available on the INRA website. Only a few key bibliographic references are cited here; a complete bibliography is included in the experts' report.

The first chapter provides background information, which is not in itself the focus of the Collective Scientific Expertise. These background data relate to copper (approved uses, regulatory restrictions and the reasons for these restrictions, actual use in agricultural production contexts, etc.) and alternatives to copper (the range of possible techniques, general rules for regulatory approval and authorization, etc.). It specifies the documentary sources available with regard to these alternatives.

The second chapter describes the various technical means available or proposed to control pathogens, either directly (by killing the pathogen or limiting its development) or indirectly (by increasing crop resistance): natural biocidal preparations, microbial biocontrol agents, plant genetic resistance, stimulators of natural plant defenses, homeopathy and isopathy, etc.

The third chapter focuses on agronomic strategies designed to limit plant health risks: prevention measures to reduce sources of contamination (removal of infected plants or crop residues, etc.); physical protection against infection (rain and/or hail protection); and crop or planting management methods (pruning and training of fruit trees, planting of mixed covers, etc.) intended to create conditions unfavorable to the development and spread of disease.

The fourth chapter considers information available at the level of the cropping systems, as well as the impediments that exist with respect to the development and adoption of innovations within these systems.

A concluding chapter summarizes the lessons that may be drawn from this analysis, including the current availability of alternatives to the use of copper, the possibilities for further implementation, and continuing research needs. In addition, it proposes a set of theoretical prototypes for integrated protection systems for the three most important agricultural uses of copper.

Box I.1. The Collective Scientific Assessment (ESCo)

The ESCo is an institutional expertise activity, governed by a national charter for expertise signed by INRA in 2011. It is defined as an activity for the assembly and analysis of scientific knowledge in diverse fields relevant to the clarification of public decision-making. The review and analysis is as complete as possible, but is not intended to provide specific advice, recommendations, or direct answers to the questions faced by public policymakers: its sole objective is to provide a critical review of scientific information, including points of debate and knowledge gaps, to support decision-makers in considering the actions available to them. The analysis is conducted by an interdisciplinary group of expert researchers from a range of institutions. For the ESCo on "Alternatives to Copper," a dozen experts from different research institutions were involved. Their work was based on a literature corpus of nearly 1000 references, primarily scientific articles, and supplemented by technical documents. The exercise concludes with the production of a report (in French) consisting of the individual experts' contributions; a synopsis (in French and in English) intended for use by decision-makers; and a short summary (in French and in English) intended for a more general audience.

1. Background

Copper: properties and uses

Biological properties and toxicological and ecotoxicological profile of copper

Copper is important for all living systems. It is a vital element involved in electron transport and thus in energetic metabolism; it also has antimicrobial properties. The precise mechanisms underlying the biocidal effects of copper on microorganisms have not been fully elucidated yet, although a number of hypotheses have been proposed: loss of electrolytes across the cellular membrane, creation of an oxidative stress, disruption of the ionic balance, blocking of normal protein functioning *via* chelation on active protein sites... A consensus is emerging among researchers that numerous organisms use the regulation of copper homeostasis – from a vital component to a cellular poison – to fight microbial infections. The antimicrobial properties of copper are the basis of a variety of applications for the management of human, animal, and plant health.

Formulations using copper

For its plant health applications, copper is used primarily in its ionic forms, in formulations based on copper salts (copper sulfate or copper hydroxide) combined with various adjuvants. The classical 'Bordeaux mixture' (copper sulfate + lime) is typical of this type of formulation. These products are generally used as sprays on above-ground plant parts; they can also be used for seed treatments (primarily for cereals) or as local applications (wound dressings for tree cuts, drenches to the seeding bed, etc.).

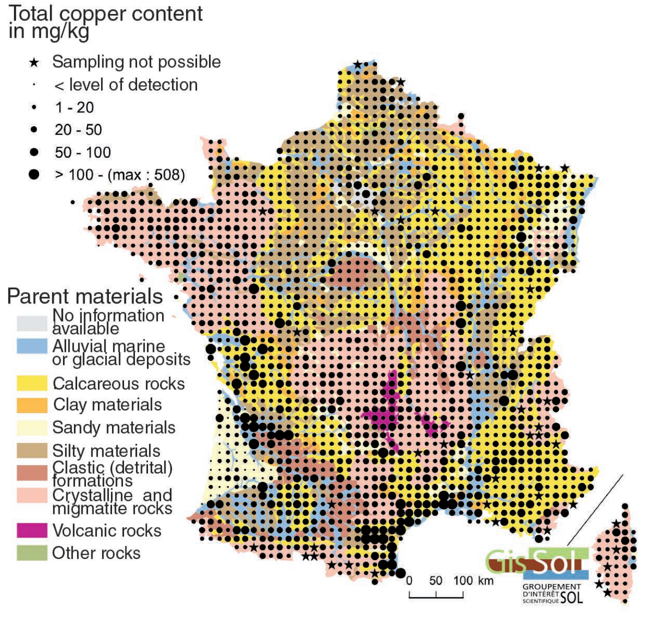
More recently, methods have been developed for the use of copper oxide nanoparticles (nano-CuO and nano-$CuCO_3$) that can be applied or incorporated into various materials (e.g. textiles)These nano-copper materials can act for instance as biocides for the treatment of wood and wood products against fungi and insects responsible for biodegradation.

Copper accumulation in soils

Copper concentrations in natural soils range from 3 to 100 mg/kg, depending on the underlying substrate and the soil type, and from 5 to 30-45 mg/kg in non-contaminated agricultural soils. In the latter, copper levels in the soil solution are generally very low (on the order of 1 to 10 μM, depending on the soil type), with an important fraction of the copper present being retained on clay-humic matrices.

Human activities, and particularly the repeated application of copper-based pesticides, are the main source for copper pollution in agricultural soils. They cause an accumulation, sometimes massive, of this metallic element in topsoil horizons (Figure 1.1). In Europe, the almost uninterrupted use of Bordeaux mixture to control grape downy mildew thus raised very strongly copper concentrations in wineyards, up to 200, and even sometimes 500 mg/kg.

Figure 1.1. Total copper contents in topsoil samples (0-30 cm) of the Soil Quality Monitoring Network.



Source: Gis Sol, RMQS, 2011; INRA, BDGSF, 1998

Phytotoxicity for crops

Excess copper concentrations have known harmful effects on the growth and development of the above-ground and below-ground parts of most plants, resulting in a decrease in total biomass. Some plant families and species, including legumes, grapevines, hops, and cereals, are particularly affected.

Copper toxicity is directly linked to the bioavailability of copper ions. Copper concentrations over 2 µM in nutrient solutions can be toxic for plants. A large part of their toxic effect comes from the inhibition of photosynthesis and the degradation of chloroplasts, resulting in more or less severe chlorosis. By disrupting the plant's oxidative metabolism, excess copper also triggers the plant general defenses, which comes at a metabolic cost.

Copper applications can also have an effect on the physiological composition and thus on the quality of harvested products. For example, they can reduce polyphenol levels and thus the anti-oxidant properties of olive leaves, and they can modify the concentration and balance of aromatic compounds in hop flowers.

Scientific research conducted in the 1990s on different plant species growing on heavily contaminated mining sites found that such accessions could be used to increase the plants' tolerance of excess heavy metals, with potential applications for the bio-remediation of contaminated soils. To our knowledge, however, the capacity to tolerate high soil copper levels has not been a focus of breeding programs in any plant species of agricultural interest.

Ecotoxicity

The deleterious effects of excess copper on soil microbial communities are well established. It is, after all, because of its antimicrobial effects that copper is used in agriculture. Given that fungi and bacteria play a critical role in trophic webs and in the completion of biogeochemical cycles, it is hardly surprising that disruption of soil microbial communities can lead to an impoverishment of locally available resources for other ecosystem consumers.

The toxicity of copper for specific components of the soil fauna, such as the springtail *Folsomia candida*, has also been shown. Impacts on other indicator species, such as earthworms, are less clear. Estimates of lethal copper concentrations for adult worms vary: some studies have found significantly increased mortality rates at concentrations of 150 mg/kg of soil, whereas others have found no effect at these levels. Copper seems to have a low acute toxicity for the earthworm test-species *Eisenia foetida*, with median lethal concentrations (LC_{50}) above 5,500 mg/kg of dry soil in laboratory conditions. At lower levels, chronic toxicity for earthworms is often observed: delayed sexual maturity, reduction in the number of cocoons, reduced hatching rates. Quantities of copper that show no measurable impact on these lifecycle parameters can still have observable effects on worm physiology. It is thus reasonable to assume that copper contamination of soils has long-term chronic effects on earthworm population dynamics and other soil fauna components that are important to the maintenance of soil structure and biogeochemical cycling. Copper applications are also toxic for fungal species used as biocontrol agents (for example, *Beauveria bassiana*, used against pest insects).

Nanoparticles containing copper can also be toxic for the plant-soil system, although it is not clear whether this toxicity is caused by the nanoparticles themselves or by an associated release of copper ions. Effects on plants are similar to those caused by an excess accumulation of copper ions in soil: a dramatic reduction in growth of the exposed plants and a modification of the ionic balance in plant tissues. Effects on soil microbial communities (attributed generally to the release of copper ions) have not been described in detail, but have been shown: reductions in microbial diversity, reductions in soil bacterial communities favorable to plant growth, reductions in iron uptake by both plants and microbes. It would appear that these nanoparticles also have a serious impact on other environmental compartments, particularly wetlands: fish, crustaceans, and algae all appear to be more sensitive than soil bacteria to the toxicity of copper oxide nanoparticles.

Uses of copper for crop protection

Approved uses

Copper is approved for crop protection uses against a variety of diseases due to fungi, bacteria and oomycetes, mainly on grape, fruit crops, and vegetable crops (Table 1.1 and Box 1.1).

Table 1.1. Currently approved uses of copper in France

	Crops	Diseases/pathogens	
		Bacterial diseases	**Fungal diseases**
Fruit trees & grapes	Citrus	*Xanthomonas axonopodis* pv. *citri*, *X. axonopodis* pv. *citrumelo*, *X. citri* subsp. *citri*	
	Trees and shrubs		Various diseases
	Cherries	*Agrobacterium tumefaciens* *Pseudomonas*	*Coryneum* and *Polystigma*
	Shell nuts (walnuts, hazelnuts, almonds)	*Pseudomonas avellanae, P. syringae* pv. *coryli* *Xanthomonas campestris* pv. *juglandis*	
	Kiwi	*Pseudomonas syringae* pv. *actinidiae*	
	Olives	Olive knot (*Pseudomonas savastanoi*)	Olive peacock spot (*Spilocaea oleaginea*), *Fusicoccum*
	Peach (+ apricot)	*Xanthomonas arboricola* pv. *pruni* *Pseudomonas*	Peach leaf curl (*Taphrina deformans*), Peach canker (*Fusicoccum* sp.) *Coryneum* and *Polystigma*
	Apples (+ pears, quince, Asian pear)	*Pseudomonas*	European canker (*Nectria galligena*) Foliar diseases Scab (*Venturia inaequalis*)
	Plum	Bacterial diseases	Scab(s) Leaf curl
	Black currant		Foliage diseases
	Raspberry		Foliage diseases
	Grapes	Crown gall (*Agrobacterium vitis*)	Phomopsis cane and leaf spot (*Phomopsis viticola*) Downy mildew (*Plasmopara viticola*)

Table 1.1. Next

	Crops	Diseases/pathogens	
		Bacterial diseases	Fungal diseases
Arable field crops	Wheat		Fungi other than Pythiaceae [seed application]: Common root rot (*Bipolaris sorokiniana*), Take-all (*Gaeumannomyces graminis*), Fusarium moulds (*Fusarium graminearum*, *F. culmorum*, *Microdochium nivale*)
	Rye		Fungi other than Pythiaceae [seed application]: Fusarium moulds (*Microdochium nivale, Fusarium* sp.)
	Potato		Late blight : *Phytophthora infestans*
Vegetable crops	Artichoke	Bacterial diseases	Downy mildew(s)
	Carrots		Oomycete pathogens (Pythiaceae)
	Celery	Bacterial diseases	
	Chicory - root	Bacterial diseases	
	Chicory - witloof	Bacterial diseases	
	Cabbage crops	*Pseudomonas fluorescens* (broccoli) *Xanthomonas campestris* pv. *campestris*	Downy mildew(s)
	Cucumber (+ pickling cucumbers, summer squash)		Downy mildew
	Strawberry	Bacterial diseases	Brown spot
	Beans	Bacterial diseases	
	Hops		Downy mildew
	Lettuce	Bacterial diseases	Downy mildew (*Bremia lactucae*)
	Melon	*Acidovorax citruli* *Xanthomonas campestris* pv. *cucurbitae*	Downy mildew
	Onion	*Xanthomonas axonopodis* pv. *allii*	Downy mildew
	Leak	*Pseudomonas syringae* pv. *porri*	Downy mildew
	Tomato	*Pseudomonas syringae* *Clavibacter michiganensis* *Pectobacterium* spp., *Dickeya* spp. *Ralstonia solanacearum* many *Xanthomonas*	Late blight (*Phytophthora infestans*)

Table 1.1. Next

	Crops	Diseases/pathogens	
		Bacterial diseases	Fungal diseases
Other uses	Indoor & balcony plants		Various diseases
	Rose		Fungal cankers
	Seed crops		Various diseases
	Seed crops – Beet (sugar and forrage)		Downy mildew
	Seed crops for PAMCP*, ornamental and vegetable crops		Downy mildew, white rust Rusts
	PAMCP*	Bacterial diseases	Fungal diseases (mildews)
	General application		Wound dressing

* PAMCP: perfume, aromatic, medicinal and condiment plants. In square brackets [ ]: applications other than aerial sprays. (sources: Ephy database and ITAB Guide 2017).

• In **perennial crops**, approved uses of copper include fungal and bacterial diseases affecting grapevines, stone fruits, pome fruits, and tree nuts. Copper treatments are also occasionally used against diseases for which they are not approved, including brown rot blossom blight in apricots and black rot in grapes.

• In **vegetable crops**, copper is approved against fungal and bacterial diseases of a dozen or so crops belonging to various botanical families.

• In **arable field crops**, approved uses of copper are limited to combating late blight in potatoes, and a few fungal diseases in wheat and rye that can be transmitted by seed.

• Finally, copper is approved against various fungal diseases affecting perfume, aromatic, and medicinal plants (PAMCP); ornamental species; and seed crops, and for diseases that develop on tree cuts.

Target pathogens

Pathogenic microorganisms targeted by the crop protection uses of copper belong to three major groups. Conditions for disease development and the methods available to fight these diseases depend on the biological characteristics of the different pathogen groups. The three groups are:

• **Fungi**, especially **Ascomycetes**. Ascomycetes are fungi capable of both sexual reproduction (producing perithecia, which overwinter in dead infected leaf material and from which ascospores emerge in the spring, leading to primary infections of new plant material) and asexual reproduction (producing conidia on above-ground plant parts; dissemination of the conidiospores cause secondary infections through the summer and fall);

• **Oomycetes**. Long considered to be related to the fungi, oomycetes have a life cycle somewhat similar to that of ascomycetes but are taxonomically very distinct from the true fungi. They are characterized by non-divided hyphae, a diploid genome, and spores that can be self-motile in water (zoospores);

Box 1.1. Major uses of copper

Some uses of copper, notably in OA, are considered “major” in terms of the land area involved, the economic importance of the crops to be treated, the yield losses occasioned by the target diseases, and/or the quantities of copper applied. Such uses are the focus of the greatest number of research studies and technical trials.

Grapewine downy mildew, caused by the oomycete *Plasmopara viticola*, is one of the two most serious diseases for this crop (the other is powdery mildew). Severely damaging and with a high epidemic potential, especially in areas with an oceanic climate, it requires a highly effective level of protection, in the absence of which harvests can be severely impacted or even entirely lost. Given the high degree of susceptibility of most grapewine varieties, controlling downy mildew with a contact product like copper requires numerous applications (up to 15 or more per year). Vineyards occupy approximately 782,700 ha in France (Agreste 2016).

Apple scab, caused by the ascomycete fungus *Venturia inaequalis*, is a disease of economic importance (scabbed fruit is unmarketable). Apple orchards receive an average of 23 applications of fungicides/bactericides per year (ranging from 15 to 29 depending on the region), nearly three-quarters of which target apple scab (Agreste). Copper can cause russetting on fruits, so the protection of organic apple trees against scab relies on a combination of copper (highly effective), sulfur, and lime sulfur (where permitted). Copper-based treatments are also used to control European canker (caused by Nectria galligena). Apple production for table fruit accounts for about 36,500 hectares in France.

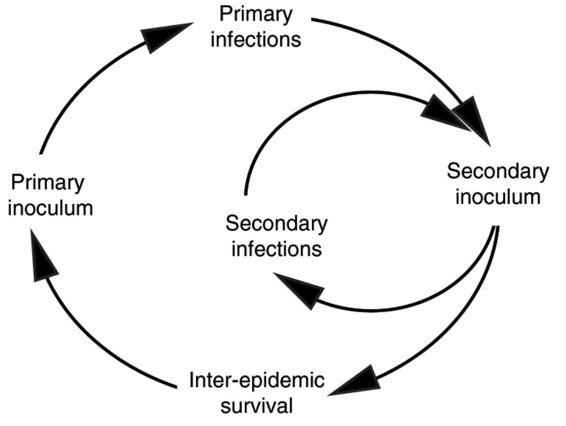
Potato late blight, caused by the oomycete *Phytophthora infestans*, is the most serious disease of potatoes. It manifests with symptoms of spreading necrosis on all plant parts (leaves, stems, and tubers), and can result in yield losses of up to 100 percent. In the case of late infestations, it can cause quality losses due to rotten areas on affected tubers. Potato late blight affects all areas of potato production, but is more regularly severe in oceanic climates. To control late blight, growers make an average of 10 to 12 applications of copper-based fungicides per year, or up to 15 to 20 in areas of high risk for late blight. Potato production accounts for approximately 180,000 ha in France.

P. infestans also causes serious damage to tomatoes (which belong to the same plant family as potatoes), particularly in field production.

- **Bacteria.** Prokaryotic organisms which in most cases rely on asexual reproduction, and which typically penetrate the plant *via* natural openings (stoma, lenticels, wounds) rather than by means of their own specialized structures.

These pathogens all have in common to generate polycyclic infections (Figure 1.2) and to depend upon liquid water (or at least saturating humidity) for the dispersal and germination of fungal spores (*sensu lato*) and bacterial dissemination.

Figure 1.2. Schematic life cycle of polycyclic fungal diseases.



Pesticides can inhibit the growth of non-reproductive tissues (hyphae) and/or the production and germination of spores (from sexual or asexual reproduction). Combating these polycyclical diseases requires beginning treatments as soon as weather conditions (rainfall, temperature) become favorable to primary infection in the spring, and continuing throughout the growing season as long as conditions are favorable to secondary infections. Existing decision-making tools (DMT) are intended primarily to optimize the timing of applications while limiting their total number. Such tools assess infection risks by using models to simulate pathogen development according to meteorological conditions.

Regulatory restrictions on the use of copper

Recognition of the negative environmental effects of copper-based products has led to regulatory restrictions on their use. In the EU, the copper re-homologation procedure of 2018 set the maximum dose of copper-based formulations allowed for crop protection purposes, both in organic and conventional production systems, at 4 kg of metal copper/ha/yr (down from to 6 kg/ha/yr before) as a 7 yr moving average. Rates recommended to producers by agricultural advisory services may be considerably lower than this maximum allowance. Furthermore, some countries have chosen to regulate copper more strictly. In Switzerland, applications are limited to 4 kg Cu/ha/yr for most crops (based on a sliding average over 5 years, with up to 6 kg permitted in the case of intense disease pressure in a given year); for small fruits, the maximum allowed amount is 2 kg/ha/yr; for stone fruits, 1.5 kg/ha/yr. Other countries (the Netherlands, Scandinavia) and some certification associations (Demeter in Germany, for example) have chosen to totally prohibit the use of copper for crop protection purposes, in both OA and in CA. The use of copper as a fertilizer is allowed, however, with no restriction on application amounts. This regulatory loophole may lead to "covert" crop protection uses of copper in some situations.

The use of copper is controversial even within OA. The copper-based products currently used in agriculture are all formulated as synthetic mineral chemistry. Their authorization for use in OA thus seems somewhat contrary to the founding principles of this type of farming, which bans synthetic products. Copper is allowed in OA primarily because the use of copper-based products in agriculture pre-dates the dramatic increase in availability of chemical pesticides in the years following the Second World War (Bordeaux mixture first came into use in the 1880s). The hiatus between the founding principles of OA and the synthetic nature of copper-based products is one of the reasons why some growers and certifying bodies, in particular those that are part of the biodynamic movement, reject its use.

Regulatory differences across different countries, production sectors, and quality standards with respect to the use of copper have led to technical dead-ends in countries that have banned its use, followed by a significant reduction in some types of organic production – for example organic potato production in the Netherlands. It also creates a competitive distortion among growers from different countries, prompting recurrent demands from countries that have banned the crop protection use of copper for an extension of this prohibition to the entire EU. This request has not been granted during the last examination of copper at the EU level (November 2018), but in part prompted the reduction from 6 to 4 kg of the maximum annual application rate per ha.

Quantities of copper currently used in OA

Copper is the only active ingredient with a strong antimicrobial effect and a wide range of action that is approved for use in OA. Finding a replacement for copper in OA is thus much more problematic than it is in CA, which can generally use synthetic pesticides as alternative solutions, at least for fungal diseases.

In OA, three recent surveys – conducted in France by ITAB (Jonis, 2009), again in France by ITAB and IFV (Berthier and Chovelon, 2013), and in Switzerland by FiBL (Speiser *et al.*, 2015) – found that the actual use of copper, while generally below maximum allowed levels, is nevertheless high. In Switzerland, copper use is around 3 kg/ha/yr for potatoes, celery and European grape varieties (which are susceptible to downy mildew); around 2.5 kg/ha/yr for cherries; and 1 kg/ha/yr in apple and pear orchards. These rates are around 60-80% of maximum allowed rates.

In France, the use of copper in organic viticulture is nearly 5 kg/ha/yr on average in years of high downy mildew pressure (around one year out of two in the first decade of the 21st century), with notable differences between regions: 1.6 kgCu/ha/yr in Alsace, 5.6 kg Cu in the Loire Valley, and up to 6 kg Cu or more in Champagne, Midi-Pyrenees, Aquitaine, and Languedoc-Roussillon. Inter-annual variations can also be significant: average consumption in France is thus 3 kg Cu/ha/yr in years of low disease pressure, *vs.* 5 kg Cu in years of high disease pressure. The same study found similar trends in tree fruit production and vegetable production. Values found in the French surveys were generally higher than those found in the study in Switzerland. These differences may be attributable to the greater diversity of production contexts in France, and/or to the fact that the French data are older.

It should be noted that the 2009 survey reported higher copper usage, both in viticulture and in tree fruit production, than that recommended by some recent production guidelines. In apples, for example, current guidelines to control apple scab recommend 7-8 applications of small amounts of copper (100-300 g/ha per application) from bud-break to harvest, for a total application amount 2500 g/ha below the maximum allowed rate. Both the 2009 survey and the 2013 survey for viticulture suggest the existence of technical dead-ends and/or multiple uses of copper, situations that could cause some growers to exceed recommended rates or even maximum allowances for copper use.

Thus, in organic peach production, the recommendation is for approximately 5 kg of metal Cu per ha per year to combat leaf curl, primarily as an early-season treatment; added to this are one to three applications at a rate of 1250 g/ha at leaf fall to control bacterial diseases, for a total use that is above the 6 kg/ha/yr limit. Finally, the surveys only count applications explicitly intended for crop protection, whereas recommendations also frequently include the use of copper as a foliar fertilizer, at low but repeated doses. For example, in-season applications of 100-200 g of copper per application are recommended in organic apricot production to control leaf rust and scab attacks on the fruit, but such applications were not included within the crop protection use totals. It is thus likely that the actual use of copper frequently falls near or above the 6 kg/ha/yr authorized limit for certain key crops such as grapes and fruit trees.

Effectiveness of copper treatments

There are several ways to evaluate treatment effectiveness. One is to measure reductions in the incidence or severity of disease in treated crops vs. non-treated controls. Another is to consider differences in yields between treated and control crops. Most published studies are based on experimental fields or plots and are thus not necessarily representative of commercial production conditions. Nevertheless, yield gains attributable to copper applications have generally been observed. According to one German review, the use of copper reduced average losses by 10-15% in vegetable and ornamental crop production, 15-20% in potato production, and 50-100% in fruit tree production.

While most uses of copper are justified by their biological efficacy, some treatments are made – and are even recommended – despite a lack of evidence as to their effectiveness. This is the case, for example, with brown rot blossom blight in apricot, which is sometimes said to benefit from applications of copper in the spring. However, a review of field trials and observations showed that such applications are ineffective, and may even be detrimental to the control of this disease, while the use of copper products is not approved for this particular indication. Nevertheless, it still is frequently recommended to growers “as a precaution.”

There have been few identified cases of the evolution of pathogen populations in response to the use of copper products. The main example is the appearance of copper-resistant strains of *Xanthomonas* on tomatoes. This finding has stimulated research to identify forms of genetic resistance to this bacterial disease.

Alternatives to copper: types and regulatory framework

Available methods and modes of action

Alternative methods belong to one of three major types, according to their underlying mode of action:

• **Methods that act directly on the pathogen itself**. These include the application of biocidal substances (primarily plant extracts with antimicrobial properties), but also the use of biocontrol organisms that act directly on the pathogen through antagonism, hyperparasitism, or ecological competition;

• **Methods that use the plants own capacity for resistance**, including the breeding of resistant varieties exploiting the genetic resources of the cultivated species or related species; the application of plant defense stimulators; or the use of plant morphology and architecture to escape or limit infections. Plant resistance can indeed be constitutive, or it can be induced by infection or other external stimuli;

• **Methods that use agronomic practices to fight primary infection (prevention) or secondary infections (avoidance)**. Prevention methods include the management of potentially infected crop material (gathering, shredding, or burying of litter; selection of disease-free seeds and plants; elimination of volunteers or refuse piles close to fields). Avoidance methods include covering crops to avoid contamination by airborne or splash-borne spores; reducing the time during which above-ground plant parts are exposed to moisture, to limit spore germination and infection; and minimizing damage to foliage and other plant parts, which can create entry points for pathogens.

"Biocontrol" includes methods with either direct or indirect effects on plant pathogens, but making use exclusively of "natural" products or substances (in other words, excluding synthetic mineral or organic compounds. It also includes the use of signaling molecules (attractant or repellent pheromones, plant odors, etc.), although these are not relevant to the control of diseases targeted by the use of copper. It is important to note that not all biocontrol methods are approved for use in OA; conversely, some methods allowed in OA (such as the use of copper) are logically excluded from the field of biocontrol.

Steps required for regulatory approval and commercialization

The market introduction of substances, formulated products, and plant varieties is subject to a series of regulations intended to ensure for the user the nature and quality of what he or she purchases, for the consumer the safety of the products, and for the company the protection of its intellectual property rights. In France, regulatory requirements are mostly the same as those in effect at the EU level, but the national framework is in some cases more strict than the rules adopted by the European Union.

Approval of biocidal substances and biocontrol organisms

The approval process for crop protection products (Box 1.2) applies to natural biocides, plant defense stimulators, and biological control organisms as well as to the standard synthetic pesticides.

EC Regulation no. 1107/2009 on the market introduction of crop protection products defines what is meant by an active ingredient (Art. 22). It also defines what are referred to as basic substances (Article 23) and low-risk crop protection products (Article 47), two categories of materials eligible for a simplified approval process.

Basic substances correspond to substances that have not been created for use as crop protection products but that may have value as such (for example, food by-products), and which have no known negative impacts on human or environmental health. These substances may be used to strengthen crop health, but are distinct from other categories of crop protection products.

In France, regulations refer to the concept of "*préparations naturelles peu préoccupantes*" (PNPP), or "natural preparations causing little concern" – which does not exist in European legislation. The 2014 *Loi d'avenir pour l'agriculture, l'alimentation et la forêt* (Law on the Future of Agriculture, Food, and Forests) defines PNPP (Article 50) as consisting exclusively of basic substances (as defined by EC Regulation no. 1107/2009) and natural substances with biostimulant effects. The effects of the latter are not considered as crop protection or pesticide effects (any claim other than for a biostimulant effect is prohibited). The simplified approval process for basic substances and PNPP is intended to accelerate market entry for this type of product, but it does have some unintended consequences, with some product developers allowing a certain ambiguity between the declared use and the actual intended use of their products. The typical case is that of biocontrol products that are marketed as "biostimulants", in order to avoid a more lengthy and expensive approval process, although their primary intended use is clearly plant protection.

