



Review

Current Scenario of Exogenously Induced RNAi for Lepidopteran Agricultural Pest Control: From dsRNA Design to Topical Application

Vívian S. Lucena-Leandro ¹, Emanuel F. A. Abreu ¹, Leonardo A. Vidal ^{1,2}, Caroline R. Torres ^{1,3}, Camila I. C. V. F. Junqueira ^{1,3}, Juliana Dantas ¹ and Érika V. S. Albuquerque ^{1,*}

¹ Embrapa Recursos Genéticos e Biotecnologia, Brasília 70770-917, DF, Brazil

² Department of Cellular Biology, Institute of Biological Sciences, Campus Darcy Ribeiro, Universidade de Brasília—UnB, Brasília 70910-9002, DF, Brazil

³ Department of Agronomy and Veterinary Medicine, Campus Darcy Ribeiro, Universidade de Brasília—UnB, Brasília 70910-9002, DF, Brazil

* Correspondence: erika.albuquerque@embrapa.br



Citation: Lucena-Leandro, V.S.; Abreu, E.F.A.; Vidal, L.A.; Torres, C.R.; Junqueira, C.I.C.V.F.; Dantas, J.; Albuquerque, É.V.S. Current Scenario of Exogenously Induced RNAi for Lepidopteran Agricultural Pest Control: From dsRNA Design to Topical Application. *Int. J. Mol. Sci.* **2022**, *23*, 15836. <https://doi.org/10.3390/ijms232415836>

Academic Editors: Alexandra S. Dubrovina and Konstantin V. Kiselev

Received: 7 October 2022

Accepted: 25 November 2022

Published: 13 December 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Invasive insects cost the global economy around USD 70 billion per year. Moreover, increasing agricultural insect pests raise concerns about global food security constraining and infestation rising after climate changes. Current agricultural pest management largely relies on plant breeding—with or without transgenes—and chemical pesticides. Both approaches face serious technological obsolescence in the field due to plant resistance breakdown or development of insecticide resistance. The need for new modes of action (MoA) for managing crop health is growing each year, driven by market demands to reduce economic losses and by consumer demand for phytosanitary measures. The disabling of pest genes through sequence-specific expression silencing is a promising tool in the development of environmentally-friendly and safe biopesticides. The specificity conferred by long dsRNA-base solutions helps minimize effects on off-target genes in the insect pest genome and the target gene in non-target organisms (NTOs). In this review, we summarize the status of gene silencing by RNA interference (RNAi) for agricultural control. More specifically, we focus on the engineering, development and application of gene silencing to control Lepidoptera through non-transforming dsRNA technologies. Despite some delivery and stability drawbacks of topical applications, we reviewed works showing convincing proof-of-concept results that point to innovative solutions. Considerations about the regulation of the ongoing research on dsRNA-based pesticides to produce commercialized products for exogenous application are discussed. Academic and industry initiatives have revealed a worthy effort to control Lepidoptera pests with this new mode of action, which provides more sustainable and reliable technologies for field management. New data on the genomics of this taxon may contribute to a future customized target gene portfolio. As a case study, we illustrate how dsRNA and associated methodologies could be applied to control an important lepidopteran coffee pest.

Keywords: biopesticide; gene target; genome; insect; silencing; topical; validation

1. Introduction

Insects play an important role in global crop loss, whether feeding on plants, acting as vectors of other diseases, or both [1]. It is estimated that these arthropods are responsible for reducing world food production by 20% [2]. They also reduce household food security at the post-harvest level [3]. Adaptive interventions are required, otherwise this damage may increase within the climate change scenario [4].

Conventional pesticides are currently used to control agricultural insect pests. However, resistant pest populations are frequently selected for by continuous exposure to a

given molecule (e.g., chlorantraniliprole) [5]. This context has prompted research on alternative ways to mitigate the negative impacts of insects on the field, which is favored by the increasing knowledge of plant–pest interactions. Pest management strategies that utilize novel modes of action (MoA), such as gene expression silencing in response to RNA interference (RNAi), may bypass pesticide resistance and avoid chemical pesticides [6].

Since the first report involving the expression knockout of two target genes in the nematode *Caenorhabditis elegans* by antisense RNA molecules [7], the RNAi pathway has been reported as highly conserved in almost all eukaryotes [8] and described as a fine-tuned form of gene regulation [9,10] and defensive barrier [11–13]. Additionally, RNA silencing contributes to suppression of transposable elements [14], DNA elimination [15], heterochromatin formation [16] and posttranscriptional repression of cellular genes [17].

The RNAi gene silencing pathways are highly conserved in insects and operate basically in three distinct ways, according to the kind of small RNA responsible for triggering the silencing effect: siRNAs in the siRNA pathway for endo/exogenous dsRNAs; miRNAs (miRNAs) in the miRNA pathway [18]; and P-element induced wimpy testis (Piwi)-interacting RNAs (piRNAs) in the piRNA pathway [19,20].

In arthropods, the first step in triggering the dsRNA-mediated RNAi pathway is the uptake of dsRNA molecules from the external environment, which depends on transmembrane channel-mediated and/or endocytosis-mediated mechanisms [21], followed by interaction of dsRNA with degradation machinery located inside the cells. Several proteins implicated in dsRNA uptake have been studied and described. In the model organism *C. elegans*, four proteins belonging to the *Sid* family (SID-1, SID-2, SID-3 and SID-5) were related to dsRNA uptake efficiency (reviewed by [22]). SID-1 is a channel protein that binds to the dsRNA molecules required for systemic interference [23]. SID-1 orthologs have been reported in lepidopteran pests [24,25]. Clathrin belongs to another class of proteins which promotes clathrin-dependent endocytosis [26]. In *Acyrtosiphon pisum*, the pea aphid, genes involved in the clathrin-dependent pathway were induced 12 h after feeding with dsRNA [27]. Pattern recognition receptors (PRRs) interact with membrane receptors, performing an important function in the uptake of dsRNA through the endocytosis pathway [28,29]. In this context, PRRs in the lepidopteran pests *Helicoverpa armigera*, *Plutella xylostella* and *Spodoptera exigua* were reviewed [30].

Once in contact with insect cellular membranes, dsRNA molecules are taken up by endocytosis [31]. In the cytoplasm environment, dsRNA attaches to a complex formed by the Dicer-2 ribo-nuclease (DCR2), dsRNA binding protein (R2D2) and other associated proteins like pre-mRNA splicing factor (SMD1), Arsenite Resistance Protein 2 (ARS2) and Nuclear Cap-Binding Complex (CBC). The interaction of dsRNA molecules with DCR2, R2D2, SMD1, ARS2 and CBC promote a conformation capable of recruiting the Argonaute protein (AGO) to assemble of a second complex, called RISC (RNA-induced silencing complex). At the mature RISC complex, part of the dsRNA sequence is used as a guide/bait to identify the target mRNA, degrade it and avoid its expression.

In terms of ability to spread the dsRNA signal, two types of events are described: the cell-autonomous route, in which a single cell undergoes the effect of dsRNA presence, and the non-cell-autonomous route, where the interfering effects travel toward tissues/cells/organs distinct from the initial point of application or production. The non-cell-autonomous route is indicated for the development of new MoA assets in RNAi-based pest control. Further details on dsRNA uptake and the progress of this knowledge over time in different species has been reviewed elsewhere [22,32].

Gene silencing is a promising technology that aims to contribute to the control management of several insects of agronomic interest, especially in its topical version, termed spray-induced gene silencing (SIGS) [25]. Compared to conventional pesticides, the SIGS approach has the advantage of high specificity towards the target organism and fast environmental degradation into innocuous compounds [33,34]. Therefore, this novel pest management procedure has the potential to reduce the employment of conventional insecticides, with intrinsic advantages related to the regulatory restrictions inherent to genetically

modified organisms (GMOs), given that dsRNAs can be delivered via topical methods while still maintaining target specificity [35]. In this paper, we highlight the siRNA pathway for exogenous dsRNA, which may represent the next phase of species-specific pest management [36].

2. dsRNA-Mediated Silencing to Control Insects

2.1. dsRNA-Mediated Silencing Core Machinery

In insects, the double-stranded RNA (dsRNA)-mediated gene silencing technique was initially used in a study on *Drosophila melanogaster* functional genomics [37]. As other insects were studied and their mechanism of gene silencing via RNAi was consequently better understood, it was clear that RNAi efficiency varied among different insect families depending on the insect's ability to trigger the gene silencing machinery. This RNAi efficiency relies on cellular uptake, dsRNA degradation, inter- and intracellular transport, and processing of dsRNA into short/small interfering RNA (siRNA) [18].

Upon uptake of dsRNA by the target insect, the first step for intracellular siRNA—the mediated pathway, in general—is the cleavage of the exogenous dsRNA by a specific endonuclease from the *Dcr2* family [18]. The fragments generated by DCR2 are used as a template by R2D2, an RNA-dependent RNA polymerase, leading to amplification of the silencing stimulus. Next, the siRNA molecules produced by the DCR2/R2D2 complex guide the activity of an RNA-induced silencing complex (RISC), formed by Argonaute 2 (AGO2) and some associated proteins, to conduct the homologous RNA degradation [38]. There is a positive co-relationship between RNAi efficiency and the core RNAi pathway gene expression pattern. Therefore, the next requirement for a successful RNAi-based strategy is to understand how the silencing machinery operates in the target insect.

The main elements of the siRNA pathway studied to elucidate the dsRNA processing are DCR2, R2D2, and AGO2. In the case of the fall armyworm (*Spodoptera frugiperda*; Lepidoptera), the basic transcription levels of these core elements of RNAi machinery are similar to those observed in western corn rootworm (*Diabrotica virgifera*; Coleoptera) and southern green stink bug (*Nezara viridula*; Hemiptera) [39]. All three taxa are considered highly efficient species on which to employ RNAi strategies [40–42], from phylogenetic orders whose response to dsRNAi varies.

The impact of dsRNA delivery via injection or feeding on the modulation of the RNAi core genes remains unclear. This was investigated in the European corn borer (ECB) (*Ostrinia nubilalis*; Lepidoptera), a low RNAi efficient species [6]. The study revealed only one transcript for each of the three core RNAi pathway genes (*Dcr2*, *R2D2* and *Ago2*) denominated *OnDcr2*, *OnR2D2* and *OnAgo2*. Expression levels of these genes after dsRNA injection remained steady during the injection assay, while *OnDcr2* alone was upregulated upon an artificial diet assay. Furthermore, analysis of the predicted domains provided functional information concerning conformational differences of *OnAgo2*, *OnR2D2* and *OnDcr2* that could justify the low efficiency of the RNAi apparatus in ECB [6].

