

Review

Tackling the Context-Dependency of Microbial-Induced Resistance

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Abstract: Plant protection with beneficial microbes is considered to be a promising alternative to chemical control of pests and pathogens. Beneficial microbes can boost plant defences via induced systemic resistance (ISR), enhancing plant resistance against future biotic stresses. Although the use of ISR-inducing microbes in agriculture seems promising, the activation of ISR is context-dependent: it often occurs only under particular biotic and abiotic conditions, thus making its use unpredictable and hindering its application. Although major breakthroughs in research on mechanistic aspects of ISR have been reported, ISR research is mainly conducted under highly controlled conditions, differing from those in agricultural systems. This forms one of the bottlenecks for the development of applications based on ISR-inducing microbes in commercial agriculture. We propose an approach that explicitly incorporates context-dependent factors in ISR research to improve the predictability of ISR induction under environmentally variable conditions. Here, we highlight how abiotic and biotic factors influence plant–microbe interactions in the context of ISR. We also discuss the need to raise awareness in harnessing interdisciplinary efforts between researchers and stakeholders partaking in the development of applications involving ISR-inducing microbes for sustainable agriculture.

Keywords: context-dependency; plant defence; microbial induced systemic resistance; environmental stressor; microbial inoculants

1. Introduction

In the last decades, the interest in using beneficial microbes in agriculture has grown significantly due to their ability to improve plant resistance against pathogens and insect pests and to increase tolerance to abiotic stressors [1,2]. Due to worldwide increasing food demands and the pressure to reduce the use of agrochemicals that are harmful to the environment and human health, environmentally friendly forms of crop protection in agriculture have become crucial to ensure crop yield and quality. In this context, the use of beneficial microbes is a promising alternative to the use of chemical pesticides and fungicides for sustainable agriculture and horticulture. The term “beneficial microbes” encompasses a wide range of microbes found in association with plants that confer positive effects on plant growth (as biofertilizers), defence (as plant protectants) or both. Some of the successful and well-studied examples of bio-fertilizers are plant growth-promoting

rhizobacteria (PGPR) [3], arbuscular mycorrhizal fungi (AMF) [4], and nitrogen-fixing bacteria (NFB) [5]. Others are used as biocontrol agents such as *Trichoderma* spp. that enhance protection against both plant-pathogenic fungi [6] and parasitic nematodes [7], and entomopathogenic fungi (EPF) [8] or bacteria (EPB) (e.g., *Bacillus thuringensis*) that enhance plant protection against insect pests [9]. The mechanisms underlying microbial effects on crop protection are diverse. They include both direct effects on plant pathogens and pests that are mediated by, e.g., competition for nutrients and space, the production of a wide array of antibiotics or the production of hydrolytic enzymes [10,11], and indirect effects mediated by enhanced attraction or efficacy of pest natural enemies [12] or by sensitization of the plant's immune system by a mechanism known as induced systemic resistance (ISR) that provides broad spectrum resistance against a variety of pathogens and pests. In this review, we will focus on beneficial effects of microbes on crop protection that are mediated through ISR and the challenges associated with their application in sustainable agriculture and horticulture.

ISR is an important mechanism by which beneficial microbes can help plants defend themselves. ISR can be triggered upon local recognition of elicitors such as microbe-associated molecular patterns (MAMPs) [13] and volatile organic compounds (VOCs) [14] and then cascade into a broad-spectrum systemic response through the plant. Effects of ISR on enhanced plant resistance or tolerance against microbial pathogens have been well documented [15], whereas ISR's potential role in defence against insect pests is gaining momentum [16]. However, ISR is highly context-dependent since it is often only triggered when a specific set of conditions is met and is conditional on environmental factors that can alter the outcome of plant-microbe interactions. The unpredictability of ISR events is one of the major bottlenecks for developing ISR-inducing microbes as a future technology for agriculture.

Currently, many of the beneficial microbes that have shown the potential to trigger ISR as one of their effects (Table 1) are not sold as ISR products per se, but, for example, as biostimulants or as biofertilizers even though a reduction of pathogen or pest damage through their activation of ISR may contribute to their enhancement of crop production. Notably, when they are registered as biopesticides, this is predominantly based on their direct fungicidal, nematocidal or pesticidal effects mediated by direct antibiotic or cell lytic effects and not necessarily on their indirect, plant-mediated effects, i.e., their potential to induce ISR (Table 1). This is not only due to the strict dichotomy in regulations between the registration of a product as a biofertilizer or as a biopesticide and the specific and costly requirements involved, but also because reliable expression of a strong ISR effect may be too unpredictable due to our limited understanding of the context-dependency of their biological effects. Thus, the aim of this paper is to highlight and promote stronger awareness of the context-dependency in the activation of ISR by microbes. In order to achieve more robust ISR activation under environmental conditions inherent in agricultural practices, we encourage researchers to move towards studies that resemble or take into consideration agricultural field conditions. Therefore, increasing knowledge about ISR mechanisms might help improve the development of ISR-based technologies and their applications with a higher degree of predictability and consistency.