The simplified approval process for '*substances considered to be of little concern*' stipulates that the product has no known harmful effects on human health (assumed for food-based products; the question is examined for other products), animal health, or environmental health. The substance must be of plant, animal, or mineral origin (except in the case of microorganisms), and obtained by a process that is accessible to all end users. Applications are reviewed in France by ANSES (Box 1.2).

As of late 2017, 18 basic substances have received EU approval (one of which, oxygenated water, is prohibited for use in OA). A majority of the requests submitted to date for plant-based preparations have been rejected, with the data submitted being judged insufficient by EFSA. The preparations that have been approved are made from nettles, horsetail, and willow bark. The list of "biostimulants" (an imprecisely defined category) approved in France is currently limited to medicinal plants that may be freely sold (Act of 27 April 2016).

Box 1.2. The approval process for crop protection products

The process applies to all crop protection products, and consists of two stages:

- At the European level, an approval stage for the **active ingredient**. The request is considered by the European Food Safety Authority (EFSA), according to three criteria: the physicochemical properties of the substance, its risk profile for human health (toxicology and residues), and its risk profile for the environment (ecotoxicology and persistence and behavior in the environment). When approved, the active ingredient is listed on Annex 1 of EU Rule 540/2011. The EU Pesticide Database (https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en) lists all authorized substances.
- At the national level, market authorization stage for the formulated **commercial product** (active ingredient + adjuvants). In addition to the information submitted for evaluation at the European level, the application dossier includes data on the product effectiveness and its proposed uses (crop, application rates). In France, AMM (market authorizations, autorisations de mise sur le marché) are awarded by ANSES (Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du travail). The list of products approved for use in France is available on the ANSES web site (https://ephy.anses.fr/).

For commercial products approved for sale in Europe, the efficacy of the product for the advertised use (as listed in the authorization application) must be demonstrated. This is not always the case outside the EU: in some countries, notably in North America, a demonstration of product effectiveness is not required for regulatory approval.

Products intended for **biocontrol** are inventoried on a separate list following approval. As mentioned above, listing of a product on the biocontrol list does not necessarily mean it will be allowed for use in OA: all new approved products receiving market authorization are subject to additional scrutiny with regard to OA certification requirements (Annex II of Rule 889/2008). The only notable exception to this rule is for macroorganisms used for biological control, which are not required to seek a market authorization.

Plant cultivar registration

Before seeds can be approved for sale and planting, new varieties must be listed on the Official Catalogue of Plant Species and Varieties (overseen in France by the CTPS, the *Comité Technique Permanent de la Sélection*). Prior to listing, the variety must satisfy a series of criteria referred to as DUS, for **Distinctiveness** (relative to existing listed varieties), **Uniformity** (among individuals within the variety), and **Stability** (of the variety's characteristics over time). For major field crop species, new varieties must also meet criteria for VCU (Value for Cultivation and Use; in French, *Valeur Agronomique, Technologique* *et Environnementale* - VATE), designed to assess the level of genetic progress the new variety represents compared to already listed ones.

To encourage the development of varieties suitable for OA, and for low-input agriculture more generally, VATE tests include an assessment of the variety's tolerance/resistance to major diseases when grown without pesticide protection.

For local or heirloom varieties, which are typically less uniform in their characteristics, requirements for listing on the Official Catalogue are given more flexibility. For major field crops and for garden crops, varieties may be designated as "conservation varieties" (those that are adapted to local conditions and at risk of genetic erosion); for garden crops, varieties may be designated as "with no intrinsic value" for commercial crop production. Both statuses can benefit from a derogation intended to facilitate their official listing and hence continued use and reproduction while also providing a framework for commercial sales.

Each EU member state is able to set its own VCU criteria and protocols for registering plant varieties onto its own national List. Any variety registered on the list of any EU member state accesses the EU catalogue, and is then eligible for trade over the whole EU territory.

Assessing the effectiveness of alternatives to copper

For substances or products that are still in the research or trial phase and have yet to receive regulatory approval, the scientific literature showed a wide range of assessment methods: tests performed *in vitro* (application of the experimental product to a pathogen grown in the laboratory), trials conducted on plants grown in pots and/or in greenhouses (following artificial infection by the pathogen), and field trials (usually at experimental stations, occasionally on farms).

In the case of copper, the efficacy of a control method can be measured in terms of its effects on: i) the frequency or severity of symptoms or other damage to leaves and/or harvested plant material; ii) yield losses caused by the pathogen; or iii) the quantity of pathogen propagules present in the environment.

Assessments typically compare the efficacy of two or more protection regimes, in OA and/or in CA. The efficacy of an alternative product or practice may be compared to the efficacy of standard copper-based treatments or to the efficacy of a reference synthetic fungicide (for example, mancozeb, which is used on many crops). Many trials also evaluate the effectiveness of the alternative method in association with a reduced application of copper. An alternative method may be judged to be of value even if it does not by itself allow for a sufficient level of protection for commercial production. Distinguishing between situations of low, moderate, or high disease pressure (different years, relative susceptibility of the cultivated variety) for a given pathogen can help identify methods that are sufficiently effective under certain conditions.

Research on alternatives to copper

Regulatory and other pressures to reduce or eliminate the crop protection uses of copper – and to reduce the use of synthetic pesticides more generally – have stimulated considerable research, including a wide variety of different approaches. This work has included, for example:

- since the beginning of the 2000s, three European research programs focused on the development and adoption of alternatives to copper (Box 1.3);
- more basic research on the relevant underlying biological mechanisms, particularly on natural plant defenses against pathogens;
- in genetics, systematic research on genes and QTL for resistance in major crop species (grapewine, apples, etc.). Such research has been facilitated by the development of methods for molecular genome analysis;
- more technical studies to test or improve the efficacy of different formulations, particularly for plant-based formulations used in OA;
- research and R&D work conducted by private companies, although such work is typically regarded as proprietary and is only minimally publicly available, if at all.

Box 1.3. Three major European research programs on alternatives to copper

- **Blight Mop** (Development of a systems approach for the Management of potato late blight [caused by Phytophthora infestans] in EU Organic Potato production in the absence of copper-based fungicides; 2000-2005), focused exclusively on potato late blight. This project (in common with the other two described below) sought to evaluate innovative techniques for disease control (PDS, agronomic practices, resistant varieties, etc.) and their integration within overall crop production systems. Trials were conducted both at experiment stations and on cooperating farms.
- **RepCo** (Replacement of copper fungicide in organic production of grapevine and apple in Europe; 2005-2009). This project involved research on grape and apple production in six countries, testing a large number of alternative products (fungicides, elicitors, antagonists, biostimulants) as well as reduced rates of copper. This project included the most complete study made to date on alternatives to copper for grapes (more than 100 substances tested on plants in pots; Dagostin et al., 2011).
- **Co-Free** (Innovative strategies for copper-free low input and organic farming systems; 2012-2017). This project addressed fruit crops, grapes, potatoes, and tomatoes, and sought to develop integrated strategies, but was obliged to devote a significant part of its work to the assessment of individual new products (plant extracts, microorganisms utilizable for biological control) that had not yet been sufficiently evaluated (lack of available references prior to the start of the project). This project was only recently completed and its results have not yet been published in full.

Establishment and characteristics of the bibliographic corpus

The bibliographic corpus for this ESCo was assembled based on a search of the international bibliographic database Web of Science (WoS), and focusing on recent publications (issued after 2000). The search request was constructed by combining two search equations: one listing different control methods (generic techniques, specific substances, etc.), the other listing the target pathogens (those for which the use of copper-based products is allowed).

The references obtained by this process were divided among the experts according to their areas of expertise. Each expert then refined and strengthened its literature corpus: removing the inevitable "noise," performing additional search requests on specific topics, supplementing based on his or her own bibliographic resources, including both broader studies (e.g., on the modes of action of specific substances) and more technical material (articles published in industry and technical journals, experimental reports, etc.). For non-academic (not peer-reviewed) references, it was left to the individual expert to retain or reject an article based on its quality and value (completeness of information as to experimental conditions, etc.). Additional on-line databases were consulted as needed: e.g., to confirm approved uses of copper in different contexts, or to verify materials allowed or prohibited in OA.

The total corpus examined by the ESCo was made up of approximately 1000 references, the large majority of which appeared in peer-reviewed scientific journals after the year 2000 (see Annex). Bibliographic corpuses can vary considerably in size depending on the topic. Results pertaining to the most important alternatives to copper were subjected to a systematic analysis (presented in table form in the full scientific report).

2. Alternative methods to the use of copper

Natural biocidal preparations

A CORPUS OF 466 REFERENCES ABOUT NATURAL BIOCIDAL SUBSTANCES/FORMULATIONS with potential as alternatives to copper was assembled and examined For the (many) substances still under development, the diversity of in vitro experimental protocols and the differences in observed activity upon transfer from controlled conditions to the field made it impossible to evaluate the actual biocidal potential of these natural products. The analysis thus focused on substances that had been the subject of field and/or greenhouse trials, and on products already commercially available. The analysis gave priority to 60 or so peer-reviewed journal articles, which were the most informative. A publication from one of the European research programs (RepCo; Dagostin et al., 2011) was particularly valuable, since it allowed the comparison of products evaluated under uniform experimental conditions (on plants in pots).

Origins and modes of action

The active ingredients and formulations of organic biocides come from plant extracts or from microbial metabolites. They consist either of extracts containing a mixture of compounds, or of purified molecules. The modes of antimicrobial action for these formulations are rarely described, but most of these biologically active substances either inhibit the growth of colonies or hyphae or prevent the formation or germination of propagules (spores, bacterial cells, etc.). The physiological and molecular modes of action involved, notably for the antifungal compounds, remain in many cases poorly understood: only a few compounds that act against strains pathogenic to humans have been studied in detail.

Natural "biocidal" formulations approved for use in France and in Europe

Among the 18 (as of the end of 2017) substances or preparations recognized at the EU level as **basic substances**, and listed as such in Annex 1 of EC Regulation no. 1107/2009, 11 claim to have a fungicidal activity, and 7 of these against pathogens targeted by copper (Table 2.1). This list is regularly expanded as applications are reviewed; many of the substances for which applications are made are plant extracts. While food-based basic substances are all allowed for use in OA; others are considered on a case-by-case basis.

Table 2.1. Natural "biocidal" preparations approved for use in France and in Europe as of 2017

Substance	Approval Category*; date	Use (crop / pathogen)	Commercial products
Basic Substances		Uses **	
Calcium hydroxide (slaked lime)	F ; 07/2015	Apples / canker (*Nectria*)	
Sodium bicarbonate	F ; 12/2015	Apples / scab	
Horsetail (*Equisetum arvense*)	F ; 07/2014	Apples / scab Peaches / leaf curl Grapewine / downy mildew	
Willow bark extract (*Salix* spp.)	F ; 07/2015	Apples / scab Peaches / leaf curl Grapewine / downy mildew	
Nettle (*Urtica* spp.)	F ; 2017	Grapewine / downy mildew Potatoes / late blight	
Vinegar (acetic acid)	B, F ; 07/2015	Wheat (seed treatment) / Fungi other than Pythiaceae	
Lecithins	F ; 07/2015	Peach / leaf curl Grapewine / downy mildew	
Active ingredients/ substances		Approved uses	
Sulfur		Apples / scab	numerous products
Potassium bicarbonate		Apples / scab	Armicarb, K-BLOC
Formulations based on essential citrus oils		Grapewine / downy mildew Lettuce / downy mildew	Essen'Ciel, Limocide, Prev-Am

* B = bactericide ; F = fungicide
** according to the "Use sheets" available at http://www.itab.asso.fr/activites/pp-dossiers-sb.php

Calcium and potassium salts. Potassium bicarbonate, two formulations of which are approved for use in OA, significantly reduces apple scab and grape downy mildew. Effectiveness is higher when the application is made near to the time of infection, and is also improved if the potassium bicarbonate is mixed with mineral oils. Activity against apple scab has also been shown for calcium hydroxide (in orchards) and sodium bicarbonate (in greenhouses). Against peach leaf curl, calcium hydroxide was shown to have comparable (or even superior) efficacy to copper in a trial conducted in orchards in 2017.

Sulfur. This mineral is primarily used against powdery mildews, but is also approved for use against apple scab, a disease targeted by copper. Sulfur and lime-sulfur are biocides that complement the use of copper for the protection of organic apples against scab, since the former can be used in orchards during periods of fruit susceptibility to russetting (which is caused by copper).

Basic substances of plant or animal origin. Horsetail, lecithin, willow bark, vinegar, and nettle are approved in Europe as basic substances with fungicidal properties. Among these, only horsetail, nettle, and willowbark have been the focus of publications describing their crop protection effects, particularly against grapewine downy mildew.

To our knowledge, only hydro-alcoholic preparations of horsetail show activity in cases of weak downy mildew pressure, in combination with reduced applications of copper. The effectiveness of macerated nettle preparations, or nettle "teas," to control a variety of plant diseases is promoted by many popular gardening publications and websites. In France, the Act of 18 April 2011 allowed the marketing of nettle teas, notably for use as a foliar or soil-level spray to control downy mildews; this Act was updated to include a list of other plants that may be used to produce "teas" for crop protection/the promotion of plant health. For regulatory purposes, nettle was first approved as a basic substance at the European level in 2017.

Since 1990, only one trial, conducted in southern France (Gard) on downy mildew and black rot in grapevine, demonstrated an "average effectiveness of teas (horsetail and nettle) under conditions of normal downy mildew pressure," suggesting a potential value of plant "teas" in combination with reduced rates of copper. None of the numerous other trials conducted on a variety of disease systems found a difference relative to non-treated controls; this was true, for example, in the Dagostin *et al.* study on downy mildew on grapewines grown in pots.

Preparations using essential citrus oils. Three commercial formulations made from essential citrus oils, primarily sweet orange oil, are approved for use in OA against downy mildews in grapewine and lettuce. These products also show activity *in vitro* against *Alternaria* in carrots, and inhibit the germination of scab conidia. The company selling these products markets them both for viticulture and for tree fruit production.

Natural biocidal preparations with significant potential

Here we review natural preparations that have shown significant antimicrobial properties against crop diseases under crop production conditions, but which have yet to receive approval for use in France or Europe. Data are summarized in Table 2.2.

Essential oils

Essential oils (EO) are natural plant extracts containing volatile compounds, typically extracted by steam distillation or by pressing. Several EO are marketed in the United States as fungicides for use in OA. Few scientific articles have sought to identify the active compounds or modes of action for these products. Most publications report on performance evaluations: over 140 papers have demonstrated antifungal properties for essential oils, particularly for post-harvest treatment against storage diseases (an application that lies outside the scope of this ESCo).

Table 2.2. Extracts or natural products showing antimicrobial activity, but not currently approved in Europe for crop protection purposes

	Product	Active compound(s)	Tested uses (crop/target)	Commercial product
Essential oils (EO)	EO of thyme (*Thymus vulgaris*)	thymol (90%) and carvacrol (8%)	Apple / scab (and fire blight) Grapewine / downy mildew	PromaxTM
	EO of summer savory (*Satureja hortensis*)	carvacrol (99%)	Apple / scab (and fire blight)	
	EO of oregano (*Origanum acutidens*)	carvacrol and thymol		
	EO of clove (*Syzygium aromaticum*)	eugenol	Tomato / bacterial wilt Peanut / 11 fungi	
	Mixture of EO of rosemary (18%), clove (15%) and thyme (5%)			Sporatec
	EO of tea tree (*Melaleuca alternifolia*)	terpine-4-ol		BM-608
	EO of dawn redwood (*Metasequoia glyptostroboides*)		Melon / Xanthomonas (greenhouse conditions)	
Plant extracts or plant metabolites	Yucca extract	saponins	Grapewine / downy mildew Sorghum / soilborne diseases; seed treatment	
	Five-seeded plume poppy extract (*Macleaya cordata*)	sanguinarine and chelerythrine (alkaloids)	Citrus / Anthracnose, powdery mildew Cucumber / downy mildew	Kimura SC
	Garlic extract (*Allium sativum*)	allicin	Tomato / Alternaria solani Carrots, sorghum / A. alternata Grapewine / downy mildew	
	Apple of Sodom (*Calotropis procera*): leaf extract			
	purified latex extract	CpOsm protein		
	Extract of Sakhalin knotweed (*Reynoutria sachalinensis*)	physcion and emodin (PDS action)	Greenhouse and ornamental plants / downy mildew	Milsana™ (Syngenta) in the approvals process in Europe; Regalia™ sold in the USA
	Sage extract (*Salvia officinalis*)	derivatives of luteolin and rosmarinic acid (phenols)	in vitro / Alternaria solani Grapewine / downy mildew	

Table 2.2. Next

	Product	Active compound(s)	Tested uses (crop/target)	Commercial product
Plant extracts or plant metabolites	Black poplar extract (*Populus nigra*)	populin (polyphenol)	Apples / scab	
	Licorice extract (*Glycyrrhiza glabra*)	flavonoids	Cucumber / downy mildew Grapewine / downy mildew	
	Extract of chinaberry tree (*Melia azedarach*)		Grapewine / downy mildew	
	Vegetable oil		Grapewine / downy mildew	Natur´l óleo® (oil sold in Brazil)
Microbial metabolites	Antibiotics produced by actinomycetes (*Streptomyces* spp.)	streptomycine and validamycine		Numerous commercial products worldwide. **Prohibited in Europe**
	Bacterial lipopeptides	surfactins, iturins and fengycins	Tomato / Alternaria alternata Grapewine / downy mildew Lettuce / downy mildew	

The effectiveness of essential plant oils against bacterial diseases is a key question, given the lack of other means of combating these crop diseases. A review of essential oils active against *Xanthomonas* (extracted from some 30 different plant species) reported promising results under greenhouse conditions.

Essential oils containing thymol and carvacrol. The EO of **thyme** (*Thymus vulgaris*) is reported to have a significant effect, in combination with Bordeaux mixture, against *Erwinia amylovora*, under both laboratory and orchard conditions. *In vitro*, it shows a capacity to reduce germination of conidia and ascospores of *Venturia inaequalis*. A formulation of thyme oil is recommendation for soil fumigation against a wide range of soil-dwelling pathogens. A thyme oil-based product was tested by Dagostin *et al*. against grapevine downy mildew under controlled conditions, without satisfactory results. The EO of **summer savory** (*Satureja hortensis*) is active *in vitro* against scab and fire blight. The EOs of thyme and summer savory both also show broad-spectrum activity against post-harvest fungi. Likewise, the EO of **oregano** *(Origanum acutidens)* has shown activity against more than 17 species of plant-pathogenic fungi and oomycetes, including *Alternaria alternata, A. solani, Botrytis sp.*, several *Fusarium* species*, Monilinia sp., Pythium ultimum, Phytophthora capsici, Rhizoctonia solani, Sclerotinia minor* and *Verticillium dahliae.*

The essential oil of clove (*Syzygium aromaticum*) has shown promise against bacterial wilt in tomatoes (*Ralstonia solanacearum*), both *in vitro* and under production conditions.

The oil and its major compound, eugenol, have also shown significant activity *in vitro* against eleven species of fungi pathogenic to peanuts. Eugenol shows broad-spectrum activity against strains of *Alternaria*; it is also reported to active *in vitro* against other species affecting apple storage. In vineyards, a commercial formulation of essential oils of clove, rosemary, and thyme has shown some effectiveness against downy mildew under low levels of disease pressure.

The essential oil of tea tree (*Melaleuca alternifolia*), rich in terpenes, is believed to disrupt the integrity of bacterial and fungal membranes. One study found some effectiveness in the field against grapewine downy mildew under low levels of disease pressure. Tea tree EO has also shown potentially significant activity (inhibition of hyphae growth *in vitro*) protecting seeds against seven species of pathogenic fungi (*Ascochyta rabiei, Colletotrichum lindemuthianum, Fusarium graminearum, F. culmorum, Drechslera avenae, Alternaria radicina* and *A. dauci*).

Plant extracts and metabolites

Yucca extract. Yucca extract is among the most effective treatments in Dagostin *et al.*'s study: a preparation based on saponins derived from yucca extract is listed as promising, with over 95% activity in some field trials against grapewine downy mildew. Yucca extract used as a seed treatment for sorghum allowed for a significant reduction in some soil-borne diseases. It is also reported to be active against apple scab, by inhibiting conidia germination and/or via a PDS effect.

Five-seeded plume poppy extract. Extracts from the roots of *Macleya cordata* (a plant used in Chinese medicine) contain alkaloids with fungicidal and insecticidal activity. Although the mode of action has not been determined, an extract of *M. cordata* is sold as a fungicide, and some commercial products based on this plant are known to induce a systemic acquired resistance. These extracts contain numerous alkaloids, including sanguinarine. Its efficacy in greenhouse conditions is comparable to the effects of synthetic fungicides, acting against various microbial plant pathogens. Extracts of *M. cordata* reduce the release and survival of oomycete zoospores *in vitro*, and inhibit the growth of *P. infestans* mycelium. The extract reduces infection levels by cucumber downy mildew by 90% at very low concentrations. Sanguinarine also shows a significant antifungal effect against *Rhizoctonia solani*. Five-seeded plume poppy extract is not considered to be toxic for non-target species.

Garlic extracts. For garlic juices evaluated as seed treatments, the protective factor varies considerably depending on the formulation. One trial, using an aqueous leaf extract, produced a significant reduction in *Alternaria solani* on tomatoes, with reductions in symptom severity of >71% in greenhouse conditions and 57% in field trials, resulting in a 66% increase in fruit yields in the field. An aqueous extract of garlic cloves inhibited the growth of *A. alternata* and *A. dauci* on carrot seeds. Vegetable oil enhanced with garlic extract showed a significant reduction in the severity downy mildew symptoms on grapewines. An extract of zimmu (*Allium cepa* x *A. sativum*) tested in the field against

Alternaria alternata on sorghum resulted in a 74% reduction in fungal growth and a significant increase in yields. Essential oil of garlic is reported to be effective *in vitro* against bacterial walnut blight (*Xanthomonas arboricola* pv. *juglandis*).

Apple of Sodom extract. Leaf extracts from this shrub, a native of the arid parts of Africa and the Middle East, have shown an efficacy against *Alternaria solani* on field-grown tomatoes that may be equivalent to mancozeb in terms of reductions in disease severity and increases in fruit yields. A protein (osmotin) purified from the latex shows antifungal activity (inhibition of spore germination and hyphae growth) against *Fusarium solani*, *Neurospora* sp. and *Colletotrichum gloeosporioides*. This protein is relatively heat stable and preserves its activity at a wide range of pH levels, making it a good candidate for product development.

Extracts rich in polyphenols. An extract of **Sakhalin knotweed**, already on the market in the United States, is in the approvals process in Europe (application submitted by Syngenta in 2012) against a wide range of fungal and bacterial diseases, in both organic and conventional agriculture. It is reported to be particularly effective against powdery mildew, and is primarily used on greenhouse and ornamental plants. It appears to act indirectly, by inducing plant defenses. Many published studies have reported the antifungal activity of **common sage** extracts, which strongly inhibit the growth *in vitro* of *Alternaria solani*. Trials on grapewines of a sage extract product developed by JKI/Safecrop have shown promising results against downy mildew: its activity was equivalent to that of copper in protecting grape clusters, both under controlled conditions and in the field (results were reported for two years). **Black poplar** extracts show a promising level of effectiveness against apple scab, both *in vitro* and in orchard conditions, with effects in the orchard being equivalent to conventional fungicides (mancozeb, copper hydroxyde, penconazole), with a lower treatment cost. These findings need to be verified, but suggest a promising avenue for future research. **Licorice** extracts showed antifungal activity against downy mildew in cucumber, grape, and greenhouse lettuce.

Several papers have reported on the potential of lignans, found in extracts of *Myristica fragrans*, against diseases of tomato and of rice, both *in vitro* and *in vivo*, and of berberine, found in extracts of *Coptis chinensi*, against *Monilia fructicola*.

Extract of chinaberry tree. On grapewine downy mildew, extracts of the fruits and seeds of chinaberry tree inhibit the germination of sporangia *in vitro*, and have been shown to be as effective as Bordeaux mixture in the field. However, some fruit extracts also show a broad-spectrum insecticidal effect; impact studies on crop pollinators and other auxiliary species are thus needed prior to any potential regulatory approval.

Fatty acids and vegetable oil. Field trials conducted over two years found that vegetable oil used alone, or in combination with Bordeaux mixture, enabled a >66% reduction in downy mildew symptoms on grapewines, suggesting a promising future for these substances in grapewine production.

Microbial metabolites

Antibiotics produced by actinomycetes. A relatively large number of products have been commercialized, mainly using *Streptomyces* spp. These are used intensively as fungicides in Japan, and to a lesser extent in other countries. Streptomycin, used against several bacterial and fungal diseases in plants, and validamycin, used against *Rhizoctonia*, are sold under a variety of commercial names. As antibiotics (with pharmaceutical uses), these compounds **are not allowed for crop protection use in Europe**. In the United States they are prohibited in OA, with the exception of streptomycin, which is allowed for use against fire blight on apple and pear trees.

Bacterial lipopeptides. The potential of natural-origin biosurfactants for use as crop protection antimicrobials has been widely studied. Among these are lipopeptides produced by some strains of *Bacillus subtilis* and *Pseudomonas* sp., the biocidal activities of which have been repeatedly demonstrated. These strains of *Bacillus* produce three families of biocidal lipopeptides (surfactins, iturins, and fengycins), which act to favor the beneficial elements of the microbiota around plant roots and foliar surfaces, inhibit the hyphal growth of pathogenic fungi, and/or induce systemic resistance. These substances are thought to be responsible for demonstrated effects against *Alternaria* on tomato, against downy mildew in grapewine and lettuce, and for wide spectrum of antagonistic activity against *Botrytis cinera, Rhizoctonia solani, Pythium aphanidermatum* and *Podosphaera*. These compounds exhibit weak toxicity (microtox and daphnia tests) compared to conventional products.

Some conclusions

Few specialized products are currently approved and effective

Among the substances currently approved for use in Europe and in organic agriculture, some, such as potassium biocarbonate, could help reduce the use copper. The basic substances that have been approved (horsetail and nettle extracts, vinegar, sodium bicarbonate, etc.) are readily available, but their efficacy against the diseases targeted by copper is limited to non-existent.

Many promising future avenues for research

Many **essential oils** have been tested *in vitro*, but few have been the focus of field trials. Essential oils rich in thymol and carvacrol show strong activity *in vitro* against a wide range of plant pathogens, and some studies have shown their potential in the field. The same is true for essential oils of clove and tea tree.

Among **plant extracts**, sage extract and licorice extract show promise against grapewine downy mildew, while yucca extract and black poplar extract show potential against apple scab.

The use of **bacterial metabolites** as surfactants, combining antifungal activity with the stimulation of plant defenses, also deserves further study.

Finally, many biocidal substances have proven effective under crop production conditions, but their ecotoxicological and toxicological effects must be tested before they can be authorized for use as active ingredients or as basic substances. Some of these substances are recognized as antibiotics (based on their nature and on their activity with respect to certain human pathogens), and will thus not be approved for use in crop protection, still less in OA.

Some key impediments to innovation persist

Product development and market introduction of materials based on the biocidal active substances described here will be determined in part by questions of intellectual property protection and the affiliations of the research groups involved. Substances that can be classified as basic substances have a greater likelihood of becoming available to farmers and gardeners, both professionals and hobbyists.

Microbiological agents for biocontrol

Biological control has been intensively studied over the past few decades. While numerous microorganisms have been identified as potential biocontrol agents against plant diseases, only a small number are currently available in commercial form. The use of a microbial agent for crop protection purposes requires its approval as a crop protection product.

The literature corpus analyzed here was made up of 181 publications, primarily from scientific journals. As a supplement to this corpus, a number of reference works and web sites were consulted to verify which microbial-based biocontrol products are approved for use in France and/or other countries, for pathogens targeted by the use copper in France. The articles reported on studies conducted in 34 countries, on all continents (mainly Europe, the Americas, and Asia). Studies related primarily to the effects of microorganisms against pathogenic bacteria and fungi affecting fruit crops (particularly apricot, peach, and apple) and various market garden crops. Downy mildews are also addressed, notably for grapewine, potatoes, and tomatoes.

Types of biological agents, modes of action, and conditions of use

Types of organisms

The microorganisms useable for biocontrol against pathogens targeted by copper belong to three major biological groups: 1) fungi, yeasts, and oomycetes – eukaryotic organisms, generally multicellular, that produce a thallus bearing sexual or asexual reproductive organs; 2) bacteria and actinomycetes – prokaryotic, unicellular organisms that generally multiply by asexual cellular division; and 3) viruses, which multiply by making use of the cellular machinery of the infected host. The specific biological properties of each group determine their manner of use (formulation, application methods, shelf life, ability to be combined with other crop protection materials or strategies, etc.) as well as their potential for practical use under crop production conditions. These factors are necessarily taken into account by the crop protection firms working to develop microbial biocontrol products. Information as to the specific composition of these products is scarce, however (nature of the formulations, etc.), since such details constitute a form of intellectual property.