2.2. dsRNA Designing

Insects present different levels of susceptibility to dsRNA. Some orders, such as Coleoptera, perform strongly, while others, including Lepidoptera and Diptera, exhibit highly variable outcomes in response to dsRNA treatment, requiring detailed tactics to deliver better-performing solutions [43].

The employment of RNAi for pest control purposes has been improved with advances in high-throughput sequencing (genomic and transcriptomic) and bioinformatic tools [44]. Deeper molecular information on the target insects has provided high specificity to the identification of essential genes for which to create silencing molecules. Hence, large datasets enable strict homology levels between the dsRNA and its corresponding mRNA target. dsRNA design for commercial purposes starts with the development of a preliminary pipeline, where software settings must be adjusted for each specific trait vs. organism analyzed [45–47]. Then, minimum off-target effects criteria are set to select functional siRNA

sequences that guarantee near-perfect matching [48]. Accordingly, carefully designed dsRNA makes it possible to obtain broad-spectrum or extremely specific molecules targeted to the same gene in different insect species, or even within species of the same genus [49].

The precaution of excluding cross-kingdom sRNAs (ck-sRNAs) helps avoid undesirable effects on non-target species. For this purpose, when preparing samples for the sequencing step, it is necessary to treat samples in order to avoid contamination with other organisms (plant cells, endogenous microbiota, parasites) [50], determine the number of biological replicates and calculate the correct amount of reads to achieve sufficient genome coverage (typically three biological replicates with 5–10 million reads each). Sequencing of sRNAs is carried out using a wide variety of high-throughput technologies [51,52], including MiSeq and HiSeq (Illumina Inc., San Diego, California, EUA), SMRT PacBio (Pacific BioSciences, Menlo Park, California, EUA), and Roche 454 technologies (Branford, Connecticut), depending on the output range and total reads per run required [53]. These NGS technologies also allow direct sequencing of cDNA produced from messenger RNA (RNA-seq), enabling the de novo construction of the transcriptome without an anchoring genome [54,55]. For instance, RNASeq (Illumina) and digital gene expression tag profile (DGE-tag) were used to screen optimal RNAi targets in Asian corn borer (ACB) (*Ostrinia furnalis*). Larval-stage specific expression genes were selected for RNAi testing by spraying dsRNA on larvae, reaching mortalities of 73% to 100% at 5 days after treatment [56]. The combination of DGE-tag with RNA-seq is a rapid way to select candidate target genes for RNAi [57].

For siRNA-mediated silencing, high pairing between the target RNA and siRNA is more critical than dsRNA-mediated methods. Furthermore, the siRNA length must be observed for more successful RNAi silencing, considering lepidopteran siRNA populations 20 nt long were observed in some species [58]. BLAST search is an important tool, although not very accurate for short sequences such as siRNAs. Other software to select functional siRNAs, such as PFRED [59] and siRNA-Finder (si-Fi) [60], is available.

2.3. Genomic Data on Lepidopteran Pests

Fully sequenced insect pest genomes support assertive gene targeting for dsRNA design [44]. According to the NCBI, the Insecta class (Figure 1a) has 3091 deposited genomes. From these data, 1831 are reference genomes, while 220 were annotated using the NCBI RefSeq.

Lepidopteran insects are holometabolous butterflies and moths whose life cycles comprise the egg, first to fourth instar larvae, pupa and sexually dimorphic adult [61]. According to [62], Lepidoptera is the second most diverse order (157,424 recognized species) and includes the most devastating agricultural pests in the world [63]. For the Lepidoptera order, we found 1611 genomes, being the Ditrysia clade (Figure 1b) represented by 1540 total genomes, 836 of which were reference genomes and 34 annotated by NCBI RefSeq.

The Yponomeutoidea superfamily (Figure 1c) contains 16 deposited genomes, with four reference genomes and the only NCBI RefSeq genome annotated in the Plutellidae family—the well-studied *Plutella xylostella* (Figure 1c), which has genome assembly at the chromosome level. However, other families lack genomic data, such as the cosmopolitan Lyonetiidae family (Figure 1c), which has about 210 described species, encompassing important pests that are usually leaf and branch mining and parasitize dicotyledons [64]. Lyonetids include miner pests which cause wilting and defoliation to: fruit shrubs, such as apple, pear, peach, apricot and cherry in Europe and Asia; and coffee in Americas [65–67]; ornamental plants [68–71]; medicinal plants [72]; trees, such as willow and poplar and bushes [73–75]. Unlike other lyonetiid miners, *Leucoptera coffeella* or coffee leaf miner (CLM) feeds exclusively on *Coffea* spp. plants in the Neotropical region [76]. The leaf damage caused by the CLM attack cause productivity losses estimated at 87% and defoliation of up to 75%, depending on the season [77,78]. Recently obtained sequences of *L. coffeella* have generated large genomic, transcriptomic and proteomic information at the molecular

level [79], contributing to the development of RNAi research in Lepidoptera by improving the knowledge of the RNAi machinery and the selection of highly specific gene targets.

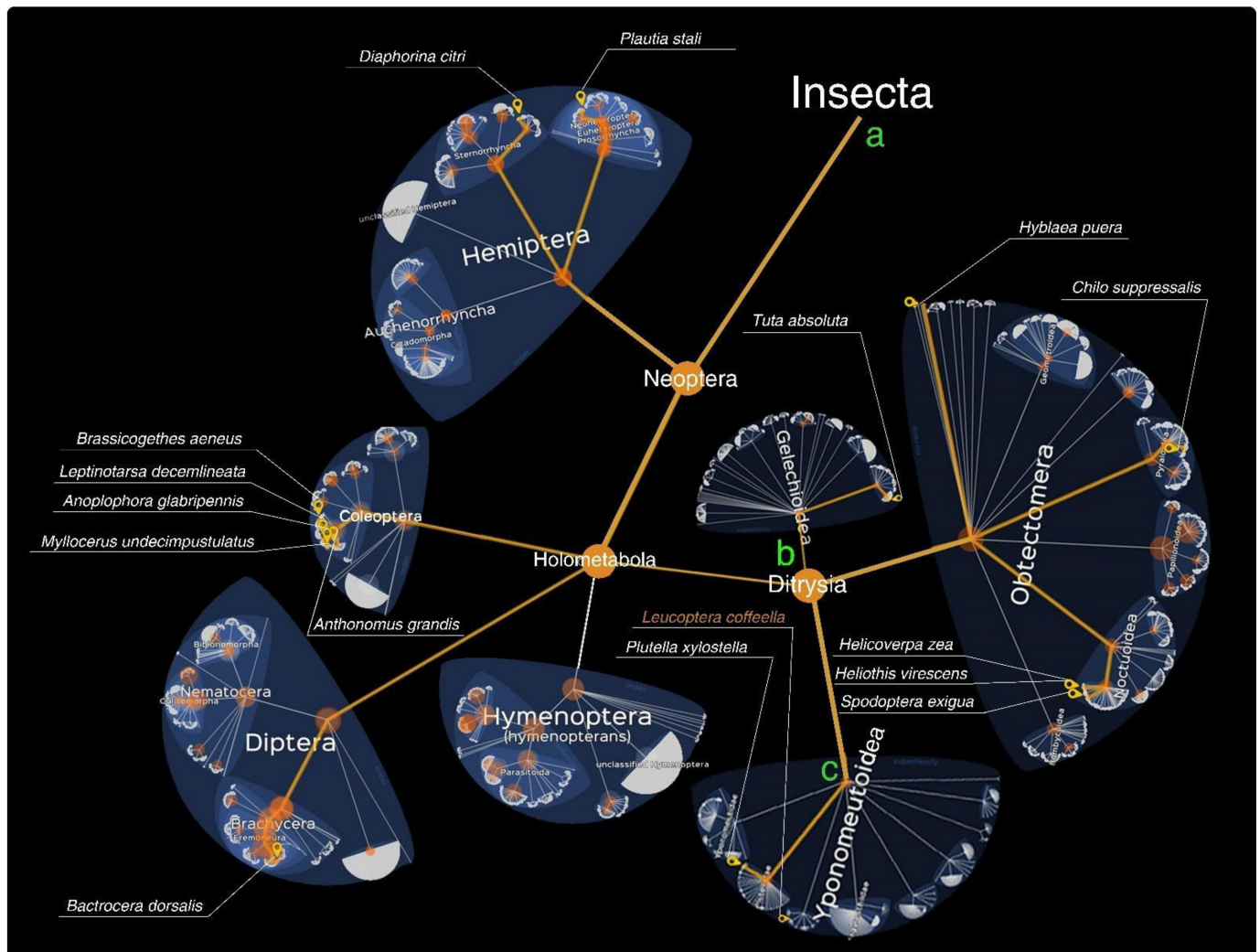


Figure 1. Main taxonomic levels are depicted in a tree, modified from <https://lifemap-ncbi.univ-lyon1.fr/> (accessed on 21 September 2022), showing the Insecta class (a), the Ditrysia clade (b) and the Yponomeutoidea superfamily (c). Orange spheres highlight the levels with fully sequenced genome data. Insect species listed in Tables 1 and 2 (written in white) are marked by a yellow tag. The *L. coffeella* in the Lyonetiidae family is written in orange.

2.4. Insect Target Genes for dsRNA Silencing

To reduce or eliminate the use of chemical pesticides harmful to health and the environment, RNAi has been increasingly developed and tested in agricultural pests. In Table 1, we show some validated genes that are not used in planta silencing (non-GMOs). Insect gene-silencing papers reported before 2017 and some related to pests occurring in the Neotropical Region has been compiled and reviewed elsewhere [49,80].

Table 1. dsRNA-validated genes for insect silencing by non-transformative methods.

Year	Species (Order)	NCBI: txid	Target	Delivery	Mortality	Reference
2017	<i>Anthonomus grandis</i> (Coleoptera)	7044	<i>AgraCHS2</i>	microinjection	100%	[81]
2019	<i>Bactrocera dorsalis</i> (Diptera)	27457	<i>Tssk1</i> <i>Tektin1</i>	artificial diet	58.99% 64.49%	[82]
2019	<i>Myloccerus undecimpustulatus undatus</i> (Coleoptera)	1811735	<i>Prosa2</i> <i>RPS13</i> <i>Snf7</i> <i>V-ATPase A</i>	injection and feeding	78.60% 64.10% 92.70% 43.10%	[83]
2020	<i>Anoplophora glabripennis</i> (Coleoptera)	217634	<i>IAP</i> <i>SNF7</i> <i>SSK</i>	artificial diet	90% 75% 80%	[84]
2021	<i>Diaphorina citri</i> (Hemiptera)	121845	<i>DcCP64</i>	soaking	72%	[85]
2021	<i>Leptinotarsa decemlineata</i> (Coleoptera)	7539	<i>PSMB5</i>	leaf soaking	50–90%	[86]
2021	<i>Plautia stali</i> (Hemiptera)	106108	<i>vATPase</i> <i>IAP</i> <i>MCO2</i> <i>Snf7</i>	injection and feeding	100%	[87]
2022	<i>Brassicogethes aeneus</i> (Coleoptera)	1431903	α COP	leaf soaking	62%	[88]

The dsRNA to silence the *AgraCHS2* gene resulted in 100% adult mortality when microinjected into the cotton boll weevil (*Anthonomus grandis*; Coleoptera). This gene is essential for peritrophic membrane biosynthesis, intestinal epithelium protection, and nutrient assimilation [81]. Subsequently, the *Tssk1* and *Tektin1* genes (important for male fertility) were silenced, causing the death of 58–64% of *Bactrocera dorsalis* (Diptera) individuals by artificial diet [82].

