

Spray-induced gene silencing in the battle against pathogens, pests and weeds on our fields – soon reality or still a sci-fi?

IVETA VACHOVÁ¹ , JHONNY STALYN HERNÁNDEZ OROZCO¹ , TEREZA KALISTOVÁ¹ ,
GUY SMAGGHE^{2,3} , MARTIN JANDA^{1*} 

¹Department of Experimental Plant Biology, Faculty of Science,
University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

²Institute of Entomology, Guizhou University, Guiyang, P.R. China

³Department of Biology, Vrije Universiteit Brussel (VUB), Brussel, Belgium

*Corresponding author: mjanda04@jcu.cz

Citation: Vachová I. Orozco J.S.H., Kalistová T., Smagghe G., Janda M. (2026): Spray-induced gene silencing in the battle against pathogens, pests and weeds on our fields – soon reality or still a sci-fi? *Plant Protect. Sci.*, 62: 303–317.

Abstract: Agriculture faces ongoing challenges from pathogens, pests and weed threats that are amplified by climate change, intensive monoculture farming practices, and regulatory restrictions limiting traditional chemical treatments. To enhance resilience, innovative solutions are urgently needed. One promising strategy is RNA interference (RNAi), a natural mechanism for gene silencing. Among RNAi-based approaches, spray-induced gene silencing (SIGS) stands out for its ability to deliver double-stranded RNA (dsRNA) directly to eukaryotic organisms, modulating gene expression to suppress harmful species. Recent research demonstrates that appropriately designed dsRNA can suppress growth or induce mortality in over fifty pestiferous species, including fungi, oomycetes, nematodes, insects, and even weeds. Importantly, the first commercial SIGS-based product received U.S. Environmental Protection Agency approval in December 2023, highlighting advances in cost-effective dsRNA production and extended shelf stability. This review emphasises the practical applications and innovations of SIGS, providing clear guidelines for translating laboratory findings to field success. By critically evaluating current progress, this review highlights SIGS as a promising tool for sustainable agriculture while identifying challenges that must be addressed to fully realise its potential.

Keywords: SIGS; RNA interference; Crop protection; dsRNA; nanoparticles; *Calantha*; sustainable agriculture; pathogens and pests

Agriculture has historically faced numerous challenges, with farmers continually striving to mitigate yield losses from pathogens, pests, and weeds. Traditional management strategies have included mechanical removal of the attacker, field crop rotation, and chemical treatments of diverse origins (including organic

and "bio-based" solutions). Since the mid-20th century, the intensification of agriculture has made high, stable crop yields increasingly dependent on herbicides, fungicides, and pesticides (Beckerman et al. 2023).

However, this reliance carries substantial drawbacks. Many chemical agents exhibit broad-spec-

Supported by the MEYS Inter-Excellence II, Inter-COST project No. LUC23146 (IV, JSHO, TK, MJ) and from exRNA-PATH COST Action CA20110 (<https://exrnath.eu/>) (MJ; JSHO; GS).

© The authors. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).

trum activity, which negatively impacts biodiversity (Rashid et al. 2010; Beckerman et al. 2023), while their residues pose potential risks to human health (Alengebawy et al. 2021; Lini et al. 2024). Consequently, regulatory authorities, particularly within the European Union, are increasingly restricting or banning certain fungicides, pesticides (including seed treatments), and herbicides (Storck et al. 2017; Stokstad 2018; Silva et al. 2022). Under the EU's "Farm to Fork" strategy, for example, the use of high-risk pesticides is targeted for a 50% reduction by 2030 relative to the 2015–2017 baseline (European Commission – Farm to Fork Strategy; Silva et al. 2022).

This regulatory shift imposes growing pressure on farmers and introduces uncertainty about future crop yields, creating an urgent need for innovative alternatives. One of the most promising approaches is the use of short double-stranded RNA (dsRNA) molecules, which exploit the natural mechanism of RNA interference (RNAi) to specifically suppress the growth or viability of pathogens, pests, and weeds (Agrawal et al. 2003; NobelPrize.org 2006). This technology, commonly referred to as spray-induced gene silencing (SIGS), offers a versatile, target-specific, and environmentally friendly strategy for crop protection (Chen et al. 2025a; Kostov & Smagghe 2025).

Recent innovations have significantly expanded the applications of SIGS. Advances in nanocarrier formulations, such as layered double hydroxides (LDHs), liposomes, and biodegradable polymeric nanoparticles, now enable efficient delivery and prolonged stability of dsRNA molecules under field conditions. These innovations enhance plant and target-organism uptake, overcome environmental degradation, and facilitate controlled release, thereby addressing previous limitations of RNA-based crop protection (Mosquera et al. 2025; Smagghe et al. 2025). Additionally, the commercialisation of the first dsRNA-based product, Calantha®, marks a critical milestone, demonstrating the feasibility of RNAi-based crop protection on a commercial scale.

This systematic review highlights the design principles, delivery strategies, and practical applications of SIGS, accompanied by a curated list of successful studies and tested nanocarriers. We also discuss the key barriers to widespread adoption, including regulatory challenges, environmental safety assessments, and scalability. By integrat-

ing recent innovations with practical guidelines, this article aims to provide a roadmap for both researchers and practitioners seeking to implement RNAi-based solutions in modern plant protection systems. For more detailed recent reviews and comprehensive discussions, we recommend Chen et al. (2025a), Mosquera et al. (2025), and the book by Smagghe et al. (2025).

RESEARCH DYNAMICS OF SPRAY-INDUCED GENE SILENCING

SIGS is a relatively recent approach under consideration for plant protection. The first notable mention of the term, appearing in the title or abstract of a peer-reviewed article indexed in the Web of Science database, dates back to 2016 (Koch et al. 2016). Since then, as illustrated in Figure 1, publication activity has grown exponentially, reflecting a corresponding increase in research intensity and interest in this topic.

Our literature survey differs quantitatively from the dataset reported by Mosquera et al. (2025), primarily due to our narrower focus. We restricted our search to publications that explicitly used the term "spray-induced gene silencing", thereby ensuring direct relevance to the scope of this concise review. In contrast, Mosquera et al. (2025) applied broader search criteria, including general RNAi-related terms and also "Host-Induced Gene Silencing" (HIGS), resulting in a dataset that extended back to 2000 and comprising more than a thousand publications. This difference aligns with historical fact: the principal idea underlying SIGS predates 2016; the concept of using exogenous dsRNA for gene silencing was initially observed in the nematode *Caenorhabditis elegans* through feeding experiments (Whangbo et al. 2017). Nevertheless, both analyses reveal a similar exponential trend in research (publishing) activity, highlighting the growing scientific focus on RNAi-based plant protection strategies.

Our survey further revealed a relatively low ratio of original research articles to review papers (1.5). For comparison, the ratio is approximately 5 for CRISPR-related publications and around 10 for "RNAi"-related studies. Except for the year 2024, the ratio in SIGS publications remains close to 1 (Figure 1). In our view, this low ratio, indicating a high proportion of review and conceptual papers, reflects substantial speculation about the potential

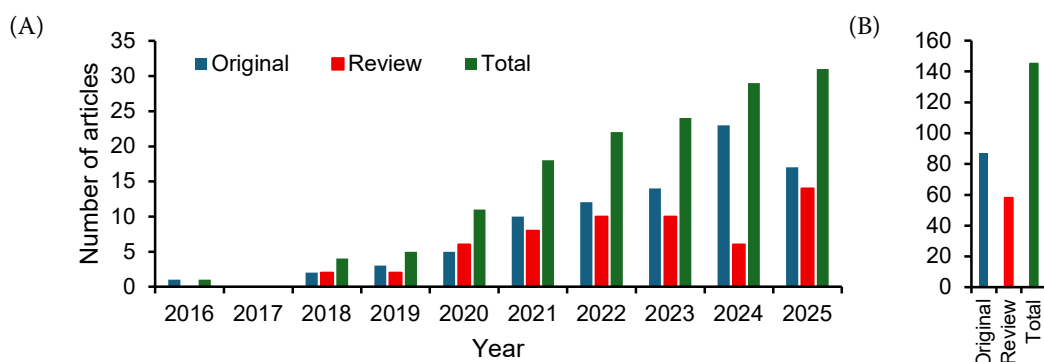


Figure 1. Number of publications on spray-induced gene silencing (SIGS) recorded in the Web of Science database. Publications were retrieved using the search terms "spray-induced gene silencing" and "spray-induced gene silencing" followed by manual curation to distinguish between reviews (red columns) and original research articles (blue columns) and to remove potential duplicates. The total number of publications in the given year is in green. (A) shows the publication history from 2016 to 2025 (September), and (B) shows the total number of publications highlighted in (A).

applications of SIGS, its underlying mechanisms, and broader biological implications. It underscores that many concepts, particularly in agricultural contexts, remain to be experimentally validated.