Modes of action

Three principal modes of action have been identified, involving diverse biological mechanisms: the destruction or direct inhibition of the pathogen; competition with the pathogenic agent; and interaction with the process of pathogenesis. For some biological control organisms, the mode of action has not been precisely determined.

Direct inhibition or destruction of the pathogenic agent

This mode of action can involve two mechanisms:

• **antibiosis**, in which the antagonist organism produces secondary metabolites that are toxic to the pathogen, inhibiting its germination, mycelium growth and/or sporulation. Antibiosis is the most extensively studied mode of action, because it is readily identified in the laboratory. However, the production of antibiotic compounds depends on numerous environmental factors (water potential, environmental pH, temperature, etc.). There are many examples of bacteria and fungi that produce antibiotic compounds, notably *Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces* sp., and *Trichoderma* sp. Substances responsible for antibiosis have been identified in several species, and the genes involved in the production of some of these substances have been identified. This mode of action is the closest to that of chemical control; it can thus be very effective in inhibiting the development of a microbial pathogen, but can also present similar risks of the toxicity of the molecules involved for the environment, for applicators, and for consumers. There is also the potential for the appearance of resistant strains of the target pathogen.

• **hyperparasitism**, in which the antagonist penetrates the tissues of the pathogen and destroys it by colonizing its organs. The fungus *Ampelomyces quisqualis*, for example, parasitizes the fungus that causes powdery mildew. The use of hyperparasites for biocontrol presents certain disadvantages, including the need for direct contact with the pathogen and the need for a rapid effect for the pathogen to be destroyed.

Competition for nutrients or space

Some microorganisms (bacteria, yeasts, filamentous fungi) can inhibit the germination of pathogen conidia *via* competition for nutritive elements such as nitrogen, carbon, or other macro- or microelements present in the environment. This mode of action is particularly effective against pathogenic fungi, spores of which require a nutrient source to initiate germination. Reduction in spore germination rates and slowing of the pathogen's mycelium growth will limit the number of infections and the growth of lesions. Nutrient competition has been shown for the antagonistic fungi *Trichoderma harzianum* T39, for example.

Interaction with the process of pathogenesis

This mode of action can involve three distinct mechanisms:

• **interference with the target's pathogenic capacity**, for example with the hydrolytic enzymes that pathogenic fungi synthesize to breakdown plant tissues during colonization. Biological control agents can affect the pathogenic capacity of fungi by destroying some of these hydrolytic enzymes or reducing their effectiveness (*via*, a modification of the environmental pH, for example);

• **modification of the surface properties of the host crop's organs.** Some bacteria have the capacity to inhibit the processes of pathogen adhesion and growth by synthesizing tensio-active compounds that alter the wettability of leaves or reduce periods of wetness that are favorable to pathogen development;

• **stimulation of host plant resistance.** Many microorganisms have a plant-defense stimulation effect (PDS; see Natural plant defense stimulators), with the microbes producing of elicitors of various types. This is true, for example, of *Bacillus subtilis*, the active bacterium in the product Serenade®. Plant-defense stimulation is a major focus of current research.

In some cases, biological control results from a **combination of different action mechanisms**. Multiple modes of action, including hyperparasitism and antibiosis, have been demonstrated for a single strain of the fungus *Trichoderma*. In these cases of combined effects, the role and relative importance of each mode of action within the overall disease control picture is generally unknown.

Effectiveness factors and barriers to use

The effectiveness of biocontrol agents is governed by complex factors related to their modes of action and to the fact that they are living organisms. In commercial crop production conditions, the survival, residence, and activity of biocontrol organisms will depend on:

• **the environmental context they encounter**. The fluctuation of microclimatic conditions (temperature, humidity, etc.) is typically identified as a key factor in the instability of biocontrol effectiveness. Even at the plant surface, biocontrol organisms are subject to variations in nutrient availability and microhabitat characteristics (leaf morphology, chemical exudates, etc.).

• **agricultural practices**. The effectiveness of a biological control agent can vary depending on the crop variety, fertilization regimes, or the application of other crop protection materials. Information on the mutual compatibility of different biocontrol agents and the effects of other pesticide products is needed.

• **the quality of the biocontrol product and its manner of application.** Biocontrol effectiveness and persistence will also be influenced by the quality of the microbial agent, its product formulation, transportation and storage conditions, and uniformity of application. All these factors can effect the number of living propagules introduced into the field, their capacity to move around and multiply, their contact with the target, etc. Ease of use depends on the mode of action: the application of a biocontrol organism that acts by contact will be more challenging than one that acts at a distance; the stimulation of natural plant resistance requires time to take effect, etc.

• **characteristics of the target pathogen.** Diseases that spread rapidly are more difficult to control than monocyclical diseases that develop more slowly. The effectiveness of a biological control agent will then depend on the quantity of pathogen inoculum present and on the genetic variability of the pathogen strains.

Products approved in France for current uses of copper

In France, **eleven microbial biocontrol products** currently have market authorization (MA) for use against diseases targeted by an allowed use of copper (Table 2.3-A). In terms of current uses for copper, these eleven biocontrol products cover:

• two of the 21 registered uses in **arboriculture** and **viticulture** (in part): control of apple scab (Serenade), and control of wood diseases in grapewines (Esquive). Serenade also has an MA for the control of brown rots in stone fruit.

• two uses in **major field crops** (in part): control of fungal diseases (*Fusarium*) in wheat and rye, applied as a seed treatment. The product here is Cerall; two other products, Prestop and Polyversum, can also be used against this type of target. One major use of copper, late blight in potatoes, is not covered.

• two of the 20 uses of copper in **market garden production** (in part). Two products (Trianum and Tusal) can be used to protect carrots against Pythiaceae (*Pythium* and *Phytophthora*); Serenade is approved for use against bacterial diseases in tomato (although it is not clear whether all bacteria affecting tomatoes are effectively controlled by this product).

• various uses for **indoor and balcony plants** and for **perfume, aromatic, medicinal and condiment plants** (very partially). Six products are involved (Asperello T34 Biocontrol, Mycostop, Prestop, Serenade, Trianum, and Tusal), with application methods being different than those used for copper. Finally, one product approved for use against wood infections in grapewines (Esquive) can partially replace the use of copper to protect wounds and cuts.

The available scientific literature has insufficient information on the effectiveness of these products in production conditions in France.

Products approved in other countries

At the European Union level, 27 strains of microorganisms are currently listed for the control of plant diseases in the EU Pesticide Database: 17 strains of fungi, yeasts, or oomycetes; 8 strains of bacteria or actinomycetes; and 2 viruses (database consulted January 31, 2017). Among these strains, 9 that are currently not approved in France are relevant for uses of copper in French production systems (Table 2.3-B; strains noted in blue). Therefore, some of these microorganisms are currently used elsewhere in the EU for situations for which there is no available alternative to copper in France. One additional strain is approved in the EU for use against brown rot in peach. Moreover, Serenade is approved in other European countries for some additional important uses, including powdery mildew in grapewines and downy mildew in lettuce.

Elsewhere in the world, 44 microorganisms have been commercially developed as biocontrol agents for the control of plant diseases: 25 strains of fungi, yeasts, or oomycetes; 14 strains of bacteria or actinomycetes; and 5 viruses. Among these, 19 strains (in addition to bacteriophages on the market in the United States) are contained in products approved outside the EU for uses that correspond to uses of copper in France (Table 2.3-C). If they were to prove effective in French production conditions, these products could make it possible to cover (at least in part) 19 uses of copper for which there is currently no approved alternative product in France, and to strengthen coverage of the 11 uses for which there is currently an alternative product. Seven of these strains (shown in blue in Table 2.3-C) are already approved in the EU for other uses, which could facilitate their approval in France.

Other microbial agents with biocontrol potential

Among the fifty or so reported uses of copper in France, twenty-four have no corresponding commercial microbial biocontrol product approved for use in France or elsewhere. For 4 of these "orphan" uses, the scientific literature suggests microorganisms that have been tested for their effectiveness against the corresponding pathogens. All are bacterial pathogens; the affected crops are olives, grapewines, onions, and pears. For the use [onion x bacterial diseases], a study in the United States on the product BlightBan C901/A506 reported efficacy in the field that was equivalent to the use of copper. For the three other uses, the reported studies used "laboratory" strains, and thus do not allow for a precise assessment of effectiveness in the field.

Some papers reviewed for this ESCo report on trials of existing commercial products (Actinovate, AgriPhage, AQ10, BlightBan, Bloomtime Biological, Bmj WP, Mycostop, Serenade, and Sonata) for alternate applications. In other words, an effort is being made to extend the data in preparation for a potential approval extension to an additional use. Available data point to good potential for using Serenade as a replacement for copper for several major uses, including [grapewine × downy mildew], [potato × late blight], and [tomato × bacterial diseases] (at least *Pseudomonas syringae*). For BlightBan, efficacy data are more mixed (tomato × *Pseudomonas syringae* or *Xanthomonas* sp.), apart from the good results against bacterial diseases in onions, as noted above.

Overall, the bibliographic analysis suggests that new strains of microorganisms could eventually be developed to cover uses of copper for which no biocontrol products are currently available, either in France or elsewhere. The development of such products requires many additional steps beyond the "simple" demonstration of effectiveness within a research trial, however. These include studies of technical feasibility, analyses of production costs, and other steps required to apply for and receive regulatory approval.

Table 2.3. Microbial crop protection products approved for use against diseases targeted by copper in France

	Product name (Manufacturer / distributor)	Microorganism Species and strain(s)	Use (crop / target)	Type of treatment
A. Products approved in France	Asperello T34 Biocontrol (Bio Control Technologies)	*Trichoderma asperellum* T34	Ornamental crops / vascular wilts	Soil
	Blossom Protect (Bio Ferm GMBH)	*Aureobasidium pullulans* DSM 14940 and DSM 14941	Apple / fire blight	Aboveground plant parts
	Cerall (Belchim)	*Pseudomonas chlororaphis* MA342	Wheat, rye / fungi other than Pythiaceae	Seeds
	Esquive (Agrauxine)	*Trichoderma atroviride* I-1237	Grapewine / Esca, black dead arm, Eutypa dieback	Aboveground parts (including wound care)
	Mycostop Verdera Oy (Lallemand Plant Care)	*Streptomyces* K61	General treatments (limited to non-food plants and aboveground vegetables not directly contacting the soil)	Soil
	Polyversum (De Sangosse)	*Pythium oligandrum* M1 and ATCC 38472	Wheat / fusariums	Aboveground parts
	Prestop Verdera Oy (Lallemand Plant Care)	*Gliocladium catenulatum* J1446	Wheat, rye / fungi other than Pythiaceae General treatments / fungi other than Pythiaceae; Pythiaceae	Soil
	Rotstop Verdera Oy (Lallemand Plant Care)	*Phlebiopsis gigantea*	Forests / root rot	Tree stumps
	Serenade (Bayer)	*Bacillus subtilis* QST 713	Ornamental crops, shell nuts, tomato, PAMCP / bacterial diseases Apple / fire blight, scab Stone fruits / leaf curl	Aboveground parts
	Trianum (Koppert)	*Trichoderma harzianum* Rifai T-22 and ITEM-908	Vegetable crops / Pythiaceae Ornamental crops, PAMCP / fungal diseases	Soil
	Tusal (Newbiotechnic SA)	*Trichoderma atroviride* T11	Vegetable, floral, and ornamental crops, green plants / mushrooms and Pythiaceae	Irrigation

Table 2.3. Next

	Product name (Manufacturer / distributor)	Microorganism Species and strain(s)	Use (crop / target)	Type of treatment
B. Products approved for use elsewhere in the EU		*Ampelomyces quisqualis* AQ10	Grapewine / powdery mildew	
		Bacillus pumilus QST 2808		
	Serenade (additional uses)	*Bacillus subtilis* QST 713	Grapewine / powdery mildew Lettuce / downy mildew	
		Pseudomonas sp. DSMZ 13134	Soil-dwelling fungi	Soil
		Saccharomyces cerevisiae LAS02	Peach / leaf crul	
		Streptomyces K61	Aromatic plants	Soil
		Streptomyces lydicus WYEC 108	Soil-dwelling fungi	Soil
		Trichoderma atroviride SC1	Grapewine / wood diseases	
		Trichoderma atroviride IMI 206040 and *T. polysporum* IMI 206039	Chondrostereum purpureum	
		Verticillium albo-atrum WCS850	Elm / verticillium wilt	
C. Products approved for use outside the EU	AgriPhage			
	Bio-Tam, Tenet	*Trichoderma asperellum* ICC012 + *T. gamsii* ICC080		
	BlightBan A506	*Pseudomonas fluorescens*		
	Bloomtime Biological	*Pantoea agglomerans* E325		
	Double Nickel 55	*Bacillus amyloliquefaciens* D747		
	Ecosom-TV	*Trichoderma viride* TNAU		
	Galltroll	*Agrobacterium radiobacter* K84		
	Nogall	*Agrobacterium radiobacter* K1026		
	Polyversum (additional uses)	*Pythium oligandrum* M1 and ATCC 38472		
	Serenade (additional uses)	*Bacillus subtilis* QST 713		
	Sonata	*Bacillus pumilus* QST 2808		
	Taegro	*Bacillus subtilis amyloliquefaciens* FZB24		

* PAMCP: perfume, aromatic, medicinal and condiment plants. In blue: product, strain, or use not allowed in France but approved elsewhere **in the EU** (possibly for uses other than those targeted by copper in France). In purple: product, strain, or use only approved **outside the EU** (possibly for uses other than those targeted by copper in France). (source: https://ephy.anses.fr/, consulted 31 Jan. 2017).

Conclusion and perspectives

Incomplete coverage for uses of copper

Among the fifty or so uses of copper in France, eleven are partially covered by microbial biocontrol products currently approved for use in France. This coverage is "partial" because many uses of copper are broad in nature (corresponding to several or even many different pathogens), whereas the effectiveness of biological control organisms is often specific to one or a few pathogens. Five of the eleven uses are covered by a single product, moreover, which could pose problems for the longevity of the product's effectiveness.

Nineteen additional uses have the potential to be partially covered if products already on the market elsewhere in the EU or elsewhere in the world receive approval for use in France. However, their actual utility as a replacement for copper in France will depend on their efficacy under French production conditions. In some countries, notably in North America, a demonstration of product efficacy is not required for regulatory approval, as is the case in Europe. It is thus possible that some products approved for use in other countries will not receive approval for market introduction in Europe.

Based on the bibliographic analysis, microorganisms could eventually cover four additional uses, depending on a variety of technical, industrial, regulatory, and commercial factors, as well as on the willingness of the firms involved to undertake the necessary market authorization applications.

Finally, 20 uses of copper have not even been a focus of published research.

Significant needs for further research

Significant scientific and commercial investments are required to develop and market microbial biocontrol materials. This fact helps explain why a considerable segment of current research is focused on extending the uses of existing products. The number of new formulations, based on new biocontrol strains or species, remains limited, including for the major uses of copper.

A further challenge is that the very nature of biocontrol materials raises a range of questions as to the compatibility between these products and other agricultural techniques and methods applied to a given crop. A significant increase in research on microbial ecology, as a means of better understanding and positioning microbiological control within agricultural systems, is thus also needed.

Varietal resistance

The development and introduction of resistant varieties is an important and promising crop protection method for limiting the use of crop protection products in general. Advances in plant genetics have enabled breeders to introduce resistance factors into cultivated varieties, for example through crosses with related wild species. Large-scale agricultural use of these varieties, however, quickly revealed pests species' ability to overcome these forms of resistance. Mechanisms of plant resistance to pests – mobilized both by breeding programs and by the use of plant defense stimulators (see Natural plant défense stimulators) – are presented in Box 2.1.

Box 2.1. Types of plant resistance

Like all living organisms, plants only survive if they can resist, by virtue of their specific immune systems, a wide range of pests, primarily pathogenic microorganisms (viruses, bacteria, and fungi) and herbivorous arthropods (insects and mites). Plants' capacity to limit the exploitation of their tissues as a nutrient source for parasites constitutes their **resistance to parasites**. This resistance may be described in terms of genetics (the inheritance of traits), plant pathology (the expression and evolution of symptoms over the course of the host-parasite interaction), biochemistry (the underlying physiological mechanisms) or ecology (the range of hosts and parasite specialization).

A typology of plant resistance

Three principal types of plant resistance may be distinguished according to the level of interaction between the plant and the pest microorganism: non-host resistances; qualitative resistances; and quantitative resistances.

- **Non-host resistances** are characterized by a total exclusion of the potential aggressor, which is thus unable to establish a parasite relationship with the plant. This is a species characteristic: all genotypes of a plant species are resistant to all genotypes of a given microbial agent. This results in a total absence of infection, and thus a total absence of symptom (total immunity). It is based primarily on constitutive defenses existing prior to any contact with the potential aggressor, and which form physical barriers to infection: an external cuticle, the composition of the plant's cellular walls, etc. Non-host resistance is the most common type of resistance among plants, and also the most durable. For this reason, a better understanding of the mechanisms and genetic determinants of non-host resistance could potentially allow for the future development of cultivars that are durably resistant to the most important pathogens.

When a pest succeeds in overcoming these initial barriers, it is able to establish a more or less effective parasite relationship with its host. The plant's capacity to limit the consequences of this interaction – more or less completely – constitute the two types of **host resistance: qualitative and quantitative**.

- **Qualitative resistance** usually results, in terms of symptoms, in what is called the "hypersensitive response," that is, a cellular necrosis limted to the site of infection, without further extension and without reproduction of the pathogen. It is thus a form of **total** resistance, in the sense that it completely prevents the development of the disease on the resistant host.

In terms of genetics, qualitative resistance is generally monogenic or oligogenic, that is, inherited via one or a few genes. The corresponding host/parasite interaction generally follows the "gene-for-gene" model: the resistance is a result of

Box 2.1. Next

a (direct or indirect) interaction between the plant's gene for resistance and the pathogen's gene for non-virulence. The resistance will thus only be active for some plant genotypes (those that carry the gene for resistance) and against some parasite genotypes (those that carry the gene for avirulence). Qualitative resistances are thus specific to these specific genetic configurations, rather than being general to the species, like non-host resistances.

Because of their very strong genetic effects (via simple inheritance) and phenotypic outcome (a total prevention of infections), genes for qualitative resistance are often referred to as "major resistance" genes, designated with a capital R[1].

- **Quantitative resistance** results in a reduction of symptom severity (size of lesions, rate of spread) relative to susceptible individuals. Quantitative resistance may also reduce parasite reproduction, although it will not generally prevent it entirely. Quantitative resistances are thus **partial** resistances, since they do not allow for a total blockage of the pathogen lifecycle.

Quantitative resistances are usually active against all pathogen genotypes, and are thus described as a non-specific form of resistance. They are generally polygenic, that is, determined by multiple (sometimes a great many) QTL[2] with weak or moderate individual effects. This complex genetic determinism makes it difficult to select for quantitative resistance, although it should also be longer-lasting. There are few examples of the loss of partial resistance, whereas there are many examples of the loss of total resistance.

The period of time needed to observe, under commercial production conditions, a loss of resistance (a sudden loss of effectiveness) or an erosion of resistance (a gradual reduction in effectiveness) is highly variable. Some resistance genes remain effective after several decades of intensive use, whereas others have been overcome in a few years, or even in a few months. The length of time required to develop and introduce a new variety carrying a new resistance gene, on the other hand, remains long. The introgression of R genes into "elite" cultivars takes

1. R genes are named according to the following code: the initials of the latin name of the pathogen, sometimes followed by 3(4) letters indicating the plant species from which each gene was identified and a number. For instance, *Rpi-ber1* designates a gene for resistance to *Phytophthora infestans* coming from *Solanum berthaultii*.

2. A QTL (for *quantitative trait locus*) is a chromosome region (more or less extended) closely associated to a quantitative trait. Each QTL thus corresponds to a chromosome segment containing one or more genes segregating together with this quantitative trait. QTLs being identified through genetic mapping methods, and therefore through correlative assessments between the presence/absence of specific molecular markers and the phenotypic variation of the trait considered (disease resistance, yield, product quality…), their size depends strongly on the resolution in the measurement of the trait itself (quality of the phenotypic data), on the number and density of genomic markers available, and on the size of the segregating population used for the mapping. The identification of genetic markers located close to the genes or QTLs of interest allows Marker Assisted Selection (MAS) and Marker Assisted Breeding (MAB).

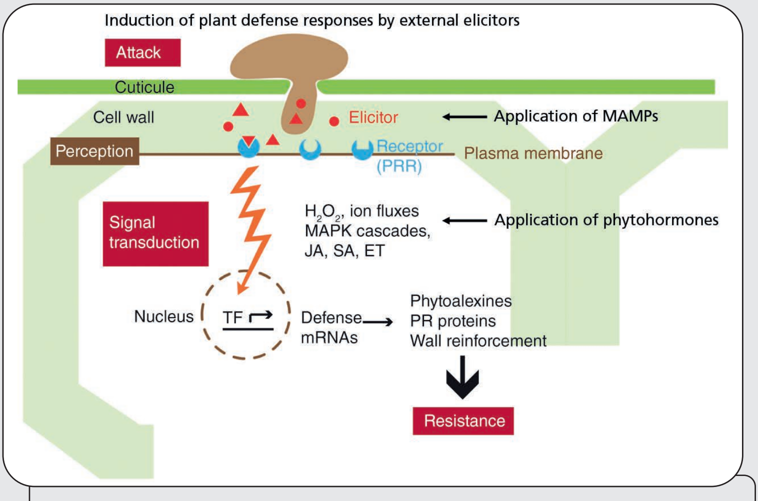
Box 2.1. Next

up to 15-20 years via traditional breeding methods. Marker Assisted Selection (MAS) has reduced the time needed for the development of new varieties by half. This technique makes it possible to rapidly test the status (resistant or susceptible) of a large number of individuals by searching for the presence of molecular markers linked to the relevant gene, rather than via phenotypic evaluation (testing for infection under controlled conditions or in the field).

Underlying physiological mechanisms: Plant resistance and defense

The first line of plant defense against microbial pests, termed **constitutive**, resides at the cuticle layer (the hydrophobic layer at the surface of aboveground plant parts) and the cellular wall. This is generally the locus of **non-host resistance**. Nevertheless, non-host resistance can also involve other mechanisms which are not well understood, but the exploitation of which could eventually allow for the creation of durably resistant plants. A second line of defense, which may be **induced**, lies in the plant's perception of the pest and subsequent defense reactions (Figure 2.1). It is these defense reactions that are made use of in the various types of host resistance being developed, according to what is known as the "zig-zag" model.

Figure 2.1. Plant defense reactions to a pathogen in the PTI framework



ET, Ethylene; MAPK, Mitogen Activated Protein Kinases; JA, Jasmonic Acid; SA, Salicylic Acid; TF, Transcription Factor; PR proteins, Pathogenesis Related proteins; MAMPs, Microbe Associated Molecular Patterns.

This model distinguishes two main steps in defense induction by pathogen attacks. The first is activated in response to elicitors, i.e. molecules produced by the pathogen itself during infection. These molecules, either secreted by the pathogen,

Box 2.1. Next

present on its cell surfaces or resulting from the degradation of infected host cells, are now designated under the generic term PAMP (Pathogen-Associated Molecular Pattern). They are most often structural components (of the host cell wall, of bacterial flagellae, etc.), common to many micro-organisms and which can be recognised by specific receptors located in the host cell cytoplasmic membranes. These molecules are therefore for the plant as many signatures of an on-going attack. The PAMP- receptor bound triggers a cascade of cell signalling events, which leads to the expression of defense genes coding the synthetis of antimicrobial compounds (phytoalexins), but also of defense proteins involved in the management of oxydative stress, pathogen degradation, the re-programming of plant metabolism towards defense, or compounds (callose, lignin...) mobilised to strengthen the host cell wall at the point of attack. This system, called **PTI** (PAMP-triggered immunity) or basal immunity, allows to prevent infection by most microbes. PTI is thought to be directly involved in **quantitative resistance**.

To continue to attack a plant following the triggering of PTI, some pests have developed other types of molecules, called effectors, which block the defense reactions prompted by PTI. In response, plants have developed specific receptors that target these effectors, and use them to activate a second wave of defense reactions, collectively termed **ETI** (effector-triggered immunity). This is the underlying mechanism of qualitative resistance, with the major genes being those that govern the specific receptors for the effectors. ETI is made up of two defense mechanisms.

One is the hypersensitive response, which is local, triggering cell death around the point of pathogen attack, blocking further development of biotropic parasites. The other, known as systemic acquired resistance, consists in the diffusion of hormonal signals from the point of attack throughout the rest of plant, telling the cells of other plant organs to activate their defenses in preparation for further attack. These mechanisms are governed by plant hormones, the best known of which are salicylic acid (SA), jasmonic acid (JA), and ethylene. The defense mechanisms associated with these different hormones are not necessarily effective against all pathogenic agents. ETI defenses thus complement PTI defenses and can be highly effective, but they are only active against some pathogen strains.

Two WoS queries were carried out: the first, targeting relevant review articles pertaining to all crops (not only those relying on the use of copper), yielded 66 citations; the second, targeting primary articles relating specifically to the crops and pathogens involved in copper applications, yielded 422 citations. In addition, the analysis included a handful of citations from the gray literature. Among the 422 primary articles, 125 were focused on apples, 118 on potato, 42 on tomato, 20 on grapewines, and 14 on lettuce. The analysis thus focused primarily on these five crops of major agronomic importance. The part of the analysis focused on the sustainable management of resistance was not limited to these five crops, since relevant articles were found from both WoS queries.

Forms of resistance among the most copper-dependent crops

Genetic resources and available varieties in apple

Resistance factors identified and used

In apple production, copper is primarily used to control **scab** (caused by *Venturia inaequalis*) and European canker (table 2.4A). Genetic selection is focused first on resistance to *V. inaequalis*, a fungal species with a strong ability to evolve.

With respect to **scab**, 17 major **resistance genes** (designated *Rvi1* through *Rvi17*) have been described to date. All function according to the gene-for-gene model. The majority of scab-resistant apple varieties currently in use carry the resistance gene *Rvi6* (also called *Vf*): over one hundred such varieties are listed in commercial catalogues. Nevertheless, scab-resistant varieties account for a very small percentage of total apple production area (<1% in Europe), although the percentage can be much higher in OA orchards. Beginning in the 1990s, this resistance was overcome in several European countries by virulent strains of scab, which was shown to have come from pathogen populations found on a wild progenitor of the *Vf* resistance. Very few varieties carry other resistance genes (*Rvi5* in the cultivars *Murray* and *Rouville*, *Rvi4* in *Regia*, *Rvi13* in *Durello di Forlì*). In addition, at least 13 **QTL** for partial resistance have been described in apples, some with a wide spectrum of target races (QTL effective against the majority of tested strains, such as F11 and F17), others with a narrow one (specific QTL, effective only against certain strains; like T1).

Researchers have also identified resistance factors for fire blight (caused by *Erwinia amylovora*), and selection programs are attempting to obtain varieties combining scab resistance with fire blight resistance, and in some cases powdery mildew resistance (caused by *Podosphaera leucotricha*). A few cultivars have been identified as a result of these efforts.

Pear, a species genetically close to apple, is also affected by a scab (caused by *Venturia pirina* or *V. nashicola*). The literature available on this crop is much less extensive. Three major resistance genes have been described, however, as well as what appear to be at least eleven QTL.

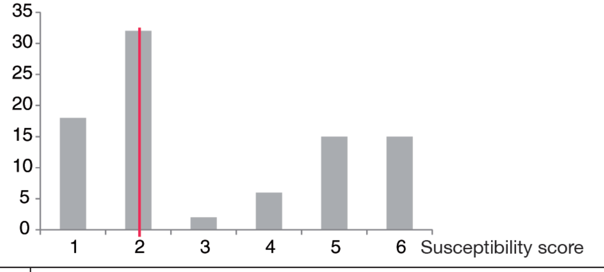
Variety susceptibility under field conditions

A number of studies have evaluated the susceptibility of different pear varieties in orchard settings. One of the most important studies (conducted in Canada and published in 2005) evaluated the susceptibility to scab of 54 cultivars and 14 breeding lines over a three-year period in an orchard receiving zero fungicide applications. The study identified 16 cultivars showing very low (or even zero) incidence of disease: *Regine*, *Rebella*, *Resi*, *Rewena*, *Akane*, *Anis Aily*, *Antonovka*, *Bramley's Seedling*, *Chehalis*, *Discovery*, *Generos*, *Golden Reinette*, *Golden Russett*, *Margil*, *Peypring Cerueuko* and *Wolf River*.