In the Sri Lanka weevil (*Myloccerus undecimpustulatus undatus*; Coleoptera) silencing was validated through injection and feeding the genes *Prosa2* (proteasome subunit alpha type 2) [89], *RPS13* (structural element of the 40S subunit) [90], *Snf7* (endosomal sorting complex required for transport III- ESCRT-III) [91] and *V-ATPase A* (transmembrane ATP-driven proton pump) [92]. The mortality rate ranged from 43.1% to 92.7%.

The Asian long-horned beetle (ALB), a polyphagous wood-boring species (*Anoplophora glabripennis*; Coleoptera). Bioassays in which larvae were fed with dsRNA resulted in the death of 75–90% of the individuals, upon silencing the inhibitor of apoptosis (*IAP*), *SNF7* and snakeskin (*SSK*) genes [84]. Still, the Asian citrus psyllid (ACP) vector (*Diaphorina citri*; Hemiptera) transmits the Citrus Huanglongbing disease (HLB). Tests with dsRNA to silence the *DcCP64* gene, responsible for the synthesis of the 64-like cuticle protein, resulted in 72% of the psyllids' deaths [85]. However, the brown-winged green stinkbug (*Plautia stali*; Hemiptera), known for infesting various fruits and crop plants, showed 100% of death when the insects were treated with RNAi directed to the genes *vATPase*, *IAP*, *MCO2* and *Snf7* [87].

The Colorado potato beetle (CPB) (*Leptinotarsa decemlineata*; Coleoptera) was subjected to *PSMB5* gene silencing, which is part of the ubiquitin/proteasome machinery, reaching between 50% and 90% mortality depending on the life stage of the CPB. The study ended up generating an RNA-based biopesticide, the Ledprona[®], which is being reviewed for registration at the United States Environmental Protection Agency (EPA) [93].

The order Lepidoptera contains several highly destructive representatives that generally show low mortality rates when subjected to RNAi-based tests [94]. Despite the limitations of the gene silencing effect in lepidopterans, potential solutions may still exist,

as there are possibilities that have not yet been tested [95]. In Table 2, we listed works published between 2018–2022 on the validation of lepidopteran pest genes silenced by exogenous application.

Table 2. Lepidopteran target genes validated for dsRNA silencing. The taxonomic positions of these genes' corresponding species are depicted in Figure 2.

Year	Species (Family)	NCBI: txid	Target	Delivery	Mortality	Reference
2018	<i>Helicoverpa zea</i> (Noctuidae)	7113	<i>TipE</i> <i>GluCl</i> <i>Para</i> <i>Notch</i>	microinjection	12–16% 12–16% 12–16% 12–16%	[96]
2018	<i>Plutella xylostella</i> (Plutellidae)	51655	<i>AChE</i>	topical foliar application	69–74%	[97]
2018	<i>Heliothis virescens</i> (Noctuidae)	7102	<i>PBAN</i>	topical foliar application and Injection	50–60%	[98]
	<i>Helicoverpa zea</i> (Noctuidae)	7113			50–60%	
2021	<i>Hyblaea puera</i> (Hyblaeidae)	268502	<i>HpEcR</i> <i>HpCHS1</i> <i>HpChi-h</i>	topical foliar application	46% 30% 32%	[99]
2021	<i>Spodoptera exigua</i> (Noctuidae)	7107	<i>GNAF</i>	feeding	48%	[100]
2021	<i>Chilo suppressalis</i> (Crambidae)	168631	<i>ND</i> <i>GPDH</i> <i>MSL3</i>	topical foliar application	50% 50% 50%	[101]
2021	<i>Tuta absoluta</i> (Gelechiidae)	702717	<i>v-ATPase B</i> <i>JHBP</i>	topical foliar application	70% 70%	[102]

The *AChE* gene was silenced via soaking in diamond back moth (*Plutella xylostella*) one of the main pests of cruciferous vegetables. This gene is responsible for the synthesis of acetylcholine esterase—the primary target of commercial insecticides—which interrupts the action of neurotransmitters. To increase stability, researchers tested a concatemerized form of the molecule. The mortality rate was up to 72%, higher than that observed with the non-concatemerized control [97].

The corn caterpillar (*Helicoverpa zea*) was subjected to tests involving RNAi using genes that are targets of commercial insecticides: *Para* (paralytic effect), *TipE* (temperature-induced paralysis), *GluCl* (glutamate chloride channel), and *Notch* (encodes proteins that make up neuronal cells). Three delivery methods were used: microinjection, egg immersion, and larval feeding. Microinjection of eggs of the *GluCl*, *Para*, and *TipE* genes reduced hatching rates, while the *Notch* gene showed no difference. None of the genes were effective for larval feeding and egg immersion methods [96].

Pinworm (*Tuta absoluta*) is the most aggressive tomato pest in South America, Africa and Asia. This lepidopteran, which feeds on mesophyll, was subjected to tests with RNAi of the genes: *v-ATPase B* (keeps the midgut lumen alkaline by increasing amino acid absorption) and *JHBP* (essential for development and reproductive maturation). Topical application on the leaf surface resulted in 70% of larval mortality for the two target genes [102].

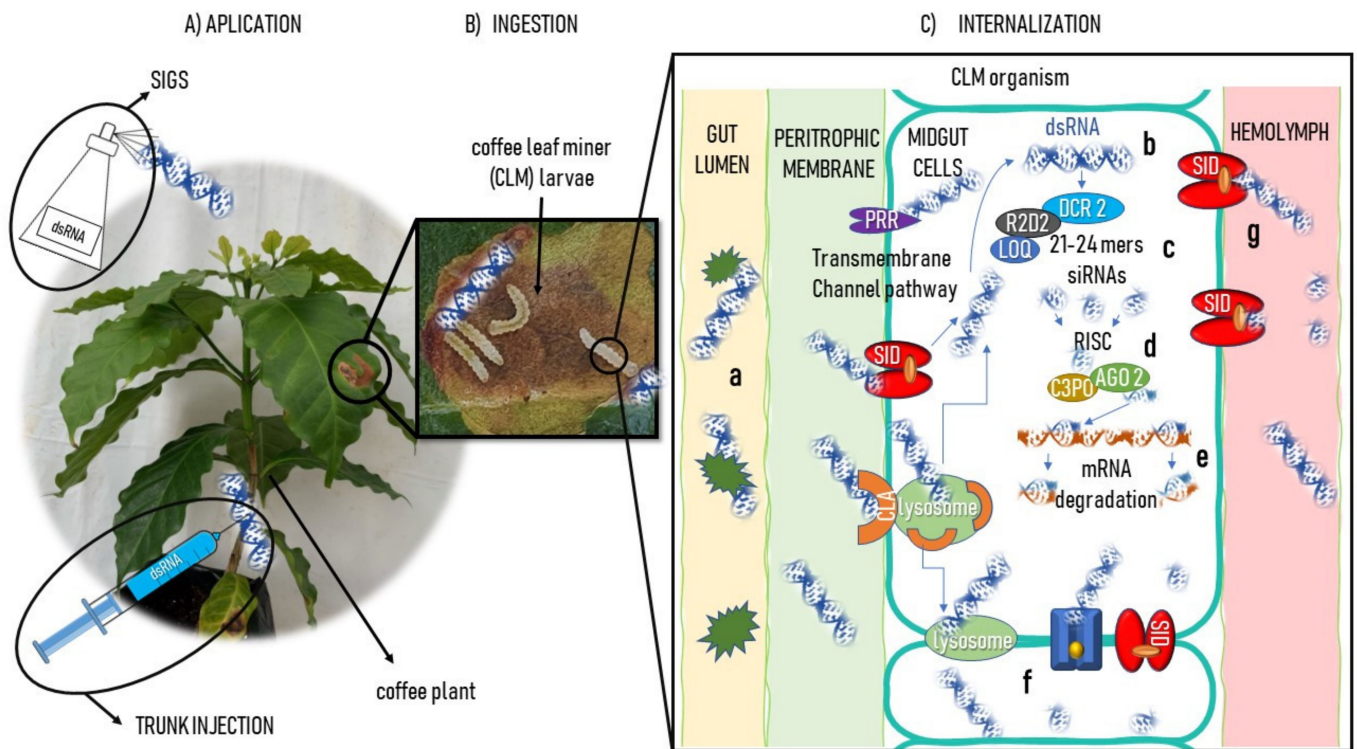


Figure 2. Schematic strategy of RNAi to control *L. coffeella* by long dsRNA-induced silencing of coffee plants. (A) dsRNA delivery by SIGS or trunk injection; (B) dsRNA oral ingestion by larva feeding the leaf parenchyma; (C) dsRNA internalization in the larva: **a** transfer from insect gut lumen to midgut cells by the clathrin-mediated endocytic pathway (CLA), pattern recognition receptors (PRRs), and/or RNA-binding proteins (RBPs); **b** long dsRNA interaction with an R2D2-DCR2 complex associated with LOQ; **c** LOQ recognition of exogenously delivered dsRNA to slicing by Dicer (*DCR2*) with *R2D2* to produce siRNAs; **d** guide-strand selection by AGO2 complexed with C3PO; **e** siRNA-guided silencing by attachment to the target mRNA passenger strand; systemic amplification of the RNAi silencing by dsRNA and siRNA; **f** dispersion through other gut cells by the cytoplasm and **g** diffusion to the hemolymph.

The Asiatic rice borer (*Chilo suppressalis*) is one of the world's main crop pests. As its chemical control is expensive, the RNAi technique has become a possible option for the borer's management. Three selected genes—*ND* (NADH dehydrogenase), *GPDH* (glycerol 3-phosphate dehydrogenase), and *MSL3* (male specific lethal 3)—were tested. Rice leaves were brushed with bacterial dsRNA solutions that contained newly hatched larvae. The insect mortality rate was 50% for each gene [101].

Corn caterpillar (*H. zea*) and tobacco caterpillar (*Heliothis virescens*) were subjected to control tests containing dsRNA of the *PBAN* target gene. dsRNA delivery by artificial larval diet or pupae injection caused a mortality rate that ranged from 30 to 60%. A delay in larval development and some interference in the development of pupae were also observed in the two agricultural pests analyzed [98].