Table 1. Examples of beneficial microbes observed to have ISR-triggering capacity and examples of conspecific strains that are currently approved and commercialized as biopesticides according to the EU Pesticide Database (<https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=search.as>; accessed on 15 June 2021). Registration categories listed as FU, BA, NE, and IN indicate Fungicide, Bactericide, Nematicide, and Insecticide, respectively. NA indicates that for a particular group of microbes, no entries were found for any of the registered categories in the EU Pesticide Database. Note that this is a non-exhaustive list that is only meant to provide examples for each group of microbes. Further note that effects of entomopathogenic fungi in the second column refer to their (indirect) ISR protective effects, even though their registration is commonly based on their direct insecticidal effects. Similarly, effects of biocontrol fungi in the second column refer to their ISR effects even though their registration is commonly based on their direct mycoparasitic effects.

ISR-Triggering Microbes	Reported ISR Protection	Ref. ¹	Registered Microbes on the EU Pesticide Database	Registered Categories
PGPR Bacteria				
<i>Bacillus</i> spp. <i>B. amyloliquefaciens</i> <i>B. cereus</i> <i>B. mycooides</i> <i>B. pasteurii</i> <i>B. pumilus</i> strain SE34 <i>B. sphaericus</i> <i>B. subtilis</i>	- - - - - - -	<i>Aspergillus niger</i> <i>Cercospora beticola</i> <i>Cucumber mosaic virus (CMV)</i> <i>Erwinia tracheiphila</i> <i>Peronospora tabacina</i>	[17,18] <i>B. amyloliquefaciens</i> MBI 600 <i>B. amyloliquefaciens</i> str. QST 713 <i>B. amyloliquefaciens</i> strain FZB24 <i>B. amyloliquefaciens</i> subsp. <i>plantarum</i> D747 <i>Bacillus firmus</i> I-1582 <i>Bacillus pumilus</i> QST 2808 <i>Bacillus subtilis</i> strain IAB/BS03	FU BA NE FU NE FU None
<i>Pseudomonas</i> spp. <i>P. fluorescens</i> WCS417r <i>P. fluorescens</i> WCS374r <i>P. putida</i> WCS358 <i>P. aeruginosa</i> 7NSK2 <i>P. fluorescens</i> CHA0 <i>P. putida</i> KT2440	- - - -	<i>Fusarium oxysporum</i> f. sp. <i>raphani</i> <i>Peronospora parasitica</i> <i>Pseudomonas syringae</i> pv <i>tomato</i>	[18–21] NA	NA