In addition, some national technical documents records data on the observed susceptibility of different fruit tree varieties. In France, a guide published by INRA-GRAB in 2016, "Fruit tree susceptibility, follow the guide!" reported scab-sensibility data for 19 apple varieties grown in an orchard in southeastern France (INRA Gotheron), with 8 of these varieties also evaluated in an orchard in western France (INRA Angers); as well as for 36 varieties, including both heirlooms and modern cultivars, in four other orchards in different parts of France (Drôme, Nord, Lot-et-Garonne, and Vaucluse). These data indicate that many heirloom varieties exhibit good resistance to leaf scab, including *Provençale rouge d'hiver*, *Pomme d'Adam*, *Pomme de Risoul*, *de l'Estre*, *Court Pendu*, *Gris du Limousin*, *Reinette Champagne*, *Reinette des Capucins* and *Cabarette*. Among recent varieties, *Reine des Reinettes* and *Honeycrisp* also have good resistance to leaf scab, but are much more susceptible to fruit scabbing. Among the varieties with the best performance on both leaves and fruit were *Belle de Boskoop*, *Akane*, and *Reinette des Capucins*. Previous research has shown the value of this resistance for reducing pesticide use. Thus, it was possible to reduce the number of fungicide applications made for scab by more than 50% on *Melrose*, and by more than 60% on *Reine des Reinettes*, relative to the reference variety *Golden*. Since many of the varieties in the orchards reported on in the INRA-GRAB guide were more resistant than *Melrose* or *Reine des Reinettes*, it seems likely that even greater reductions in treatment levels could be achieved with the most resistant cultivars.

In Canada, the guide "Integrated pest management for apple trees," published by the Ministry for Agriculture of Ontario, included a partial list of commercially available, scab-resistant cultivars; of the 26 apple varieties listed, all but one (*Rouville*) were described as resistant. In the United States, in 2006, Purdue University compiled data on the susceptibility of 88 apple varieties to scab and fire blight. The data on scab show low levels of resistance for most of the varieties (Figure 2.2), but identify 15 varieties with high levels of resistance (*CrimsonCrisp*, *Entreprise*, *Florina*, *Freedom*, *GoldRush*, *Liberty*, *Macfree*, *Nova Easygrow*, *Novamac*, *Nova Spy*, *Prima*, *Priscilla*, *Pristine*, *Sir Prize* and *William's Pride*). With respect to fire blight, 19 varieties were noted as "resistant" and two as "very resistant." The two very resistant varieties were *Freedom* and *Novamac*, which were also described as "very resistant" to scab.

Figure 2.2. Susceptibility to scab of different apple varieties: distribution of the "scab" score across 88 apple varieties.



A score of 1 indicates that the variety is highly susceptible; 9 means the variety is highly resistant. In red: the median of these scores. Numbers on the y axis correspond to the number of varieties in each resistance class (source: Data compiled by Purdue University, 2006).

Genetic resources and available varieties in grapewine

In viticulture, copper-based products are only approved for use against downy mildew (caused by *Plasmopara viticola*) and bacterial diseases. Breeding programs for grapewines generally focus on the crop's two most important diseases: downy mildew and powdery mildew (caused by *Erisyphe necator* and managed in OA with sulfur-based products).

Resistance factors identified and in use

For downy mildew, at least 14 resistance factors have been described (according to a summary from 2011), with 6 of these currently in use in European breeding programs (references dating from 2003 to 2012) (Table 2.4B).

For powdery mildew, at least 11 resistance factors (designated *Run* and *Ren*) have been described (according to a review from 2015), 4 of which are in use in European breeding programs: *Run1*, *Run2.2*, *Ren1* and *Ren3*.

The most active entities in grapewine breeding are all European (WBI in Germany, IGA in Italy, Agroscope in Switzerland, etc.). In France, INRA, along with the *Institut Français de la Vigne* (IFV), has been working since 1974 to introduce resistance factors from *Muscadinia rotundifolia*, a species highly resistant to both powdery mildew and downy mildew, into European grapewines (*Vitis vinifera*). In the year 2000, this effort produced a series of genotypes (named "Bouquet") which were then, in a new breeding program, crossed with wild species of American and Asian grapes. The resulting material, named "Resdur," should improve the durability of this resistance by stacking several resistance genes. Among the "Resdur" varieties, four received official variety listing in 2017 (*Artaban*, *Vidoc*, *Floreal* and *Voltis*), combining the genes *Rpv1* and *Rpv3* (+ *Run1* and *Run3* for powdery mildew). A second set of 19 varieties associating the genes *Rpv1* and *Rpv10* (+ *Run1* and *Ren3.2*) will be introduced in 2020.

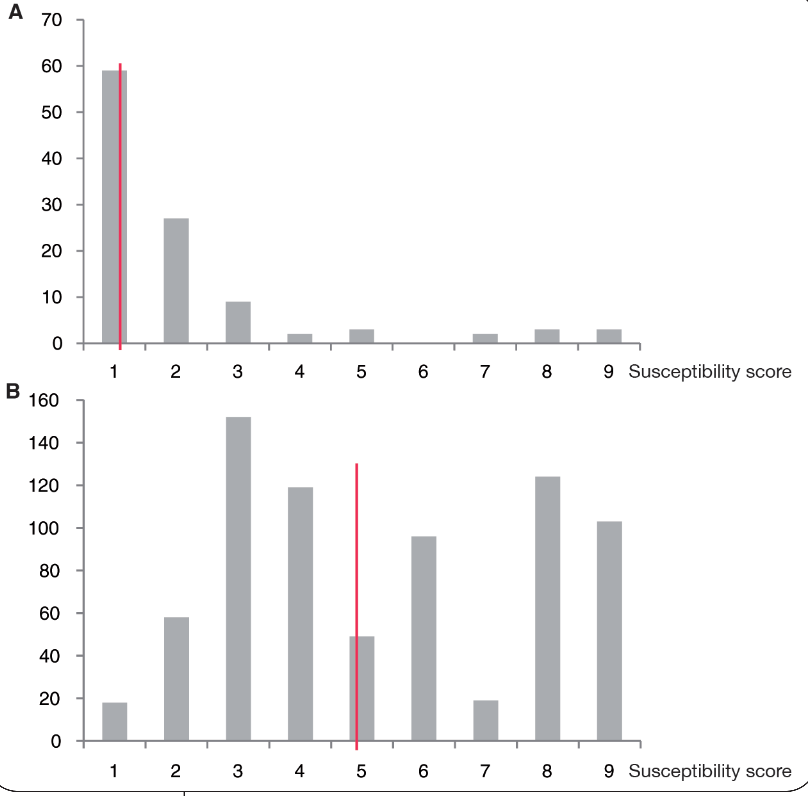
The powdery mildew and downy mildew populations pathogenic to grapewines have a strong evolutionary potential, as shown by the rapidity with which they have responded to the selective pressures exerted by fungicides - developing resistance to almost every molecule in use. There would thus appear to be a real potential of these pathogens to adapt to resistant varieties as well. Several cases of the breakdown or loss of resistance have indeed already been reported: for downy mildew, to the QTL *Rpv3*, *Rpv1* and *Rpv10* (each individually); for powdery mildew, to the QTL *Ren3* and *Run1*.

Variety susceptibility in vineyards

A 2013 guide published by the ICV (Institut Coopératif du Vin), "Varieties resistant to cryptogamic pathogens – A European overview" provides technical survey of current breeding programs and the status of variety resistance as an objective within these programs. It describes agronomic traits (including resistance to downy mildew, botrytis, and powdery mildew) and other characteristics for 200 varieties in six European countries. Since 2017, the national monitoring program OsCar (http://observatoire-cepages-resistants.fr/) has been collecting and distributing data on the observed in-vineyard susceptibility of a large number of resistant varieties across multiple wine-producing regions.

The *Vitis* International Variety Catalogue (www.vivc.de) describes the characteristics of several thousand grapewine varieties, including (where applicable) information on susceptibility to the most important diseases. Analysis of the 847 grapewine varieties included in the catalogue shows that downy mildew resistance is relatively rare among varieties of *Vitis vinifera*, but relatively widespread among varieties resulting from interspecific crossings (Figure 2.3, A and B).

Figure 2.3. Downy mildew-susceptibility of grape varieties used in winemaking: distribution of the "leaf downy mildew" score for 108 varieties of *Vitis vinifera* (A) and for 739 varieties resulting from interspecific crossings (B).



A score of 1 means the variety is highly susceptible; 9 means the variety is highly resistant. In red: Median of the susceptibility scores. Nulbers on the y axes correspond to the number of varieties in each resistance class (source: International Catalogue of Grape Varieties; www.vivc.de, consulted Feb. 27, 2017).

Genetic resources and varieties available in potato

Late blight (caused by *Phytophthora infestans*) can affect all parts of the potato plant (aboveground and belowground). The use of resistant varieties is considered to be a good solution for limiting yield losses to late blight, although *P. infestans* shows a strong capacity for adaptation to forms of plant resistance (table 2.4C).

Breeding programs for potato (*Solanum tuberosum*) have a wealth of material at their disposal within the genus *Solanum*, which includes some 1,500-2,000 species adapted to a wide array of habitats and thus to a variety of biotic and abiotic stresses. Numerous collections of the *Solanum* genus, including key alleles for different selection objectives, are maintained in various international gene banks and are accessible to researchers and breeders. In France, for example, INRA Rennes holds more than 10,000 accessions. The genetic improvement of potato is a long and complex process, however; the species is polyploid and strongly heterozygote, which creates barriers to interspecific hybridization, requiring special techniques (e.g., protoplast fusion) to successfully cross certain genotypes. The time required to create a variety, from the initial crosses to the final listing, is typically 15-30 years.

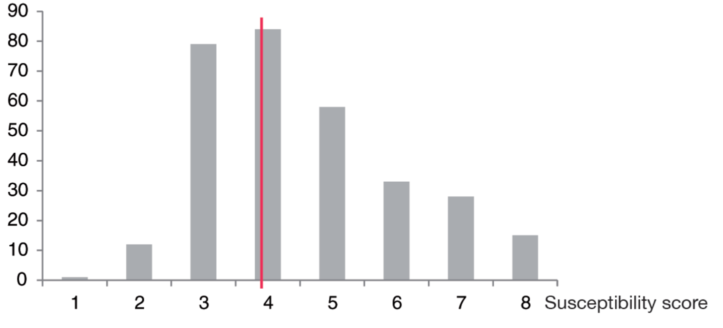
A list of **major resistance genes** identified as of 2013 includes 63 genes from 27 species of *Solanum*. The first of these genes (*R1* through *R11*) were identified in a wild Mexican species, *S. demissum*. *R1*, *R2*, *R3*, *R4* and *R10* were introgressed into cultivated varieties, but the resistance they conferred was rapidly overcome in the field, beginning in the 1950s. Other major genes were subsequently identified. The genes currently being developed have a wide range of action, including some identified in *S. bulbocastanum* (*Rpi-blb1*, *Rpi-blb2*, *Rpi-blb3* and *Rpi-abpt*) and in *S. phureja* (*Rpi-phu1*). Marker assisted selection is possible for many of the major resistance genes to *P. infestans*. The fact that more than 1,300 potential resistance genes have been identified in the genome of *S. phureja* (a diploid species closely related to potato, used as a reference species) and the large number of *Solanum* species overall suggests that many potentially useful genes remain to be discovered.

Quantitative resistances, showing varying levels of effectiveness, have also been identified in a number of *Solanum* species. There is a strong association between QTL for partial resistance and the trait of lateness (agronomically undesirable). Genes for partial resistance that are independent of maturity have been identified more recently, however. Field trials have demonstrated that that number and strength of fungicidal treatments can be reduced by using partially resistant varieties. Although the examples of the loss of partial resistance are rare, it has been shown that populations of *P. infestans* are capable of adapting to partial resistance when it is used extensively or for long periods of time.

In contrast to foliar late blight, the genetics for resistance to **late blight on tubers** has received little study. Tuber resistance appears in general to be inherited independently of foliage resistance, the *R1* resistance gene being one exception. Tuber resistance appears to be controlled by several QTL for partial resistance; four such QTL have been identified on the potato genome.

Despite the large number of available resistance factors (R and QTL) and in-field resistance performance that can be very good, surveys report that resistant varieties are not widely adopted and by growers. Decision-making tools exist that can account for the resistance performance of different varieties and adjust chemical treatment schedules accordingly. Nevertheless, an analysis of 310 potato varieties grown in France for which a late blight-resistance score was available found that the median resistance level remains low (Figure 2.4).

Figure 2.4. Susceptibility of potato varieties to late blight: distribution of the "foliar late blight" score for 310 varieties of potato grown in France.



A score of 1 indicates the variety is highly susceptible; 9 means the variety is highly resistant. In red: median of the susceptibility scores. Numbers on the y axis correspond to the number of varieties in each resistance class (source: Semences et Progrès, n° 173, Dec. 2015 – Jan. 2016).

Genetic resources and varieties available in tomato

In tomato production, copper is used to control late blight (caused by *Phytophthora infestans*) and bacterial foliar diseases associated with various species of *Xanthomonas* (table 2.4D). Resistances to these two pathogens have been identified in a number of wild tomato species (*Lycopersicum pennelli*, *Solanum pimpinellifolium, S. peruvianum,* and *S. habrochaites*).

Several resistance genes (for example, *Ph-1*, *Ph-2* and *Ph-3*) and QTL for partial resistance have an effect on *Phytophthora infestans*. The major resistance gene *Ph-1* was introduced into one variety (*Rockingham*, listed in 1962), and then into several others (*Nova*, *New Yorker*, etc.), but was rapidly overcome by new genotypes of *P. infestans*. The resistance conferred by *Ph-2*, mapped on a single part of the tomato genome, slows disease development; it is thus a gene for partial resistance.

Expression of this resistance depends on environmental conditions (temperature), plant age, and of course on the strains of *P. infestans* that are present. *Ph-2* has nevertheless been introduced into many varieties, including *Legend*, *Centennial*, *Macline*, *Pieraline*, *Herline*, and *Fline*. The resistance gene *Ph-3* has a strong effect, and has been introduced into several cultivars, but strains of *P. infestans* that remain virulent in the presence of this gene have been reported. In Brazil, *Ph-1*, *Ph-2* and *Ph-3* have each been overcome individually, and some strains of *P. infestans* are infectious even in the presence of all three genes. More recently, the gene *Ph-5* was identified, which controls many strains of *P. infestans*, including those that are virulent to *Ph-3*. *Ph-5* is being used alone and in combination with *Ph-2* and *Ph-3*.

Bacterial diseases caused by ***Xanthomonas*** were initially controlled by copper, but resistant bacterial populations rapidly appeared, rendering this control strategy ineffective. The search for resistance genes began in the 1960s and the focus of considerable effort. Four major sources of resistance have been identified: one is a total resistance conferred by three genes; the three others are monogenic. The identification of molecular markers makes it possible to use marker assisted selection. Virulent pathotypes exist for each of these resistances, however, limiting the longevity of these hypersensitive-response resistances. More recently, research on bacterial effectors has led to the identification of two effectors that could be used to develop new forms of resistance using an effectoromics approach (see below). Quantitative polygenic resistances have also been identified, and could presumably be longer-lasting.

Genetic resources and varieties available in lettuce

Among the many diseases that affect lettuce, two are controlled by copper: downy mildew (caused by *Bremia lactucae*), a major disease of lettuce, and bacterial diseases (for example, those caused by *Pseudomonas cichorii* and *Xanthomonas campestris*). Disease resistance, particularly to downy mildew, is a priority for lettuce breeding programs (table 2.4E).

The genus *Lactuca* includes a hundred or so wild species, mostly native to Asia and Africa. Resistance genes for downy mildew have been identified in *Lactuca sativa* and in nine wild species of *Lactuca*. A 2016 summary of resistance factors against *B. lactucae* listed 51 genes (28 of which, designated *Dm*, have been mapped to a single locus on the lettuce genome) and 15 QTL (designated qDMR).

The overcoming of these gene-for-gene type resistances by virulent strains of *B. lactucae* are common under field conditions, leading to a rapid replacement of lettuce varieties. This in turn leads to a rapid evolution of the frequency of virulence factors in populations of *B. lactucae*, which can be monitored to identify resistance genes that are still locally effective. Numerous strategies have been suggested to improve the durability of these resistances, including the regular introduction of new genes, the combination of several genes within a single genotype, the introduction of partial resistances, and the in-field association of several varieties carrying complementary resistance factors.

Table 2.4. Resistance factors identified, described, and in use in European breeding programs

Species	Disease	Level of resistance	Locus	Species of origin	Genes introduced into varieties	Resistant varieties useable in OA
A. Apple	Scab (*Venturia inaequalis*)	Total	17 major genes described (as of 2011)	Wild apples, including *Malus floribunda*	*Rvi6 (=Vf)* in many varieties; now often overcome *Rvi5* in *Murray* and *Rouville*, *Rvi4* in *Regia*, *Rvi13* in *Durello di Forli* (in 2009)	
		Partial	at least 13 QTL described		T1, F11, F17	
B. Grapewine	Downy mildew (*Plasmopara viticola*)	at least 14 resistance factors described (as of 2011), including:				
		Total	*Rpv2*	*Muscadinia rotundifolia*	the 6 cited as in use in European selection programs (as of 2013) (*Rpv1* + *Rpv3*) or (*Rpv1* + *Rpv10*) in the INRA varieties 'Resdur'	*Artaban*, *Vidoc*, *Floreal*, *Voltis* (listed 2017)
		Partial	*Rpv1*	*M. rotundifolia*		
			Rpv3	*Vitis rupestris*		
			Rpv8, Rpv10, Rpv12	*Vitis amurensis*		
C. Potato	Late blight (*Phytophthora infestans*)	Total	63 major genes identified (as of 2013)	27 species of *Solanum*, including *S. demissum*, *S. bulbocastanum*, *S. phureja*	R1, R2, R3, R4, R10 RpiBlb 1 RpiBlb2 Rpi-abpt	*Allians*, *Eden*, *Coquine*, *Passion*, *Makhai*
		Partial	A large number of QTL			
D. Tomato	Late blight (*Phytophthora infestans*)	Total	*Ph-1*	*Lycopersicum pennelli*, *Solanum pimpinellifolium*, *S. peruvianum* and *S. habrochaites*	introduced into several varieties; rapidly overcome	
			Ph-3			
		Partial	*Ph-2*			
		monogenic	*Ph-5*		more recently identified; used alone or in combination with *Ph-2* and *Ph-3*	
	Bacterial spot (*Xanthomonas*)	Total	4 sources identified		documented instances of loss of resistance exist	
			some QTL identified			
E. Lettuce	Downy mildew (*Bremia lactucae*)		51 genes identified, of which 28 have been mapped (as of 2016)	*Lactuca sativa*, *L. saligna*, *L. serriola*, *L. virosa*, *L. indica*, *L. quercina*, *L. aculeata*, *L. biennis*, *L. tatarica* and *L. viminea*		rapid replacement of lettuce varieties
			15 QTL reported (as of 2016)			

Sustainable management of genetic resistance

Over the course of the 2000s, the concept of "durable resistance" was gradually replaced by the quest for "sustainable resistance management." Historically, resistances were considered long-lasting if they maintained their effectiveness when in use for many years and over large areas, in environments conducive to the development of the pathogen. Defined in this way, resistance durability could only be identified *a posteriori*, after it had been overcome. It was also understood as an intrinsic property of the resistance gene, whereas in fact it results from a combination of effects operating from the scale of the gene to that of the production region and system. Resistance durability is therefore now regarded as a property of the entire host-parasite interaction rather than a property of the resistance gene alone.

The goal of "sustainable resistance management" is to design management and deployment strategies for the introduction of resistance factors that will reduce disease incidence in the short term while minimizing the likelihood of pathogen adaption in the long term. Such strategies require a focus on both breeding programs, which must identify resistance genes or combinations of genes with potential for greater durability), and on resistance deployment in space and time. In some cases, sustainable resistance management thus implies some type of collective organization, with resistance genes being recognized as a "common good."

Breeding strategies for more sustainable resistance

Several strategies have been tested or may be imagined as ways of improving resistance durability.

To identify resistance factors or combinations with the potential for greater durability

A pathogen becomes virulent when the molecule(s) it produces (called *effector(s)*), rather than being detected by the plant and thus triggering the plant's defense reactions (ETI), escape recognition by the plant. Changes in a pathogen's effector molecules result from mutations or deletions in the corresponding gene sequence (a gene for *avirulence*). While allowing the pathogen to overcome the host plant's resistance, these genetic changes may also alter the initial function of the gene: this is the "cost" of virulence, paid for by the "mutant" parasite on susceptible hosts lacking the resistance gene. The fitness of the virulent mutants may thus be less than that of avirulent individuals on plant genotypes devoid of the resistance gene.

These costs to fitness in association with virulence are difficult to measure, but have been effectively quantified in viruses, bacteria, and nematodes, as well as in some oomycetes targeted by copper, such as *P. infestans* in potatoes. The costs of virulence can be gradually compensated for by subsequent mutations that restore the gene function. For resistance to be long-lasting, the costs to fitness associated with virulence must be high and not easily compensated for by the pathogen.

Major resistances that recognize effectors not essential to pathogen fitness can thus be easily overcome *via* a rapid change in the allele sequence or even the loss of the effector. For this reason, under field conditions, the most durable forms of plant resistance are those targeting avirulence gene products (*i.e*. effectors) that are essential to pathogen fitness. New approaches, combining genetic engineering and a detailed understanding of the underlying mechanisms, are now being developed to identify resistance genes with the greatest potential for longevity. **Effectoromics** is a resistance selection strategy based on first identifying within the pathogen an avirulence protein (effector) essential to its survival, and then identifying within the host the corresponding gene for resistance. In theory, this approach should ensure a long-lasting resistance. Several INRA teams are exploring this new approach in their work on *Venturia inaequalis, Phytophthora infestans* and *P. capsici*. For *P. infestans*, this approach has led to the identification of a new resistance protein and the corresponding genes in *Solanum*.
Partial (quantitative) resistances are believed to be intrinsically more durable than total (qualitative) resistances, since adapting to the resistances conferred by several QTL will require a greater number of genetic modifications (recombinations and/or mutations) in the pathogen genome. Nevertheless, some cases of adaptation to quantitative resistance have been observed, particularly in diseases of cereals but also in grapewine downy mildew (adaptation to the partial resistances possessed by the varieties *Regent*, *Prior* and *Bronner*) or in potato late blight (partial resistance of the cultivar *Désirée*). The breadth of the QTL action spectrum is an important factor: whereas broad-spectrum QTL exert only a weak selection pressure on pathogen populations, narrow-spectrum QTL, because they are more specific, actively select for adapted strains. Nevertheless, in the case of apple scab, orchard monitoring over eight years showed a reduction in the effectiveness of broad-spectrum QTL (F11 and F17), but a very slight loss in effectiveness of a narrow-spectrum QTL (T1).

Breed for broad-spectrum or complementary genes

Current breeding programs seek to exploit the mechanisms of host resistance in plants. Whenever available, they seek to make use of resistance genes that interact with pathogens according to the gene-for-gene model, which in most cases will be specific to specific strains of the pathogen.

By contrast, non-host resistance refers to the resistance of all genotypes of a plant species to all genotypes of a given pathogen. These mechanisms include passive defenses (e.g., physical barriers to infection provided by the plant cuticle) and active defense reactions induced by general pathogen elicitors (referred to as *pathogen-associated molecular patterns*, or PAMP, such as bacterial flagellin or fungal chitin), which are detected by the plant's pattern recognition receptors (PRR). As the most common form of plant resistance, non-host resistance is believed to be more durable than host resistance. An improved understanding of the mechanisms and genetic determinants of non-host resistance could eventually make it possible to develop varieties with more durable resistance to major pathogens.

The use of biotechnology, particularly genome editing, is cited in the literature as a way of introducing non-host resistances into the most important crop plants. Similar ambitions were cited for transgenic techniques in the early 2000s. Several such strategies are described in recent publications: transferring PRR receptors into plant species that lack them (used to obtain tomatoes resistant to several bacterial pathogens); designing transgenic plants that produce small RNA segments, which interfere with the transcription of key pathogen genes essential to virulence (ongoing trials targeting viruses, *P. infestans* or *B. lactucae*); deactivating plant genes for susceptibility to a pathogen as a way of inhibiting infection.

Combining multiple resistance genes in a single variety

Combining (also called "stacking" or "pyramiding") resistance genes within a single variety can improve resistance durability. Gene stacking should be more effective against pathogens i) with limited capacities for dispersion (soil-dwelling parasites) and ii) with obligate asexual reproduction (so unable to use recombination to gather several virulence factors into the same genome). It should also be more effective if the costs of virulence associated with each resistance gene are high and their combined costs are additive or multiplicative. For this strategy to be effective, the stacked genes must not have been previously used individually; if they have, pathogens are more likely to be able to overcome the resistance gene by gene. This condition is almost impossible to guarantee, however, due to the lack of coordination among different breeding programs.

Gene stacking is the currently favored strategy within breeding programs for downy mildew-resistant lettuce and grapewines. It may also be useful for controlling late blight in potato and tomato. In apples, stacking the QTL *T1*, *F11*, and *F17* is a promising avenue: by acting against different stages in the infection cycle of *V. inaequalis*, this association is more effective than the individual QTL.

Another form of gene stacking is to combine a major resistance gene with QTL for partial resistance. This kind of combination generally proves more durable than introducing a major resistance gene alone into a susceptible genetic background. The partially resistant genetic background appears to reduce the size of the pathogen population enough to prevent, or at least considerably slow down, pathogen adaptation to the major resistance gene. The long-lasting resistance to *P. infestans* of the potato varieties *Sarpo Mira* and *Bzura* are believed to be due to their association of partial and total resistance factors.

Some varieties (of tomato, potato, grapewine, etc.) combine resistance factors targeting different pathogens and/or pests. Combining multiple resistances within a single variety can be problematic, however, since plant defenses to biotrophic pathogens (those that feed on living tissues, and often have a limited range of hosts) and necrotrophic pathogens (which rapidly kill host tissues, and often have a wide range of hosts) are physiologically antagonistic. It is rare that a plant can simultaneously mobilize one set of defense reactions depending on salicylic acid and another controlled by jasmonic acid.

Create heterogenous varieties

Increasing the genetic diversity of resistance factors within crops improves the durability of major resistances. Breeding programs can emphasize intra-specific genetic diversity in two ways. The first consists of practicing massal selection within open-pollinated populations. Research can contribute to this by improving methods of participatory selection that allow for the dynamic management of diversity.

The second way is through the creation of multiline varieties; that is, varieties made up of genotypes that are phenotypically identical but carry different major resistance genes. This approach has been used with success against rusts in cereals. Selecting for multiline varieties is however slow and painstaking. Gene-editing approaches (using systems such as CRISPR/Cas9) make it possible to construct true isogenetic lines that differ only in their resistance genes, but these types of genetic engineering techniques are controversial in many countries, and are currently prohibited in OA because they are considered transgenic.

Strategies for the use of resistant varieties in the field: organizing genetic diversification

Growing mixtures of resistant and susceptible cultivars together can limit the spread of disease in three ways: the resistant component can act as a physical barrier to the spread of inoculum among susceptible plants; it can reduce pathogen reproduction and thus the quantity of inoculum present in the field; and finally, the presence of avirulent pathogen genotypes, capable of infecting the susceptible host plants but not the resistant plants, can trigger plant defense mechanisms that may then be effective against the virulent strains. The genetic specialization imposed on the pathogen by the diversity of the host plants can reduce the risk of emergence of multi-virulent strains.

Theoretical studies using mechanistic models of disease occurrence suggest that the effectiveness of cultivar mixtures is strongly dependent on the size of the contiguous land area occupied by a single genotype: the smaller this is (mixtures of many genotypes, plants with smaller leaves, low planting densities), the greater the predicted effectiveness of the association. Effectiveness will thus be lower for a simple juxtaposition of fields planted to susceptible or resistant varieties (landscape mosaics, which in any case are relatively difficult to organize) than for a mixture of the same varieties within a single field. This theoretical finding is confirmed by observations made in orchards: the association in equal amounts of two apple varieties, one scab-susceptible (*Smoothee*®, *Melrouge*) and the other scab-resistant by virtue of the gene *Vf* (*Baujade*, *Pitchounette*), is more effective in controlling scab on the susceptible variety when the two variety types are mixed within rows than when they are planted in alternate rows.