The teak defoliator (*Hyblaea pueria*) causes severe defoliation to teak, a tree of paramount commercial importance in forestry. Sequences from the *HpCHS1*, *HpChi-h*, and *HpEcR* genes, related to chitin metabolism, were used in the construction of dsRNAs that were administered to the larvae via topical application on the leaves. It was found that 30–46% of the treated larvae died; a large number of deformed pupae were also observed, in addition to the deformed pupae [99].

Assuming that gene silencing can occur in Lepidoptera species, we believe that the CLM (*Leucoptera coffeella*) can also be controlled by exogenous dsRNA technologies. Aiming to develop biopesticide solutions to this important coffee pest, our research group has performed the full genome PACBio and paired-end Illumina combined DNA sequencing

from pupae samples. The generated data allowed for nuclear genome, transcriptome and proteome analyses as a basis for the discovery of RNAi mechanisms specific to the Lyonetiidae family, as well as the selection of target genes. This information is essential to RNAi development, as the taxonomically closest complete genome is in the very distant Yponomeutoidea taxon [79].

3. Validation Tests of the dsRNA Candidates for Proof of Concept

After synthesizing the dsRNA molecules in the laboratory, validation in a controlled rearing environment is required before field application, either by direct delivery to the insect or indirect application on the plant for subsequent ingestion. About general assay aspects, an interesting study provided important insights into how to apply dsRNA exogenously to plants in *Arabidopsis thaliana* [103]. Under different physiological conditions (plant age, time of day, soil moisture, high salinity and heat and cold stress) and via various application media (brush spreading, spraying, infiltration, inoculation, needle injection and pipetting), the best results were associated to application at night, low moisture soil, brush spreading, spraying and pipetting.

Aiming to further test the small-sized *Leucoptera coffeella* larvae (1-4 mm in length), we found some useful techniques that could be applied to testing larvae and pupae in vitro or ex vitro. In this way, we discuss some dsRNA application techniques used in other insects that can be tested according to our reality, considering the size and development stages of *L. coffeella* and the characteristics of the coffee plant.

To insects depending on the living plant tissue to complete the life cycle, as *L. leucoptera*, the plant infiltration seems to be very suitable. This procedure is performed by syringe without a needle, pressing the dsRNA-containing solution under the abaxial face of the leaf. It may be a viable method for validating the dsRNA in plants, as it is possible to visualize the entry of the solution into the leaf. Sometime later, the liquid diffuses inside the plant tissues and is no longer visible. The infiltration process is carried out in sunflowers [104]. Even if it is an easy method, it is quite variable in effectiveness, depending on plant anatomy.

Microinjection is an option proved to work even to 1 mm insect developmental stages. Tests performed with dsRNA in the cotton boll weevil (*Anthonomus grandis*) showed a 93% reduction in oviposition and 100% of adults death to 1mm microinjected larvae [81]. With *H. zea* 1mm diameter eggs, microinjection resulted in 12–16% mortality of larvae after silencing five genes [96]. Nine dsRNA molecules specific to *Nezara viridula* (southern green stink bug) showed an average mortality rate of 90% after microinjection into 3.2mm nymphs [105,106].

Immature stages could also be tested by detached leaves, as the 48% mortality reported to the beet armyworm (*Spodoptera exigua*) (Table 2) fed with dsRNA treated leaves [100]. Also, worth of testing is the soaking method used in *Diaphorina citri* (Asian citrus psyllid) nymphs [85,107].

4. dsRNA-Based Products

4.1. Formulation with Nanocarriers

Once the target genes that impair insect proliferation are chosen and preliminary assays are realized in laboratory and greenhouse conditions, other constraints inherent to the dsRNAi approach should be addressed. The stability of dsRNA molecules after field application and in insect gut conditions is crucial to extending the material's half-life length.

It is known that to control lepidopteran pests via dsRNA-based products, avoiding dsRNA degradation by RNases and high pH levels is a sine qua non situation [108]. After application to the plants, the dsRNA molecule must get to the target gene inside the insect cells [109]. The primary constraint of using molecules ingested by insects is their degradation in the gut. Most of the dsRNases present in lepidopterans' guts are basophilic, presenting an optimum pH of around 9.0 [26]. dsRNA stability is influenced by high pH

values due to its chemical structure. In alkaline conditions, dsRNA can be hydrolyzed in some regions [26]. Noticeably, the drawbacks in Lepidoptera are even more significant [110] because pH in the lepidopteran gut can reach 12 [111]. This factor is probably the biggest challenge to making dsRNA applicable to lepidopteran insect control [110]. Beyond the gut, dsRNases present high activity in other body fluids such as saliva and hemolymph, which cause the degradation before the dsRNA can be processed by the insect [23].

The fate of dsRNA is short both in soil and aquatic environments [34,112]. Microbial nucleases present in the soil and on leaves, UV-radiation and run-off due to dew and rain can significantly limit the availability of dsRNA to the pest [112,113].

Fortunately, most of those problems have been gradually overcome with improvements and recent findings in chemistry and nanotechnology. Thus, dsRNA formulations containing protection agents, surfactants, and diluents may be required [114]. Many nano-materials are used to form complexes with dsRNA, as presented below—especially the cationic ones that present positive extremities, able to bond with the negative parts of dsRNA molecules.

4.2. Peptides

The peptide transduction domain (PTD) may be fused to dsRNA binding domain (DRDB) molecules, allowing a more efficient internalization by the cell and consequently enhancing gene silencing in *Anthonomus grandis*. Although the PTD-DRDB complex made the dsRNA more stable and efficient in the presence of nucleases in the *A. grandis* gut [115], the dsRNA was harder to synthesize. An alternative is using branched amphiphilic peptide capsules (BAPCs), which are more easily synthesized than PTD, to protect dsRNAs [116]. These nano capsules are made of natural amino acids, and are water soluble and resistant to detergents, proteases and chaotropic agents. The association of BAPCs to dsRNA led to premature deaths of pea aphid (*Acyrtosiphon pisum*) when fed with a diet containing BAPCs-dsRNA complex [117].

4.3. Transfection Reagent

Lipofectamine[®] 2000 (Reagent Catalog number: 11668030, Thermo Fisher Scientific, Life Sciences Solutions, 5781 Van Allen Way, Carlsbad, CA, USA 92008, Invitrogen) is a transfection reagent able to inhibit nuclease activity and characterized by the presence of cations at the phospholipid bilayer. These characteristics allow lipofectamine to overcome the repulsion relation between cell membranes and nucleic acids, becoming a good candidate to be added to dsRNA formulations [116]. When used to treat the brown stink bug (*Euschistus heros*; Hemiptera), lipofectamine was able to increase the mortality of second-instar nymphs by 15% after 14 days of artificial feeding, in comparison with the naked dsRNA molecules, which caused 33% mortality [118].

Another transfection reagent, Cellfectin[®] II Reagent (CFII) (Catalog number: 10362100, Thermo Fisher Scientific, Life Sciences Solutions, 5781 Van Allen Way, Carlsbad, CA, USA 92008), was tested complexed with dsRNA against *Spodoptera frugiperda*. One group of larvae was fed with CFII-dsRNA complex and the other group received naked dsRNA. The diet without CFII caused 25% mortality, while the group that received CFII-dsRNA registered 55%. These results indicate that CFII was able to protect dsRNA molecules from the high pH and the nucleases of the hemolymph and midgut lumen, increasing dsRNA's final efficiency [119].

4.4. Macromolecular Polymers

Star polycation (SPc) is a cationic amino acid dendrimer nanocarrier that is able to enhance gene transfection efficiency. Its dsRNA association capability, due to its positive charge, allowed RNAi silencing of the *CYP6CY3* gene up to 84.3% mortality in *Aphis gossypii* [120]. SPc was also used in association with dsRNA to verify its capability in the control of polyphage aphid (*Myzus persicae*; Hemiptera) [121]. To avoid water repellency a 0.1% detergent solution was added to the formulation. The penetration efficiency at the

aphid, measured by fluorescence tests, showed that with SPc, dsRNA molecules were able to penetrate the aphid's whole body. The *vestigial* (*vg*) and *Ultrabithorax* (*Ubx*) genes were downregulated 44.0% and 36.5%, respectively, 24 h after treatment.

SPc polymerized with DMAEMA (2-N-(dimethyl aminoethyl) methacrylate) was made in association with dsRNA to control *Chilo suppressalis* by artificial feeding. The SPc protection showed an increase of 60% in larvae mortality [122]. In *S. frugiperda*, the SPc complexed with dsRNA was tested in Sf9 cells, hemolymph and midgut lumen contents collected from *S. frugiperda* larvae. The performance of the complex and naked molecules was measured by UV tests at 488 nm, showing that SPc was able to enhance dsRNA molecules' stability. After 12 h of incubation, the UV signal was much stronger in cells that absorbed the complex dsRNA/SPc than in naked dsRNA, showing that SPc can promote dsRNA uptake by cells. Also, SPc was able to protect dsRNA molecules from being degraded by RNase A and the insect hemolymph [123].

Polymers containing guanidine were developed to stabilize dsRNA molecules in very alkaline environments and to protect these molecules from nucleases. Since dsRNA molecules are very sensitive to high pH environments because of the hydrolyzation caused to double-stranded molecules [26], it is necessary to prevent degradation in lepidopteran's guts, which can reach pH 12 [111]. dsRNA can form complexes with the guanilate polymer due to its positive charge. In laboratory ex vivo tests, a guanilate polymer was able to avoid dsRNA degradation for over 30 h at pH 11, while the naked molecules were degraded after 10 min in *Spodoptera exigua* gut juice. In vivo bioassays showed that the mortality associated with the dsRNA complexed with the guanilate polymer was around 53.3%, while the naked molecules caused 16.7% of mortality [124].

Chitin can be deacetylated and become a natural material called chitosan. This cationic nanopolymer is composed of biodegradable and biocompatible molecules [125]. Chitosan nanoparticles (CNPs) were complexed to dsRNA molecules and sprayed over chickpea leaves to control the lepidopteran *Helicoverpa armigera*. The CNPs/dsRNA complex led to a reduction in length and weight of the larvae when compared to naked-molecule silencing [126]. Chitosan (CS) can be further improved by other materials, such as sodium tripolyphosphate (STPP), a nanosized cross-linker able to increase the protection of the chitosan-dsRNA complex, forming a CS-STPP-dsRNA complex [127].

4.5. Other Materials

Layered double hydroxide (LDH) clay nanosheets have a flat, hexagonal, positively charged structure that interacts with dsRNA molecules, forming a complex named BioClay. This structure allows for dsRNA detection on the surface of leaves even 30 days after application via foliar spray. Moreover, it also enables improved permanence of the molecules over the leaves, avoiding removal by the application of other products [128].