Notably, recent studies are moving beyond conceptual frameworks toward practical applications. Advances in nanocarrier formulations, including LDH clays, chitosan nanoparticles, liposomes, and biodegradable polymeric carriers, have enhanced the stability, uptake, and field persistence of dsRNA molecules. These innovations enable targeted delivery to pathogens, pests, or weeds, improving silencing efficiency under variable environmental conditions (Mosquera et al. 2025; Smagghe et al. 2025). In parallel, the development of field-ready dsRNA formulations, exemplified by commercial products such as Calantha[®], demonstrates that SIGS can now move from laboratory-scale experiments to scalable agricultural applications. Together, these technological advances highlight a clear shift from theoretical potential to actionable strategies, indicating that SIGS is becoming a feasible, environmentally friendly alternative to conventional chemical crop protection agents.

PRINCIPLE OF SIGS

SIGS is a technological approach based on RNAi, a biological process for which Andrew Fire and Craig Mello were awarded the Nobel Prize in Physiology or Medicine in 2006 (NobelPrize.org 2006). The mechanism of RNAi has been extensively described, including in the context of SIGS (Nobel-

Prize.org 2006; Kostov & Smagghe 2025), and will be briefly summarised here.

RNAi begins with the formation of dsRNA, which is recognised by the Dicer protein and cleaved into small fragments approximately 20–25 base pairs (bp) in length. These fragments associate with proteins to form the RNA-induced silencing complex (RISC), which includes, among others, Argonaute, an enzyme responsible for the precise cleavage of target messenger RNA (mRNA) (Rand et al. 2004; Redfern et al. 2013; Nakanishi 2016). The complex is guided to the target mRNA by a single-stranded small interfering RNA (siRNA) derived from the original dsRNA. High specificity of RNAi is ensured by sequence complementarity between the siRNA and the target mRNA, leading to mRNA degradation (Agrawal et al. 2003).

A key breakthrough in the development of SIGS as an agricultural application occurred in 2013, when Weiberg et al. (2013) demonstrated that RNA can mediate communication between a host plant (*Arabidopsis thaliana*) and a fungal pathogen (*Botrytis cinerea*). Specifically, *B. cinerea* siRNAs were shown to modulate *A. thaliana* immunity (Weiberg et al. 2013). Since then, RNA-based bidirectional communication has been documented between plants and a wide range of organisms, including bacteria (Ren et al. 2019; Jang et al. 2024), fungi, which are both pathogenic (Weiberg et al. 2013; Cai et al. 2018) and symbiotic (Roth et al. 2019), oomycetes (Hou et al. 2019; Dunker et al. 2020), nematodes (Chow et al. 2019), insects (Ghosh et al. 2017), and even other plants

(Shahid et al. 2018) see also reviews (Zhang et al. 2017; Cai et al. 2019; Huang et al. 2019; Wang & Dean 2020; Chen & Rechavi 2022).

The origin of the dsRNA or siRNA distinguishes two main strategies for using RNAi against pathogens and pests. When the siRNA is produced endogenously by the plant, the approach is referred to as host-induced gene silencing (HIGS). This method involves the development of transgenic crops, which remains controversial, particularly in the European Union. Although HIGS can offer highly effective and environmentally friendly solutions, this review focuses on the second strategy: SIGS.

In SIGS, dsRNA is synthesised externally and applied to the plant, typically via foliar spraying, hence the term "spray-induced" (Qiao et al. 2021). This exogenous application eliminates the need for transgenic plants. Studies have shown that dsRNA can be delivered through various methods, including foliar sprays, soil application, soaking of plant tissues, or direct infiltration into leaves (for detailed discussion, see reviews (Chen et al. 2025a; Germing et al. 2025; Kostov & Smagghe 2025; Mosquera et al. 2025)).

DOCUMENTED SUCCESSFUL APPLICATIONS OF SIGS TO PROTECT CROPS AGAINST ATTACKERS

Recent reviews (He et al. 2024; Chen et al. 2025a) provided what initially appears to be an exhaustive list of 41 distinct pathogens (including viruses) and pest species against which SIGS has been successfully applied under laboratory conditions [included in electronic supplementary material (ESM Table S1)]. However, we found that not all entries in their lists can be unequivocally considered SIGs, and in some cases, the original literature did not report a clear effect. Our literature survey focused exclusively on SIGS and identified at least 23 additional species for which SIGS has been tested with positive results (ESM Table S1). Consistent with Chen et al. (2025a) and He et al. (2024), we excluded studies on nematodes, the first organisms for which the "SIGS" principle (e.g., feeding or flooding with dsRNA) was applied, as these studies did not explicitly use SIGS.

The compiled list (ESM Table S1) is dominated by fungi (27 species) and insects (21 species) with mites (2), oomycetes (4), viruses (8) and plants (2)

representing smaller proportions (Figure 2A). Among fungi, the most frequently studied species are *Fusarium graminearum* (10 studies) and *B. cinerea* (8 studies), followed by *Fusarium oxysporum*, *Magnaporthe oryzae*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (each with 3 studies) (ESM Table S1, Figure 2A). Insect studies are more evenly distributed across species, with most being tested in only one or two studies (ESM Table S1, Figure 2A).

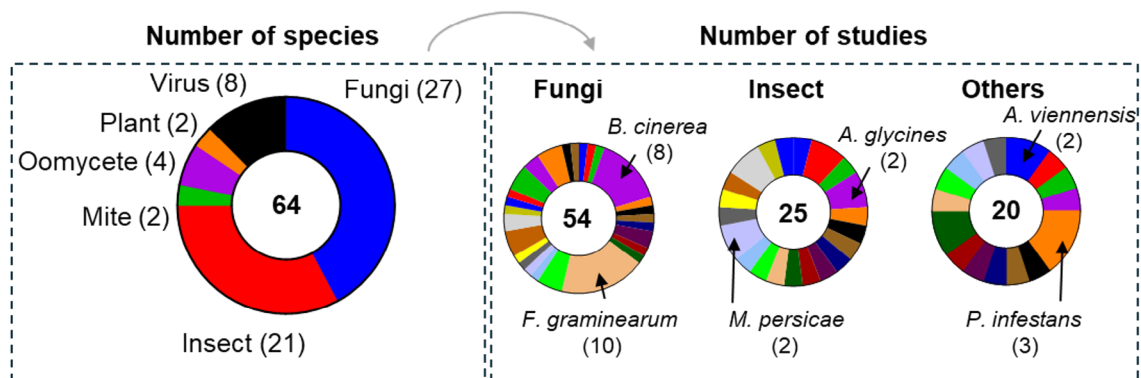
As shown in Figure 2B and ESM Table S1, SIGS studies are not limited to model host plants, reflecting the method's agricultural potential. We identified 43 distinct plant species, of which 42 are angiosperms: 35 eudicots and 7 monocots. These angiosperms are further divided into three clades: rosids (25), asterids (10) and commelinids (6) (ESM Table S1, Figure 2B). Among model plants and crops for SIGS, *Triticum aestivum* (13 studies), *Solanum lycopersicum* (8), *Vitis vinifera* (7), *Hordeum vulgare*, *Oryza sativa* and *Arabidopsis thaliana* (6), as well as *Glycine max*, *Lactuca sativa*, *Musa* and *Nicotiana benthamiana* (5) are most frequently tested (ESM Table S1, Figure 2B). Despite fewer monocot species being tested, the number of studies per species is comparable to that for eudicots, reflecting the agricultural significance of monocot crops.

This overview highlights the continuously growing number of studies validating SIGS since its discovery. From our own laboratory experience, we additionally report successful experiments using dsRNA against *S. sclerotiorum* and *Alternaria papavericola*, with *Papaver somniferum* (breadseed poppy) as the host plant (data not shown).

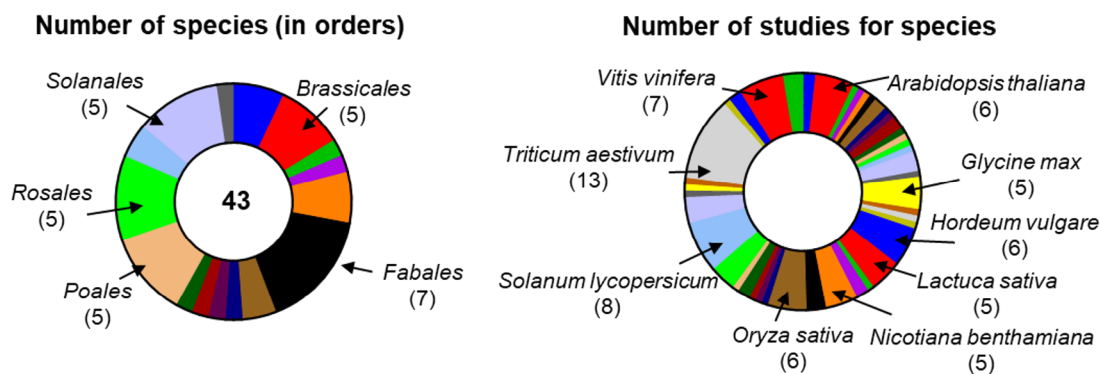
A critical factor determining the efficacy and specificity of SIGS is the selection of target genes for siRNA (dsRNA) design. Effective design of dsRNA sequence requires that the target organism be sufficiently genetically characterised. Ideally, selected genes should be essential for the survival or pathogenicity of the organism, particularly those involved in successful infection or infestation of the crop. For a comprehensive summary of target genes and design strategies, see (Mosquera et al. 2025; Smagghe et al. 2025).