<i>Streptomyces</i> spp. <i>S. enissocaealis</i> strain IC10 <i>S. rochei</i> strain Y28 <i>S. vinaceusdrappus</i> SS14 <i>Streptomyces</i> sp. strain AcH 505	- - -	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> <i>Microspheera alphitoides</i>	[22–24] <i>Streptomyces lydicus</i> WYEC 108 <i>Streptomyces</i> K61 (formerly <i>S. griseoviridis</i>)	BA/FU FU
Entomopathogenic fungi ²				
<i>Beauveria bassiana</i> <i>B. bassiana</i> BG11 <i>B. bassiana</i> FRh2	- - - - -	<i>Bemisia tabaci</i> <i>Botrytis cinerea</i> <i>Phytium myriotylum</i> <i>Rhizoctonia solani</i> <i>Sclerotinia sclerotiorum</i> <i>Zucchini yellow mosaic virus (ZYMV)</i>	[25–29] <i>Beauveria bassiana</i> IMI389521 <i>Beauveria bassiana</i> PPRI 5339 <i>Beauveria bassiana</i> strain 147 <i>Beauveria bassiana</i> strain ATCC 74040 <i>Beauveria bassiana</i> strain GHA <i>Beauveria bassiana</i> strain NPP111B005 <i>Beauveria bassiana</i> strains ATCC 74040 and GHA	None None IN None None IN IN
<i>Metarhizium</i> spp. <i>M. robertsii</i> <i>M. brunneum</i>	- - -	<i>Fusarium solani</i> <i>Fusarium solani</i> f. sp. <i>phaseoli</i> <i>Plutella xylostella</i>	[30–32] <i>Metarhizium anisopliae</i> var. <i>anisopliae</i> strain BIPESCO 5/F52	IN
Biocontrol Fungi				
<i>Trichoderma</i> spp. <i>T. asperellum</i> <i>T. atroviride</i> <i>T. ghanense</i> T10 <i>T. harzianum</i> <i>T4, T7, T22, T39, T78</i> <i>T. hamatum</i> T17 <i>T. virens</i>	- - - - - - -	<i>Botrytis cinerea</i> <i>Colletotrichum graminicola</i> <i>Colletotrichum lindemuthianum</i> <i>Meloidogyne incognita</i> <i>Pseudomonas syringae</i> <i>Rhizoctonia solani</i> <i>Venturia inaequalis</i>	[7,33–36] <i>Trichoderma afroharzianum</i> strain T-22 <i>Trichoderma asperellum</i> <i>Trichoderma asperellum</i> strain T34 <i>Trichoderma atroviride</i> strain ITEM 908 <i>Trichoderma atroviride</i> strain T11 and IMI 206040 <i>Trichoderma atroviride</i> strain I-1237 <i>Trichoderma atroviride</i> strain SC1 <i>Trichoderma gamsii</i> strain ICC080	None None FU None None FU FU FU
Arbuscular Mycorrhizal Fungi				
<i>Funneliformis mosseae</i> <i>Gigaspora</i> sp. <i>Glomus</i> sp. MUCL 41833 <i>Glomus versiforme</i> <i>Rhizophagus irregularis</i> <i>Rhizoglomus irregulare</i>	- - - - - -	<i>Gaeumannomyces graminis</i> <i>Meloidogyne incognita</i> <i>Phytophthora infestans</i> <i>Phytophthora parasitica</i> <i>Ralstonia solanacearum</i> <i>Rhizoctonia solani</i> <i>Tetranychus urticae</i> <i>Xanthomonas campestris</i> <i>Xiphinema index</i>	[37–42] NA	NA