Carbon Quantum Dots (CQD) are another possible formulation reagent. When associated with dsRNA molecules, CQD was able to improve gene depression by 41% in the gut, 45% in other tissues and 43% in the whole organism. In contrast, control naked dsRNA did not show any expression level reduction. The downregulation caused by silencing resulted in 70% mortality 6 days after diet feeding in *C. suppressalis*. The CQD also showed a very good stability result in comparison to chitosan, Lipofectamine® 2000 and naked molecules of dsRNA when tested on midgut homogenates [129].

5. dsRNA Production

In the last few years, dsRNA production methods have been continuously optimized to promote the wider application of this technology. dsRNA can be produced using either in vitro transcription or in vivo expression in bacteria, yeast, microalgae and other species [130–134]. Bacteria are a low-cost alternative for the production of large amounts of dsRNA [135]. *Escherichia coli* HT115 (DE3) were first used as an heterologous dsRNA production system by [136]. Since then, it has become a useful tool for functional studies in invertebrate physiology [134,137–139]. Still, other kinds of bacteria, such as *Bacillus*

thuringiensis (Bt) [130,133,140], *B. subtilis* [141,142], symbiotic bacteria [143–147], *Pseudomonas syringae*, *Corynebacterium glutamicum* and *Chlamydomonas reinhardtii* are useful biopesticide production strains that have been widely used for the control of several pests.

In addition, eukaryotic species *Saccharomyces cerevisiae* has also been used as a relevant dsRNA production system. *S. cerevisiae* does not contain the core genes *Dicer2* and *Argonaute-2* of the RNAi pathway [132], which allows efficient dsRNA synthesis in *S. cerevisiae* compared with *E. coli* and other bacterial species [148]. Fungi [131] and viruses [149,150] have also been engineered to produce dsRNA.

In experimental settings, three main approaches are used to produce dsRNA: chemical synthesis of NTPs, in vitro synthesis through RNA-dependent RNA polymerases and fermentation through microorganisms. To this goal, in vitro synthesis is best suited for the synthesis of short dsRNAs [151] and to reach high-purity dsRNA standards, despite its relatively high production cost [152].

For field application, the main requirement is the establishment of large-scale production at low cost. Bacterial systems are the most used alternative [138] and promising methods to biologically synthesize long dsRNAs for the control of Lepidoptera species and other insect pests [42,153]. Although in vivo production supplies low-cost dsRNA in high yields, this strategy requires later purification of the product and inactivation of the engineered microbial strain [154,155]. Efficient production involves the proper release of dsRNA from the cells, without affecting dsRNA integrity during the extraction process. Methods for the Cost-Effective Production of Bacteria-Derived Double [138]. Despite the risk of compromised integrity inherent to this method, dsRNA extracted from bacteria efficiently induced knockdowns in a lepidopteran cell line [138].

In 2008, the cost of synthetic dsRNA was approximately 12,000 USD/g, dropping to 60 USD/g in 2018 [156]. RNAGri had the ability to produce tons of dsRNA at a cost of 1 USD/g, while Greenlight's GreenWorX™ system can further reduce the cost of dsRNA synthesis to < 0.5 USD/g [49,152,157–159]. In 2009, a study showed that producing 30 mg of dsRNA in vivo was approximately one-third the cost of the in vitro methodology [160].

6. Field Application of dsRNA Assets

6.1. Foliar Spray

Foliar application is a very practical and convenient SIGS method that has been reducing in cost over time [161]. Reliable dsRNA market products must present convincing low-cost and high-efficiency characteristics. Foliar spray application fits the synthesis price, as the costs of this method are around 0.5 and 1 USD per gram on a cell-free bioprocessing platform [159], with about 2 to 10 g per hectare required [156,162,163]. The convenience of pulverization is that it is a method already used in most production areas, which represents a shortcut to application on farms.

The most advanced commercial foliar spray product launched is the dsRNA-based Ledprona®. Its active ingredient is a dsRNA artificially synthesized and applied via foliar spray. It has been demonstrated to be a very promising product in field trials [86].

Another factor that makes RNAi a difficult strategy to be applied is the uptake of the product when applied via foliar spray. Many leaf surfaces are re-covered, mostly by wax, or have a hydrophobic cuticle, which makes absorption more difficult; the presence of trichomes and stomata density and position also interfere directly with the dsRNA absorption by the plants [161].

Naked dsRNA molecules require some kind of protection or association with other compounds to be used as an active principle to any product, in order to avoid being degraded before they reach their final destination and allow the product to reach its maximum potential [159]. The dsRNA strategy currently faces other drawbacks to application, such as the low pH that many plants present on the leaf surface, which causes dsRNA degradation. Furthermore, UV light can reduce the biological activity of dsRNA. Rain, which is usually an excellent factor for dsRNA application, can become a threat to the stability of dsRNA

molecules. These are crucial factors that dsRNA strategies must overcome to reach a good market position [164,165].

Using bacteria to deliver dsRNA presents advantages compared to in vitro dsRNA synthesis [29]. Silencing of five different target genes caused significant larval mortality to the CPB after 12 days feeding on DE3 *E. coli* expressing dsRNAs [166]. RNAi effects were also observed by ingestion of bacteria expressing dsRNA in *Spodoptera exigua* [167,168], *Chilo infuscatellus* [57] and *Tuta absoluta* [169].

Feeding with bacteria expressing a homologous dsRNA was effective to silence an immune gene of the noctuid moth *Spodoptera littoralis* [130], although dsRNA delivery to the hemocoel in lepidopteran insects needs high amounts of dsRNA to achieve significant knockdowns. In field conditions, the quantity required is even higher [42,170].

6.2. Trunk Injection

Trunk injection is another application method that can be especially useful to apply in the field, particularly for those crops that are perennial plants and those that present woody trunks, such as citrus, coffee and pine [49]. The low efficiency of dsRNA delivery methods can be circumvented by trunk injection, a promising method that reduces environmental impacts. This process relies on the phloem as a channel where dsRNA may remain stable for a long time due to the presence of sap. Besides being a medium free of RNase, the sap has the advantage of facilitating the injected material's dissipation throughout the plant [171].

The feasibility of the trunk injection process was demonstrated for the first time in citrus trees and vines that were exposed to a single injection of 2 g of dsRNA diluted in 15 L of water [172]. The delivery was confirmed after the detection of dsRNA in the treated plants. Additionally, the authors demonstrated that two hemipteran insects that fed on sap also absorbed dsRNA, suggesting that this method would be an excellent choice for sucking pests. It is important, however, to note that the trunk injection strategy may be costly, as it requires the massive production of dsRNA and a specific injection apparatus [29].

Some products for injection delivery are currently available: Arbojet® <https://arborjet.com/> (accessed on 19 September 2022), which proposes injection by the insertion of a drilling plug [171] and ChemJet® (<https://www.chemjet.com.au/> (accessed on 19 September 2022) a rechargeable injector to be inserted within a previously pierced hole

Depending on the characteristics of the insect pest to be controlled, it is important to evaluate which injection type favors dsRNA delivery to the tissues on which the pest feeds—in the case of the CLM, for example, to the palisade parenchyma of leaf mesophyll. It is also important to evaluate the orifice size and the plug fixation options, since the open orifice becomes an entrance port for pathogens. In addition, there is a risk of generating embolisms in the vessels or compartmentalization of the vascular system after piercing. Despite this, efficient trunk injection for systemic conduction in perennials was demonstrated in apple trees with essential oil emulsions applied through 1 mm-wide and 1 cm-deep perforations [173].

A perennial large crop that could benefit from the trunk injection method is coffee. Similar to the apple tree, coffee is a perennial shrub that could be treated by trunk injection to direct dsRNA to the leaf mesophyll, aiming at CLM larval stage feeding, as illustrated in Figure 2.

7. Limitations of RNAi Technology in Lepidoptera

During the last decade, many studies have proven that RNAi technology is efficient in pest control mainly via transgenic plants, feeding, trunk injection, or spraying [56,57,174,175]. Lepidopteran insects, however, present variable RNAi efficiency for several reasons, even though their successful uptake of dsRNA has been evidenced in injection, transfection, or transgenic assays that reported good responses to dsRNA stimuli [29,94]. Studies concerning persistence of dsRNA in the digestive tract of lepidopterans indicate that dsRNA can be quickly degraded by nucleases present in the saliva, hemolymph and

gut juice [176,177]. This means that the successful usage of dsRNA in a SIGS approach relies on the protection of the dsRNA from degradation in the field and the insect gut. Formulation technologies can also be used to improve cellular internalization of dsRNA and to protect dsRNA against nucleolytic degradation, hence improving overall delivery to the pest [108,124]. Nevertheless, formulation contents might present a risk on their own and the impact of the formulation itself on the environment and non-target organisms (NTO) should be assessed as well.

Other aspects to consider for commercial application of RNAi are cost-efficiency, safety, the delivery to the site of action in the target organism and adverse effects in NTOs. The exposure of NTO is dependent on several parameters, including application rate, timing of application, application method, number of applications, off-site movement of the dsRNA and stability and persistence of the dsRNA [178,179].

Hence, RNAi efficiency varies greatly among different insect species and the major limitations for efficient RNAi include dsRNA instability, refractory gene targets, low efficiency of dsRNA cellular internalization, deficient core RNAi machinery and impaired systemic spreading of dsRNA, which constrains the application of RNAi-based pest management [123].

8. Final Considerations

RNAi-based biopesticides have fewer harmful effects than most conventional chemical pesticides and no pest resistance development is expected in the field when using long dsRNA strategies. These molecules are processed in many different siRNAs to silence the target genes, minimizing the probability of acquired RNAi-resistance. Moreover, non-transformative strategies likely prevent insects from adapting and circumventing resistance to dsRNA silencing, perhaps because the intermittent presence of the dsRNAs—in contrast to a constant supply provided by HIGS—presents lower selection pressure [180].

RNAi-based pesticide control of lepidopteran pests encompasses environmental stability and low silencing efficiency limitations that may be surmounted by nanocarriers and adjuvants associated to dsRNAs [181].

From a regulatory perspective, exogenous delivery methods are more likely to be accepted for commercialization due to their biosafety appeal. These products offer pest gene control without introducing GMO plants into the environment. Moreover, the topical application of dsRNAs is expected to be minimally impactful due to their fast environmental fate, low non-target and off-target risks and other advantages over HIGS solutions. As the importance of research and commercial interests in exogenous RNAi technology has risen strikingly in recent years, many forums for discussing the regulatory frameworks for pesticide authorization in the United States (US) and European Union (EU) have formed within the European Food Safety Authority (EFSA), the Organization for Economic Co-operation and Development (OECD) European Co-operation in Science and Technology (COST) and the scientific community [44]. Currently, while dsRNA-based insecticides are being generated, documents from these initiatives are gathering knowledge and outlining the classification and authorization procedures for the environmental risk assessment (ERA) guidelines to this new MoA, considering factors such as the introduction and mobility of dsRNA within target species, its environmental fate, prediction and determination of off-target and non-target effects and resistance development [44,181].