As evident from ESM Table S1, many authors have drawn inspiration from previous studies when selecting target genes. Orthologs of genes effective in one organism were often tested in others, resulting in a recurring focus on genes with predicted functions related to pathogenicity and development. Functional sequences are often considered

A – pathogens and pests



B – plants (nr. of studies)



C – nanocarriers

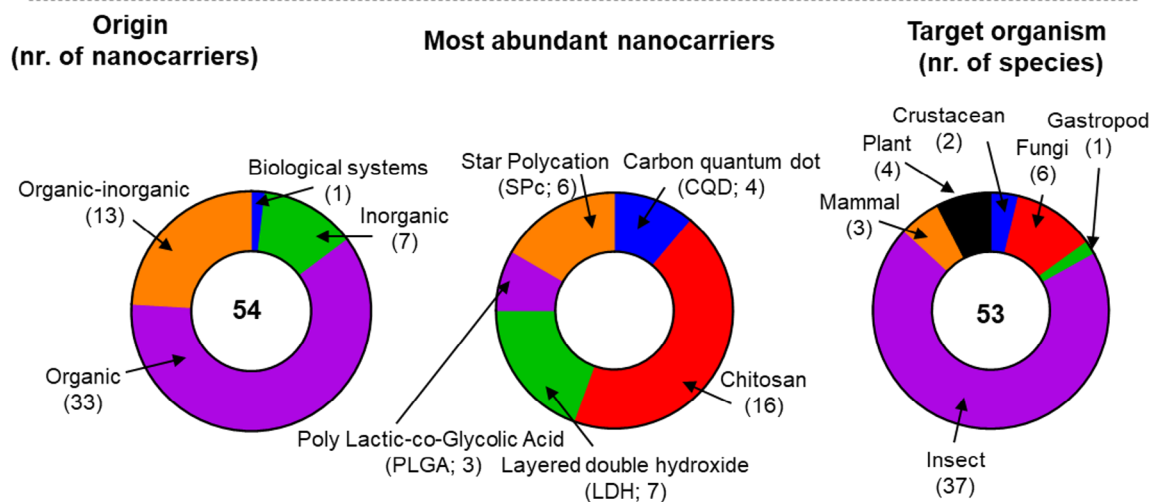


Figure 2. Graphical overview of comprehensive ESM Tables S1 and S2

(A) range of pathogens and pests targeted by SIGS (spray-induce gene silencing); (B) range of host plants for which SIGS was successfully demonstrated; (C) overview of nanocarriers used to enhance SIGS effectiveness and dsRNA stability. Numbers in the centre of the graphs indicate the total number of species or studies. In some graphs, displaying the total can be confusing because one study covers more than one plant species (right graph in panel B) and nanocarriers (middle graph in panel C), so they were not highlighted. For most graphs, only the most abundant values are indicated with text and arrows. Full details are provided in ESM Tables S1 (A, B) and S2 (C); the data represent the analysis till September 2025

for potential future commercial products (Germing et al. 2025). In fungi, oomycetes and insects, commonly targeted genes include those involved in RNAi machinery, such as Dicer-Like (DCL) and Argonaute genes (ESM Table S1), as well as genes encoding cytoskeletal proteins (actin and tubulin).

A crucial task for near-future research will be to determine which target gene properties influence the development of resistance to SIGS. Resistance has been observed in laboratory experiments with dsRNA-based control of the Colorado potato beetle (*Leptinotarsa decemlineata*), although these experiments were conducted under highly favourable artificial conditions that differ from field applications (Mishra et al. 2021; Mishra & Jurat-Fuentes 2025; Narva et al. 2025). Understanding these dynamics, along with proactive anti-resistance strategies (Gao et al. 2024), will be key to ensuring the long-term effectiveness and sustainability of SIGS-based crop protection.

Recent innovations in target gene selection and delivery strategies have further advanced the practical potential of SIGS. Modern approaches integrate bioinformatic pipelines and comparative genomics to prioritise genes that are highly conserved, essential for survival or pathogenicity, and exhibit minimal off-target effects in non-target organisms (NTOs) (Mosquera et al. 2025; Smagghe et al. 2025). Functional prioritisation now often combines transcriptomic and proteomic data to identify genes expressed during critical infection or infestation stages. These advances are closely linked with improvements in dsRNA delivery, including nanocarrier systems that enhance stability, uptake, and environmental persistence. Together, optimised target selection and advanced delivery formulations are enabling SIGS to transition from laboratory proof-of-concept studies to field-ready, crop-protection solutions, providing a more precise, sustainable alternative to conventional chemical pesticides (Germing et al. 2025; Mosquera et al. 2025; Smagghe et al. 2025).

OPTIMISATION OF DSRNA EFFICACY

While selecting appropriate target genes is crucial for the success of RNAi-based approaches, it is not the only determinant of dsRNA efficacy. The design of the dsRNA sequence itself is critical and should be tailored to the intended biological

outcome. Sequence design influences specificity, depending on the uniqueness of the selected DNA region. For example, targeting a conserved gene across a taxonomic group (typically genes involved in development) may allow broad-spectrum control, but it increases the risk of off-target effects. In contrast, targeting a species-specific gene requires identifying unique sequences, which is more challenging, but typically focuses on genes connected with virulence or pathogenicity.

Another important factor is the length of the dsRNA, typically ranging from 300 to 900 bp. Longer sequences generally tend to be more effective and reduce the risk of resistance development in the targeted organism, although this is not universal, as shown in a study controlling *Austropuccinia psidii* infection (Höfle et al. 2020; Werner et al. 2020; Degnan et al. 2023a). Designing longer dsRNAs increases the difficulty of avoiding off-target effects, though the likelihood of off-target effects is more strongly influenced by target gene selection (Wang et al. 2023). High sequence complementarity between dsRNA and the target mRNA significantly enhances silencing efficiency (Höfle et al. 2020; Mosquera et al. 2025).

Stability of dsRNA is another critical consideration. Several studies report that dsRNA is unstable when exposed to environmental factors such as UV radiation, ubiquitous nucleases, or rainfall (San Miguel & Scott 2016; Mitter et al. 2017; Dhandapani et al. 2019; Bennett et al. 2020; Niño-Sánchez et al. 2022; Yang et al. 2022; Qiao et al. 2023; Wang et al. 2023). While this highlights its biodegradability and environmental safety, reduced stability can compromise efficacy under field conditions. Stability can be improved through thoughtful sequence design, considering secondary structures which can be optimised manually or computationally (Werner et al. 2020). Additionally, tertiary structures such as hairpin loops or tetra-U motifs have been associated with enhanced efficacy and stability (Wu et al. 2024). Chemical modifications, such as altering terminal hydroxyl groups, can further improve RNA stability (Boo & Kim 2020). Interestingly, N1-methyl-pseudouridine used in mRNA COVID-19 vaccines could potentially enhance dsRNA stability, though no studies have tested this in SIGS. Regulatory limitations may arise, as this is not a naturally occurring compound (Morais et al. 2021). A rapidly growing area of SIGS research involves the use of nanocar-

riers to deliver dsRNA. Key considerations, including cost, ease of dsRNA incorporation, production scalability, and toxicity, have been reviewed by (Mosquera et al. 2025). Table S2 summarises tested nanocarriers in combination with dsRNA, classified into four main groups by origin: inorganic (e.g., clay-based particles; carbon dots); organic (e.g., liposomes, extracellular vesicles); organic-inorganic hybrids (e.g., coated dextran); and synthetic polymers (e.g. polyethylenimine), with some biological systems such as genetically modified yeast cells. In this review, we identified 54 nanocarrier types, predominantly organic (33) with an additional 13 organic-inorganic types and 7 inorganic types (Figure 2C, ESM Table S2). The most frequently used nanocarriers are chitosan-based (16 studies); layered double hydroxide (LDH) (7) and star polycation (6), with liposome-based strategies also common (ESM Table S2).