¹ References represent both reviewed and primary data. The authors apologize to all the researchers whose work could not be included because of space limitations. ² For some entomopathogenic fungi, plant-mediated protective effects of these microbes have been shown, although it has not been corroborated whether they are ISR-related events.

2. ISR Context-Dependency: Plants, Microbes, and the Environment

The induction of systemic resistance is not an inherent microbial trait by itself, but rather depends on the microbe's interaction with the host plant (plant's genotype) and its environment (biotic and abiotic) [43,44]. Below, we review the different phases in the activation of ISR during which context-dependency can be generated: during microbial establishment and early interactions with the host in the rhizosphere, during integration of signals involved in ISR and responses of plants to other abiotic challenges, and during top-down effects of biotic interactions aboveground on ISR (Figure 1).

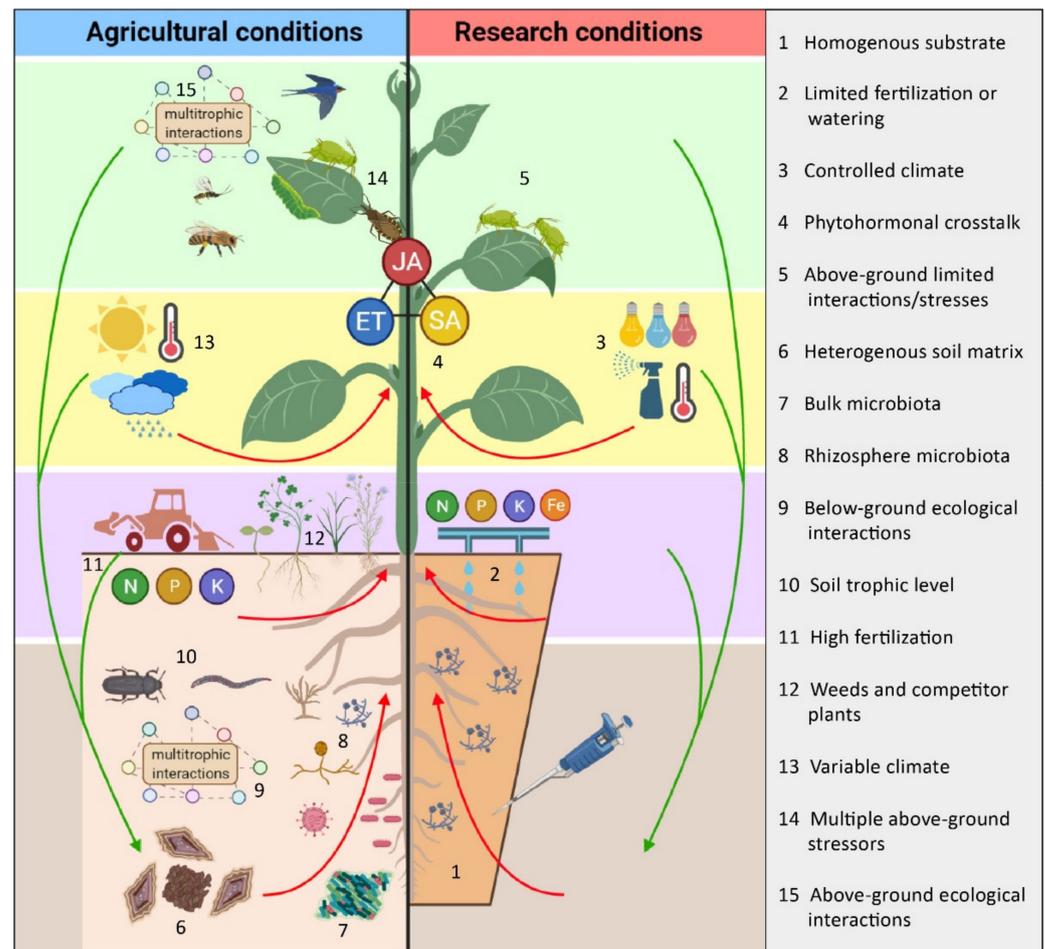


Figure 1. The context-dependency of ISR. Simplified representation of biotic and abiotic factors that can impact successful ISR activation under agricultural field conditions (**left**) and controlled research conditions (**right**). From bottom to top, red arrows indicate the progression of ISR activation from the onset at the root level to systemic transmission. Colored boxes indicate possible factors impacting activation of ISR at different stages of the process. Following the process from bottom to top: (brown) belowground triggering of ISR, (purple) impact of soil nutrient availability and plant nutritional status, (yellow) integration of systemic responses through crosstalk between signaling pathways activated by the beneficial microbes and abiotic environmental stressors, and (green) multiple above-ground stressors. Green arrows indicate cascading top-to-bottom effects that impact plant–microbe interactions belowground, and red arrows indicate bottom-up effects and environmental factors that potentially alter plant–microbe interactions and therefore ISR. Differences between the two environmental conditions (**left**, **right**) lead to variation in plant-beneficial microbe interactions and therefore differences in the potential to activate ISR, although microbial ISR activation under agricultural conditions is less explored. This image was made with © BioRender-biorender.com.

2.1. Factors Impacting the Onset of ISR in the Rhizosphere

For soil microbes, the molecular mechanisms involved in the onset of ISR are often studied at the root level where the plant recognises the beneficial microbe through microbe-associated molecular patterns (MAMPs) and a local signal is generated that cascades to the rest of the plant tissues. However, beneficial microbes inoculated into the soil go through a series of challenges such as competition for resources and production of antibiotics while interacting with the local microbiota [45] that will determine their ability to establish, proliferate, and trigger ISR in the plant. The colonization of plants by root-beneficial microbes (specifically PGPR) has been shown to be crucial for reducing or suppressing aboveground plant diseases [46,47]. The formation of root-associated biofilms are regulated by the plant's signalling and exudation patterns, but also by microbial population-dependent processes such as quorum sensing [46]. It has been observed that beneficial bacteria can activate ISR only once a minimum concentration of cells has been reached (often around 10^5 – 10^7 colony-forming units (CFU) per gram of root) for several days [48].