Considering all the aspects discussed above, one can infer that in the medium-term future the control of lepidopterans by topical dsRNA will be widely adopted in world agriculture.

Author Contributions: Conceptualization, E.F.A.A. and É.V.S.A.; writing—original draft preparation, V.S.L.-L., E.F.A.A., L.A.V., C.R.T., C.I.C.V.F.J., J.D. and É.V.S.A.; writing—review and editing, V.S.L.-L., E.F.A.A., L.A.V., J.D. and É.V.S.A.; supervision, E.F.A.A., J.D. and É.V.S.A.; project administration, É.V.S.A.; funding acquisition, J.D. and É.V.S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the CONCAFE—Consórcio Pesquisa Café to project funding and APC Brazilian Agricultural Research Corporation (grant number 10.18.20.004.00.00) and the fellowships of V.L.L. (Embrapa/FUNAPE SICONV N° 851.999/17) and C.T. (Embrapa/FUNAPE SICONV No 851.999/17); by Embrapa to the fellowship of L.A.V. (SAIC/Embrapa 10.200.21/0011-9-13111); by CNPq—Coordenação de Aperfeiçoamento de Pessoal de Nível Superior to the fellowship of C.J. (139618/2022-8).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. García-Lara, S.; Serna-Saldivar, S. Insect Pests. In *Encyclopedia of Food and Health*; Caballero, B., Finglas, P.M., Toldrá, F., Eds.; Academic Press: Cambridge, MA, USA, 2016; pp. 432–436. ISBN 9780123849533. [[CrossRef](#)]
2. Sharma, S.; Kooner, R.; Arora, R. Insect Pests and Crop Losses. In *Breeding Insect Resistant Crops for Sustainable Agriculture*; Arora, R., Sandhu, S., Eds.; Springer Singapore: Singapore, 2017; pp. 45–66. ISBN 978-981-10-6055-7.
3. Savary, S.; Willocquet, L.; Pethybridge, S.J.; Esker, P.; McRoberts, N.; Nelson, A. The Global Burden of Pathogens and Pests on Major Food Crops. *Nat. Ecol. Evol.* **2019**, *3*, 430–439. [[CrossRef](#)] [[PubMed](#)]
4. IPPC Secretariat. *Scientific Review of the Impact of Climate Change on Plant Pests: A Global Challenge to Prevent and Mitigate Plant-Pest Risks in Agriculture, Forestry and Ecosystems*; FAO on behalf of the IPPC Secretariat: Rome, Italy, 2021; ISBN 978-92-5-134435-4.
5. Gould, F.; Brown, Z.S.; Kuzma, J. Wicked Evolution: Can We Address the Sociobiological Dilemma of Pesticide Resistance? *Science* **2018**, *360*, 728–732. [[CrossRef](#)] [[PubMed](#)]
6. Cooper, A.M.W.; Song, H.; Shi, X.; Yu, Z.; Lorenzen, M.; Silver, K.; Zhang, J.; Zhu, K.Y. Characterization, Expression Patterns, and Transcriptional Responses of Three Core RNA Interference Pathway Genes from *Ostrinia Nubilalis*. *J. Insect Physiol.* **2021**, *129*, 104181. [[CrossRef](#)] [[PubMed](#)]
7. Fire, A.; Albertson, D.; Harrison, S.W.; Moerman, D.G. Production of Antisense RNA Leads to Effective and Specific Inhibition of Gene Expression in *C. Elegans* Muscle. *Development* **1991**, *113*, 503–514. [[CrossRef](#)] [[PubMed](#)]
8. Hannon, G.J. RNA Interference. *Nature* **2002**, *418*, 244–251. [[CrossRef](#)]
9. Carthew, R.W.; Sontheimer, E.J. Origins and Mechanisms of miRNAs and siRNAs. *Cell* **2009**, *136*, 642–655. [[CrossRef](#)]
10. Gebert, L.F.R.; MacRae, I.J. Regulation of MicroRNA Function in Animals. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 21–37. [[CrossRef](#)]
11. Ding, S.-W.; Han, Q.; Wang, J.; Li, W.-X. Antiviral RNA Interference in Mammals. *Curr. Opin. Immunol.* **2018**, *54*, 109–114. [[CrossRef](#)]
12. Ding, S.-W. RNA-Based Antiviral Immunity. *Nat. Rev. Immunol.* **2010**, *10*, 632–644. [[CrossRef](#)]
13. Bronkhorst, A.W.; van Rij, R.P. The Long and Short of Antiviral Defense: Small RNA-Based Immunity in Insects. *Curr. Opin. Virol.* **2014**, *7*, 19–28. [[CrossRef](#)]
14. Chapman, E.; Tagliani, F.; Bayne, E.H. Separable Roles for RNAi in Regulation of Transposable Elements and Viability in the Fission Yeast *Schizosaccharomyces Japonicus*. *PLoS Genet.* **2022**, *18*, e1010100. [[CrossRef](#)]
15. Kataoka, K.; Mochizuki, K. Programmed DNA Elimination in Tetrahymena: A Small RNA-Mediated Genome Surveillance Mechanism. *Adv. Exp. Med. Biol.* **2011**, *722*, 156–173. [[CrossRef](#)]
16. Martienssen, R.; Moazed, D. RNAi and Heterochromatin Assembly. *Cold Spring Harb. Perspect. Biol.* **2015**, *7*, a019323. [[CrossRef](#)]
17. Ashfaq, M.A.; Dinesh Kumar, V.; Soma Sekhar Reddy, P.; Anil Kumar, C.; Sai Kumar, K.; Narasimha Rao, N.; Tarakeswari, M.; Sujatha, M. Post-Transcriptional Gene Silencing: Basic Concepts and Applications. *J. Biosci.* **2020**, *45*, 128. [[CrossRef](#)]
18. Zhu, K.Y.; Palli, S.R. Mechanisms, Applications, and Challenges of Insect RNA Interference. *Annu. Rev. Entomol.* **2020**, *65*, 293–311. [[CrossRef](#)]
19. Koliopoulou, A.; Santos, D.; Taning, C.N.T.; Wynant, N.; Vanden Broeck, J.; Smagghe, G.; Swevers, L. PIWI Pathway against Viruses in Insects. *WIREs RNA* **2019**, *10*, e1555. [[CrossRef](#)]
20. Zhou, X.; Liao, Z.; Jia, Q.; Cheng, L.; Li, F. Identification and Characterization of Piwi Subfamily in Insects. *Biochem. Biophys. Res. Commun.* **2007**, *362*, 126–131. [[CrossRef](#)]
21. Barnard, A.-C.; Nijhof, A.M.; Fick, W.; Stutzer, C.; Maritz-Olivier, C. RNAi in Arthropods: Insight into the Machinery and Applications for Understanding the Pathogen-Vector Interface. *Genes* **2012**, *3*, 702–741. [[CrossRef](#)]
22. Vélez, A.M.; Fishilevich, E. The Mysteries of Insect RNAi: A Focus on DsRNA Uptake and Transport. *Pestic. Biochem. Physiol.* **2018**, *151*, 25–31. [[CrossRef](#)]
23. Liu, S.; Jaouannet, M.; Dempsey, D.A.; Imani, J.; Coustau, C.; Kogel, K.-H. RNA-Based Technologies for Insect Control in Plant Production. *Biotechnol. Adv.* **2020**, *39*, 107463. [[CrossRef](#)]
24. Meng, F.; Yang, M.; Li, Y.; Li, T.; Liu, X.; Wang, G.; Wang, Z.; Jin, X.; Li, W. Functional Analysis of RNA Interference-Related Soybean Pod Borer (*Lepidoptera*) Genes Based on Transcriptome Sequences. *Front. Physiol.* **2018**, *9*, 383. [[CrossRef](#)] [[PubMed](#)]