The target organisms in these studies are primarily insects (37 species), with only 3 mammalian species tested. However, when measured by the number of studies, rather than species, mammalian cells rise to 22, mostly human or mouse models. Insects remain the leading target group (43 studies) (ESM Table S2). Most tested nanocarriers enhanced dsRNA stability and uptake, subsequently improving silencing efficacy (ESM Table S2).

Some studies suggest that dsRNA can remain sufficiently stable without additives and still retain efficacy for a reasonable period (Bai et al. 2023; Degnan et al. 2023; Narva et al. 2025). For example, dsRNA applications controlling rust disease (Degnan et al. 2023a, 2023b) or the first commercial product, Calantha[®], reportedly do not require stabilising additives (Narva et al. 2025).

Uptake of dsRNA by the target organism is another critical factor for SIGS efficacy. Fungi such as *B. cinerea*, *Verticillium dahliae*, *Aspergillus niger*, *S. clerotiorum* and *Rhizoctonia solani* readily absorb dsRNA (Qiao et al. 2021). Nematodes also absorb dsRNA efficiently (Yadav et al. 2006; Opdenstein et al. 2024), although they are not the focus of this review. Insects are more challenging, as dsRNA must be ingested and bioavailable in the correct tissue (e.g., phloem for aphids) (Liu et al. 2021). However, insects are promising targets for SIGS, as demonstrated by the first commercial dsRNA product (Calantha[®]) targeting the Colorado potato beetle (Rodrigues et al. 2021; GreenLight Biosciences 2024; Narva et al. 2025).

Some organisms show limited or no uptake of dsRNA, including beneficial fungus *Trichoderma virens*, the oomycete pathogen *Phytophthora infestans*, certain Lepidoptera species and the fungi *Zymoseptoria tritici* and *Colletotrichum gloeosporioides* (Qiao et al. 2021).

The mechanism of dsRNA uptake varies by organism. In fungi, endocytosis is the only confirmed uptake mechanism (Wytinck et al. 2020; Qiao et al. 2021; Liu et al. 2025). Nematodes absorb dsRNA via soaking or feeding, and specific dsRNA transport channels have been identified (McEwan et al. 2012; Whangbo et al. 2017). In insects, dsRNA must first be ingested and then internalised via endocytosis (Huvenne & Smagghe 2010; Vélez & Fishilevich 2018; Ye et al. 2021). Although orthologs of nematode dsRNA transport genes (of SID-1-like proteins, SIL) have been identified in insects, their functional role in uptake remains unclear, with conflicting evidence reported (Miyata et al. 2014; Wang et al. 2014; Cappelle et al. 2016; Vélez & Fishilevich 2018; Wytinck et al. 2020; Zhu & Palli 2020; Ye et al. 2023; Luo et al. 2024).

Finally, the method of dsRNA application must be chosen carefully, as it directly affects both efficacy and cost. Direct SIGS involves pathogens or pests absorbing dsRNA from foliar sprays, which can be enhanced with nanocarriers. Indirect methods involve uptake via the host plant, typically through infiltration or root soaking, although foliar sprays may also be effective depending on the target organism.

In summary, optimising dsRNA efficacy for SIGS requires a holistic approach that integrates multiple factors. Careful target gene selection ensures specificity and reduces off-target effects, while thoughtful sequence design, which considers length, complementarity, and secondary/tertiary structures, maximises silencing efficiency. Nanocarrier-based delivery systems can enhance stability, uptake, and persistence under environmental conditions, complementing the intrinsic properties of dsRNA. Understanding uptake mechanisms in the target organism is critical for selecting the appropriate application method, whether direct foliar sprays, infiltration, or root-soaking approaches. Together, these strategies provide a cohesive framework to advance SIGS from laboratory studies toward field-ready, sustainable crop protection solutions, ensuring both effectiveness and environmental safety (Mosquera et al. 2025; Narva et al. 2025; Smagghe et al. 2025).

REQUIREMENTS FOR FIELD APPLICATION OF SIGS

At the beginning of this section, we recommend that readers consult the recent and detailed review "A Step-by-Step Guide to SIGS Against Fungi" by Mosquera et al. 2025 and for more comprehensive insights, the recently published book focusing on RNAi use in agriculture edited by co-author of this review, Guy Smagghe and his colleagues (Smagghe et al. 2025). These publications provide invaluable guidance for researchers, especially beginners interested in the practical implementation of SIGS.

As demonstrated in previous sections, significant progress has been made over the past decade in exploring the potential of SIGS in agricultural practice (ESM Tables S1 and S2). However, laboratory success does not automatically mean viable translation to the field conditions. Field application must contend with environmental variability, competition from existing (often cheaper) solutions, regulatory frameworks, and public perception, both from end consumers and from farmers, whose trust is essential (Figure 3).

A recent successful example illustrates the pathway from research to market. In 2024, GreenLight Biosciences launched the first commercial dsRNA-based product, Calantha[®], targeting the Colorado potato beetle (*L. decemlineata*) (Narva et al. 2025). The active ingredient, Ledprona[®] (CAS# 2433753-68-3), comprises 0.8% of the formulation, with the remainder undisclosed. Ledprona[®] targets a specific gene in the beetle responsible for proteasome production (Proteasome Subunit Beta Type-5) (Rodrigues et al. 2021) and has been extensively studied (Rodrigues et al. 2021; Pallis et al. 2022; Pallis et al. 2023a, 2023b; Buzza & Alyokhin 2025). Calantha[®] has undergone global testing and received approval from the U.S. Environmental Protection Agency (US-EPA) and other regulatory agencies (Rodrigues et al. 2021; US-EPA 2023; GreenLight Biosciences 2024; Narva et al. 2025).

The Colorado potato beetle causes annual crop losses exceeding 500 mil USD (GreenLight Biosciences 2025) and is difficult to control due to multiple generations per year and rapid development of pesticide resistance (Mota-Sanchez et al. 2006; Alyokhin et al. 2008). Calantha[®] is applied via foliar spray or soil drench, with a recommended dosage of approximately 10 g/ha. The product is effective

across all life stages, with mortality occurring within 7–10 days, a slower rate than with conventional insecticides (US-EPA 2023; GreenLight Biosciences 2024; Narva et al. 2025). It is reportedly compatible with other insecticides commonly used in integrated pest management programs. Calantha[®] requires no special storage conditions and has a shelf life of more than 2 years at room temperature. Specificity tests required for US-EPA approval confirmed that there were no adverse effects on selected non-target insects or mammals (US-EPA 2023; Wenninger et al. 2025). Field trials spanned from 2018 to 2024 (Narva et al. 2025). The US-EPA also evaluated potential additives in Calantha[®], ensuring that the product remains biodegradable, as expected for dsRNA (Narva et al. 2025).

A critical milestone for field deployment is large-scale, cost-effective dsRNA production. GreenLight Biosciences reports the capacity to produce dsRNA at an industrial scale (t per year) at costs as low as 1 USD/g using cell-free synthesis technology (GreenLight Biosciences 2024). This is essential as SIGS, like other crop protection treatments, require repeated application due to the transient nature of dsRNA activity.

The development pipeline of Calantha[®] is detailed by Narva et al. (2025). Briefly, it involved: (i) Target organism selection: Identifying an economically important, dsRNA-susceptible, genetically characterised pest. (ii) Transcriptomic analysis of target organism: Generating unique transcriptomic data to identify optimal target genes using machine learning/AI, followed by *in silico* off-target analysis to maximise specificity. The PSMB5 (proteasome subunit $\beta 5$; Graser et al. 2025) gene was selected as the primary target. (iii) dsRNA production and laboratory testing: Assessing efficacy, kinetics, dosage, and longevity of effect. (iv) Scale-up: Developing cost-effective, cell-free dsRNA production for field application. (v) Field trials: Extensive trials supervised by the EPA, including treatment number and application timing, culminating in a stable, safe, and effective product. (vi) Protection and approval: Patents were secured (Rodrigues et al. 2021; Stewart et al. 2023), followed by regulatory approval and integration into existing pest management programs.