Broadly similar results have been observed for late blight in potato, while at the same time underscoring the importance of inoculum pressure on the effectiveness of variety mixtures. Trials suggest that variety mixtures can be effective in conditions of low or moderate late blight pressure (alternating rows of partially resistant cultivars reduce the incidence of late blight on the susceptible *Bintje*); whereas in conditions of strong late blight pressure, cultivar mixtures had no measurable effect. If mixtures have good effectiveness where disease pressure is lower or where it is artificially reduced by pesticide applications, their use in combination with reduced fungicide treatments could be effective in climatic environments strongly favorable to pathogen development. In apples, low-dose fungicide use resulted in a significant reduction in the incidence of scab (up to a 75% reduction on leaves and up to a 70% reduction on fruits) on a susceptible variety when grown together with a resistant variety (mixed varieties within rows). Similar results were obtained for late blight in potatoes under conditions of high disease pressure.

Cultivar mixtures have no effect, however, once strains of the pathogen virulent on the resistant variety had become well established in the field. Thus, a mixture of susceptible and resistant apple genotypes showed no measurable advantage when virulent strains of *V. inaequalis* were present in the orchard; unfortunately, this loss of resistance for the *Vf* gene is now common in France. The use of **monitoring programs** to study virulence evolution in the field is thus important to help guide breeding programs for resistant varieties and to inform growers' variety selections. One such initiative based in Switzerland, "Virulence monitoring for *Venturia inaequalis*," has been monitoring the incidence and severity of scab on a range of apple varieties, primarily in European orchards, since 2009.

Some conclusions

An abundance of genetic resources for variety development

For the majority of the relevant disease systems – including downy mildews, late blight, and scab – a plethora of genetic resources are available for use in breeding programs. Generally obtained from species closely related to the crops in question, this material often includes specific resistance genes with limited longevity in the field (one to several years maximum); in some cases, QTL for quantitative resistance can also be found. Resistance to other pathogens targeted by copper (particularly bacterial diseases) are more frequently of the quantitative type.

Breeding for resistance is a relatively new priority

Selecting for resistance has only recently emerged as a priority within breeding programs. Previously, other objectives (yield, agronomic traits, crop quality characteristics) were considered more important. In some cases, resistance genes were found to be genetically linked to undesirable agronomic traits (lateness, for example); in other cases, difficulties existed with respect to the genetics of the resistant trait (e.g., a polygenic trait difficult to introgress, or low fertility of resulting crosses, requiring the use of cell biology techniques such as embryo rescue).

Breeding efforts can become focused on one or a few major specific resistance genes as soon as these become available. Gene stacking is often emphasized (as with downy mildew in grapewines and lettuce, for example), sometimes including QTL for partial resistance, which can prolong the durability of the stacked genes.

The use of new genomic techniques, in tandem with an improved understanding of the underlying biological mechanisms (identification of effectors and the use effectoromics for rapid assessment of large populations; gene editing), have the potential to greatly reduce the time necessary to obtain new varieties or other interesting genetic combinations. Nevertheless, questions persist as to the acceptability of these techniques (especially in OA) and as to the importance of different traits depending on the production system (relative value of different criteria, introduction of new traits into specific ideotypes).

Limited breeding efforts specifically for OA

Plant breeding intended specifically for OA remains rare: 95% of cultivars currently used in OA were selected for the management regimes of conventional agriculture, with high levels of inputs. In practice, however, the most important traits for selection are different for the two types of production systems.

In Europe, a few breeding programs include an OA management trial within the final selection phases. These is true for cereals breeding programs in Austria, in Germany, and in France (by INRA). Very few programs include OA management from the onset, however. One such program in Switzerland has been breeding for organic wheat production for the past 25 years, including an effort to develop resistance to diseases that are particularly problematic in OA (Septoria blotch, Fusarium rots, rusts).

Participatory plant breeding programs, which seek to directly involve farmers in the development of new cultivars, are well suited to the values and requirements of breeding for OA. Two examples exist (in Brittany, France, and Oregon, USA) of participatory breeding programs for broccoli that include conditions specific to OA. These types of programs are more likely to take into account the existence of strong *genotype x environment x OA management* interactions.

The availability of resistant cultivars within catalogues is highly variable

The number of commercially available resistant varieties varies widely depending on the disease system: it is very high for downy mildew in lettuce, for example, but remains low for downy mildew in grapewines. A significant increase in the latter is anticipated in the coming decade, however.

Adoption of resistant varieties is generally limited; nevertheless, examples of the loss of resistance under production conditions are fairly common. Significant work thus remains to be done to promote the adoption of resistant varieties by farmers, their understanding by distributors, and their appreciation by consumers. Tools to facilitate this work are being developed, including the French national monitoring network OsCar, which since 2017 has organized experience-sharing on the performance of resistant grapewine varieties under different production systems. A goal of the network is to assist grapewine growers in developing management regimes to minimize the use of copper.

Managing resistance for sustainability is a key challenge

The major challenge for a more systematic use of resistant varieties is the sustainable management of their durability. Resistance durability can be determined by the types and sources of resistance selected for use (non-host resistances being *a priori* very long-lasting but difficult to introduce into "foreign" genetic backgrounds; specific or non-specific host resistances), by the manner in which genotypes are assembled (stacking, diversification, multiline), and finally by the use of strategies for the introduction of new cultivars (spatial and temporal diversification) as a function of their resistance characteristics.

Qualitative and quantitative resistances should ideally be used in combination with other disease-control methods in order to reduce the size of pathogen populations and thus their adaptive capacity. While the use of resistance alone can certainly allow for reductions in copper use, only combinations of solutions (e.g., total and partial resistances in a single cultivar, treated with an effective biocontrol product) will make it possible, in some cases, to totally abandon the use of copper. Too little research has been devoted to the gains in resistance durability that could be achieved from the coordination of genetic approaches to disease control with agronomic approaches, including crop protection products, despite the worldwide emphasis on integrated pest management (IPM) over the past thirty years.

Finally, interactions between resistant plants and microbial communities, including both pathogens and non-pathogens, is an underexamined but potentially critical question to consider when developing strategies for the introduction of resistant varieties. How do resistant plants influence microbial communities, and how do microbial communities influence resistance longevity? This is an entirely new frontier for research, exploration of which has barely begun.

Natural plant defense stimulators

The bibliographic corpus on this topic was obtained in two phases. An initial corpus of 30 articles was established using a WoS query to search for review articles on elicitor-induced resistance induction and plant defense mechanisms (for any and all plant-pathogen interactions). A second corpus of approximately 600 primary articles was obtained from a WoS query focused on elictor-induced resistance to diseases treated with copper. The second corpus proved much richer. Articles in this category having been appearing at a steady rate since 2000. Most concentrate on in diseases of major economic importance: late blight in potatoes and tomatoes; downy mildew in grapewines; scab in pip fruit; and bacterial diseases in tomatoes. Other diseases addressed include bacterial diseases in kiwi, peacock spot in olives, etc.

Elicitor-induced resistance

A brief history

The discovery of plant defense stimulators (PDS), also known as natural defense stimulators (NDS), or elicitors, is linked to research on plant defense mechanisms against pests, now jointly understood as components of plant immune systems. The existence of plant immunity was hypothesized in the early 20th century by researchers studying the phenomenon of plant resistance to diseases.

In the 1970s, the study of these mechanisms using biochemical methods made it possible to demonstrate that molecules of microbial origin or from phytopathogenic fungi were capable of triggering the production of antimicrobial metabolites in plants. These molecules were thus termed "elicitors."

Today, the term "elicitor" refers more generally to any product that triggers defense reactions. It is synonymous with the term "resistance inducer," often used in English. In France, the acronym "SDP" (*stimulateur des défenses des plantes*) has been adopted, notably among crop protection specialists.

Using elicitors for plant protection was first proposed in the 1980s. The first PDS product (Bion®, of synthetic origin, released by Ciba-Geigy) was approved for crop protection purposes in the 1990s. In more recent years, numerous publications have reviewed elicitors of various chemical natures (saccharides, proteins, etc.), origins (microbial or plant cell wall materials, phytopathogenic oomycetes, etc.), and modes of action within the plant. The late 1990s and early 2000s saw further research seeking to increase PDS efficacy under production conditions, as well as the commercialization of several natural PDS (including laminarin, chitosans, harpin, or plant extracts), with varying effectiveness.

Scientific studies on PDS and their effects continue to be published regularly; but the range of PDS on the market remains limited, as do their practical applications.

Plant defenses and PDS action

The application of external elicitors aims to imitate a pest or pathogen attack and thus to trigger plant defenses artificially, as a preventive measure. Most non-hormonal elicitors are considered to be PAMP and thus should elicit a PTI. It is believed that PAMP are recognized by most plant species, although there are many exceptions. Hormonal elicitors can activate a powerful response similar to that mobilized by an ETI, but this has only been verified in a few cases.

Note that microorganisms used in the biological control of diseases often have a mode of action that involves elicitor-induced resistance (see above).

Elicitors and their applications

Most PDS that have been studied are either experimental products or products that have yet to receive approval for use as crop protection products in France. PDS that have received market authorization in France to date are Bion (benzothiadiazole, Syngenta), Vacciplant (laminarin, Arysta Goëmar), Régalis (prohexadione, BASF), Stifénia (fenugreek powder, SOFT), and, most recently, an oligosaccharides-based product (COS-OGA) distributed by Syngenta. Stifénia is not used for the plant diseases of interest to this study.

Table 2.5 summarizes the principal PDS examined in the literature (primarily in the scientific literature) and specifies their observed effectiveness against the major diseases targeted by copper (late blight, downy mildews and bacterial diseases).

Synthetic PDS

These products are not allowed for use in OA, but are interesting to consider as a point of comparison or as reference products. They have received the most study and in some cases show high levels of effectiveness.

Analogues of natural plant hormones

Benzothiadiazole (BTH), by far the most studied molecule, is a synthetic functional analogue of salicylic acid, invented by Ciba-Geigy and approved in France under the trade name Bion®. Its efficacy is highly variable depending on the disease system and on environmental conditions; as expected, it appears to be active specifically against diseases triggering the SA pathway in plants. BTH has partial efficacy against various fungal and bacterial diseases (Table 2.5). It allows for a reduction in copper applications against *Xanthomonas* in tomato, and may be used against strains of these bacteria that are resistant to copper.

β-aminobutyric acid (BABA) is a non-protein amino acid, rare in nature, produced at low concentrations by plants in response to parasite attack. It is relatively effective against downy mildews and has a strong synergistic effect with mancozeb. It also allows for a reduction in fungicide applications, for example against late blight in potatoes. However, its efficacy depends on the potato cultivar, and decreases on downy mildew in grapewines when climate conditions are more favorable to disease development. Despite its apparent potential, BABA has not been developed commercially. It is not patentable, its ecotoxicological profile is unknown, and it is slightly phytotoxic.

Anti-gibberellics (growth regulators), including prohexadione-Ca (used as a growth regulator for pome fruit), have also been shown to be resistance inducers against fire blight. This molecule is approved in France under the name Régalis®.

Phosphites (salts of phosphorous acid)

Many products containing these salts (most often in the form of potassium salts) are available on the market. They are especially active against downy mildews and late blight. Field trials generally show that they have near-total efficacy in situations of average disease pressure, but are insufficiently effective in cases of high disease pressure and thus must then be used in combination with a reduced-rate application of fungicide. While phosphites definitely have elicitor activity, at the rates typically used they are primarily biocides. Their favorable ecotoxicological profile suggests good potential for limiting the use of synthetic fungicides and/or copper. Nevertheless, being a mineral product but of synthetic origin (like copper preparations), they are not allowed for use in OA.

Natural PDS

Purified plant hormones

Salicylic acid (SA), a key hormone for different types of plant defense, is little used in practice. There are a few situations in which it has shown convincing results, however, such as in olives against peacock eye disease. Its phytotoxicity makes SA difficult to use; BTH is less toxic and is thus generally preferred. The methyl ester of salicylic acid (methyl salicylate), the primary compound in the essential oil of wintergreen, is also a PDS and may see future development.

Jasmonic acid (JA) and **methyl jasmonate** are likewise little used in practice. These two plant hormones have strong physiological effects, and can disrupt plant development. JA provides partial protection against cryptogamic diseases in softwood tree species and against powdery mildew in grapewines.

Oligosaccharides and polysaccharides

These complex sugars are the structural constituents of the cell walls of microorganisms, arthropods (chitin) and plants (pectin). Various studies have shown some effectiveness of **simple sugars** (monosaccharides) in activating general plant defenses. These are mostly likely the same pathways mobilized by the destruction of plant cell walls during a pest or pathogen attack. Intellectual property on these molecules is difficult to protect, however, which limits their development potential as crop protection products.

Chitosan, a derivative of chitin, has been the focus of many studies. Chitosan is edible and has multiple agrifood and cosmetic uses, although it may be an allergen for some people. It is an elicitor, but it also has a relatively strong direct biocidal effect, which seems to be its primary mode of action at higher application rates. It has been tested against many different diseases, with results that are variable (Table 2.5) but among the best of those obtained with natural products. Comparison of results is difficult due to the number of different commercial formulations, including some for which the type of chitosan is not specified. Little use of this product has so far been made in France.

COS-OGA (oligochitosan and oligogalacturonic acid) is a formulated mixture perfected by a Belgian start-up, recently approved for use and distributed by Syngenta in France. It has an efficacy of 40-50% against powdery mildew in grapewines and also has some effect against downy mildew. According to Syngenta data on trials made in vineyards in 2016, COS-OGA allows for a reduction of the copper rates required to control downy mildew, but this result remains to be confirmed over several years and in different geographic and environmental conditions.

Glucans are polysaccharides of glucose, formed as reserve sugars of certain algae and as the structural constituents of the cell walls of some mushrooms. Laminarin, a glucan extract from algae, is the active ingredient in several products marketed by Goëmar in France and approved for use against a number of diseases (Table 2.5). Its efficacy on apples and major field crops is controversial, however. A recent article suggests that

Table 2.5. Major PDS active under production conditions against diseases targeted by copper

	Active ingredient	Target pathogen	Efficacy* (under production conditions)	Commercial development**
Synthetic compounds (not allowed in OA)	Benzothiadiazole (BTH) = Acibenzolar-S-Methyl (ASM) Functional analogue of salicylic acid, but less phytotoxic	Scab / apple Bacterial diseases / tomato Bacterial blight / kiwi	+ ++ ++	Bion® (*Syngenta*), approved in France against powdery mildew in wheat, bacterial diseases in tomato, rust in chrysanthemum, etc.
	β-aminobutyric acid (BABA)	Downy mildews *Alternaria*	++ +/++	No commercial development; impossible to patent as such
	Phosphites (phosphoric acid salts) Elicitors and fungicides	Downy mildews Scab / apple	+++ +/++	Numerous products available on the market
Natural products	Salicylic acid (SA) Plant hormone; phytotoxic	Peacock spot disease / olive	+++	Methyl salicylate (wintergreen oil, a compound from the EO of Gaultheria) may see future development
	Chitosan Elicitor and fungicide	Downy mildew / grapewine Late blight / potato Mildew / millet (seed treatment) Bacterial diseases / tomato Bacterial blight / kiwi	 weak +/++	Various commercial formulations (type of chitosan not specified)
	Laminarin (glucan extracted from brown algae)	Scab / apple Powdery mildew / grapewine, cereals Downy mildew / grapewine	controversial no	Vacciplant® (*Arysta Goëmar*), approved in France against powdery mildews, downy mildew in lettuce, fire blight and scab in apple
	Chitooligosaccharide and oligogalacturonic acid (COS-OGA)	Downy mildew / grapewine	+	COS-OGA (distributed by *Syngenta*), approved in France but not authorized in OA
	Harpin (bacterial protein)	Scab / apple	controversial	Initially marketed as a PDS (Messenger®), now marketed as a biostimulant
	Extract of penicillin (Pen)	Scab / apple Downy mildew / grapewine Late blight / potato	+/++ +/++ 0	Product considered promising, no commercial development
	Rhamnolipids	Downy mildew / grapewine	+	
	Yeast cell walls	Downy mildew / grapewine	+	MA obtained by *Agrauxine*
	Compost extracts	Bacterial diseases / tomato	+	

* + : less than 50%. ++ : between 50 and 75%. +++ : above 75%
** In **bold**: products currently approved for use in France. In italics: companies marketing the product

the association of laminarin and reduced rates of copper shows promise against downy mildew in grapewine under conditions of moderate disease pressure.

Extracts of microorganisms, proteins and metabolites obtained from microorganisms

Extracts or fractions of microorganisms. Filtrates from the production of mushrooms, oomycetes, and phytopathogenic or beneficial bacteria, as well as parietal extracts that contain elicitors, have been the focus of various publications reporting on their potential as PDS, although mostly in laboratory settings. Thus, an **extract of *Penicillium*** *sp.* (Pen) was shown to have PDS activity against various bacterial and fungal diseases (Table 2.5), but this apparently promising product does not seem to be a target for commercial development. **Extracts of yeast cell walls** from *Saccharomyces cerevisiae,* a by-product of the industrial production of yeast, are also PDS; the company Agrauxine has obtained a market authorization for a version of this extract for use against downy mildew in grapewines, with distribution to be undertaken by BASF.

Proteins from microorganisms. Many MAMP (*microbe-associated molecular pattern*, that is, molecules from any type of microorganism, pathogenic or not) are protein-like: bacterial flagellin, oomycete elicitins (cryptogein, oligandrin, etc.), bacterial harpin, etc. For now, however, most of these are still the focus of basic research. Several studies have shown that oligandrin is a resistance-inducer in various laboratory scenarios. Others have shown that elicitin-like substances obtained from oomycete cell walls have a partial, but very significant efficacy against Cercospora leaf spot in sugar beet. A Spanish company (Plant Response) is working to develop a product based on MAMP proteins from plant-pathogenic fungi (*Sclerotinia sp.*, for example).

Harpin is the active ingredient in a commercial product called Messenger®, with various applications. Its effects in the field against apple scab or fire blight in pear are disputed. Harpin is currently being sold as a biostimulant and no longer as a PDS.

Microbial metabolites. Research on the mechanisms by which beneficial microorganisms increase plant resistance has led to the identification of several microbial metabolites that play a major role in this phenomenon. This work suggests that microorganisms may be an important source of PDS in the future. The application of products that have been well described and well formulated is usually easier than the application of live microorganisms.

Other compounds: vitamins, liposaccharides, lipopeptides, fatty acids

Vitamins such as riboflavin and thiamin show PDS activity against grapewine downy mildew under laboratory conditions, but their usefulness in field conditions has not yet been demonstrated. Rhamnolipids and surfactins (surfactants of microbial origin) have a distinct PDS effect in the laboratory, but the few trials that have been made of rhamnolipids in vineyards have not shown consistent effectiveness against downy mildew. Hexanoic acid (a fatty acid) shows some efficacy against fungal and bacterial diseases of citrus, notably in applications to the roots (*via* irrigation of the substrate); it also has an effect against botrytis in tomato.

Various products

PDS from various **plant extracts** have shown relatively high efficacy against several diseases: an extract of ivy against apple fire blight, extracts of rhubarb and Solidago against downy mildew in grapewine, etc. These formulations definitely activate plant defenses, but frequently also act directly by blocking pathogen germination. It is thus difficult to clearly distinguish the importance of each mode of action in the overall level of efficacy. This could be a source of variability in the results that have been observed, if the conditions for expression of one or both mechanisms are variable.

Some **composts** used as crop substrates induce disease resistance, as do water-based extracts of these composts ("compost teas"). The determinants of this effect have not been clearly elucidated, and appear to vary depending on the compost.

Several **commercial fertilizer products** based on mineral salts (NPK, micronutrients) with or without added organic compounds, have shown partial but significant efficacy against grapewine downy mildew and apple scab (according to the Joint Technology Network Elicitra). The precise composition of these products is not known, however, and their mode of PDS action remains to be determined. Information on these products is currently more available in the gray (unpublished) literature than in the scientific literature. Their use may increase if they allow for a reduction in fungicide treatments at a reasonable cost.

Some conclusions

PDS: somewhat effective, but less than copper

Analysis of the scientific literature suggests that there is currently no PDS that is as effective as copper for controlling the major pathogens targeted by copper treatments. Some synthetic PDS (particularly elicitors of the salicylic acid pathway, like BTH) can be as effective as the best fungicides against certain diseases not targeted by copper. In contexts where BTH does have an effect against diseases targeted by copper, the effect is often better than that of natural PDS. In the case of bacterial diseases, for example, some reports suggest that BTH is almost as effective as copper.

All PDS of natural origin show a partial efficacy, in the range of 20-70%, but usually much below than 50% under production conditions; they are thus much less effective than copper. There are fewer published results from field trials with these products than for synthetic substances. Chitosans are often classed among the most effective in the published trials, but these are also biofungicides.

Phosphites (salts of phosphorous acid): a different kind of PDS?

These products have a remarkable efficacy against oomycetes, at least 70 to 80%. Their classification as PDS has been a point of debate: while their elicitor effect has been shown, at the concentrations typically used it is their fungicidal effect that is predominant. Whether phosphites should be considered synthetic or natural is another point of discussion: are they really any more synthetic than the copper salts currently used in OA? Phosphites were approved for use in OA in a number of European countries (Germany, Greece, Austria, Spain, Hungary, Czech Republic) up until September 2013, when this authorization was revoked in conjunction with their classification as a crop protection product rather than as a fertilizing or biostimulant product. They are now prohibited in OA in all of the EU. It should be noted as well that phosphites can accumulate in harvested crops.

Efficacy: Conflicting results, significant variability

PDS have a reputation for variable efficacy; this reputation is confirmed both by a review of the literature and by expert opinion. Field data to qualify this question are relatively scarce, however. In addition, the many biotic and abiotic factors likely to affect plant response to PDS, and thus PDS efficacy, remain poorly understood. A few studies suggest that plant genotype, disease pressure, mineral nutrition, and the developmental stage of different plant organs can affect plant response, but these are questions that require further investigation. Research on the mode of PDS action under production conditions is also incomplete, making it difficult to optimize PDS treatments.

While some published studies report significant effectiveness for PDS, expert opinion (e.g., from the Elicitra network) consider them to be ineffective for the same plant diseases. These contradictions relate, for example, to the effects of laminarin and harpin against fire blight and scab in apples. One can surmise that the published efficacies correspond to tests conducted under "optimal" conditions, both in terms of the experimental conditions and the physiological condition of the plants.

Research for new PDS is ongoing

New PDS of various types are regularly reported in the scientific literature: metabolites or extracts from beneficial or food-related microorganisms, "simple" fatty acids (such as hexanoic acid, which shows promise against citrus diseases), secondary plant metabolites (methyl salicylate), other more or less familiar plant extracts, etc. Nevertheless, the relatively long period surveyed by the bibliographic analysis for this ESCo (2000-2016) allows one to observe that PDS that appeared valuable and even of proven efficacy in the field a dozen years ago have since been abandoned, following the example of "Pen." This situation highlights the barriers to commercial PDS development, which involves a wide array of factors: the influence of environmental conditions, persistence, plants' capacity to respond, the impact of disease pressure, bioavailability within the plant, the importance of product formulation, commercial profitability, etc.

Let us note that the present expertise is based primarily on the published scientific literature, with only a limited view of the scope of private research – mainly carried out by SME, which generally do not provide access to their results. Private research is clearly active and ongoing, however, and is likely to result in new PDS being brought on to the market.

As with other biocontrol materials, increased societal pressure to reduce pesticide use combined with the recent entry of the major crop protection product companies into the PDS field can only accelerate their integration into existing crop protection practices. The example of COS-OGA, which was invented by a startup and is now being marketed by a major firm, illustrates this dynamic. Potential public incentives (of the Ecophyto type) and the continued regulatory review of existing pesticides, with several molecules being withdrawn from the market, constitute two major reasons for the further development of PDS. On the other hand, the need to stimulate plant defenses *prior* to pest attacks suggests a need for improved integration with locally adapted tools for early pathogen detection – an area in which there has been very little published research.

PDS will not replace copper, but may allow for reduced copper rates

The value of using PDS in combination with reduced levels of fungicides (usually as a mixture) has been understood for at least the past 15 years (combination of BTH and synthetic fungicides, synergies between BABA and mancozeb, etc.). Some trials using combinations of PDS and copper products have shown encouraging results against downy mildew in grapewines. A useful goal now would be to develop a theoretical basis for identifying what are likely to be the most effective combinations, including PDS-PDS combinations and PDS-biocontrol organism combinations. This recommendation is in line with the conclusions of the large-scale study made by Dagostin et al. (2011), which identified natural products with partial efficacy against grapewine downy mildew, including both PDS and biocides, as candidates for subsequent trials of reduced-rate copper treatments.

Isotherapy, homeopathic and biodynamic preparations

Definitions and principles of action

Isotherapy, based on the principle of "treating like with like," is employed in various forms in human and veterinary medicine (vaccination, for example), and sometimes in the management of plant health. Its primary application with respect to plant health consists in using highly diluted preparations of the pathogen itself or of infected plant tissues which are then "potentized" (that is, agitated for an extended period). These preparations, which can be made using living or dead organisms (ashes of incinerated pests, for example), are then sprayed on the plants to be treated. Other forms of isotherapy have also been used in plant protection, occasionally with some success, such as protocols to provide plants with relative immunity using weakened strains of a virus, or inoculating plants with "hypovirulent" strains of a fungus.

Homeopathy is based on a similar idea, although the nature of the active principles involved is somewhat different. It uses highly diluted natural extracts (of plants, soils, etc.), some of which can be highly toxic at higher concentrations, again potentized *via* prolonged agitation. Another idea sometimes invoked is that of "the memory of water." Some homeopathic preparations are so dilute that they can no longer statistically contain a single molecule of the original substance; advocates maintain that the activity of these preparations comes from the capacity of water to retain the molecular imprint of these substances. As with isotherapy, this type of alternative of "gentle" medicine is relatively widespread in human and animal health.

Biodynamics uses a group of nine preparations (designated by the numbers 500 through 508), which recipes were outlined by the founder of the biodynamic movement, R. Steiner. Among the ingredients are cow manure and finely ground quartz incubated in a horn (preparations 500 and 501) or plant extracts (502 through 508). Again used in highly diluted form, these preparations are supposed to favor plant growth, development, and resistance to pests and pathogens. Biodynamic preparations can be made by the farmer him- or herself or purchased from specialized suppliers.

Lastly, we should note the use of "**proteodies**," musical sequences that are said to interfere with the amino acid sequences of proteins. This approach has received widespread media attention, but its scientific fundamentals have not been established. A search of the *Web of Science* on the keywords "genodics" (the term proposed by J. Sternheimer, originator of the idea) or "proteod*" turned up no references.

Crop protection effects

These practices have been the focus of very few academic publications. The analysis offered here is thus based for the most part on a small number of technical articles and other works addressing these methods of crop protection.

Isotherapy

The small number of available references on the efficacy of highly diluted and potentized preparations of diseased plant tissues and/or target pathogens are all from the "gray" literature (technical journals, summary reports, websites intended for a general audience). Most of these do not clearly differentiate between the methods of isotherapy and those of homeopathy.

With respect to the diseases targeted by copper applications:

- Several repeated trials on apricot blossom brown rot all concluded that the use of isotherapy preparations at 2, 4, 8, and 12 DH had negative effects, increasing the severity of the disease.
- A report on a number experiments made by an homeopath and amateur gardener found a neutral or negative effect of isotherapy treatments to cure late blight and *Corynebacterium* in tomato. Nevertheless, the author states that he believes the treatments did have an effect, although recognizing that most of the trials could not be analyzed statistically due to an insufficient number of plants or the absence of untreated controls. The experiments did not seek to test the use of preventive isotherapy treatments.
- A "personal report" by a Belgian farmer growing cereal crops and using preparations described as isotherapy recorded promising results. His treatments also included unspecified "trace nutrients and hydro-alcoholic extracts," however, making it impossible to attribute the observed effects solely to the "isotherapy" component. In addition, the article notes that use of a supplemental chemical treatment was sometimes necessary.

Homeopathy

With respect to homeopathy (highly diluted plant or mineral extracts), the only reference found in the *Web of Science* reported no statistically significant positive effect of these preparations in combating pests of tomatoes in the field, and sometimes reported negative effects on disease management (particularly septorium on the foliage). More encouraging results were obtained in a greenhouse trial, but the experiment was not repeated and the findings thus require further confirmation.

A recent study surveyed the principal homeopathic remedies used against the most common diseases of tree fruits, market garden crops, and flower crops. The study lists a series of experiments and observations, but does not provide the underlying data that would allow for a scientific assessment of the efficacy of the suggested "recipes."