25. Saxena, S.; Reddy, K.R.K.; Rajam, M.V. DsRNA-Mediated Silencing of Chitin Synthase A (CHSA) Affects Growth and Development of Leucinodes Orbonalis, Brinjal Fruitand Shoot Borer. *J. Asia-Pac. Entomol.* **2022**, *25*, 101908. [[CrossRef](#)]
26. Kunte, N.; McGraw, E.; Bell, S.; Held, D.; Avila, L.-A. Prospects, Challenges and Current Status of RNAi through Insect Feeding. *Pest Manag. Sci.* **2020**, *76*, 26–41. [[CrossRef](#)] [[PubMed](#)]
27. Ye, C.; Hu, X.-S.; Wang, Z.-W.; Wei, D.; Smagghe, G.; Christiaens, O.; Niu, J.; Wang, J.-J. Involvement of Clathrin-Dependent Endocytosis in Cellular DsRNA Uptake in Aphids. *Insect Biochem. Mol. Biol.* **2021**, *132*, 103557. [[CrossRef](#)] [[PubMed](#)]
28. Brubaker, S.W.; Bonham, K.S.; Zanon, I.; Kagan, J.C. Innate Immune Pattern Recognition: A Cell Biological Perspective. *Annu. Rev. Immunol.* **2015**, *33*, 257–290. [[CrossRef](#)] [[PubMed](#)]
29. Joga, M.R.; Zotti, M.J.; Smagghe, G.; Christiaens, O. RNAi Efficiency, Systemic Properties, and Novel Delivery Methods for Pest Insect Control: What We Know So Far. *Front. Physiol.* **2016**, *7*, 553. [[CrossRef](#)]
30. Lin, Z.; Wang, J.-L.; Cheng, Y.; Wang, J.-X.; Zou, Z. Pattern Recognition Receptors from *Lepidopteran* Insects and Their Biological Functions. *Dev. Comp. Immunol.* **2020**, *108*, 103688. [[CrossRef](#)]
31. Arraes, F.B.M.; Martins-de-Sa, D.; Noriega Vasquez, D.D.; Melo, B.P.; Faheem, M.; de Macedo, L.L.P.; Morgante, C.V.; Barbosa, J.A.R.G.; Togawa, R.C.; Moreira, V.J.V.; et al. Dissecting Protein Domain Variability in the Core RNA Interference Machinery of Five Insect Orders. *RNA Biol.* **2021**, *18*, 1653–1681. [[CrossRef](#)]
32. Huvenne, H.; Smagghe, G. Mechanisms of DsRNA Uptake in Insects and Potential of RNAi for Pest Control: A Review. *J. Insect Physiol.* **2010**, *56*, 227–235. [[CrossRef](#)]
33. Albright III, V.C.; Wong, C.R.; Hellmich, R.L.; Coats, J.R. Dissipation of Double-Stranded RNA in Aquatic Microcosms. *Environ. Toxicol. Chem.* **2017**, *36*, 1249–1253. [[CrossRef](#)]
34. Dubelman, S.; Fischer, J.; Zapata, F.; Huizinga, K.; Jiang, C.; Uffman, J.; Levine, S.; Carson, D. Environmental Fate of Double-Stranded RNA in Agricultural Soils. *PLoS ONE* **2014**, *9*, e93155. [[CrossRef](#)]
35. Zhang, H.; Zhang, H.; Demirel, G.S.; González-Grandío, E.; Fan, C.; Landry, M.P. Engineering DNA Nanostructures for SiRNA Delivery in Plants. *Nat. Protoc.* **2020**, *15*, 3064–3087. [[CrossRef](#)]
36. Darlington, M.; Reinders, J.D.; Sethi, A.; Lu, A.L.; Ramaseshadri, P.; Fischer, J.R.; Boeckman, C.J.; Petrick, J.S.; Roper, J.M.; Narva, K.E.; et al. RNAi for Western Corn Rootworm Management: Lessons Learned, Challenges, and Future Directions. *Insects* **2022**, *13*, 57. [[CrossRef](#)]
37. Kennerdell, J.R.; Carthew, R.W. Use of DsRNA-Mediated Genetic Interference to Demonstrate That Frizzled and Frizzled 2 Act in the Wingless Pathway. *Cell* **1998**, *95*, 1017–1026. [[CrossRef](#)]
38. Liu, S.; Geng, S.; Li, A.; Mao, Y.; Mao, L. RNAi Technology for Plant Protection and Its Application in Wheat. *aBIOTECH* **2021**, *2*, 365–374. [[CrossRef](#)]
39. Davis-Vogel, C.; Allen, B.V.; Hemert, J.L.V.; Sethi, A.; Nelson, M.E.; Sashital, D.G. Identification and Comparison of Key RNA Interference Machinery from Western Corn Rootworm, Fall Armyworm, and Southern Green Stink Bug. *PLoS ONE* **2018**, *13*, e0203160. [[CrossRef](#)]
40. Dhandapani, R.K.; Gurusamy, D.; Palli, S.R. Protamine–Lipid–DsRNA Nanoparticles Improve RNAi Efficiency in the Fall Armyworm, *Spodoptera frugiperda*. *J. Agric. Food Chem.* **2022**, *70*, 6634–6643. [[CrossRef](#)]
41. Souza, D.; Christensen, S.A.; Wu, K.; Buss, L.; Kleckner, K.; Darrisaw, C.; Shirk, P.D.; Siegfried, B.D. RNAi-Induced Knockdown of White Gene in the Southern Green Stink Bug (*Nezara viridula* L.). *Sci. Rep.* **2022**, *12*, 10396. [[CrossRef](#)]
42. Wan, X.-S.; Shi, M.-R.; Xu, J.; Liu, J.-H.; Ye, H. Interference Efficiency and Effects of Bacterium-Mediated RNAi in the Fall Armyworm (*Lepidoptera*: Noctuidae). *J. Insect Sci.* **2021**, *21*, 8. [[CrossRef](#)]
43. Cooper, A.M.; Silver, K.; Zhang, J.; Park, Y.; Zhu, K.Y. Molecular Mechanisms Influencing Efficiency of RNA Interference in Insects. *Pest Manag. Sci.* **2019**, *75*, 18–28. [[CrossRef](#)]
44. Christiaens, O.; Sweet, J.; Dzhambazova, T.; Urru, I.; Smagghe, G.; Kostov, K.; Arpaia, S. Implementation of RNAi-Based Arthropod Pest Control: Environmental Risks, Potential for Resistance and Regulatory Considerations. *J. Pest Sci.* **2022**, *95*, 1–15. [[CrossRef](#)]
45. Dai, X.; Zhuang, Z.; Zhao, P.X. Computational Analysis of MiRNA Targets in Plants: Current Status and Challenges. *Brief. Bioinform.* **2011**, *12*, 115–121. [[CrossRef](#)] [[PubMed](#)]
46. Li, P.; Piao, Y.; Shon, H.S.; Ryu, K.H. Comparing the Normalization Methods for the Differential Analysis of Illumina High-Throughput RNA-Seq Data. *BMC Bioinform.* **2015**, *16*, 347. [[CrossRef](#)] [[PubMed](#)]
47. Srivastava, P.K.; Moturu, T.R.; Pandey, P.; Baldwin, I.T.; Pandey, S.P. A Comparison of Performance of Plant MiRNA Target Prediction Tools and the Characterization of Features for Genome-Wide Target Prediction. *BMC Genom.* **2014**, *15*, 348. [[CrossRef](#)] [[PubMed](#)]
48. Naito, Y.; Ui-Tei, K. SiRNA Design Software for a Target Gene-Specific RNA Interference. *Front. Genet.* **2012**, *3*, 102. [[CrossRef](#)]
49. Cagliari, D.; Dias, N.P.; Galdeano, D.M.; dos Santos, E.Á.; Smagghe, G.; Zotti, M.J. Management of Pest Insects and Plant Diseases by Non-Transformative RNAi. *Front. Plant Sci.* **2019**, *10*, 1319. [[CrossRef](#)]
50. Willow, J.; Taning, C.N.T.; Cook, S.M.; Sulg, S.; Silva, A.I.; Smagghe, G.; Veromann, E. RNAi Targets in Agricultural Pest Insects: Advancements, Knowledge Gaps, and IPM. *Front. Agron.* **2021**, *3*, 794312. [[CrossRef](#)]
51. Conesa, A.; Madrigal, P.; Tarazona, S.; Gomez-Cabrero, D.; Cervera, A.; McPherson, A.; Szczesniak, M.W.; Gaffney, D.J.; Elo, L.L.; Zhang, X.; et al. A Survey of Best Practices for RNA-Seq Data Analysis. *Genome Biol.* **2016**, *17*, 13. [[CrossRef](#)]

52. Zanini, S.; Šečić, E.; Jelonek, L.; Kogel, K.-H. A Bioinformatics Pipeline for the Analysis and Target Prediction of RNA Effectors in Bidirectional Communication During Plant–Microbe Interactions. *Front. Plant Sci.* **2018**, *9*, 1212. [CrossRef]
53. Andrews, S. FastQC: A Quality Control Tool for High Throughput Sequence Data. 2010. Available online: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc> (accessed on 19 September 2022).
54. Haas, B.J.; Zody, M.C. Advancing RNA-Seq Analysis. *Nat. Biotechnol.* **2010**, *28*, 421–423. [CrossRef]
55. Wang, X.-B.; Wu, Q.; Ito, T.; Cillo, F.; Li, W.-X.; Chen, X.; Yu, J.-L.; Ding, S.-W. RNAi-Mediated Viral Immunity Requires Amplification of Virus-Derived siRNAs in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 484–489. [CrossRef]
56. Wang, Y.; Zhang, H.; Li, H.; Miao, X. Second-Generation Sequencing Supply an Effective Way to Screen RNAi Targets in Large Scale for Potential Application in Pest Insect Control. *PLoS ONE* **2011**, *6*, e18644. [CrossRef]
57. Zhang, H.; Li, H.-C.; Miao, X.-X. Feasibility, Limitation and Possible Solutions of RNAi-Based Technology for Insect Pest Control. *Insect Sci.* **2013**, *20*, 15–30. [CrossRef]
58. Santos, D.; Mingels, L.; Vogel, E.; Wang, L.; Christiaens, O.; Cappelle, K.; Wynant, N.; Gansemans, Y.; Van Nieuwerburgh, F.; Smagghe, G.; et al. Generation of Virus- and DsRNA-Derived siRNAs with Species-Dependent Length in Insects. *Viruses* **2019**, *11*, 738. [CrossRef]
59. Sciabola, S.; Xi, H.; Cruz, D.; Cao, Q.; Lawrence, C.; Zhang, T.; Rotstein, S.; Hughes, J.D.; Caffrey, D.R.; Stanton, R.V. PFRED: A Computational Platform for siRNA and Antisense Oligonucleotides Design. *PLoS ONE* **2021**, *16*, e0238753. [CrossRef]
60. Lück, S.; Kreszies, T.; Strickert, M.; Schweizer, P.; Kuhlmann, M.; Douchkov, D. siRNA-Finder (Si-Fi) Software for RNAi-Target Design and Off-Target Prediction. *Front. Plant Sci.* **2019**, *10*, 1023. [CrossRef]
61. Motta, I.O.; Dantas, J.; Vidal, L.; Bílio, J.; Pujol-Luz, J.R.; Albuquerque, É.V.S. The Coffee Leaf Miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae): Identification of the Larval Instars and Description of Male and Female Genitalia. *Rev. Bras. Entomol.* **2021**, *65*, e20200122. [CrossRef]
62. Mitter, C.; Davis, D.R.; Cummings, M.P. Phylogeny and Evolution of *Lepidoptera*. *Annu. Rev. Entomol.* **2017**, *62*, 265–283. [CrossRef]
63. Sree, K.S.; Varma, A. (Eds.) *Biocontrol of Lepidopteran Pests: Use of Soil Microbes and Their Metabolites*; Soil Biology; Springer International Publishing: Cham, Switzerland, 2015; Volume 43, ISBN 978-3-319-14498-6.
64. Fazekas, I. Distribution and Bionomy of *Lyonetia Ledi* Wocke, 1859—A Review with Notes (*Lepidoptera*, Lyonetiidae). *Lepidopterol. Hung.* **2022**, *18*, 5–21. [CrossRef]
65. Choi, H.-S.; Kim, G.-J.; Shin, H.-J. Biocontrol of Moth Pests in Apple Orchards: Preliminary Field Study of Application Potential for Mass Trapping. *Biotechnol. Bioproc. E* **2011**, *16*, 153–157. [CrossRef]
66. Davis, E.E.; Schober, N.; Gómez, N.N.; Venette, R.C. *Mini Risk Assessment: Pear Leaf Blister Moth, Leucoptera malifoliella* Costa [Lepidoptera: Lyonetiidae]; University of Minnesota/USDA Forest Service: St. Paul, MN, USA, 2009.
67. Yuan, S.; Chenyihang, L.; Zhen, Z.; Zhijie, S.; Tao, L.; Jianyu, D. Advances in Biological Characteristics and Control of *Lyonetia clerkella*. *J. Zhejiang AF Univ.* **2022**, *39*, 687–694.
68. Dello Jacovo, E.; Valentine, T.A.; Maluk, M.; Toorop, P.; Lopez del Egidio, L.; Frachon, N.; Kenicer, G.; Park, L.; Goff, M.; Ferro, V.A. Towards a Characterisation of the Wild Legume Bitter Vetch (*Lathyrus linifolius* L. (Reichard) Bässler): Heteromorphic Seed Germination, Root Nodule Structure and N-fixing Rhizobial Symbionts. *Plant Biol.* **2019**, *21*, 523–532. [CrossRef] [PubMed]
69. Kaila, L.; Wikstroem, B. *Leucoptera lathyriifoliella* (Stainton, 1866) and *L-Orobi* (Stainton, 1870): Two Distinct Species (Lyonetiidae). *Nota Lepidopterol.* **2004**, *27*, 187–192.
70. Lindquist, O.H. The Cottonwood Leaf Miner, *Leucoptera albella* Cham. Canada Department of Forestry, Forest Entomology and Pathology Branch Bi-Monthly Progress Report 19. 1963. Available online: https://digitalcommons.usu.edu/aspens_bib/6048 (accessed on 19 September 2022).
71. Mey, W. Phyllobrostis Minoica Sp. n. from Crete (Greece)—An Expected Discovery (*Lepidoptera*, Yponomeutoidea, Lyonetiidae). *Nota Lepidopterol.* **2014**, *37*, 161–165. [CrossRef]
72. Van Nieukerken, E. Records of Mining *Lepidoptera* in Belgium with Nine Species New to the Country (*Nepticulidae*, *Opostegidae*, *Tischeriidae*, *Lyonetiidae*). *Phegea* **2006**, *34*, 125–144.
73. Baryshnikova, S.V. A Review of the Lyonetiid Moths (*Lepidoptera*, Lyonetiidae): II. The Subfamilies Lyonetiinae and Bedelliinae. *Entomol. Rev.* **2007**, *87*, 361–367. [CrossRef]
74. Sandoval, A.; Ide, S.; Rothmann, S.; Zuñiga, E.; Bosch, P.; Peragallo, M. Detección de *Leucoptera sinuella* (Reutti) (Lepidoptera: Lyonetiidae) en Chile, con la identificación de algunos parasitoides asociados. *Rev. Chil. Entomol.* **2019**, *45*, 65–77. Available online: <https://www.biotaxa.org/rce/article/view/46595> (accessed on 19 September 2022).
75. Syrett, P.; Fowler, S.; Coombs, E.; Hosking, J.; Markin, G.; Paynter, Q.; Sheppard, A. The Potential for Biological Control of Scotch Broom (*Cytisus scoparius*) (Fabaceae) and Related Weedy Species. *Biocontrol. News Inf.* **1999**, *20*, 17N–34N.
76. Dantas, J.; Motta, I.O.; Vidal, L.A.; Nascimento, E.F.M.B.; Bilio, J.; Pupe, J.M.; Veiga, A.; Carvalho, C.; Lopes, R.B.; Rocha, T.L.; et al. A Comprehensive Review of the Coffee Leaf Miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae)—A Major Pest for the Coffee Crop in Brazil and Others Neotropical Countries. *Insects* **2021**, *12*, 1130. [CrossRef]
77. De Souza, J.C.; Reis, P.R.; de Oliveira Rigitano, R.L.; Ciociola Júnior, A.I. Eficiência de thiamethoxam no controle do bicho-mineiro do cafeeiro. II—Influência na época de aplicação via irrigação por gotejamento. 2006. Available online: <http://www.sbicafe.ufv.br/handle/123456789/3892> (accessed on 19 September 2022).
78. Neves, M.F. Análise dos benefícios econômicos e sociais da utilização do carbofurano no controle de nematoides, Bicho Mineiro (*Leucoptera coffeella*) e Cigarra do Cafeeiro (Quesada gigas e Fidicina pronoe) na cultura do café 29. *Univ. Sao Paulo* **2006**, *29*, 1–29.