The experience with Calantha[®] provides practical lessons for future SIGS products. Beyond technical success, achieving societal acceptance and farmer trust is essential for adoption. The pathway demon-

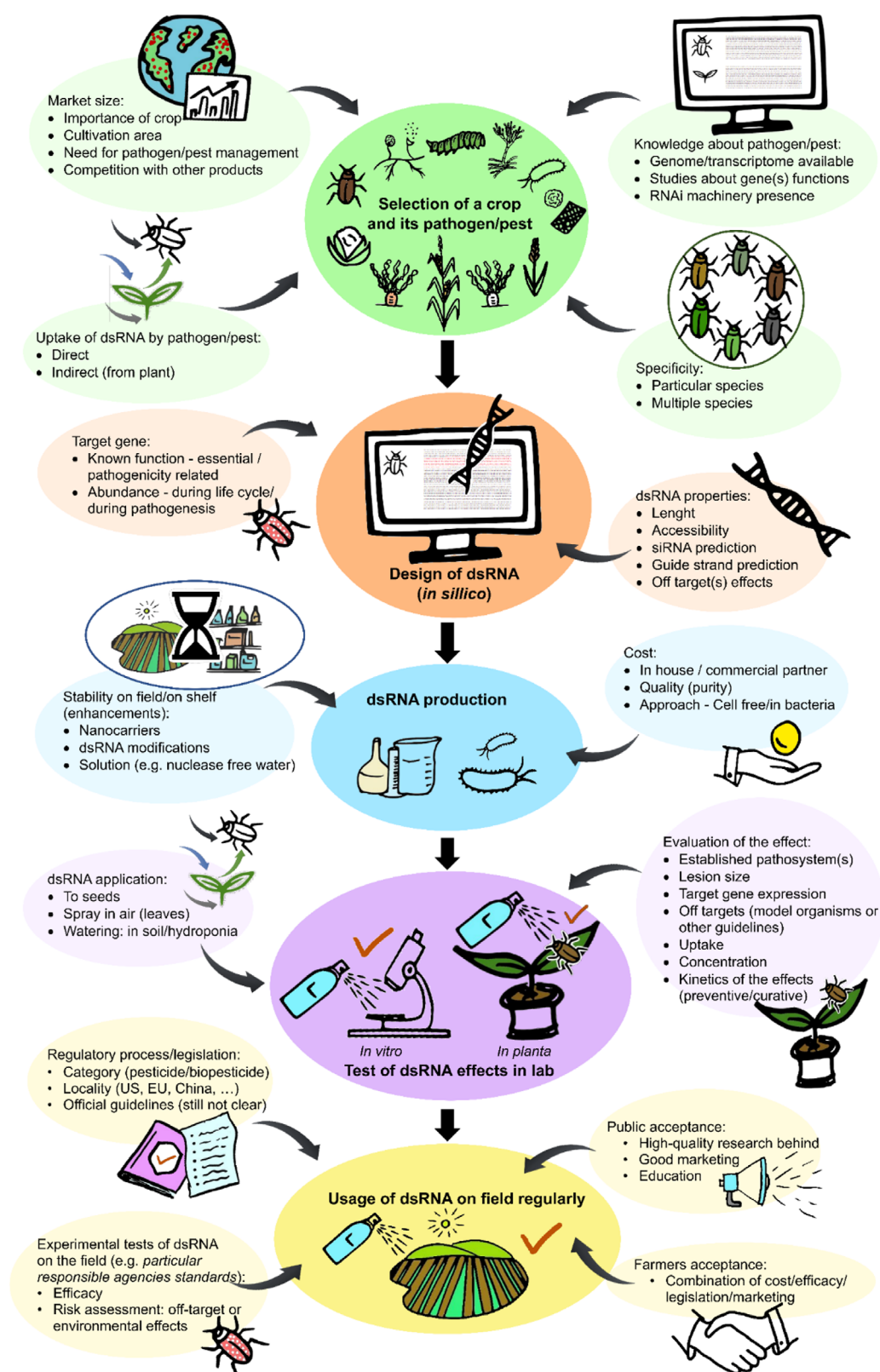


Figure 3. Graphical guidelines for developing the SIGS approach, highlighting its potential for success in the field. This scheme was inspired by the review (Mosquera et al. 2025), the book chapter detailing the path of the *Calantha*[®] biopesticide to market (Narva et al. 2025) and our own laboratory experience combined with insights from negotiations with commercial partners; SIGS – spray-induced gene silencing

strated by Calantha[®] paves the way for subsequent dsRNA-based crop protection products to reach the market safely and effectively (Narva et al. 2025; Smagghe et al. 2025).

In summary, successful field deployment of SIGS requires an integrated approach combining technical, regulatory, and practical considerations. Key requirements include: selecting economically relevant and genetically characterised targets, designing dsRNA for high specificity and stability, and choosing appropriate delivery methods, including nanocarriers if needed. Large-scale, cost-effective production of dsRNA is essential to cover extensive crop areas, and application strategies must match the biology and uptake mechanisms of the target organism. Regulatory compliance, environmental safety, and thorough field efficacy testing are mandatory. Finally, effective communication with farmers and the public is critical for building trust and ensuring adoption (Figure 3). Following these guidelines provides a roadmap for translating laboratory SIGS research into reliable, sustainable, and commercially viable crop protection solutions (Mosquera et al. 2025; Narva et al. 2025; Smagghe et al. 2025).

RECENT DEVELOPMENTS IN SIGS (2024–2025)

In the past two years, SIGS research has accelerated significantly, with several notable innovations and proof-of-concept applications. For example, Knoblich et al. (2025) engineered effective dsRNAs (edsRNAs) by selecting highly functional siRNAs against Cucumber mosaic virus (CMV); these multivalent edsRNAs were processed more efficiently and provided cross-protection across CMV variants, substantially increasing silencing specificity and potency. In parallel, advances in nanocarrier design are improving, and prolonging, the efficacy of RNA sprays: recent reviews highlight formulations based on chitosan nanoparticles, LDH clays, and bacterial minicells, which confer enhanced environmental stability, uptake, and persistence of dsRNA under field conditions (Chen et al. 2025a; Imran et al. 2025; Mamun et al. 2025). Relatedly, a new "step-by-step" protocol for the application of SIGS in plant-fungal systems has just been published, providing practical guidance on delivering dsRNA to fungal pathogens via foliar sprays and

ensuring efficient uptake and gene silencing (Mosquera et al. 2025).

On the application side, SIGS is being extended into weed control: a 2025 study demonstrated topical dsRNA silencing of the EPSPS gene (5-enolpyruvylshikimate-3-phosphate synthase) in the weed *Digitaria insularis*, reducing growth and biomass, and underscoring the potential of RNA-based bioherbicides (Cintra et al. 2025). Moreover, SIGS has been used to functionally characterise genes in *Blumeria graminis* (the powdery mildew fungus) during its pre-penetration stages, using exogenous dsRNA to silence actin, which revealed its crucial role in pathogen establishment (Zhang et al. 2025).

From a mechanistic standpoint, work on *Fusarium* species demonstrates that sprayed dsRNAs (and small RNAs) can be mobilised through the plant vascular system and enter fungal hyphae for gene silencing. However, the precise uptake routes (e.g., endocytosis, vesicle trafficking) remain under investigation (Huang et al. 2025). Finally, on the frontier of delivery technology, a recent Nanoscience-RNAi review (2025) outlines how modern nanoparticle platforms, including solid-lipid nanoparticles, lipid-based vesicles, carbon nanotubes, and others, are being optimised for agricultural RNAi applications, offering better protection of dsRNA against degradation, controlled release, and improved biocompatibility (Mathur et al. 2025).

Regarding the biosafety of RNAi-biopesticides, we believe that tailor-made risk assessment frameworks can help address data requirements for unintended risks to NTOs while also establishing best practices for agricultural applications (Taning et al. 2021; He et al. 2022; Kleter & Smagghe 2025). However, the data needed to evaluate the environmental impact of sprayable RNA molecules and their potential risks to NTOs remain poorly defined (Chen & De Schutter 2024; Willow & Smagghe 2025). Today, different bioinformatic analyses, enabled by expanding genomic data libraries, improved algorithms, and increasingly targeted research, play a crucial role in risk assessment by identifying NTOs' whole genes that show sequence homology to the pest or pathogen target sequence. Example web platforms supporting these analyses include dsRIP (Cedden et al. 2025), dsRNAEngineer (Chen et al. 2025b), and dsOMG (Lyu et al. 2025). Such tools can detect poten-

tial off-target effects, supported by mechanistic knowledge of RNAi, comprehensive sequencing data, and fit-for-purpose algorithms.

Taken together, these advances illustrate how SIGS is rapidly maturing from proof-of-concept to practical, field-ready technologies: improved molecular design (edsRNAs), more robust delivery systems, expanding applications (weeds), and better mechanistic understanding are all converging to push SIGS closer to real-world adoption.

OUTLOOK: BRIGHT OR DARK FUTURE FOR SIGS?

As illustrated in Figure 1 and supported by the expanding literature on SIGS and RNAi in general (Chen et al. 2025a; Mosquera et al. 2025; Smaghe et al. 2025), knowledge on spray-induced gene silencing is growing rapidly. These advances open previously unimagined possibilities for the use of dsRNA in sustainable agriculture. A breakthrough has been the reduction in dsRNA production costs, which, until recently, was considered a primary barrier to field-scale application. With this limitation being gradually overcome, the prospects for SIGS are increasingly promising.