Biodynamics

A meta-analysis of various trials published on the effects of each of the nine biodynamic preparations found that none of them showed demonstrable biological activity in classic factorial experiments (Chalker-Scott, 2013). The author emphasizes that these overall negative findings do not necessarily disqualify all biodynamic practices: they only show that none of the preparations on its own had a measurable effect on crop health. It may be noted that Steiner himself did not make reference to a scientific approach, but rather a spiritual one, to support his practices; he believed that his methods did not need to be confirmed by scientific trials.

Several of the biodynamic preparations, including nettle tea (504) and horsetail tea (508), are sometimes used alone in non-biodynamic contexts. Nevertheless, as noted above (section Natural biocidal preparations), it is difficult to demonstrate a replicable crop health effect from their use in classic factorial trials.

A detailed analysis of the composition of several sources of preparation 500 found that they contained elevated microbial populations, rich in *Bacillus spp*., with strong fermentation activity, and had a significant auxinic effect on test plants under controlled conditions, comparable to what could be expected in the field at the concentrations used in biodynamic preparations. It is thus possible that this preparation has a growth-promoting effect without a specific crop protection effect or any impact on the physical structure of the soil.

Some conclusions

Methods with little overall effect, and in some cases a negative effect

Based on the small number of publications addressing the crop protection applications of highly diluted and potentized preparations of various types (diseased tissues, homeopathic preparations, biodynamic preparations), one can conclude that none of those that have been tested to date has any demonstrated efficacy. Worse, some seem to have negative effects, with the application of these preparations (particularly those making use of live pathogens) leading to more severe infection – especially with isotherapy, where applications of living pathogenic material can indeed be regarded as direct inoculations. These types of preparations would thus appear to have very little potential use for crop protection.

Experimental conditions for evaluation should be reconsidered

With the possible exception of trials with biodynamic preparations, the majority of the available reports deal with curative rather than preventive treatments. It is thus possible that better results could be obtained with a different application schedule, aforing preventative sprays. It is also possible (and even likely) that the few successes reported after application of these preparations should actually result from either to post-treatment meteorological conditions that were unfavorable to the pathogen, or to other, adequate management practices or conditions (early observation of symptoms, immediate intervention, etc.). Given that the latter are insufficiently documented in the available references, it is not possible to examine this hypothesis further, nor to take a position with regard to the possible plant-defense elicitor activity of some of these preparations.

3. Agronomic management of crop health risks

In addition to the alternative methods to the use of copper-based products examined in the previous chapter, there are indirect methods of crop protection that can influence either the availability of disease inoculum (prophylactic methods) or the receptivity of the crop.

The bibliography assembled for this portion of the study included approximately one hundred references, a majority of which were scientific articles returned by a WoS search on different criteria for the agronomic management of diseases targeted by copper. Additional technical articles and project reports were identified by the participating experts.

Prophylactic methods

Prevention (prophylaxis) seeks to reduce primary contaminations, mainly by acting on the survival and availability of the primary inoculum of the pathogens. Preventive strategies are organized around three major objectives: 1) to eliminate inoculum reservoirs in or near crop production areas; 2) to limit the survival of any inoculum that is present; 3) to avoid external additions of inoculum. Prevention makes use of a wide range of methods, the effectiveness of which is often good due to the fact that they are implemented prior to disease outbreaks (*i.e.*, on small pathogen populations). However, since the success of prevention manifests as a “non-effect” (lack of disease outbreaks), it is more difficult to measure than curative interventions, with the result that the value of prevention is generally underestimated.

The bibliographic analysis focused on preventive methods useable in OA; those making use of products not allowed in OA, such as urea, are thus not presented here. The analysis prioritized trials conducted *in situ* and those that assessed prevention efficacy in terms of reduced crop damage.

Eliminating sources of active primary inoculum from field areas

Removing, shredding or burying infected crop residues

Apple scab is the most extensively studied disease in this regard. In temperate regions, *V. inaequalis* survives the winter in its dormant phase, primarily in dead leaves on the ground. In the following spring, ascospores formed on these leaves become airborne during rain events, resulting in primary contamination of leaves and fruits. Various studies have accordingly explored ways of reducing this supply of ascospores by intervening with respect to leaf litter on the orchard floor.

Trials conducted in both conventional and organic orchards, in several countries, have measured the effectiveness of gathering and removing dead leaves versus various methods of shredding and/or burying leaf litter so as to accelerate leaf decomposition and reduce the formation of ascospores. All of these trials (Table 3.1) confirm the value of managing leaf litter at the end of the growing season. The complete elimination of leaves is the most effective solution. A "coarse" shredding of leaves, performed with a hammer mill of a type that apple growers typically already have (for chipping tree prunings) is less effective than a "fine" shredding (requiring the purchase of a specialized shredder at additional cost).

Table 3.1. Efficacy of various preventive methods in controlling apple scab

Experimental design	Preventive technique	Reduction relative to no-prevention control		
		Inoculum	Incidence [or severity] on leaves	Incidence [or severity] on fruits
Northeastern **USA**: 8 conventional orchards (var. *McIntosh* and *Cortland*), 3 years	Shredding of leaves in the fall at 95% leaf drop	71%	[79%]	59%
France, Indre and Loire: 1 conventional orchard (*Gala*), year 2007-08	Shredding of leaves in the fall	90%	50%	76%
Hungary: 2 orchards in OA; copper use limited to 2 applications at budburst	Shredding of leaves		26 to 36%	
	Removal of leaves		42 to 47%	
	Burying of leaves		7 to 26%	
	Black plastic on the soil		56 to 69%	
France: OA orchard; 1 application of copper at budburst	Burying of leaves in the rows/ removal of leaves between the rows	95%	40 to 70% [61 to 67%]	55 to 83% [68 to 73%]
France, Limousin	Coarse shredding of leaves	60%		
	Fine shredding of leaves	80%		
Belgium: OA orchard (*Initial*), 2 years	Raking and shredding of leaves	42%		13%
	Raking and removal of leaves, burying in the row	75%		74%
Canada: two varieties, two years	Spraying of an antagonist (*M. ochracea*) on the canopy in the fall	71 to 80%		
Germany: 4 orchards in OA (*Jonagold*), 4 years (2011-2014)	Spraying of sugar beet extract on the canopy in the fall	44 to 70%	18 to 49%	7 to 88%

Burying leaves lying within the tree row is another very effective method that can complement the removal or shredding of leaves from the grass strips between the rows. Burying leaf litter requires tilling the soil along the row, which thus must be combined with operations to mechanically control weeds. Trials have demonstrated the importance of managing leaves within the tree row, where they are less easily raked and shredded. Burying leaves can be performed with a disk. It can be facilitated by digging a channel at the base of the trees (opening a furrow); the leaves collected in the furrow are then buried in the fall by hilling (closing the furrow).

Various strategies combining shredding or elimination of leaf litter between the rows and soil tillage in the rows have been developed and approved in OA. In a trial conducted in the Drôme (southeastern France), removing the leaves between the rows (with a leaf rake or vacuum) and burying those in the row (disking) reduced the airborne concentration of ascospores by 95% relative to management without these preventive methods. In association with an OA fungicide protection (one application of 2.5 kg Cu/ha at budburst, followed by applications of sulfur), preventive methods reduced the number of scab spots per fruit at harvest by approximately 70%, independently of the level of disease pressure.

A similar strategy of burying or shredding leaf litter is strongly recommended to control **anthracnose in walnuts** (caused by the ascomycete *Gnomonia leptostyla*), which overwinters in contaminated leaves on the soil surface.

Surprisingly, this preventive method of gathering and removing leaf litter does not appear to be scientifically documented against **downy mildew in grapewines**, although oospores present in leaf litter are understood to be the major source of primary inoculum leading to disease outbreaks. A search of the WoS found no articles on the removal of leaf litter as a preventive method for combating downy mildew in grapewines, even in the most recent summaries. Most research on this topic relates to predicting oospore germination in order to determine the timing of fungicide treatments.

Elimination of infected plants or plant parts

Crop residues on or in the soil are not the only possible sources of primary inoculum. For many perennial species, lesions on the branches or infected plant parts remaining on the plant through the winter are also important reservoirs for inoculum. Thus, the pathogen causing brown rot on cherries (*Monilinia* spp.) overwinters in the form of a mycelium within mummified fruit that can remain attached to the tree or fall to the ground, and within small cankers on the branches. Prevention thus consists of removing the mummified fruit, as well as pruning and removing diseased branches. In Hungary, trials in OA have shown that these operations (completed in August) allowed for a significant reduction in the rate of infected branches the following year, even within management regimes including fungicide protection.

The most extreme form of this type of preventive treatment is the complete elimination of infected or dead plants from the orchard or field (roguing). This approach is widespread in some types of fruit production, such as peach production in the United States, and can also be prescribed as part of the mandatory measures for combating certain regulated bacterial or viral pathogens. Such methods are also central for disease control in the production of seeds and other planting material (see below). Its effectiveness depends on several factors: the percentage of infected plants, the ease of symptom observation/disease detection and whether symptoms are actually fully diagnostic (no healthy carriers), the characteristics of pathogen dispersal, the amount of land area involved. For diseases where the inoculum arrives each year primarily from outside the plot (as with leaf curl in apricots, which is transmitted by an insect vector), use of this prevention measure within the parcel will have only a limited effect, and cannot effectively reduce new infections.

Limiting pathogen survival

Through the introduction of antagonists

Reduction in inoculum levels can also be achieved *via* a reduction in survival rates. In the case of dormant forms present on crop residues (leaves, for example), reduced survival rates can also be obtained by applying antagonistic organisms. For apple scab, the antagonist that has shown the best potential for reducing ascospore production in orchards in the fungus *Microsphaeropsis ochracea*. A Canadian trial (Table 3.1) found that spraying this antagonist on the orchard canopy at 10% leaf drop significantly reduced the number of ascospores the following spring. Subsequent trials conducted in European orchards, however, including in France, using a pre-market product based on *M. ochracea*, did not show satisfactory results. No product containing this antagonist is currently approved for use in orchards in France.

By accelerating the decomposition of infected leaf litter

Since leaf litter frequently serves as the nutrient support for dormant forms of pathogens, accelerating its decomposition by introducing nitrogen-rich material can help limit the formation of primary inoculum for the following season. In a four-year trial conducted in Germany (Table 3.1), spraying a product made from sugar beet processing wastes onto the canopy allowed for a nearly 40% reduction in average scab incidence on leaves and fruits.

Through crop rotation

In annual crops, rotations are a mainstay of disease prevention, particularly for diseases caused by soil-dwelling pathogens. In major field crops and in market garden production, crop rotations can prevent pathogens present in crop residues or volunteers from finding susceptible hosts in the same field in the subsequent crop year. Risks to crop health are accordingly greater in simplified cropping systems using short rotations and/or other, frequently associated practices (for example, no-till) that reduce the use of preventive measures (no burying of infected crop residues, etc.).

Despite its effectiveness, crop rotation is rarely fully exploited in contemporary production systems, because economic considerations take precedence in the choice of crop successions. From this point of view, the use of cover crops in-between primary crops has significant potential for improving crop health conditions, and deserves more attention from researchers. The practice of using "cleansing crops" between two primary crops in greenhouse production, however, is relatively well understood and utilized.

Preventing the arrival of external inoculum

This objective is achieved primarily through the selection of certified disease-free planting material. This prevention method is critical, particularly with respect to chronic diseases (viruses, certain bacterial diseases) and for crops that are propagated vegetatively (tubers, fruit trees, grapevines). Used systematically for the certification of planting material, it helps prevent the spread of viral diseases in potatoes, for example.

Sanitary selection is based on the visual inspection of nursery plots and the removal of visibly affected plants – which must not be left on site in order to avoid possible contamination of vector insects. However, this procedure, while generally effective against many pathogens, particularly viruses and bacteria, cannot guarantee the absence of latent infections (the asymptomatic presence of the pathogen). It is thus important to combine visual inspection with post-harvest testing, especially using new molecular detection tools, to manage latent infections.

Creating production and distribution systems for certified seeds is thus critical to the development of integrated crop health protection systems. Such systems are also a key element in programs for the obligatory control of pathogens regulated by quarantine. However, organic agriculture often favors informal seed markets, based on "participatory" or "cooperative" approaches to certification rather than on the sale of certified seed according to the rules of conventional agriculture. It remains to be seen to what extent such alternative practices allow to maintain high standards of seed health. Answering this question would require an interdisciplinary research effort linking biological and agronomic sciences (examination of seed and plant health across several generations, analysis of pathways for disease contamination or elimination) with human and social sciences (examination of exchange networks, economic value, social organization of producers and sectors).

Physical protection against infection

The idea of physical protection methods to combat disease is to create a microclimate at the level of the plant that is unfavorable to infection by the pathogen, and/or to block or restrict pathogen access to susceptible plant parts by means of a physical obstacle. These methods generally involve the use of various types of protective covers (rain protection, hail protection) intended to limit moisture on crop foliage, modify the microclimate more generally (greenhouses and cold frames), inhibit the introduction of pathogens (preventing wounds), or prevent pathogen dispersal in the environment (covering debris piles).

The protective effect of growing crops within greenhouses and other shelters is well known. This ESCo thus focused instead on more recent techniques: the use of high tunnels or other forms of protection to shield perennial plants from rain; and covering sources of potential inoculum (piles of harvest waste or culled material in immediate proximity to crop fields). Relatively few scientific articles have been published specifically on these methods, with most relating to perennial crops (fruit trees). The corpus was accordingly supplemented with technical reports and other publications targeted at producers, which often contain useful information on the efficacy and use of these strategies.

Rain covers for perennial crops

The primary objective of these covers is to create a physical barrier against rain above trees or grapevines, preventing water runoff within the foliage area and reducing the amount of time flowers, leaves, and fruits are subjected to surface moisture. Not having rain falling directly on plants can also reduce the spread of inoculum *via* splashing. Covers are usually made of polyethylene or some other translucent, impermeable material, spread above the rows to allow water to run down to the soil between the rows. This arrangement is often extended on the ends with insect netting and/or combined with hail protection. Rain protection systems used in trials were either prototypes developed by growers or researchers or commercial systems marketed by companies such as Voen or Filpack. The quality of the rain protection, its wind resistance, and the microclimate and light intensity underneath can vary markedly from system to system.

First developed in the 1990s for the protection of **cherry** crops against bursting after a rain event just prior to harvest, the technique has spread to other fruit production sectors. Its effectiveness against disease development has been tested in **apples**, especially with respect to scab: trials conducted in Europe (France, Germany, Denmark) showed 90 to 100% effectiveness against scab on fruits (covered orchards without the use of crop protection products).

Systems for rain protection have shown very good effectiveness in reducing the incidence of numerous diseases whose development requires a period of moisture on plant surfaces. Many such diseases are targeted by copper applications in OA: apple scab, grapewine downy mildew, kiwi bacterial canker... In many cases, physical protection makes it possible to achieve very good commercial quality without pesticide treatments. However, it does not control - and may even favor- other diseases with lower moisture requirements, such as powdery mildews on strawberries, apples, and grapewines. They may also lead to increased pest damage from pests that are shielded from their usual predators. Additional research is therefore needed to identify how different plant-pathogen complexes are affected by the use of rain protection. No references were found relating to peaches, particularly for leaf curl, which is treated with copper in OA.

Despite their effectiveness, these systems have not been widely adopted by producers. Although they are relatively expensive, they can be combined with other forms of protection against various risks (hail, insects, etc.).

Covering/containing external sources of inoculum

The accumulation of damaged or infected plant material (culls, diseased plants) in the vicinity of cropping areas is common for root crops like potatoes. These piles of waste material constitute one of the main sources of primary inoculum for subsequent crops, particularly for airborne pathogens such as late blight. The complete destruction of large piles is difficult, so one recommendation is to cover them with a black tarp to accelerate their decomposition (solarization) and prevent the dispersal of spores. Despite its effectiveness and moderate cost, and notwithstanding the existence of local regulations to fine growers who fail to comply, this recommendation is not sufficiently followed, in conventional as in organic production.

Management of the structure of crop plant and canopies

The architecture of crop plants and canopies

The architecture of crop plants and canopies is an important but widely neglected factor in crop susceptibility to diseases. It depends on the genetic characteristics of the species and the variety, as well as on plant management (pruning, training, choice of root stock, etc.) and other characteristics of field layout and crop management (planting density, fertilization, etc.).

Plant architecture affects the microclimate within the foliage zone (including moisture levels, a key parameter in the development of many diseases), as well as the dispersal, distribution, and deposition of inoculum on plant surfaces. Plant architecture also affects how crop protection products are deposited on plants. A number of studies, many of them now relatively ancient, suggest that the impact of plant architecture on the spread of disease can be significant. Differences of 25-35% in the rate of epidemic development can be attributed to favorable *vs* unfavorable plant architectures.

Genetics and plant architecture

Genetic mapping has revealed that loci relating to growth habit and developmental traits (flowering date, earliness, branching, etc.) are often associated with QTLs for partial disease resistance. Growth habit characteristics that create unfavorable microclimates for disease development (high and infrequent branching, reduced leaf area) have often been selected against in the process of variety creation, since dense, low growth provides better photosynthetic efficiency and thus higher yield potential (but greater disease susceptibility).

Management of plant architecture via training

For woody perennial crops (fruit trees, grapewines), planting density, vigor (as determined by rootstock selection), and the type and intensity of pruning will all influence plant structure within the field and thus microclimate within the foliage. Plant management can also influence plant phenology, physiology, and the growth pattern of shoots, which can likewise encourage or discourage pathogen development. In apple trees, more severe pruning can reduce scab development by improving fungicide deposition. The so-called "open-vase" pruning, which brings light into the center of the tree, is thought to result in a more open, ventilated canopy that dries more quickly and is thus less favorable to scab. A significant reduction in disease incidence is indeed observed with this pruning style during the primary contamination period in the spring. However, because it leads to a prolonged growth period in the summer, and thus the presence of young leaves susceptible to scab, this method may also be more favorable to secondary infections.

In apricots, rootstock choice and grafting height have been shown to be key determinants in preventing the bacterial disease caused by *Pseudomonas syringae*, a disease that is difficult to control even with copper and which can weaken and even kill many trees.

Leaf pruning and green fruit pruning (grapewines, hops) can also reduce the severity of major foliar diseases (downy mildew, powdery mildew, etc.).

Species and cultivar mixtures

As described in the section on plant resistance, monocultures of genetically identical plants, currently the norm in most cropping systems, encourage pathogen adaptation and thus disease development. Increasing the spatial diversity of plant resistance to pathogens *via* the use of mixed-variety or mixed-species plantings, and/or increasing the temporal diversity of crops are thus additional methods for limiting disease outbreaks and strengthening system robustness.

Cultivar mixtures can both slow down the overcoming of specific resistances and create barrier effects to limit the spread of disease. They can extend the useful life of a commercially valuable but disease-susceptible variety by "protecting" it with resistant varieties. The issue of managing multiple harvest dates and marketing pathways can be significant, however (feasibility, additional costs, etc.). One of the primary impediments to a more widespread use of cultivar mixtures is the difficulty of managing plantings with a range of different agronomic characteristics, including maturation date and harvest quality.

Findings as to the effectiveness of cultivar mixtures, presented in the preceding chapter, also suggest that efficacy is improved when local disease pressure is reduced. It would thus be interesting to evaluate cultivar mixtures in OA systems also making use of prevention, the selection of disease-free plant material, and/or biocontrol methods.

Crop associations (mixed-species plantings) within a field or orchard are also effective, but are rarely used in current agricultural systems (with the notable exception of forage crops, which are rarely treated with copper). They are gaining interest within agricultural systems that place a high value on biodiversity (agroforestry, permaculture), but these have rarely been the focus of research evaluating their potential for reducing the use of copper.

Some conclusions

Prevention: an effective but often neglected method

Methods designed to eliminate – or at least sharply reduce – the presence of primary inoculum in field areas (removing infected plants, shredding or burying of crop residues, pruning of infected plant parts) are used with success in fruit production. They are rarely used in other types of perennial crops, such as viticulture, although the reasons for this are not clear.

Other techniques can help limit the survival of disease inoculum: the addition of organic material to assist in the decomposition of infected leaf litter, the application of antagonists prior to the dormant phase of the pathogen species, crop rotation in the case of annual crops. While these methods have been generally shown to be effective, they are rarely adopted in practice. Organic production systems make use of them more often than conventional production systems.

Selection and certification of disease-free planting material for effective sanitation

The selection of disease-free seeds is a key element in disease prevention, especially against viruses and bacteria tht transmitted through plant material used for propagation (seeds, tubers, cuttings). Disease-free seed production can be favored by supplementing visual controls with the use of molecular or serological tests to detect latent infections. In OA, this lever remains controversial and under-utilized, with preference often given to alternative models for the production and distribution of genetic resources and planting materials, with as yet unevaluated consequences on seed and crop health.

Physical protection, an effective strategy for woody perennial crops

Protection against rain has been shown to be highly effective in reducing the incidence of many diseases targeted by copper in OA, notably apple scab and grapevine downy mildew. In many cases, such methods allow for high-quality commercial production without the use of fungicides. However, rain protection has no effect on or may even favor other diseases, such as powdery mildews. The crop-pathogen pairs and contexts that can most benefit from rain protection systems have yet to be fully identified. Despite their effectiveness, and the possibility of combining them with protection systems for other risks (hail, insects, etc.), these systems remain relatively under-utilized by growers. Rain protection and other forms of physical protection are also relatively expensive, and can require changes in crop management methods and/or changes in crop varieties. They can also present acceptability challenges due to the “artificial” look they create in agricultural fields. As a result, these elements must be considered in conjunction as part of an overall integrated protection, and even production, system.

Using plant architecture and plant cover characteristics to limit disease outbreaks

Plant growth habits and canopy structure can determine both crop microclimates (temperature, periods of moisture at the plant surface) and the spatial distribution of susceptible plant material, and thus can have a major impact on microbial infection and the development of disease outbreaks. Plant architecture characteristics that are unfavorable to pathogens are frequently genetically co-located with QTL for quantitative resistance, but have often been selected against within contemporary cultivars, since they are associated with traits that are *a priori* unfavorable to high crop yields (limited leaf area, tall plants, large internodal spacings).

It is possible to find genotypes with growth habits that are less favorable to infection, and to use them in the development of new varieties. In the case of woody perennial species, pruning methods can also be used to shape plants to be less favorable to disease spread. For instance, open-vase pruning of apple trees can help reduce scab, and leaf pruning can help reduce against downy mildew and powdery mildew in grapewines and hops.

Mixed crops: multiple benefits, but challenges ahead

Growing several cultivars or several species together within the same field can reduce the vulnerability of each component of the mixture to pathogen attack. When resistant genotypes are included, mixed plantings can also delay pathogen adaptation to resistances (dilution effect and cross-resistance effect), and thus extend resistance longevity. The effectiveness of mixed plantings is strongly dependent on local inoculum pressure, and so should be greater if mixed plantings are used in conjunction with other preventive methods and/or the use of biocontrol methods.

Nevertheless, although some processors (including millers and maltsters) are beginning to appreciate them, the use of mixed plantings remains very limited in today's agriculture (with the exception of forage production) due to the challenges of managing heterogeneous crops (mechanization issues, harvest dates) and/or constraints on their uses. Mixed plantings are central to cropping systems like agroforestry and permaculture, but their performance in terms of pest management remains to be fully evaluated.

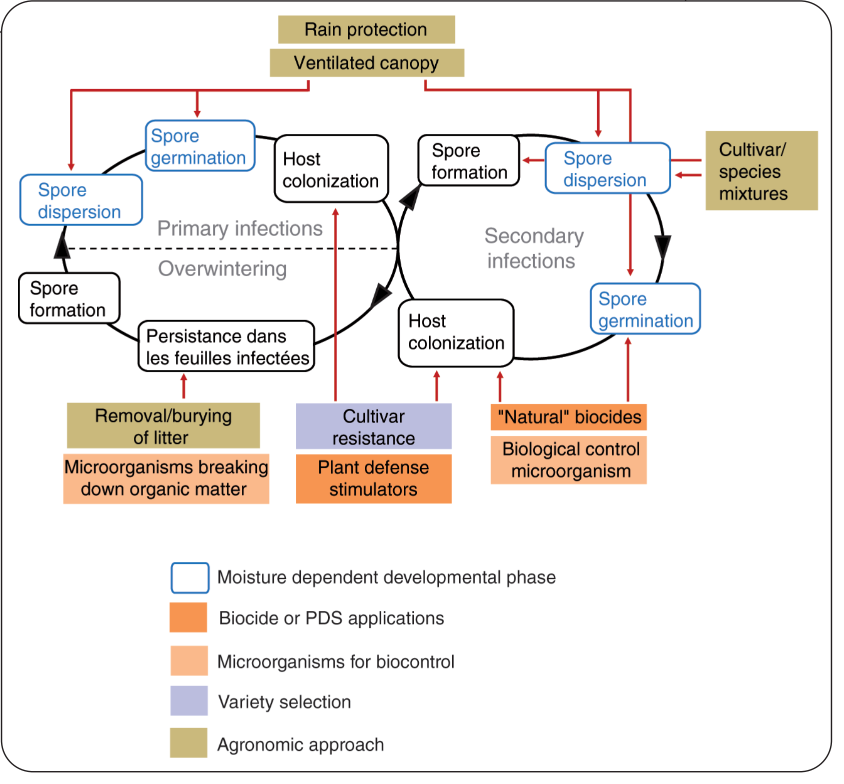
4. Introducing alternative levers and practices into integrated protection systems

Developing crop protection systems that use little or no copper requires at least two conditions: i) the availability of alternative technical solutions (preventive or curative methods, agronomic practices) together with varieties that are resistant or less susceptible to disease and useable within systems of demonstrable efficacy; and ii) making these new systems acceptable to farmers and production chains.

The previous chapters indeed show that based on current evidence, it will be difficult to replace copper products with a single, 'silver bullet' alternative technique while retaining the same efficacy and the same persistence at a similar price. Given this reality, it makes sense to consider the possibility of combining multiple partial efficacies (in time or in space) to obtain not an individual alternative solution (the logic of substitution), but rather a sequence of protection choices and actions, amounting to a new integrated production system (a logic of partial or total reconception). The goal is assure a level of efficacy and sustainability that is at least equivalent to that of current systems using copper, but without the use (or with only minimal use) of copper products. For this "systems" approach, we have chosen to consider the three "major uses of copper" mentioned above, which are also those for which the necessary amount of research information is available. Table 4.1 summarizes the different alternative methods identified in the preceding chapters for these three cases. Figure 4.1 illustrates the complementarity of these methods, which act on different phases of the pathogen lifecycle.

In a subsequent section, we will consider the determinants and barriers to the conception and/or adoption of integrated systems of this type, and in particular the role of agricultural input suppliers and their commercial strategies regarding the diffusion of key innovations necessary to these systems. This is, indeed, a major factor in gauging to what extent promising experimental results may or may not translate into future changes in agricultural practice, and thus in considering what kinds of recommendations are most likely to facilitate these transitions.

Figure 4.1. Alternative methods to copper treatments, and their action on the lifecycle of ascomycete and oomycete pathogens.



Evaluations and comparisons of cropping systems

Research on the assessment of crop protection systems as a whole, as opposed to the evaluation of individual components, are remarkably rare. Published studies of this type, and relating to question addressed by this ESCo, have emerged primarily from two types of research or research-and-demonstration projects, either specialized or multi-sectorial:

• Three **European research projects** on alternatives to copper were described as including "systems" experiments – ***Blight Mop***, focusing on potatoes; ***RepCo***, focused on combating downy mildew in grapewine and scab in apples; and ***Co-Free***, which sought to develop protection strategies without copper for OA in apples, grapewines, tomatoes, and potatoes. Some were included as part of *Blight Mop* and *Co-Free*, but they were implemented late in the project process and were thus generally not replicated. Moreover, or as a result, the findings were not publicized other than in project reports, and in the case of *Co-Free* these project reports are not yet available.

• **In France**, nearly 400 DEPHY cropping systems with reduced use of crop protection products, covering the range of crop production systems, have been tested within the DEPHY EXPE network, part of the Ecophyto program, with the goal of gradually promoting their transfer to farmers. Some of these systems, in tree fruits, viticulture, major field crops and vegetable production, are managed organically. Running for 5 or 6 years, the projects began in 2012 or 2013 and thus have not yet been completed. "Summaries of results at the midway point at the national level" are currently available for the Viticulture and Major Field Crop sectors.

Viticulture

A relatively extensive literature exists on strategies for improved crop protection efficacy in organic viticulture (better protection with less copper) and on substituting alternative methods in the place of copper (see Chapter 2, and Table 4.1). Nevertheless, few publications tackle the rethinking of cropping systems to totally eliminate copper through the coordinated use of genetic mechanisms, prevention methods, and product substitution.