Whether or not successful establishment of the beneficial microbes in the rhizosphere results in activation of ISR is strongly dependent on the genetic background of the plant and microbe. The plant genetic background is a crucial factor since plants from different species or cultivars often show differences in the extent to which they express ISR in response to a single microbial strain [49]. For example, *Pseudomonas fluorescens* WCS374r has been shown to induce resistance in radish (*Rhaphanus sativus*) [50] but not in *Arabidopsis thaliana* [51] while *Pseudomonas putida* strain WCS358r can elicit ISR in *Arabidopsis* but not in radish [51]. Interestingly, *P. fluorescens* strain WCS417r elicited resistance in both radish and *Arabidopsis* plants [51]. Although ISR activation has been observed in a wide range of commercial crop species (tomato, cucumber, tobacco, bean, etc.) against different biotic stresses [17], the molecular mechanisms underlying ISR activation in local tissues and systemic signalling have been mainly studied in only a few model plant species, such as *A. thaliana* for PGPR, and *Solanum lycopersicum* as well as *Medicago truncatula* for arbuscular mycorrhizal fungi. Hence, our knowledge of ISR metabolic pathways in commercial crops is still limited, and it is often unknown whether molecular mechanisms observed in model species can be extrapolated to other crop species.

2.2. ISR Activation in the Plant: Interactions with Plant Responses to Other Abiotic Factors in the Environment

Upon recognition by the plant, microbial elicitors cause physiological changes in the plant by activation of a network of signalling molecules including, e.g., reactive oxygen species (ROS) [52] and reactive nitrogen species (RNS) [53], as well as by affecting the levels of various phytohormones, mainly jasmonic acid (JA) and ethylene (ET), that act as central players in regulating ISR [15]. Despite the fact that JA and ET are considered the main phytohormones involved in the regulation of ISR response, other phytohormones are also known to take part in the ISR response. For example, salicylic acid (SA) is also involved, but not by triggering the hypersensitive response typical for systemic acquired resistance (SAR) [54]. Recently, other phytohormones, including members of the oxylipins family (to which JA belongs), have been found to be involved in signalling as well. Importantly, plants integrate information about the different aspects of their abiotic and biotic environment through cross-talk between the different signalling pathways that transduce information about these various aspects of their environment [55,56]. This allows them to prioritize and fine-tune their responses to the most imminent challenges posed by their environment as a whole [57]. However, it also means that the mere presence of microbes that have the potential to activate ISR is no guarantee that plants will actually activate ISR in response to the detection of their presence [58]. Therefore, further knowledge on phytohormonal regulation is crucial to understand how responses to other environmental factors are integrated in the plant's phytohormonal system and thus how they impact ISR activation. A wide variety of abiotic factors have been shown to impact ISR, including interactions with

nutrient availability [59,60], soil organic matter content [61,62], soil moisture [63–65], soil pH [66], and light (quality and intensity) [67,68]. Below, we focus on nutrient availability as recent studies have highlighted the importance of nutrient deficiencies in governing patterns of root exudation of plant secondary metabolites involved in the regulation of plant–microbe mutualistic interactions [69].

The most studied nutrient-deficiency responses are for phosphorus (P), iron (Fe), and nitrogen (N). Phosphorus deficiency in plants triggers the activation of the phosphate starvation response (PSR) and the production of strigolactones [70], which are important in regulating the plant’s interaction with symbiotic microbes such as AMF and other endophytic fungi [71]. Under iron deficiency, it has been observed that *Arabidopsis* plants produce coumarins [72], which are defence-related secondary metabolites that are also involved in re-shaping the plant root microbiome. Under nitrogen deficiency, legumes release flavonoids in the rhizosphere that attract rhizobia and induce *nod* genes in rhizobia to synthesize Nod factors [73]. Hence, nutrient deficiency can impact ISR activation by altering the recruitment of beneficial microbes through modified root exudation patterns [72] or by leading to changes in plant immunity that alter plant–microbe interactions. Although the recruitment of beneficial microbes under nutrient deficiency often benefits plants, it should be noted that this is not always the case. For instance, under P-deficiency, a recruited PGPR strain, *Bacillus amyloliquefaciens*, induced hypersensitivity to P-deficiency in *A. thaliana* through its response to an emitted volatile compound, diacetyl [74].

Although the link between nutrient deficiency responses and immunity is still not fully understood, it is crucial for understanding how plants regulate their microbiome and whether or not they prioritize microbial relationships as a way of alleviating stress. In particular, the link between the PSR and immunity has been well documented in *A. thaliana* [71,74,75] where P-deficiency triggers the expression of the PSR master transcriptional regulators PHR1 and PHL1. This leads to changes in the phytohormonal balance; expression of JA-inducible genes is enhanced while that of SA-inducible genes is repressed [75]. As a consequence, PSR signalling has been shown to result in induction of the JA signalling pathway and enhanced defence against a generalist leaf chewing insect herbivore in *A. thaliana*, tomato, and tobacco [76], but is associated with enhanced susceptibility to a bacterial and an oomycete pathogen [75]. The complexity of the interaction between phosphate availability, ISR, and immunity is illustrated by the work of Spagnoletti et al. [77]. These authors showed that low P, as expected, resulted in a 2.5-fold increase in susceptibility to charcoal rot in soybean. However, in the presence of AMF, low P enhanced AMF colonization, resulting in a 5-fold AMF-induced reduction in disease susceptibility that more than compensated the observed increase in disease susceptibility caused by low P in their absence. This indicates that whereas low P itself increased disease susceptibility of plants, it decreased susceptibility of plants in the presence of AMF through AMF-induced systemic resistance. There are also increasing reports evidencing a relationship between iron (Fe) deficiency and induced systemic resistance [78–80].