Challenges remain, including dsRNA stability and uptake by target organisms. Innovations such as nanocarriers are addressing these challenges, enhancing persistence and delivery efficiency, although regulatory considerations may require careful navigation. Resistance development, as with any pest management strategy, is possible; however, the high specificity and flexibility of SIGS, particularly when integrated with other control strategies, can significantly reduce this risk.

Public perception and regulatory frameworks are additional considerations. Transparent communication with the public and stakeholders is essential to foster understanding and trust. Initiatives like the COST Action CA20110 "ExRNA-PATH" (exrnath.eu) exemplify the collaborative efforts needed to promote societal acceptance of RNA-based crop protection.

While HIGS represents a related approach, it requires generating transgenic plants, making it less flexible and more controversial, particularly in regions with strict regulations, such as the European Union. Although HIGS may offer cost advantages and environmental benefits under certain circumstances, its widespread adoption remains limited.

Pioneering products such as Calantha® demonstrate that SIGS can transition from laboratory research to practical field applications. Major companies, including Bayer, BASF, and Syngenta, are actively investing in this area (Germing et al. 2025), signalling growing confidence in the technology. With continued research, regulatory support, and proactive public engagement, we believe that SIGS has the potential to become a core strategy in sustainable, integrative agriculture, offering effective, environmentally friendly solutions for the management of plant pathogens, pests, and weeds in the relatively near future.

AUTHORS CONTRIBUTION

IV – Contributed to the concept of the review, did the literature survey and contributed to creation of tables, commented final text of the manuscript; JH – contributed to tables production and edited the final version of the manuscript; TK – did the literature survey, contributed to creation of figures, commented final text of the manuscript; GS – substantially edited the manuscript; MJ – created the concept and wrote the main text of the manuscript.

REFERENCES

- Agrawal N., Dasaradhi P.V.N., Mohammed A., Malhotra P., Bhatnagar R.K., Mukherjee S.K. (2003): RNA interference: Biology, mechanism, and applications. *Microbiology and Molecular Biology Reviews*, 67: 657–685.
- Alengebawry A., Abdelkhalek S.T., Qureshi S.R., Wang M.-Q. (2021): Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics*, 9: 42.
- Alyokhin A., Baker M., Mota-Sanchez D., Dively G., Grafius E. (2008): Colorado potato beetle resistance to insecticides. *American Journal of Potato Research*, 85: 395–413.
- Bai M., Liu Z., Zhou Y., Xu Q., Liu T., Tian H. (2023): Influence of diverse storage conditions of double-stranded RNA *in vitro* on the RNA interference efficiency *in vivo* insect *Tribolium castaneum*. *Pest Management Science*, 79: 45–54.
- Beckerman J., Palmer C., Tedford E., Ypema H. (2023): Fifty years of fungicide development, deployment, and future use. *Phytopathology*, 113: 694–706.
- Bennett M., Deikman J., Hendrix B., Iandolino A. (2020): Barriers to efficient foliar uptake of dsRNA and molecular

<https://doi.org/10.17221/138/2025-PPS>

- barriers to dsRNA activity in plant cells. *Frontiers in Plant Science*, 11: 816.
- Boo S.H., Kim Y.K. (2020): The emerging role of RNA modifications in the regulation of mRNA stability. *Experimental and Molecular Medicine*, 52: 400–408.
- Buzza A.M., Alyokhin A. (2025): Control of Colorado potato beetle on potato by ledprona, 2024. *Arthropod Management Tests*, 50: tsaf078.
- Cai Q., He B., Jin H. (2019): A safe ride in extracellular vesicles – small RNA trafficking between plant hosts and pathogens. *Current Opinion in Plant Biology*, 52: 140–148.
- Cai Q., Qiao L., Wang M., He B., Lin F.-M., Palmquist J., Huang S.-D., Jin H. (2018): Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science*, 360: 1126–1129.
- Cappelle K., De Oliveira C.F.R., Van Eynde B., Christiaens O., Smagghe G. (2016): The involvement of clathrin-mediated endocytosis and two Sid-1-like transmembrane proteins in double-stranded RNA uptake in the Colorado potato beetle midgut. *Insect Molecular Biology*, 25: 315–323.
- Cedden D., Güney G., Rostás M., Bucher G. (2025): Optimising dsRNA sequences for RNAi in pest control and research with the dsRIP web platform. *BMC Biology*, 23: 114.
- Chen X., Rechavi O. (2022): Plant and animal small RNA communications between cells and organisms. *Nature Reviews Molecular Cell Biology*, 23: 185–203.
- Chen Y., De Schutter K. (2024): Biosafety aspects of RNAi-based pests control. *Pest Management Science*, 80: 3697–3706.
- Chen C., Imran M., Feng X., Shen X., Sun Z. (2025a): Spray-induced gene silencing for crop protection: recent advances and emerging trends. *Frontiers in Plant Science*, 16: 1527944.
- Chen Y., Shi Y., Wang Z., An X., Wei S., Andronis C., Vontas J., Wang J.-J., et al. (2025b): dsRNAEngineer: a web-based tool of comprehensive dsRNA design for pest control. *Trends in Biotechnology*, 43: 969–983.
- Chow F.W.-N., Koutsovoulos G., Ovando-Vázquez C., Neophytou K., Bermúdez-Barrientos J.R., Laetsch D.R., Robertson E., Kumar S., et al. (2019): Secretion of an Argonaute protein by a parasitic nematode and the evolution of its siRNA guides. *Nucleic Acids Research*, 47: 3594–3606.
- Cintra L.A., Da Rosa J., De Assis R., Marin S.R.R., Adegas F.S., Rech E.L., Nepomuceno A.L., Mertz-Henning L.M. (2025): RNAi spray-induced gene silencing of EPSPS by topical application of dsRNA in the weed *Digitaria insularis*. *Frontiers in Plant Science*, 16: 1688755.
- Degnan R.M., McTaggart A.R., Shuey L.S., Pame L.J.S., Smith G.R., Gardiner D.M., Nock V., Soffe R., et al. (2023a): Exogenous double-stranded RNA inhibits the infection physiology of rust fungi to reduce symptoms in planta. *Molecular Plant Pathology*, 24: 191–207.
- Degnan R.M., Shuey L.S., Radford-Smith J., Gardiner D.M., Carroll B.J., Mitter N., McTaggart A.R., Sawyer A. (2023b): Double-stranded RNA prevents and cures infection by rust fungi. *Communications Biology*, 6: 1234.
- Dhandapani R.K., Gurusamy D., Howell J.L., Palli S.R. (2019): Development of CS-TPP-dsRNA nanoparticles to enhance RNAi efficiency in the yellow fever mosquito, *Aedes aegypti*. *Scientific Reports*, 9: 8775.
- Dunker F., Trutzenberg A., Rothenpieler J.S., Kuhn S., Pröls R., Schreiber T., Tissier A., Kemen A., et al. (2020): Oomycete small RNAs bind to the plant RNA-induced silencing complex for virulence. *eLife*, 9: e56096.
- European Commission (not dated): Farm to Fork Strategy. https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en. (visited 2025)
- Gao Y., Alyokhin A., Zhang R., Smagghe G., Palli S.R., Jurat-Fuentes J.L., Tabashnik B.E. (2024): Proactive resistance management for sustaining the efficacy of RNA interference for pest control. *Journal of Economic Entomology*, 117: 1306–1308.
- Germering K., Navarrete C.A.D., Schiermeyer A., Hommen U., Zühl L., Eilebrecht S., Eilebrecht E. (2025): Crop protection by RNA interference: A review of recent approaches, current state of developments and use as of 2013. *Environmental Sciences Europe*, 37: 15.
- Ghosh S.K.B., Hunter W.B., Park A.L., Gundersen-Rindal D.E. (2017): Double strand RNA delivery system for plant-sap-feeding insects. *PLoS ONE*, 12: e0171861.
- Graser L., Gordon E.R., Jamison M., Talton W., Chen Y., Knorr E., Windfelder A., Narva K., et al. (2025): Targeting the proteasome subunit PSMB5 by RNA interference induces proteasome dysfunction and mortality in the Colorado potato beetle (*Leptinotarsa decemlineata*). *Scientific Reports*, 15: 41183.
- GreenLight Biosciences (2024): Calantha. <https://www.calanthaag.com/about-calantha>. (visited 2025)
- GreenLight Biosciences (2025): Non-Chemical Pesticide Alternatives to Colorado Potato Beetles. <https://www.greenlightbiosciences.com/in-the-pipeline-colorado-potato-beetle>. (visited 2025)
- He L., Huang Y., Tang X. (2022): RNAi-based pest control: Production, application and the fate of dsRNA. *Frontiers in Bioengineering and Biotechnology*, 10: 1080576.
- He L., Zhou Y., Mo Q., Huang Y., Tang X. (2024): Spray-induced gene silencing in phytopathogen: Mechanisms, applications, and progress. *Advanced Agrochem*, 3: 289–297.
- Höfle L., Biedenkopf D., Werner B.T., Shrestha A., Jelonek L., Koch A. (2020): Study on the efficiency of dsRNAs with increasing length in RNA-based silencing of the *Fusarium CYP51* genes. *RNA Biology*, 17: 463–473.