Agronomic trials seeking improved efficacy of copper or alternative methods

The use of a **predictive model** to anticipate the appearance and spread of downy mildew outbreaks (based on climate data, agronomic information, and pathology characteristics) makes it possible to reduce the number of copper treatments. Trials conducted in Italian vineyards in 2009 and 2010 found that this approach allowed for a 50% reduction in copper use with no significant increase in disease incidence or severity on leaves or fruit. Similar reductions were achieved by using the decision-making tools *Coptimizer* (in OA) and *Mildium* (research not specific to OA). Carefully planned treatment schedules thus allow for significant reductions in copper use, although still falling short of a zero-copper objective.

In general, the many trials that have been conducted on **alternatives to copper**, in Europe and elsewhere, have reported efficacy levels that are sometimes equal but more often inferior to the use of copper. The efficacy of these substitution products can be improved by using them in combination with reduced rates of copper, although publications do not always specify the total reduction achieved over the full growing cycle for grapewines.

The most ambitious trial on alternatives to copper against grape downy mildew was completed within the ***RepCo*** project (reported in Dagostin *et al.*, 2011). This trial evaluated 112 formulations on two sites in Italy and Switzerland over a four-year period. The study did not include the use of preventive or curative agronomic measures, however, and the need to include partially effective alternative products within an integrated protection

Table 4.1. Alternative methods to copper treatments, potentially or currently available, for three major uses of copper

Methods	Availability	Apple scab	Grapewine downy mildew	Potato late blight
Biocidal substances	Available	Sweet orange essential oil [ESSEN'CIEL®, LIMOCIDE®]; horsetail extract; potassium bicarbonate [ARMICARB®, K-BLOC®] (+/++ in association with Cu)	Horsetail extract, nettle extract; lecithin (?)	Nettle (--)
	Potential	Essential oil of thyme and summer savory; black poplar extract (++), yucca extract (++)	Essential oil of thyme, of tea tree (in vitro +++); sage extract (++), licorice extract (++), yucca extract, garlic extract, chinaberry tree extract; vegetable oil; bacterial lipopeptides	
PDS substances	Available	Laminarin [Vacciplant®] (!) Not allowed in OA: BTH [Bion®] (+/++); phosphites (+/++) [Kendal]	Not allowed in OA: COS-OGA, phosphites (PDS & biocide)	Not allowed in OA: BABA (+/-); phosphites (++)
	Potential			
Microbiological biocontrol agents		Bacillus subtilis QST 713 [Serenade®] (PDS)		none
	Potential			Serenade® (?)
Genetic resistance	Available	Varieties possessing total resistance Vf (+++ but rapidly circumvented) and/or partial resistance (++ but longer-lasting)	Regent, Bronner (but longevity appears to be weak) Varieties 'ResDur' listed in 2017: Artaban, Vidoc, Floreal, Voltis	Passion, Makhai (+++) Allians, Eden, Coquine, (++) Désirée and many other varieties with partial resistance (+/++)
	Potential	Genes or QTL identified but not developed	Resistant INRA varieties in test phase	Stacking of resistance genes (+++ but longevity debateable)
Agronomic prevention and physical protection	Available	Removal or burying of dead infected leaves (++) Rain protection (covering) (+++)		Longer and more diverse crop rotations (+++) Disease-free planting materials (+++) Covering of piles of infected crop wastes
	Potential		Removal of dead infected leaves, but no references in Europe Rain protection, but not tested in Europe	

Table 4.1. Next

Methods	Availability	Apple scab	Grapewine downy mildew	Potato late blight
Management of plant cover	Available	Tree pruning to promote ventilation and bring light into the tree canopy Mixtures of susceptible varieties and partially resistant varieties		Mixed plantings of susceptible and resistant varieties (+; effective if mildew pressure is moderate)
Current practices		The most effective measures are rarely utilized (resistant varieties, elimination of dead leaves) or not utilized (covering)		The average resistance of currently grown varieties is low

Efficacy in the field: high (+++), average (++), low (+), variable (+/-), not evaluated (?), controversial (!).

strategy is only mentioned in passing. Similarly, a very detailed review of control measures for grape downy mildew (Gessler *et al.* 2011) considered various uses of copper, synthetic fungicides, and biocontrol, but did not make reference to the use of agronomic methods for downy mildew prevention, nor did it discuss the use of partially effective methods within a broader integrated strategy.

Agronomic trials aimed at rethinking cropping systems

Systems designed around downy mildew-resistant varieties were tested as part of the ***Co-Free*** project, but the full results have not yet been published. Multiple treatments for downy mildew were compared across multiple sites over a period of 2-3 years: 100% copper, alternative products combined with reduced rates of copper, alternative products alone. The alternative products alone do not appear to have provided sufficient protection, but their use in combination with reduced copper applications (0.6 to 1.5 kg/ha/yr) seems to have provided satisfactory protection for the grapewines, at least during years of moderate downy mildew pressure.

The most complete experiment on rethinking viticultural systems with low levels of crop protection inputs was launched in France in 2013 as part of the **DEPHY EXPE viticulture** network. Out of a total of 48 cropping systems in six viticultural regions, 13 were managed according to OA requirements, including making an effort to reduce copper use. In Alsace (northeastern France), very careful use of copper based on multiple observations combined with a strict management of plant development (grass strips between the rows, pneumatic leaf-pruning) and the use of essential oils and propolis made it possible to limit copper applications to between 466 and 745 g/ha/yr from 2013 to 2015, with good control of downy mildew. At Gaillac (Tarn, southern France), control of plant development and the use of decision-making rules allowed for a reduction in copper applications to between 350 and 600 g/ha/yr from 2013 to 2015, with good control of downy mildew but low yields.

Two other cropping systems in the network (not in OA) were designed around the use of downy mildew- and powdery mildew-resistant varieties developed by INRA (these varieties were still in their test phase). At a site in Bordeaux, no fungicides were applied in 2013 and 2014, resulting in the expression of secondary diseases, such as black rot, to which the varieties were not resistant. In 2015 and 2016, one to two applications of synthetic fungicides were made. In Alsace, resistant varieties also provided very good control of downy mildew in 2015 and 2016; up to two fungicide treatments per year were allowed to combat black rot and to limit the risk of the downy mildew resistance being overcome. Thus, the use of downy mildew-resistant varieties allowed for the most significant reduction, although still not the total elimination, of copper use in organic viticulture.

Fruit production

In apple production, a handful of studies have combined and integrated different pest management strategies within cropping systems. Established from the late 1990s onwards, these orchard "systems" trials have generally compared organic agriculture systems management with integrated fruit production (IFP) systems and/or conventional systems, although the reduction of copper was not always designated as a priority, even for the OA systems. The four trials conducted in Europe that have been described in scientific publications all made use of variety resistance as a key component, particularly against scab.

In **Switzerland**, a trial compared IFP and OA systems with varieties with either moderate (*Boskoop*, *Idared*) or high resistance to scab (*Vf* gene). In the OA systems, annual protection against disease consisted (on average from 1995 to 2002) in a maximum of one application of copper, 6 to 11 applications of sulfur, and 9 to 11 applications of clay. Good control of scab was observed, except on *Idared*.

In **Hungary**, in a seven-year study (1999-2005) including 27 apple varieties (9 scab-resistant, 9 commercial, and 9 heirloom), the final average scab frequency was considerably higher in the OA system than in the IFP system, except for the resistant varieties, which showed no scab symptoms on the fruits. In another trial in Hungary (conducted over two years), the resistant variety *Prima* showed its value in OA systems relative to the moderately resistant variety *Jonathan*, with *Prima* allowing for a reduction in the length of time covered by copper-based treatments.

In **western France**, where the climate is highly favorable to scab, a study published in 2016 compared integrated protection strategies associating (i) resistant varieties (*Reine des Reinettes*, which is partially resistant, and *Ariane*, a carrier of the major gene *Vf*); (ii) prevention practices (shredding of leaf litter); and (iii) fungicide treatments in the case of a high risk of scab infection (low-level risks were not treated). A 50% reduction in fungicide treatments was obtained while maintaining the partial resistance of *Reine des*

Reinettes and slowing, by two years, the overcoming of the *Vf* gene. The value of *Reine des Reinettes* had previously been shown in another study in the same region, with this variety showing virtually no fruits affected by scab (versus 22% of fruits for the variety *Gala*) with no fungicide protection, in years of low scab pressure.

In the **Drôme**, the apple initiative BioREco, established in 2005 at INRA-Gotheron, included several levers in its crop protection strategy, with the goal of reducing the total number of treatments even in OA management. For disease management, the levers employed were variety resistance, the reduction of primary scab inoculum through the removal/burying of leaf litter, and scheduling fungicide treatments using a DMT and orchard monitoring. The three varieties tested were: *Golden Smoothee* (disease-susceptible), *Melrose* (moderately susceptible), and *Ariane* (scab-resistant through the *Vf* gene). In OA, disease damage was greatest on *Golden Smoothee*, which also received the greatest number of fungicide treatments. This trial demonstrated the importance of variety resistance (Table 4.2) in combination with other levers to reduce fungicide use (including copper) in OA.

Table 4.2. Annual number of fungicide treatments in OA (average for the period 2006 to 2009), for the three varieties tested in the apple program BioREco

Variety	***Golden Smoothee*** **(disease susceptible)**	***Melrose*** **(moderately susceptible)**	***Ariane*** **(scab-resistant by the gene *Vf*)***
Annual number of fungicide treatments	18	8	6.25
of which copper treatments	1.75	1	0.75

* In the absence of strains of *Venturia inaequalis* virulent for *Vf*.

Potato late blight

The most ambitious studies on potato late blight have been conducted as part of the European research projects *Blight Mop* and *Co-Free*, both of which sought to develop and test crop protection systems that could eliminate the use of copper. In both cases, the "systems" trials were initiated towards the end of the projects, so their results have only appeared in final project reports (which in the case of *Co-Free* have not yet been released), not in scientific publications.

The trials conducted during *Blight Mop* found that the most promising levers for integration into "zero-copper" strategies to control potato late blight were the use of resistant varieties, the use of cultivar mixtures (as either alternating rows or random mixtures) or species mixtures, and, to a lesser extent, various agronomic practices such as earlier planting dates and a reduced use of nitrogen fertilizers. These trials also highlighted possible synergies among these levers. Thus, the effectiveness of mixtures between resistant and susceptible cultivars increases when inoculum pressure is reduced, for example by the use of reduced rates of copper.

It thus seems possible to imagine alternative strategies providing a good level of effectiveness without the use of copper. Various combinations of levers within overall cropping systems have been tested in the field in the seven countries participating in *Blight Mop*, under agricultural production conditions (blocks of at least 0.5 ha, with practices implemented by farmers), and compared with the management sequences normally used on these farms. These trials have generally shown a significant improvement in technical performance (reduction in late blight severity) and economic performance (gross profit) relative to the farms' standard system, including for systems without copper or with strongly reduced rates of copper. This was especially true for systems using varieties with good resistance to late blight on leaves, alone or in combination with other levers (planting density, mixed-variety plantings, adjusted fertilization rates).

Nevertheless, results were highly uneven depending on the strategies adopted and/or on the locations, with these effects being undistinguishable in this study. The improvements seem to have been better overall in oceanic and temperate climates (France, Great Britain, Norway, Denmark) than in continental climates (Switzerland, Germany). Moreover, the control of late blight is clearly not the only performance factor to be considered for these management strategies: variety selection also influences yield potential, and even the commercial value of the product; some interventions that were ineffective against late blight did show a benefit against other diseases (such as *Alternaria*), or even other stresses (nutritient conditions), resulting in a gain in yield without improved control of late blight.

Implementing these types of strategies can be challenging in terms of workload (particularly for planting and harvest), equipment, and planning (for example, with respect to variety selection, harvesting of mixed plantings, or other agronomic management decisions), and can significantly impact production costs. Finally, strategies targeted at one disease alone (in this case, late blight) can be difficult to incorporate into integrated protection strategies intended to address the full range of potato diseases. These strategies thus confront problems of the commercial acceptability of innovations, notably new cultivars (see Section below).

Actors' strategies and the availability and acceptability of innovations

The realization that existing, available innovations – for example, resistant varieties and biocontrol materials – are not being adopted by most farmers leads to a more detailed consideration of how different innovations arrive on the market and to what extent they are embraced by their target audience.

Availability of innovative solutions and the commercial strategies of agribusiness firms

An innovation can be defined as "an invention that has found a market." From this perspective, the market introduction of products or services by commercial enterprises is a critical phase in the innovation process. Understanding the market introduction strategies of companies deploying these innovations is thus essential, particularly in the case of new, emerging, or niche markets, such as those for biocontrol products.

A survey conducted in 2016 among members of IBMA France (the French Association of Biocontrol Product Companies) found that 60 research and development projects were underway, with a goal of 50 new product introductions by 2018 (17 for grapewines, 14 for vegetables, 9 for fruits, 7 for cereals, 3 for flowers, and 2 for sugar beets). While the survey did not provide specific information as to the nature of these products or solutions, their reported effectiveness, or their stage of development, it suggests the significance of the biocontrol product pipeline and thus the potential future scope for development within this market. The nature of these products and the costs associated with research and regulatory procedures (*i.e.*, intellectual property considerations) are such that the scientific literature does not reflect the state of private research (often the most promising) conducted within the biocontrol sector.

The current biocontrol industry is essentially made up of two types of actors: specialized companies, often small (annual turnover of less than €1-2 million), working on one or a few products; and large crop protection companies, to which biocontrol is one of several potential avenues for diversification and which usually invest in biocontrol through sector concentration (*i.e.,* buying up the most promising smaller companies). This picture is changing rapidly, however, as the market is undergoing a period of rapid growth.

It seems reasonable to assume that the strategies of different industrial actors with respect to the market introduction of biocontrol products vary depending on their level of specialization, their business structure, and their financial resources, and that these strategies in turn help determine the arrival of innovative products on the market. The appearance of new biocontrol products will thus depend on private companies' capacities for R&D, including their financial capacity to negotiate the approval process. Two possible scenarios can thus be imagined:

1. The smaller companies seek to position themselves on the market with new products, including products for niche markets, but have limited financial resources to develop these new products. The big agrochemical companies have greater financial capacity to support R&D on promising but risky new products, but are more likely to focus on existing, known products intended for large markets.
2. The small companies operate in "start-up" mode, working intensively on new products in tandem with upstream research entities (academic laboratories for instance). Once these innovations have been worked out, the start-ups expect to be acquired by larger companies in order to pursue the market development phase.

The literature review conducted for this ESCo found no specific studies of the biocontrol sector, and thus did not allow for a detailed analysis of the industrial strategies of biocontrol sector actors or the impact of those strategies on the market introduction of new products. It would be interesting to consider how research on other health sectors (for example, the biotech sector or the med-tech sector) might apply to the biocontrol sector as a way of beginning to address these questions. This area undoubtedly deserves further study.

We should note, too, a lack of information as to the current or future market price of the various alternatives to copper. It seems unlikely that any new product will be less expensive than one or several applications of copper. Insofar as they are niche products, prices are likely to remain relatively high unless and until manufacturing processes can be made more efficient. Regulatory and policy approaches are thus likely to be key factors in promoting the adoption of such alternatives by farmers.

Practical acceptability of innovative solutions and systems

Regardless of its effectiveness in terms of crop protection, any change in agricultural practices, cropping regimes, or production systems (lengthening and diversification of rotations, use of mixed crops, etc.) involves both technical and economic risks – risks that not all actors will be prepared to take. Research suggests, however, that organic agriculture has many features favorable to the adoption of innovative systems: farmers who convert to OA tend to be innovators, ready to experiment and to take risks, even in the absence of an established corpus of technical references. OA itself, moreover, can be considered as an example of "software innovation," fundamentally grounded in information and information-sharing. From this standpoint, the availability of information is essential to OA's processes of diffusion, and challenges associated with information accessibility, including training and advisory services, are frequently cited as barriers to OA conversion.

In addition to personality characteristics (risk tolerance, interest in technological developments, etc.) influencing individual farmer behavior, deciding whether or not to adopt a given innovation will depend on the economic, structural, and institutional context of each agricultural enterprise. This has been well documented in the case of resistant varieties, the crop protection benefits of which are recognized but which remain little used in practice; and more recently in the case of agroecological principles. New methodological approaches, such as the construction and interconnection of "mental maps," can offer new insights into the relationships and interdependencies of different actors in the innovation process.

The example of resistant varieties offers a good demonstration of the various possible barriers to innovation, creating situations of socio-technical "lock-in" that can prevent the implementation of major change. These barriers are of at least four different types, sometimes acting together, sometimes individually. They are:

- **skepticism on the part of users as to the performance or longevity of the proposed solutions.** The fact that many growers who use resistant varieties follow the same crop protection regimes as for susceptible genotypes suggests a lack of confidence among growers as to the real capacity of these varieties to resist pathogen attacks over the long term. These doubts may be removed or reduced by involving users in cultivar development and evaluation, for instance by helping to define varietal ideotypes or *via* participatory or collaborative breeding approaches.

• **skepticism as to the compromises made between agronomic and organoleptic characteristics on one hand and resistance characteristics on the other hand.** Many resistant varieties have lower productivity or nutritional (or appearance) characteristics relative to standard, susceptible varieties. There is thus no strong incentive to use them so long as chemical pesticide solutions (including copper) are available, in particular for markets that are strongly organized around well-established standard varieties (fruits and vegetables and viticulture, for example). In this case, reluctance to adopt new varieties can also come from the markets themselves, which sometimes struggle to promote resistance as an argument in favor of the corresponding agricultural products. A case study of two cooperatives engaged in agroecological initiatives provided a good illustration of the gap that can exist between the affirmation of such strategies and their actual implementation. Here again, however, there are effective means of overcoming these barriers *via* serious engagement on the part of each group of actors within the supply chain. A useful recent example, studied in detail by the *Co-Free* project, relates to the adoption of late blight-resistant potato varieties that can be grown in OA without the use of copper. Such studies underline the essential role of consumer information in shifting purchasing practices toward unfamiliar but more resistant varieties, and the need to involve distributors as well as growers in the implementation of innovative systems.

• **conflicting values leading to the rejection not of products themselves, but of the processes by which those products were obtained.** This is the situation in OA with respect to synthetic products (leading to the rejection of phosphites as an alternative to copper). It likewise underlies OA's rejection of genetic engineering techniques (including genome editing), based on the principle of respect for plant integrity, and thus barring the use of resistant varieties obtained, or even suspected to have been obtained, through these techniques. This type of blockage can only be removed, if at all, by a complete transparence as to the origin of the proposed varieties and the techniques employed to create them.

• finally, **blockages resulting from the nature of research and innovation systems themselves.** As has been shown, for example, in a recent case study of wheat, researchers' activities are often oriented toward technological engineering approaches within existing modes of production. As a result, many of the technical innovations that are developed only serve to reinforce existing system lock-ins. This tendency to focus on improving the efficiency of existing systems, rather than on developing new systems, can be readily shown to have limitations, particularly in the area of crop protection.

The same is true for all agricultural development agendas based on a single "remedy" imposed from above. Analysis of actual situations in the field shows that top-down methods of promoting innovation frequently have the effect of denying local knowledge and local know-how and ignoring, rather than harnessing, local and sectorial economic and social realities. As a result, they often encounter strong resistance on the part of the farmers or other individuals they are supposed to support.

Some conclusions

A deficit of systems experiments to evaluate copper-free management strategies

Although a large number of factorial trials have been conducted to evaluate individual alternatives to copper, the literature review for this ESCo found only a small number of "systems" experiments designed to assess the efficacy and other performance criteria (labor requirements, energy requirements, economic return, etc.) of complete crop protection regimes without copper. This lack of systems investigations is unfortunate given that (as shown by the trials of individual methods) the discontinuation of copper will require a sophisticated integration of multiple, partially effective methods followed by an optimization of the resulting combinations.

Nevertheless, the few studies that have been made towards the adoption of a more systemic approach and the evaluation of truly integrated strategies for crop protection all show strong potential for the total or partial elimination of copper applications, in particularly in systems making use of varieties with a good level of resistance.

Moreover, it is interesting to note that reduced applications of copper (or of other fungicides in conventional systems) are compatible with several alternative levers (genetic resistance, of course, but also some PDS and biocontrol agents), reinforcing their efficacy by limiting pathogen pressure. Existing studies have only rarely sought to assess the secondary effects, beneficial or otherwise, of these integrated strategies. At a minimum, evaluating and making use of the synergistic effects between partially effective practices would assist in developing combined management strategies.

Significant potential for the development of innovations...

A review of the scientific literature, supplemented by surveys of commercial biocontrol product companies, suggests that a large number of new biocontrol products and formulations are currently in the pipeline. Information on these R&D efforts is nevertheless too partial (given confidentiality and intellectual property protections) to provide a full picture of the new applications (including new active ingredients and new targets) that can be expected to appear on the market over the next five years.

... but more research is needed on the underlying economic models...

Future development of the biocontrol market depends on industrial actors of various sizes and structures, but a majority of these are small companies with limited financial resources. This raises the question of business strategies and capital resources for the technological development and market introduction of innovative products. No economic study specific to the biocontrol sector was found among the literature reviewed for this ESCo, so our assessment relied on more general analytical elements obtained from research on other agricultural and health care sectors. It would nevertheless be useful to conduct more specific studies, particularly with regard to the economic strategies of small biocontrol companies, to better understand the barriers to and drivers of innovation within this rapidly developing sector. The potential role of industry and trade organizations in supporting and sharing this risk could also be explored.

... and thus on modes of adoption for innovations in this sector

Once introduced to the market, new solutions can only become innovations if they are adopted. The case of resistant varieties illustrates how adoption can face many challenges, both economic (socio-technical lock-in linked to pesticide availability, compromises between resistance and other crop qualities, return on investment for solutions developed for niche markets), and linked to processes of innovation research itself (particularly in “top-down” models that take scant account of local needs and know-how). This suggests that the co-conception of innovative methods and procedures in association with the intended audience, currently rarely practiced, is an important avenue to explore to achieve more rapid and fundamental changes in crop protection systems.

5. Overall conclusions

This analysis of the existing scientific knowledge and knowledge gaps relating to reducing or eliminating the use of copper for crop protection allows us to draw several important conclusions. Although most of the available studies on this topic place an emphasis on organic agriculture – which is more strongly affected by restrictions on the use of copper and thus is more actively searching for alternatives –, the lessons of this expertise equally apply to other modes of agricultural production. The national Ecophyto plan, for example, offers one suitable framework for transferring the conclusions to conventional agriculture. Thus, in some cases, alternatives to copper could benefit from the system of "certificates for reduced use of crop protection products" (*Certificats d'économie de produits phytosanitaires*), intended to provide a financial incentive and increased visibility for farmers' efforts to reduce their use of crop protection inputs.

A considerable quantity of available information...

Initial queries of the *Web of Science* returned thousands of scientific references relating to alternatives to the use of copper treatments. Refining and targeting the search query resulted in a final corpus of nearly 1000 scientific citations and technical documents. This abundance of scientific and technical publications suggests that academic and applied research to find alternatives to the massive use of copper is widespread and ongoing. The results from this research are potentially transposable or generalizable to other pesticides targeting the same pathogens.

... but very unevenly divided between the areas of research and development

It should be noted, however, that a majority of this research relates to the characterization of individual levers or practices (as opposed to combined effects or performance within production systems). These levers are thus understood primarily as substitutes for chemical treatments, while research to design, verify, and evaluate integrated protection systems based on multiple criteria remains all too rare. Most research currently adopts a logic of simple substitution (replacing copper with an alternative product or practice) rather than a fundamental reconception of crop production and protection systems.

Individual, partially effective solutions...

This ESCo produced a complete inventory of available alternatives that may be considered as candidate substitutes for copper, assembling all the existing data on their levels of observed effectiveness. In doing so, it underscores both the potential and the current limitations of these alternative methods, which can be divided into three broad groups:

Methods acting directly on the pathogen itself, including:

The use of **microbial biocontrol agents**. These microorganisms, which have been the focus of considerable research, can act directly on pathogens *via* antagonism, hyperparasitism or ecological competition. In addition to their direct effect, some also act as plant defense stimulators. Because of their specific characteristics (as living organisms), the use of microbial biocontrol agents is more complex than the application of chemical fungicides, making them more challenging to adopt and sometimes resulting in variable efficacy in the field. Recent research has thus focused on determining the optimal conditions for the use of these products, and on identifying the most promising strains based on an analysis of all microbiota present near the plants or plant parts to be protected.

The crop protection use of microbial biocontrol products requires a long and costly process for market introduction. To date, very few products have been approved for use against the pathogens targeted by copper, and the species and strains currently in the research pipeline are far from covering all the remaining crop protection contexts. It is thus difficult to imagine, at this stage, that microbial biocontrol products will fully take the place of copper within crop protection systems in the foreseeable future.

The use of **natural preparations or extracts with biocidal properties.** These are also a focus of considerable research. Often of complex composition, these preparations frequently have a plant-defense stimulator effect in addition to their biocidal effect (this is true of many essential oils, for example). Strong antimicrobial activity (under controlled conditions) makes for some promising candidates to take the place of copper, but product formulation remains a challenge. Other potential challenges include some undesirable effects on harvested crops and questions as to the status of some preparations with respect to organic certification.

Methods making use of plants' own capacities for resistance, either constitutive or induced by infection or other external stimuli

Resistant cultivars, developed by specialized plant breeding programs using the genetic resources of the cultivated species and/or related species, are available and effective against many of the diseases targeted by copper, including those that account for the majority of copper-based pesticide use (potato late blight, grapewine downy mildew, apple scab). These cultivars may possess either total resistance, usually controlled by one or a few genes and resulting in a total absence of symptoms, or in small necrosis at the points of infection (hypersensitive reactions); or partial resistance, usually under a complex genetic determinism (numerous loci or QTL) and resulting in a reduction or delay of disease symptoms rather than a total absence of disease development.

Despite this availability of resistant plant material, the adoption by growers of resistant cultivars is often limited. This apparent paradox is explained by a number of reservations on the part of users, including: 1) uncertainty as to **the performance and longevity of resistance in the field,** particularly in the case of quantitative resistance, even if these traits can be reinforced by complex genetic constructions at the plant level (gene or QTL stacking within a single genotype) or at the plant population level (mixed plantings); 2) **concerns about the negative effects of selection for resistance on other agronomic** (yield, earliness) or **use criteria** (taste, food value) of these varieties; 3) possible **conflicts of values with respect to the origins or selection methods used to develop resistant genotypes**, notably (but not exclusively) in OA with regard to genetic engineering (genetic modification, genome editing), which limits the use of varieties obtained or even suspected to have been obtained through the use of these technologies; and 4) **skepticism as to the need to change variety types so long as other solutions** (particularly pesticides, including copper) **are available manage crop health**, particularly in situations where variety selection is determined by quality programs (such as AOP). This last type of "lock-in" exists in all agricultural systems, including those of emerging and developing countries.

Plant defense stimulators (PDS) are a very active area of current research. A large number of products or molecules have been identified that show proven biological activity under laboratory conditions. Many of these (phosphites, extracts of microorganisms) seem to have multiple modes of action, with both defense-inducing effects and biocidal effects (particularly phosphites). Although these molecules are active under controlled laboratory conditions, transferring this activity to the field is generally challenging, with the protection provided often proving weak or unpredictable. This may arise from difficulties in formulation (products must penetrate the plant in order to be recognized and become bioavailable), application (defense stimulators must be applied *prior* to infection, whereas most biocides are most effective when applied in the presence of the target pathogen), signal perception by the plant, persistence of activity, or even assessment methods. Research to address these issues has barely begun, with most work still focused on the identification of molecules or products with demonstrable effects in the laboratory. It should be noted that, as with other biocontrol products, not all PDS are useable in organic agriculture; phosphites, for instance, are currently prohibited.

Methods based on **homeopathy** or **isotherapy** are of debateable efficacy, and do not seem to provide a viable alternative to other options. They have been the focus of very few academic or technical publications, and little or no scientific data are available as to their effectiveness.

Implementation of agronomic practices...

... to combat primary infections. A range of physical methods can be used to reduce the survival of residual inoculum in the field (eliminating infected crop residues, management of volunteers) and/or inhibit its access to the next crop (burying, covering, use of disease-free planting material). These methods are highly effective, but are often inconvenient or expensive to implement for the grower. For example, providing rain protection for fruit trees is relatively expensive (cost of material) and labour intensive, although these costs can be reduced when combined with protection against hail or against insects, which are already widely used.

... to combat secondary infections and limit disease outbreaks. The spatial and temporal diversification of hosts within fields (open-pollinated varieties, mixed-variety or mixed-species plantings to inhibit secondary infections), and crop arrangement at the landscape level (landscape mosaics, crop rotations) can be used to reduce secondary infections. They are important for the management of many plant diseases that can travel long distances.