Finally, it should be noted that there is an immediate link between nutrient availability and defense, since downstream of defense activation, the actual production of defense metabolites is often a costly process [81,82] requiring sufficient amounts and appropriate stoichiometry of the elements involved in the production of the defense metabolites in the activated biosynthetic pathways.

2.3. Impact of Biotic Factors on the Activation and Effectiveness of ISR

In addition to abiotic factors such as nutrient availability, biotic factors can also impact the activation and effectiveness of ISR. Moreover, not only the bottom-up effects of, e.g., soil nutrients and microbial competition can have an impact on ISR, but also top-down effects. For instance, herbivore-induced changes in root exudation can alter the recruitment of ISR-inducing microbes via changes in belowground microbial communities, plant phytohormones or resource competition [83] and subsequently feedback on the extent to which ISR is activated. Herbivory or pathogen infection can trigger plant responses that change

the root exudate profile and affect the structure of the rhizosphere community [46,84] and the recruitment and root colonization of beneficial microbes [85–87]. Several studies have reported effects of herbivory on mycorrhizal colonization, ranging from an increase to no effect to a decrease [88,89]. To date, these findings have not yet been tested in the context of ISR, even though they could potentially open up new insights into top-down effects on ISR when using a community approach [90]. Not much is known about the effects of natural enemies of insect herbivores on ISR. For example, parasitoids can alter interactions between herbivores and their host plant through changes in herbivore oral secretions, affecting the expression of genes involved in the JA signalling pathway [91]. Similarly, facultative endosymbionts in sap-feeding insects, such as *Hamiltonella defensa*, can alter plant defence, suppressing JA signalling [92]. It would be interesting to explore whether such responses to higher trophic levels could impact ISR activation when beneficial microbes are present.

Furthermore, the identity of the pathogen or pest species at which ISR is aimed is an important determinant of the effectiveness of ISR. Depending on the context, the outcome of a plant-beneficial microbe interaction can range from a positive to a neutral or negative impact on plant growth and resistance to stresses [93,94], also described as a “continuum from mutualism to parasitism” [95]. The effectiveness of ISR will, amongst others, be determined by the nature of the biotic stressor. In particular, focusing on ISR against insect pests, Pineda et al. [94] concluded that the degree of specialization and feeding guild of the insects can impact the outcome of plant–microbe–insect interactions. For instance, it has been observed that specialist herbivore insects are less affected when feeding on plants colonized by ISR-triggering microbes than generalists [96]. Other studies have shown that leaf chewing insects appear to be negatively affected when feeding from plants colonized by AMF and entomopathogenic fungi (EPF), whereas sap sucking aphids respond positively to AMF [93]. This large response variation among feeding styles can be related to susceptibility to the particular secondary metabolites that are induced in the feeding plant tissues [93,96] as a result of ISR activation. Furthermore, plant–microbe–insect interactions should be regarded within the context of a multi-trophic system, acknowledging it as a result of eco-evolutionary forces that can have either a negative or positive impact on the herbivores as well as on their natural enemies [97].

3. Activation of ISR under Agricultural vs. Controlled Environmental Conditions

The activation of ISR under agricultural conditions is unpredictable because it depends on the interaction between the host plant, the microbial elicitor, and the particular environment. Among studies of plant–microbe interactions, research on the effects of ISR-inducing microbes in agricultural environments is still under-represented compared to research performed under controlled conditions. For example, Berruti et al. [4] showed that out of 157 studies on AMF, only 24% were performed under agricultural field conditions while results obtained under controlled greenhouse or growth chamber conditions were much more numerous (69%). Here, we aim to highlight a few examples of agricultural factors that are currently studied within the general framework of plant–microbe interactions and that could particularly influence the ISR process (Figure 1), and approaches to enhance the efficacy of ISR inducing microbes.