<https://doi.org/10.17221/138/2025-PPS>

- Hou Y., Zhai Y., Feng L., Karimi H.Z., Rutter B.D., Zeng L., Choi D.S., Zhang B., et al. (2019): A phytophthora effector suppresses trans-kingdom RNAi to promote disease susceptibility. *Cell Host & Microbe*, 25: 153–165.
- Huang C.-Y., Wang H., Hu P., Hamby R., Jin H. (2019): Small RNAs – big players in plant-microbe interactions. *Cell Host & Microbe*, 26: 173–182.
- Huang X.-N., Wang Y., Li Y.-T., Xiao Y., Ouyang S.-Q. (2025): Trans-kingdom sRNA silencing in the prevention and control of crop Fusarium wilt disease. *Phytopathology Research*, 7: 18.
- Huvenne H., Smaghe G. (2010): Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: A review. *Journal of Insect Physiology*, 56: 227–235.
- Imran M., Feng X., Sun Z., Al Omari H., Zhang G., Zhu J., Aldayel M.F., Li C. (2025): Nanotechnology-driven gene silencing: advancements in SIGS–dsRNA technology for sustainable disease management. *Chemical and Biological Technologies in Agriculture*, 12: 31.
- Jang S., Kim D., Lee S., Ryu C.-M. (2024): Plant-induced bacterial gene silencing: A novel control method for bacterial wilt disease. *Frontiers in Plant Science*, 15: 1411837.
- Kleter G.A., Smaghe G. (2025): Safety of oligonucleotide-based active ingredients used as crop protection agents. In: Smaghe G., Palli S.R., Swevers L. (eds): *RNA interference in Agriculture: Basic Science to Applications*. Springer Nature Switzerland, Cham: 51–71.
- Knoblich M., Gursinsky T., Gago-Zachert S., Weinholdt C., Grau J., Behrens S.-E. (2025): A new level of RNA-based plant protection: dsRNAs designed from functionally characterised siRNAs highly effective against *Cucumber mosaic virus*. *Nucleic Acids Research*, 53: gkaf136.
- Koch A., Biedenkopf D., Furch A., Weber L., Rossbach O., Abdellatef E., Linicus L., Johannsmeier J., et al. (2016): An RNAi-Based control of *Fusarium graminearum* infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. *PLOS Pathogens*, 12: e1005901.
- Kostov K., Smaghe G. (2025): RNA Interference: View on its current status since the 2006 Nobel prize and impact on the agriculture looking to the future. In: Smaghe G., Palli S.R., Swevers L. (eds): *RNA Interference in Agriculture: Basic Science to Applications*. Springer Nature Switzerland, Cham: 25–50.
- Lini R.S., Scanferla D.T.P., De Oliveira N.G., Aguera R.G., Santos T.D.S., Teixeira J.J.V., Kaneshima A.M.D.S., Mossini S.A.G. (2024): Fungicides as a risk factor for the development of neurological diseases and disorders in humans: A systematic review. *Critical Reviews in Toxicology*, 54: 35–54.
- Liu S., Ladera-Carmona M.J., Poranen M.M., Van Bel A.J.E., Kogel K.-H., Imani J. (2021): Evaluation of dsRNA delivery methods for targeting macrophage migration inhibitory factor MIF in RNAi-based aphid control. *Journal of Plant Diseases and Protection*, 128: 1201–1212.
- Liu C., Cui C., Zhou G., Gao F., Zhao J., Guo H., Jin Y. (2025): The endocytic pathway for absorption of exogenous RNAs in *Verticillium dahliae*. *mLife*, 4: 45–54.
- Luo X., Nanda S., Zhang Y., Zhou X., Yang C., Pan H. (2024): Risk assessment of RNAi-based biopesticides. *New Crops*, 1: 100019.
- Lyu Z., Cai Y., Li S., Liu J., Lei X., Liu B., Zhang W. (2025): dsOMG: A web-generator to minimise off-target risk in double-stranded RNA-mediated RNAi. *Entomologia*, 45: 1017–1028.
- Mamun M.A., Occhialini A., Islam T. (2025): From stability to persistence: Insights into nanoparticle-formulated dsRNA in RNAi-based fungal disease control. *Science of the Total Environment*, 1001: 180493.
- Mathur S., Chaturvedi A., Ranjan R. (2025): Advances in RNAi-based nanoformulations: Revolutionising crop protection and stress tolerance in agriculture. *Nanoscale Advances*, 7: 1768–1783.
- McEwan D.L., Weisman A.S., Hunter C.P. (2012): Uptake of Extracellular double-stranded RNA by SID-2. *Molecular Cell*, 47: 746–754.
- Mishra S., Dee J., Moar W., Dufner-Beattie J., Baum J., Dias N.P., Alyokhin A., Buzza A., et al. (2021): Selection for high levels of resistance to double-stranded RNA (dsRNA) in Colorado potato beetle (*Leptinotarsa decemlineata* Say) using non-transgenic foliar delivery. *Scientific Reports*, 11: 6523.
- Mishra S., Jurat-Fuentes J.L. (2025): Resistance to RNAi: Mechanisms, current status, and prospects. In: Smaghe G., Palli S.R., Swevers L. (eds): *RNA interference in Agriculture: Basic Science to Applications*. Springer Nature Switzerland, Cham: 367–382.
- Mitter N., Worrall E.A., Robinson K.E., Li P., Jain R.G., Taochy C., Fletcher S.J., Carroll B.J., et al. (2017): Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nature Plants*, 3: 16207.
- Miyata K., Ramaseshadri P., Zhang Y., Segers G., Bolognesi R., Tomoyasu Y. (2014): Establishing an *in vivo* assay system to identify components involved in environmental RNA interference in the western corn rootworm. *PLoS ONE*, 9: e101661.
- Morais P., Adachi H., Yu Y.-T. (2021): The critical contribution of pseudouridine to mRNA COVID-19 vaccines. *Frontiers in Cell and Developmental Biology*, 9: 789427.
- Mosquera S., Ginésy M., Bocos-Asenjo I.T., Amin H., Diez-Hernando S., Diez J.J., Niño-Sánchez J. (2025): Spray-induced gene silencing to control plant pathogenic fungi: A step-by-step guide. *Journal of Integrative Plant Biology*, 67: 801–825.