... but still insufficiently integrated within integrated crop protection systems

Although trials of new products and formulations are becoming more common, very few decision-making and management tools specific to the objective of reducing copper are currently available or under development. This is true for decision-making tools (DMT) specifically focused on biocontrol (see above), as well as for assessing the response of different plant genotypes to these new preparations. Among such tools, priority should be given to the early detection of the initial phases of infection in the field (sensors, disease-monitoring devices), given that early detection will often determine the effectiveness of risk-assessment modeling and indeed the effectiveness of some management methods (e.g., partially resistant varieties, mixed-variety plantings).

Moreover, the partial levels of efficacy of most of these methods and products requires that they be used together within integrated crop protection strategies, and not as single elements individually substitutable for copper applications. However, only very few scientific references or data are available on integrated systems (including landscape-level systems, such as agroforestry). Finally, given the absence of suitable and accurate models, the design and assessment of such systems remains challenging, but is absolutely necessary.

Giving up copper: considerable room for improvement

A significant reduction in copper application rates can be achieved without drastic changes to cropping systems

A large number of studies on a wide variety of diseases (potato late blight, grapewine downy mildew, apple scab, etc...) have demonstrated that reductions in copper application rates of up to 50% (from 6 kg /ha.year to 3 kg/ha.year), and sometimes even more (usually maintaining the same application schedule but cutting the dose for each application), can, in most cases, provide identical or comparable protection as that provided by full application rates. Very satisfactory protection against these diseases could repeatedly be obtained by the use of 1.5 kg of copper per hectare per year vs. 3 kg/ha/yr in “standard” treatment programs and 6 kg/ha/yr under maximum allowances. It should thus be understood that a significant reduction in the maximum total amount of applied copper allowed would not produce a general crop protection crisis or threaten yields, except in situations of very high disease pressure.

Experimental systems without copper are effective…

A few pilot experiments, particularly those conducted within the European research projects *Blight Mop*, *RepCo*, and *Co-Free*, have shown that complex systems associating several levers (resistant cultivars, PDS, agronomic practices such as mixed plantings and prevention), under experiment-station conditions and in some cases on farms, can show levels of disease control equivalent to those of a classic protection program based on copper. Success with these alternative management systems appears easier to achieve and reproduce in annual than in perennial crops (fruit trees, grapewines), and/or where there are fewer obstacles to the use of resistant varieties (non-AOP systems, for example). It should be noted, too, that reported efficacies are highly variable, and that these conclusions are necessarily preliminary due to the small number of relevant studies.

… but their effectiveness is strongly dependent on system components…

These experiments show that cultivar resistance is indispensable to the effectiveness of any protection system not using copper. Host resistance can also be combined with strategies to strengthen and/or extend its effectiveness (mixed plantings, landscape mosaics). It can be reinforced by prevention methods intended to limit inoculum surviving in the fields (removing or shredding infected plant debris), or inhibiting inoculum access to susceptible plant parts (tarping or covering). On the other hand, adjusting fertilization strategies (form or quantity), or the use of biodynamic or isotherapy preparations, have generally been shown to be fully or broadly ineffective.

… and extending them will require coordination throughout the supply chain

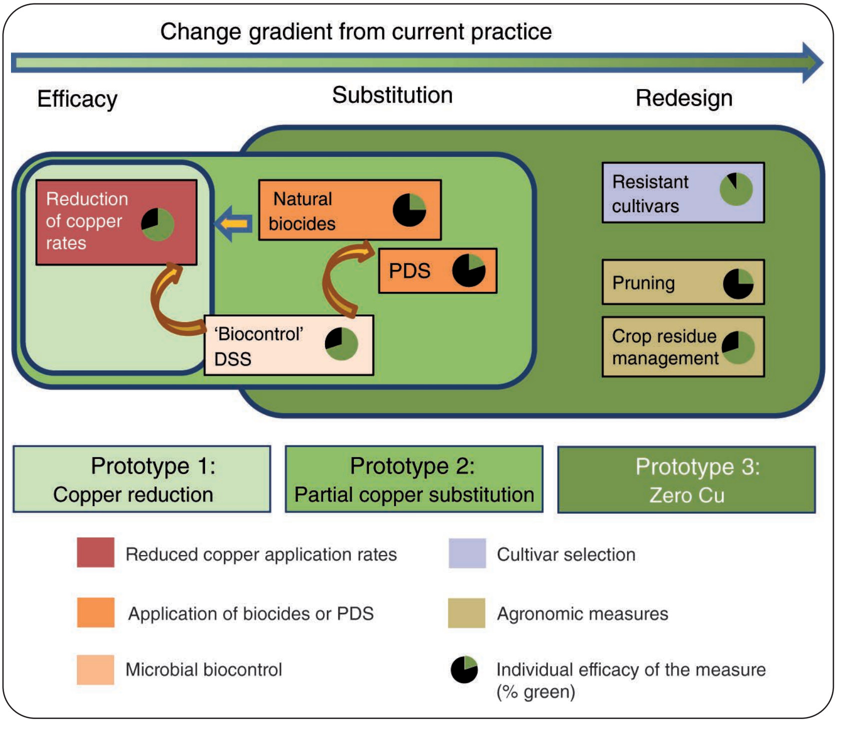
Such systems, which potentially involve major changes, will require major accommodation all along the production chain in order to be successfully adopted (outlets for new crops introduced to lengthen rotations; new supply networks; labeling or other value-added strategies for products grown without the use of copper, etc.). Some interesting initiatives in this regard – such as the creation of “variety clubs” among producers to promote resistant varieties – certainly merit further attention. The same is true for changes to AOP guidelines (for example, to allow for the use of resistant varieties).

Designing (and testing?) prototypes

The elements assembled for this ESCo make it possible to construct a series of hypothetical, prototype protection systems based on a range of specific objectives: e.g., replacing copper products without modifying other system elements; designing for maximum protection; designing for maximum sustainability; etc. The exercise was attempted for the three disease systems with the largest number of available references, and made use of a conceptual framework known as ESR, for *Efficacy* of inputs (use optimization within a logic of integrated agriculture or precision agriculture), *Substitution* with “natural” inputs or single methods such as varietal resistance, and *Reconception* of the cropping system within a logic of integrated protection.

To construct these prototypes, we adopted the following approach: i) organize, along a gradient of change relative to current practices, all alternative solutions either available (*efficacy* or *substitution*) or potentially available based on laboratory tests or preliminary field trials (*reconception*), indicating for each one its individual anticipated efficacy relative to no intervention (untreated control); ii) specify the desired objectives for each pathosystem, developing three scenarios of progressively increasing ambition relative to the overall goal of eliminating the use of copper; and iii) identify feasible combinations to meet these different objectives. Due to an absence of data, neither the costs of implementing these different prototypes nor their consequences for the management of other potential diseases were considered so far.

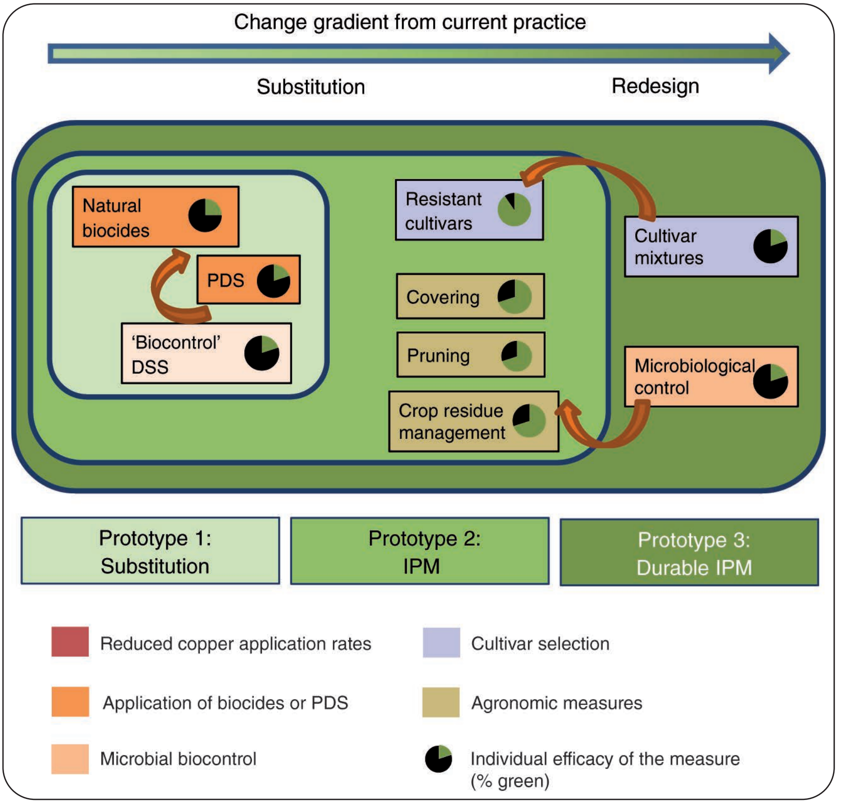
Case 1: Grapewine downy mildew



This is without question the most difficult of the three systems because of the relatively small number of available alternative levers. Some of these (resistant cultivars, for example) are difficult to rapidly introduce into the growing system.

Prototype 1 seeks to provide protection with a low or very low use of copper. It is based primarily on a direct reduction in copper application rates, supported by a DMT (such as Mildium) to help determine optimum application rates and timing and use of a high-performance sprayer to improve application effectiveness. Reduced use of copper could be also strengthened by the use of PDS or biocide products, and/or these could be substituted for some copper treatments (**Prototype 2**, partial substitution). Finally, in addition to the biocontrol methods in Prototype 2, the goal of "zero-copper" protection (**Prototype 3**) absolutely requires the use of resistant cultivars, in addition to preventive methods such as microclimate management through pruning techniques and the removal of infected leaf litter.

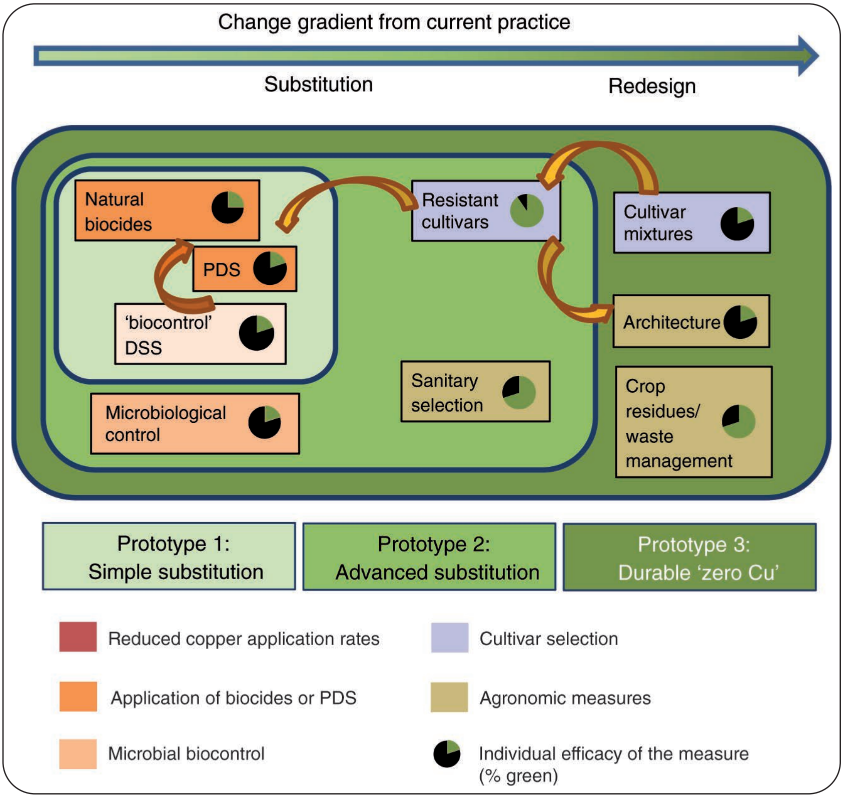
Case 2: Apple scab



This is the case for which the greatest number of levers is available. For this reason, all three prototypes were designed to make no use of copper.

In **Prototype 1**, the strategy is simply to replace copper treatments with biocontrol products (PDS or biocides), with application dates determined using a specialized DMT. Since each of these levers has only limited effectiveness, it is likely that the system would only provide satisfactory protection in years of very low disease pressure. **Prototype 2**, designed to provide integrated protection without copper, would combine biocontrol products with the use of preventive methods to limit inoculum pressure in the orchard (rain protection, open-vase pruning, removal or burying of infected leaf litter) and the use of resistant varieties. Finally, **Prototype 3** (integrated long-term protection) would seek to strengthen the potential weak spots of Prototype 2, including the risk of resistance loss (by using mixed-variety plantings within the row) and the introduction of external inoculum (by using microbiological control for hyperparasitism).

Case 3: Potato late blight



As in the preceding case, all three prototypes were based on zero use of copper.

In **Prototype 1**, the strategy is simply to replace copper treatments with biocontrol products (PDS or biocides), with application dates determined using a specialized DMT. As with scab, the limited individual effectiveness of these methods would probably make this prototype inadequate in terms of protection efficacy, especially in climates strongly favorable to late blight. A higher level of substitution (**Prototype 2**), including use of the most resistant varieties available and a strict use of disease-free planting material, especially for non-certified, farm-grown tubers, would improve protection effectiveness, but would remain vulnerable to the loss of varietal resistance. **Prototype 3**, "long-term zero copper," would thus seek to strengthen this resistance with additional methods (plant architecture unfavorable to infection, mixed-variety plantings, reduction of pathogen pressure by effective management of waste plant materials near fields, etc.).

Questions critical to the elimination of copper but insufficiently explored by current research

As detailed above, achieving adequate crop protection without the use copper will in most cases require a total re-thinking of protection systems, or even of crop production systems. The analysis provided by this ESCo points to three major areas of research that will be indispensable to this project of system redesign, but which are currently significantly under-invested in by the relevant scientific communities.

The first area relates to plant pathology. Research needs include: i) the **development of guidance tools specific to alternative methods** (for example, Decision-Making Tools tailored to the specific characteristics of plant defense stimulators and microbiological control organisms), and risk prediction (sensors and monitoring devices to detect primary infections, for example); ii) integrated protection systems that **take into account not only single pests, but groups of pests for a given crop**; and iii) **assessments of the durability of alternative methods and/or strategies**.

The second area of research requiring additional investment relates to systems agronomy. Research needs here include the development of **methods and tools for the design of innovative protection systems making little or no use of synthetic and copper pesticides** (rules for combining different technical levers for different strategic objectives, decision-making rules for tactical interventions), as well as for the **long-term assessment of these integrated systems.** A handful of pioneering studies, including those within the DEPHY network, have begun to address these questions, but such studies remain the exception and are restricted to relatively specific situations (mostly perennial crops or industrial/major field crops, rarely market garden crops or specialty crops). Much work thus remains to be done to apply these approaches to the development of zero-copper systems.

Finally, a third area that has received insufficient attention relates to the economic sciences. Analyses are needed of the **economic consequences for farmers seeking to adopt alternative crop protection methods** (changes in costs, labor requirements, etc.). Also needed are studies of the **business strategies** involved farther up the supply chain, **the impacts of these strategies on the availability and diffusion of innovations**, their **variability as a function of market structures** (mass markets vs. niche markets) **and the relative importance of the different commercial companies involved** (large agrichemical companies vs. small companies and startups). One can hypothesize that the limited financial resources of startups – which generally develop biocontrol products based on public (and thus non-patentable) research material – mean they have limited R&D and marketing capacity and are thus led to pursue minimum regulatory approval status (for example as a “biostimulant” or “fertilizer” rather than as a plant protection product) or to focus on the distribution of products whose effectiveness is already well established. Conversely, the large crop protection companies, which have only recently entered the biocontrol products market *via* the acquisition of startups and/or specialized SMEs, have the financial, human and technical resources to oversee the long-term development and promotion of these types of solutions. It would be useful for economists and sociologists of innovation to closely examine this question, to consider if these hypotheses can be confirmed, theoretically and empirically, in the case of biocontrol, or if other more important factors are at work. The potential role of industry and trade associations in promoting the use of alternative to copper also deserves more study.

Lessons for and from "conventional" systems

The opportunities and impediments to the development of alternatives to the use of copper in OA are identical to those relating to alternatives to synthetic pesticides in other types of agriculture. Many of the proposed solutions are the same (resistant varieties, biocontrol, increased use of preventive measures, etc.). Many of the key questions involved are also similar, including the degree of change required within crop protection systems and the possibilities and challenges of combining several partially effective levers within an integrated protection system. Impacts on the larger supply chain, issues as to acceptability of innovations and the capacity to overcome sociotechnical lock-ins are also similar. Given these similarities, organic agriculture and non-organic agriculture could both benefit from more coordinated research approaches to these questions, provided the general findings are then tailored to the specific conditions of each production system.

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Annex. The literature corpus analyzed

By definition, an ESCo is based on a critical analysis of international scientific publications referenced in global databases. It is thus to be distinguished from “expert opinion” reports, which are based on the pre-existing knowledge of the said experts. For an ESCo, the origin of the information used should be stated and verifiable, and a question can only be examined if there are publications available on the subject.

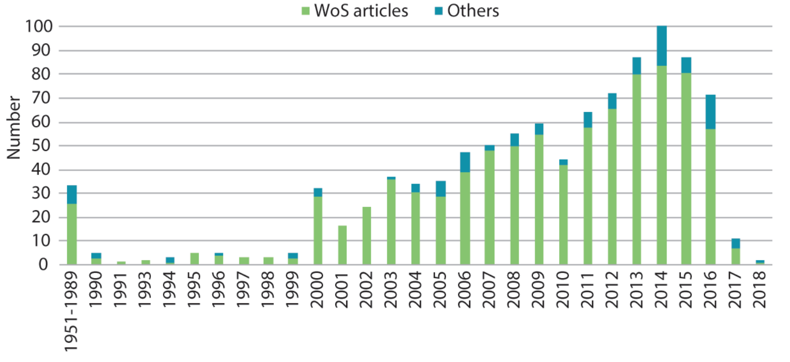
The ESCo thus supplies to the expert group a body of references extracted from ***Web of Science***. Produced by Thomson Scientific, *Web of Science* is “the” database for scientific fields worldwide; it includes all disciplines within the biophysical and social sciences. The experts then select from this initial corpus the references they consider to be relevant. They can also add publications from their own bibliographic resources, as well as technical or institutional documents they consider useful for the topic under consideration.

At the end of the exercise, the total body of references cited in the contributions of all the experts is analyzed. For this ESCo, it consisted of 992 referenced documents.

Chronological distribution of the cited references

Cited references were published between 1951 and 2018 (Figure A1). A majority of the documents cited by the experts were published after 2000, amounting to over 93% of the references. This is consistent with the decision that was made, at the beginning of the ESCo, to search the WoS database only between 2000-2016.

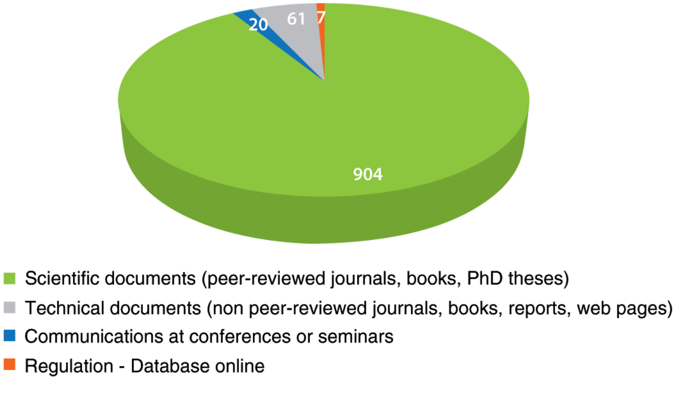
Figure A1. Temporal distribution of the 992 references cited in the ESCo report (of which 878 papers present in the WoS).



Types of references cited

The experts primarily made use of scientific documents, which represented 91% of the references cited (Figure A2). This included primarily articles published in peer-reviewed journals (over 89%). The experts also made use of technical documents (6%), as well as four regulatory texts and three online reference databases relating to approved crop protection products.

Figure A2. Types of documents cited in the ESCo report.



Countries and institutions represented by publication authors

The analysis focused on the 878 articles referenced in the WoS. A majority of the authors of these publications were from European countries (646 publications), North America (225 publications, 188 of which were from the USA), Asia (148 publications, 69 of which were from China), and South America (90 publications, 40 of these from Brazil). Within Europe, authors based in France were most numerous (149 articles), followed by Italy, the Netherlands, Germany, the UK and Switzerland (74 to 56 publications for each of these countries).

In terms of institutions, European organizations were very well represented, with INRA (128 publications), the University of Wageningen (51), CNRS (27), the BBSRC John Innes Center (20), the University of Saclay (16), and others.

Source journals

The corpus included 878 references corresponding to articles published in peer-reviewed journals (245 journals) and non-peer-reviewed technical journals (20 journals, including *Phytoma*, *Info Ctifl*, etc.). Among the main journals cited is *Acta horticulturae*, which publishes the communications to ISHS (*International society for horticultural science)* congresses. Other highly cited sources among peer-reviewed journals (Table A1) were topical journals in the field of plant pathology (*Phytopathology*, *European Journal of Plant Pathology*, *Plant Disease*), of crop protection (*Biological Control*, *Crop protection, Pest Management Science*), or on a specific plant species (*Potato Research, etc.*).

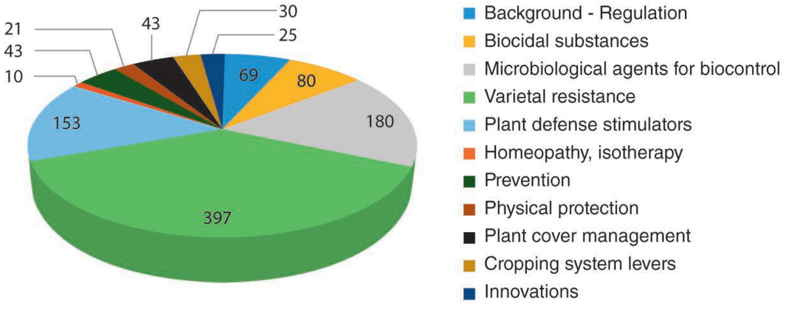
Table A1. Main peer-reviewed journals as sources of the ESCo citations

Journal	Number of articles	Journal	Number of articles
Acta Horticulturae	68	Molecular Plant-Microbe Interactions	14
Phytopathology	47	Euphytica	14
European Journal of Plant Pathology	37	Annual Review of Phytopathology	13
Plant Disease	32	Plos One	12
Plant Pathology	29	Molecular Plant Pathology	12
Biological Control	26	Potato Research	11
Crop Protection	25	American Journal of Potato Research	11
Theoretical and Applied Genetics	21	Pest Management Science	10
Molecular Breeding	15	New Phytologist	10

Distribution of references across study themes

The analysis included the 992 references cited in the different chapters of the ESCo. Three chapters had the most references: Varietal resistance, Microbial biocontrol organisms, and Plant defense stimulators (Figure A3), representing the most active research fields relevant to the ESco.

Figure A3. Number of references cited in each chapter of the ESCo report.



ESCo authors and editors

Scientific experts

Scientific leader

Didier ANDRIVON: INRA-SPE*, Rennes, UMR1349 Institut de génétique environnement et protection des plantes (IGEPP). Research Director in population biology, plant disease epidemiology and integrated pest management of potato diseases. Member of the INRA Internal Committee for Organic Agriculture (CIAB). *Scientific coordination of the work and drafting of the report parts on context, regulation and innovation.*

Contributors

Marc BARDIN: INRA-SPE, Avignon, UR0407 Pathologie végétale. Plant pathologist and microbiologist, specialising in microbial biocontrol of plant pathogenic fungi. Co-writer with P. Nicot of the report section on microbial biocontrol agents.

Cédric BERTRAND: University of Perpignan, USR3278 Centre de recherches insulaires et observatoire de l'environnement (CRIOBE). Phytochemist, expert in environmental metabolomics. President of the French-speaking group for the Study of organic pesticides of natural origin. President of the Academy for Biocontrol and Integrated Biological Protection. Scientific Director of AkiNaO. *Writer of the report section on natural biocides.*

Laurent BRUN: INRA-SPE, PACA, UE0695 Unité expérimentale recherches lintégrées – Gotheron. Agronomist and fruit tree pathologist. One of the promotors of the system trial BioREco. Writer or co-writer of the report sections on copper use, physical and agronomic control and integrated disease management systems in fruit trees.

Xavier DAIRE: INRA-SPE, Dijon, UMR1347 Agroécologie. Phytopathologist, specialist of the resistance induction by elicitors in grapes, and of PDS modes of actions and use in actual cropping conditions. Scientific co-leader of the Joint Technology Network Elicitra. Co-writer with P. Reignault of the report section on PDS.

Frédéric FABRE: INRA-SPE, Bordeaux, UMR1065 Santé et agroécologie du vignoble (SAVE). Epidemiologist and modeller, specializing in sustainable management of host resistances. Co-writer with J. Montarry of the report sections on cultivar resistances and their uses.

Christian GARY: INRA-EA*, Montpellier, UMR1230 Fonctionnement et conduite des systèmes de culture tropicaux et méditerranéens (SYSTEM). Agronomist, specialising in the design of low-pesticide cropping systems. Co-writer of the report sections on agronomic management of diseases and on systemic integration.

Josselin MONTARRY: INRA-SPE, Rennes, UMR1349 Institut de génétique environnement et protection des plantes (IGEPP). Biologist and population geneticist, with a focus on pathogen adaptation to host resistances. Co-writer with F. Fabre of the report sections on cultivar resistances and their uses.

Philippe NICOT: INRA-SPE, Avignon, UR0407 Pathologie végétale. Plant pathologist, specializing in integrated pest management and biological control of fruit and vegetable crops. President of OILB-SROP. Co-writer with M. Bardin of the report section on microbiological control.

Philippe REIGNAULT: université du Littoral Côte d'Opale, Calais, Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV). Professor in plant pathology, specialist of plant pathogen interactions and induced resistance. Co-writer with X. Daire of the report section on PDS.

Lucius TAMM: FiBL (*Forschungsinstitut für biologischen Landbau* – Institut de recherche de l'agriculture biologique), Frick (Suisse). Specialist of integrated pest management systems in organic agriculture. Head of the Plant Science Division and Deputy Director of FiBL. Contributor to the report section on integrated pest management systems.

Project management

Isabelle SAVINI: INRA-DEPE*, Paris; member of the INRA CIAB. *Project management and editorial coordination.*

Kim GIRARD: INRA-DEPE, Paris. *Project logistics.*

Documentation

Véronique DECOGNET: INRA-SPE, Avignon, UR0407 Pathologie végétale. Set-up and management of literature corpus, bibliometrics.

Anne-Sophie GRENIER: INRA-SPE, Rennes, UMR1349 IGEPP. *Set-up and management of literature corpus, bibliometrics*.

* DEPE (*Délégation à l'expertise scientifique collective, à la prospective et aux études*): Delegation for Collective Scientific Assessment, Foresight and Advanced Studies. EA (*département Environnement et Agronomie*): Environment and Agronomy division. SPE (*département Santé des plantes et Environnement*): Plant Health and Environment division.

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Copper is used to control various fungal or bacterial diseases, mainly in grapes, in fruit production and in vegetable crops. It is the only active substance approved in organic farming with a strong fungicidal effect and a wide range of action. However, the demonstration of the negative environmental effects of copper, in particular on soil and water organisms, led to regulatory restrictions on use (capping of authorized doses), and even to its ban as a pesticide in some Northern European countries.

These increasing restrictions on the use of copper, which put growers who cannot use synthetic fungicides under severe constraints, led to a recurrent demand for 'alternatives'. Numerous experimental studies have therefore been carried out to identify and test other techniques: the use of disease-resistant varieties, the application of naturally-occurring substances that have a biocidal effect and/or stimulate the plant's natural defenses, the use of microbiological control agents, the adoption of prophylactic management, and the installation of physical protection. However, results remain scattered, and these control methods are rarely implemented in the field.

Resulting from a collective scientific assessment, this volume, first published in French in 2019, is a multidisciplinary and critical synthesis of the knowledge available on the subject. It describes and assesses the different techniques potentially effective against pathogens controlled by copper treatments, and insists upon the need to combine them in integrated crop protection systems.

Didier Andrivon is Director of research at the Institute of Genetics, Environment and Plant Protection (IGEPP, Rennes); he is a former member of INRAE's Internal Committee on Organic Agriculture (CIAB), and a current member of the METABIO Steering committee.

Isabelle Savini is engineer; she works at the Delegation for Collective Scientific Assessment, Foresight and Advanced Studies (DEPE, Paris); she was also a member of the CIAB.

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