3.1. Agricultural Practices Affecting Activation and Efficacy of ISR

Several studies have shown that cultivation practices such as fertilization, pest management, and tillage regime can strongly affect soil microbial dynamics and diversity, as well as microbe–plant interactions [98,99]. Microbial inoculants are often used in substrate-grown horticultural crops (i.e., not grown in soil), but also in agricultural soils, which are heterogeneous in physical, chemical, and biological composition. Crops in conventional agricultural systems typically receive high fertilizer inputs to achieve maximum yield, whereas ISR research is often intentionally carried out under low fertilization regimes to promote the establishment of the plant–microbe association. Nutrient abundance in a system might make the plant less inclined to invest in microbial relationships [71,100]

and as discussed before, can also impact ISR activation. For example, under long-term P-fertilization, the percentage of root length colonised by AMF in maize is reduced [101] and the protection against herbivorous insects is often lost when the soil is supplemented with phosphorus [102] or nitrogen [103]. However, in some cases, fertilization results in enhanced rather than reduced resistance. For example, Vesterlund et al. [104] observed that nitrogen fertilization boosted the negative effect of fungal endophytes on herbivore performance in tall fescue. Thus, the fertilization regime can potentially alter the soil microbial community composition and ecological functions [105] but also the plant's interactions with beneficial microbes in terms of selective recruitment [60,106]. Not only N- and P-fertilization but also crop rotation and tillage may have profound effects on the dynamics of rhizosphere microbes, including the ones involved in inducing resistance. In some organically managed agricultural soils, it has been shown that the resident microbial community that is established by the long term effect of organic management enhances the induction of resistance in subsequently grown plants [107]. Tillage can disturb or reduce such a build-up of disease- or pest-suppressive soils [108].

3.2. Approaches to Enhance the Exploitation of ISR-Inducing Microbes in Sustainable Agriculture and Horticulture

The context-dependency of the induction of ISR has led to the question whether there is an optimal set of agricultural environmental conditions and management practices that reduces this context-dependency and that leads to a more reliable expression of ISR in response to application of bioinoculants capable of inducing ISR. Currently, research efforts are focused, amongst others, on improving the formulation of microbial inoculants (encapsulation, seed coating, gel, etc.) [109], their composition (i.e., using consortia of microbes rather than single species), and the mode and dose of application [110,111]. In addition, development of plant cultivars for more efficient plant–microbe interactions or crop management can help optimize the efficacy of bioinoculants for crop growth and protection [112]. However, alternatively, we might consider that ISR is inherently context-dependent and that there is no generalizable set of conditions that will reduce its context-dependency. This could lead to re-thinking our current approaches to exploit microbially induced ISR.

Several recent studies have argued for alternative approaches to harness beneficial microbes for crop growth and protection that are not (only) based on bioinoculations, but that are based on steering the local rhizosphere microbiome [113–116]. For example, crop rotation which is mostly designed to minimize the build-up of crop-specific pathogens (i.e., avoiding negative legacies), could be used for building up positive soil legacies that can steer soil microbial communities towards ones that benefit crop growth, e.g., by harbouring a larger fraction of biota able to induce ISR. Proof of concept of the successful steering of pest and disease suppression by previous crops has been provided by several recent studies [107,117–119]. Such soil legacies could in principle be created by previous crops in a crop rotation or cover crops. Steering of the soil microbiome for enhanced pest and disease suppression can also be mediated by other agricultural practices such as soil amendments [120]. The advantage of these approaches is that they recruit ISR-inducing bacteria under the prevailing crop cultivation conditions, enhancing the chances that they are indeed functional under these conditions. In addition, several studies have argued for new crop breeding strategies that enhance the ability of crops to better exploit associations with beneficial microbes by enhanced attraction of beneficials, enhanced symbiosis, or enhanced plant responses to this symbiosis (e.g., [121]). Several studies have revealed the plant genetic underpinnings of enhanced microbially induced pest suppression (e.g., [122]), making this a viable prospect that can be tailored specifically to ISR-inducing microbes.