<https://doi.org/10.17221/138/2025-PPS>

- Mota-Sanchez D., Hollingworth R.M., Grafius E.J., Moyer D.D. (2006): Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Pest Management Science*, 62: 30–37.
- Nakanishi K. (2016): Anatomy of RISC: How do small RNAs and chaperones activate Argonaute proteins? *WIREs RNA*, 7: 637–660.
- Narva K., Otto E., Sridharan K., Flannagan R., Barnes E., Mézin L., Manley B. (2025): Calantha™: The first commercialized sprayable dsRNA product for insect control. In: Smagghe G., Palli S.R., Swevers L. (eds): *RNA Interference in Agriculture: Basic Science to Applications*. Springer Nature Switzerland, Cham: 679–715.
- Niño-Sánchez J., Sambasivam P.T., Sawyer A., Hamby R., Chen A., Czislawski E., Li P., Manzie N., et al. (2022): BioClay™ prolongs RNA interference-mediated crop protection against *Botrytis cinerea*. *Journal of Integrative Plant Biology*, 64: 2187–2198.
- NobelPrize.org (2006): <https://www.nobelprize.org/prizes/medicine/2006/advanced-information/>. (visited 2025)
- Opdensteinen P., Charudattan R., Hong J.C., Roskopf E.N., Steinmetz N.F. (2024): Biochemical and nanotechnological approaches to combat phytoparasitic nematodes. *Plant Biotechnology Journal*, 22: 2444–2460.
- Pallis S., Alyokhin A., Manley B., Rodrigues T.B., Buzza A., Barnes E., Narva K. (2022): Toxicity of a novel dsRNA-based insecticide to the Colorado potato beetle in laboratory and field trials. *Pest Management Science*, 78: 3836–3848.
- Pallis S., Alyokhin A., Manley B., Rodrigues T., Barnes E., Narva K. (2023a): Effects of low doses of a novel dsRNA-based biopesticide (Calantha) on the Colorado potato beetle. *Journal of Economic Entomology*, 116: 456–461.
- Pallis S., Alyokhin A., Manley B., Rodrigues T.B., Barnes E., Narva K. (2023b): Baseline susceptibility to a novel dsRNA-based insecticide across US populations of Colorado potato beetle. *Agriculture*, 13: 2283.
- Qiao L., Lan C., Capriotti L., Ah-Fong A., Nino Sanchez J., Hamby R., Heller J., Zhao H., et al. (2021): Spray-induced gene silencing for disease control is dependent on the efficiency of pathogen RNA uptake. *Plant Biotechnology Journal*, 19: 1756–1768.
- Qiao L., Niño-Sánchez J., Hamby R., Capriotti L., Chen A., Mezzetti B., Jin H. (2023): Artificial nanovesicles for dsRNA delivery in spray-induced gene silencing for crop protection. *Plant Biotechnology Journal*, 21: 854–865.
- Rand T.A., Ginalska K., Grishin N.V., Wang X. (2004): Biochemical identification of Argonaute 2 as the sole protein required for RNA-induced silencing complex activity. *Proceedings of the National Academy of Sciences of the United States of America*, 101: 14385–14389.
- Rashid B., Husnain T., Riazuddin S. (2010): Herbicides and pesticides as potential pollutants: A global problem. In: *Plant Adaptation and Phytoremediation*. Springer Netherlands, Dordrecht: 427–447.
- Redfern A.D., Colley S.M., Beveridge D.J., Ikeda N., Epis M.R., Li X., Foulds C.E., Stuart L.M., et al. (2013): RNA-induced silencing complex (RISC) proteins PACT, TRBP, and Dicer are SRA binding nuclear receptor coregulators. *Proceedings of the National Academy of Sciences of the United States of America*, 110: 6536–6541.
- Ren B., Wang X., Duan J., Ma J. (2019): Rhizobial tRNA-derived small RNAs are signal molecules regulating plant nodulation. *Science*, 365: 919–922.
- Rodrigues T.B., Mishra S.K., Sridharan K., Barnes E.R., Alyokhin A., Tuttle R., Kokulapalan W., Garby D., et al. (2021): First sprayable double-stranded RNA-based biopesticide product targets proteasome subunit Beta type-5 in Colorado potato beetle (*Leptinotarsa decemlineata*). *Frontiers in Plant Science*, 12: 728652.
- Roth R., Hillmer S., Funaya C., Chiapello M., Schumacher K., Lo Presti L., Kahmann R., Paszkowski U. (2019): Arbuscular cell invasion coincides with extracellular vesicles and membrane tubules. *Nature Plants*, 5: 204–211.
- San Miguel K., Scott J.G. (2016): The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide. *Pest Management Science*, 72: 801–809.
- Shahid S., Kim G., Johnson N.R., Wafula E., Wang F., Coruh C., Bernal-Galeano V., Phifer T., et al. (2018): MicroRNAs from the parasitic plant *Cuscuta campestris* target host messenger RNAs. *Nature*, 553: 82–85.
- Silva V., Yang X., Fleskens L., Ritsema C.J., Geissen V. (2022): Environmental and human health at risk – Scenarios to achieve the Farm to Fork 50% pesticide reduction goals. *Environment International*, 165: 107296.
- Smagghe G., Palli S.R., Swevers L. (2025): *RNA Interference in Agriculture: Basic Science to Applications*. Springer Nature Switzerland, Cham.
- Stewart W., Aulisa L., Villiard D., Gillian J., Owens K., Dodwadkar N., Boucher P., Eldridge J. (2023): *Stabilisation of RNA for Exogenous RNAi Agricultural Applications and Formulations*.
- Stokstad E. (2018): European Union expands ban of three neonicotinoid pesticides. *Science*.
- Storck V., Karpouzas D.G., Martin-Laurent F. (2017): Towards a better pesticide policy for the European Union. *Science of The Total Environment*, 575: 1027–1033.
- Tanig C.N.T., Gui S., De Schutter K., Jahani M., Castellanos N.L., Christiaens O., Smagghe G. (2021): A sequence complementarity-based approach for evaluating off-target

<https://doi.org/10.17221/138/2025-PPS>

- transcript knockdown in *Bombus terrestris*, following ingestion of pest-specific dsRNA. *Journal of Pest Science*, 94: 487–503.
- US-EPA (2023): EPA Registers Novel Pesticide Technology for Potato Crops. <https://www.epa.gov/pesticides/epa-registers-novel-pesticide-technology-potato-crops>. (visited 2025)
- Vélez A.M., Fishilevich E. (2018): The mysteries of insect RNAi: A focus on dsRNA uptake and transport. *Pesticide Biochemistry and Physiology*, 151: 25–31.
- Wang H., Gong L., Qi J., Hu M., Zhong G., Gong L. (2014): Molecular cloning and characterisation of a sid-1-like gene in *Plutella xylostella*. *Archives of Insect Biochemistry and Physiology*, 87: 164–176.
- Wang M., Dean R.A. (2020): Movement of small RNAs in and between plants and fungi. *Molecular Plant Pathology*, 21: 589–601.
- Wang Y., Yan Q., Lan C., Tang T., Wang K., Shen J., Niu D. (2023): Nanoparticle carriers enhance RNA stability and uptake efficiency and prolong the protection against *Rhizoctonia solani*. *Phytopathology Research*, 5: 2.
- Weiberg A., Wang M., Lin F.-M., Zhao H., Zhang Z., Kaloshian I., Huang H.-D., Jin H. (2013): Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science*, 342: 118–123.
- Wenninger E.J., DeGrey S.P., Insinga J., Knopf E., Alyokhin A., Barnes E.R., Bradford B., Groves R.L., et al. (2025): Responses of non-target arthropods to the dsRNA Bioinsecticide CalanthaTM and conventional insecticides targeting colorado potato beetle, *Leptinotarsa decemlineata* (Say). *American Journal of Potato Research*, 102: 129–151.
- Werner B.T., Gaffar F.Y., Schuemann J., Biedenkopf D., Koch A.M. (2020): RNA-spray-mediated silencing of *Fusarium graminearum* AGO and DCL genes improve barley disease resistance. *Frontiers in Plant Science*, 11: 476.
- Whangbo J.S., Weisman A.S., Chae J., Hunter C.P. (2017): SID-1 domains important for dsRNA import in *Caenorhabditis elegans*. *G3 Genes|Genomes|Genetics*, 7: 3887–3899.
- Willow J., Smagghe G. (2025): RNAi applications toward environmentally sustainable food security. *Current Opinion in Environmental Science & Health*, 45: 100612.
- Wu F., Yan L., Zhao X., Lv C., Jin W. (2024): Development of an RNA nanostructure for effective *Botrytis cinerea* control through spray-induced gene silencing without an extra nanocarrier. *Journal of Fungi*, 10: 483.
- Wytinck N., Sullivan D.S., Biggar K.T., Crisostomo L., Pelka P., Belmonte M.F., Whyard S. (2020): Clathrin mediated endocytosis is involved in the uptake of exogenous double-stranded RNA in the white mold phytopathogen *Sclerotinia sclerotiorum*. *Scientific Reports*, 10: 12773.
- Yadav B.C., Veluthambi K., Subramaniam K. (2006): Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. *Molecular and Biochemical Parasitology*, 148: 219–222.
- Yang W., Wang B., Lei G., Chen G., Liu D. (2022): Advances in nanocarriers to improve the stability of dsRNA in the environment. *Frontiers in Bioengineering and Biotechnology*, 10: 974646.
- Ye C., An X., Xie B., Ding B., Niu J., Wang J. (2023): The involvement of systemic RNA interference deficient-1-like (SIL1) in cellular dsRNA uptake in *Acyrtosiphon pisum*. *Insect Science*, 30: 1393–1404.
- Ye C., Hu X.-S., Wang Z.-W., Wei D., Smagghe G., Christiaens O., Niu J., Wang J.-J. (2021): Involvement of clathrin-dependent endocytosis in cellular dsRNA uptake in aphids. *Insect Biochemistry and Molecular Biology*, 132: 103557.
- Zhang J., Khan S.A., Heckel D.G., Bock R. (2017): Next-generation insect-resistant plants: RNAi-mediated crop protection. *Trends in Biotechnology*, 35: 871–882.
- Zhang M., Zhou M., Jia Y., Li X., Wang A., Liu T., Zhou Y., Liu W., et al. (2025): Spray-induced gene silencing enables the characterisation of gene function during pre-penetration stages in *Blumeria graminis* f. sp. *tritici*. *Frontiers in Plant Science*, 16: 1628068.
- Zhu K.Y., Palli S.R. (2020): Mechanisms, applications, and challenges of insect RNA interference. *Annual Review of Entomology*, 65: 293–311.

Received: September 17, 2025

Accepted: March 10, 2026

Published online: August 23, 2026