4. Fostering Cooperation and Synergies Bridging the Gap between Science and Industry

Despite the increasing amount of applied research on ISR-inducing microbes, the questions of how to create environmental conditions that favour ISR and how to mitigate factors

that cause unpredictability in the activation of ISR are still a major knowledge gap. More interdisciplinary research focusing on the mechanisms underlying the context-dependency of plant–microbe interactions will enhance the predictability of the effects of not only ISR-based products but also microbial inoculants in general. Experiments performed under conditions that (1) better mimic the complex set of conditions of agricultural systems or (2) test targeted sets of environmental conditions should be the next step moving forward in ISR research. Ideally, collaboration processes would, on the one hand, be research-driven, but on the other hand, would consider the reality of current agricultural practices. Various areas of cooperation may entail, for instance: (1) partnership between the commercial sector and academia; (2) interdisciplinary studies across different countries evaluating context-dependency using a common study system, and (3) long-term experiments that can account for variabilities and uncertainties observed under field conditions with the involvement of farmers or land managers. Such interdisciplinary experiments will require great efforts from the interested parties in terms of agreeing on clear objectives and agricultural practices to produce reliable and comparable data. Interdisciplinary experiments along with field surveys and parallel research activities under controlled conditions can provide an excellent overall understanding of ISR mechanisms, yielding more holistic knowledge that could speed up the development of ISR-based technology.

Fostering cooperation between academia, companies, and research institutions would be beneficial for the development of innovations based on current challenges in crop protection [119,123]. One example for synergies can be found in current large field-scale studies and microbial screening processes that require time and financial investments but generate vast amount of data. The majority of tested microbial candidates are discarded if they do not produce the required outcomes across multiple conditions; however, the data generated during the screening processes, including data from unsuccessful ISR events, can provide valuable experimental information. Therefore, screening experiments, in which large numbers of microbial strains are tested for ISR activation, are a suitable scenario to delve into the context-dependency of ISR. The lack of reported unsuccessful ISR events in the literature creates a bias towards positive effects of inoculants and the risk of duplicated efforts. Just as multidisciplinary studies and data availability should broaden other roads for ISR-based technology, implementation should be considered for adjustment, such as regulatory frameworks.

Regulatory frameworks can be an additional obstacle for innovation processes and can hamper further research and development by manufacturers and biotechnology companies if compliance is disproportional to the cost of product development [124]. This could create a delay in the technological responses to current challenges. The European Union regulatory framework requires specific information, tests, and standards for certain product categories. However, the mandatory procedures are not tailored to assess the potential of ISR-inducing microbes [125]. This is because required tests for products such as biostimulants are focused on tolerance of the plant to abiotic stress and/or efficiency of uptake of available nutrients, rather than on biotic stress resistance. Malusa & Vasillev [126] state that it is important to evaluate a product such as a biofertiliser and its efficacy while taking into account the external conditions. However, at the moment, the legislative framework does not fully account for the specific processes and effects that can be achieved by using innovation based on ISR.

Currently, under the definition of biofertilizers or biostimulants, a large variety of products that are widely different in composition and action are offered on the market. In fact, under the new European regulation on fertilizers (reg. 2019/1009), some microbial-based biostimulants have “multifunctional” features, including ISR, that are not taken into consideration (Table 1) [31]. Thereby, the authors argue for a consideration of the wider context and to harness available synergies whenever possible. All in all, the challenge of legislation is to, on the one hand, allow manufacturers to develop a product under a fitting framework, and on the other hand, to provide enough structure to ensure the quality and reproducibility of these innovations.

5. Conclusions

Despite almost 30 years of microbial ISR research, one of the major bottlenecks for its success as a strategy to provide crop protection through microbial inoculation is its low effectiveness under agricultural conditions. Today, the challenge is to understand how to minimize the high ISR context-dependency: to mitigate its unpredictability by unravelling the mechanisms that enable the plant to trigger ISR and decipher the specific set of biotic and abiotic conditions that enable it. This knowledge gap is becoming crucial for the successful development of ISR-microbe based agricultural products and to provide a novel biotechnological solution that performs adequately according to farmers' needs. Currently, researchers have put major efforts into understanding the role of nutrient deficiency as a key driver of plant-microbe mutualisms impacting ISR activation and also on the plants' systemic communication between above- and below-ground plant parts. However, such studies under controlled conditions often miss the complex agricultural reality where cropping systems, fertilization, and multi-trophic interactions are variable. Looking into the future, further steps should be taken among researchers and stakeholders promoting synergies and more applied ISR research that considers the complexity of agricultural conditions. Although its implementation as a technology still poses practical challenges, microbial-induced resistance should not be underestimated as an innovative alternative to chemical plant protection. Additionally, further studies are needed to increase our knowledge of plant-microbe interactions and in particular of conditions leading to unsuccessful ISR events, which could be a source of predictability and knowledge to improve its potential application as a technology.

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