79. Martins, N.; Nascimento, E.; Vidal, L.; Lucena-Leandro, V.; Junqueira, C.; Soares, F.; Viana, M.; Nobrega, P.; Fontes, W.; Luz, I.; et al. Paving the Way for Gene Silencing in Lepidoptera: Integrated Sequencing Data Unveil the RNAi Core Machinery of *Leucoptera coffeella*. 2022. Available online: <https://www.preprints.org/manuscript/202208.0465/v1> (accessed on 19 September 2022).
80. Dias, N.P.; Cagliari, D.; dos Santos, E.A.; Smagghe, G.; Jurat-Fuentes, J.L.; Mishra, S.; Nava, D.E.; Zotti, M.J. Insecticidal Gene Silencing by RNAi in the Neotropical Region. *Neotrop. Entomol.* **2020**, *49*, 1–11. [[CrossRef](#)]
81. Macedo, L.L.P.; Antonino de Souza Junior, J.D.; Coelho, R.R.; Fonseca, F.C.A.; Firmino, A.A.P.; Silva, M.C.M.; Fragoso, R.R.; Albuquerque, E.V.S.; Silva, M.S.; de Almeida Engler, J.; et al. Knocking down Chitin Synthase 2 by RNAi Is Lethal to the Cotton Boll Weevil. *Biotechnol. Res. Innov.* **2017**, *1*, 72–86. [[CrossRef](#)]
82. Sohail, S.; Tariq, K.; Zheng, W.; Ali, M.W.; Peng, W.; Raza, M.F.; Zhang, H. RNAi-Mediated Knockdown of Tssk1 and Tektin1 Genes Impair Male Fertility in *Bactrocera dorsalis*. *Insects* **2019**, *10*, 164. [[CrossRef](#)]
83. Pinheiro, D.H.; Taylor, C.E.; Wu, K.; Siegfried, B.D. Delivery of Gene-Specific DsRNA by Microinjection and Feeding Induces RNAi Response in Sri Lanka Weevil, *Mylocerus undecimpustulatus undatus* Marshall. *Pest Manag. Sci.* **2020**, *76*, 936–943. [[CrossRef](#)]
84. Rk, D.; Jj, D.; Sr, P. Orally Delivered DsRNA Induces Knockdown of Target Genes and Mortality in the Asian Long-Horned Beetle, *Anoplophora glabripennis*. *Arch. Insect Biochem. Physiol.* **2020**, *104*, e21679. [[CrossRef](#)]
85. Yu, H.; Yi, L.; Lu, Z. Silencing of Chitin-Binding Protein with PYPV-Rich Domain Impairs Cuticle and Wing Development in the Asian Citrus Psyllid, *Diaphorina citri*. *Insects* **2022**, *13*, 353. [[CrossRef](#)]
86. Pallis, S.J. Effects of a Novel DsRNA-Based Insecticide on the Colorado Potato Beetle. Ph.D. Thesis, The University of Maine, Orono, ME, USA, 2021.
87. Nishide, Y.; Kageyama, D.; Tanaka, Y.; Yokoi, K.; Jouraku, A.; Futahashi, R.; Fukatsu, T. Effectiveness of Orally-Delivered Double-Stranded RNA on Gene Silencing in the Stinkbug *Plautia stali*. *PLoS ONE* **2021**, *16*, e0245081. [[CrossRef](#)]
88. Willow, J.; Soonvald, L.; Sulg, S.; Kaasik, R.; Silva, A.I.; Taning, C.N.T.; Christiaens, O.; Smagghe, G.; Veromann, E. RNAi Efficacy Is Enhanced by Chronic DsRNA Feeding in Pollen Beetle. *Commun. Biol.* **2021**, *4*, 444. [[CrossRef](#)]
89. Sümeği, M.; Hunyadi-Gulyás, É.; Medzihradzky, K.F.; Udvardy, A. 26S Proteasome Subunits Are O-Linked N-Acetylglucosamine-Modified in *Drosophila melanogaster*. *Biochem. Biophys. Res. Commun.* **2003**, *312*, 1284–1289. [[CrossRef](#)]
90. Rugjee, K.N.; Chaudhury, S.R.; Al-Jubran, K.; Ramanathan, P.; Matina, T.; Wen, J.; Brogna, S. Fluorescent Protein Tagging Confirms the Presence of Ribosomal Proteins at *Drosophila* Polytene Chromosomes. *PeerJ* **2013**, *1*, e15. [[CrossRef](#)] [[PubMed](#)]
91. Ramaseshadri, P.; Segers, G.; Flannagan, R.; Wiggins, E.; Clinton, W.; Ilagan, O.; McNulty, B.; Clark, T.; Bolognesi, R. Physiological and Cellular Responses Caused by RNAi-Mediated Suppression of *Snf7* Orthologue in Western Corn Rootworm (*Diabrotica virgifera virgifera*) Larvae. *PLoS ONE* **2013**, *8*, e54270. [[CrossRef](#)] [[PubMed](#)]
92. Forgac, M. Vacuolar ATPases: Rotary Proton Pumps in Physiology and Pathophysiology. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 917–929. [[CrossRef](#)] [[PubMed](#)]
93. Rodrigues, T.B.; Mishra, S.K.; Sridharan, K.; Barnes, E.R.; Alyokhin, A.; Tuttle, R.; Kokulapalan, W.; Garby, D.; Skizim, N.J.; Tang, Y.; et al. First Sprayable Double-Stranded RNA-Based Biopesticide Product Targets Proteasome Subunit Beta Type-5 in Colorado Potato Beetle (*Leptinotarsa decemlineata*). *Front. Plant Sci.* **2021**, *127*, 28652. [[CrossRef](#)] [[PubMed](#)]
94. Terenius, O.; Papanicolaou, A.; Garbutt, J.S.; Eleftherianos, I.; Huvenne, H.; Kanginakudru, S.; Albrechtsen, M.; An, C.; Aymeric, J.-L.; Barthel, A.; et al. RNA Interference in Lepidoptera: An Overview of Successful and Unsuccessful Studies and Implications for Experimental Design. *J. Insect Physiol.* **2011**, *57*, 231–245. [[CrossRef](#)]
95. Bramlett, M.; Plaetinck, G.; Maienfisch, P. RNA-Based Biocontrols—A New Paradigm in Crop Protection. *Engineering* **2020**, *6*, 522–527. [[CrossRef](#)]
96. Wang, J.; Gu, L.; Knipple, D.C. Evaluation of Some Potential Target Genes and Methods for RNAi-Mediated Pest Control of the Corn Earworm *Helicoverpa zea*. *Pestic. Biochem. Physiol.* **2018**, *149*, 67–72. [[CrossRef](#)]
97. Sharath Chandra, G.; Asokan, R.; Manamohan, M.; Krishna Kumar, N. Enhancing RNAi by Using Concatemered Double-Stranded RNA. *Pest Manag. Sci.* **2019**, *75*, 506–514. [[CrossRef](#)]
98. Choi, M.-Y.; Vander Meer, R.K. Phenotypic Effects of PBAN RNAi Using Oral Delivery of DsRNA to Corn Earworm (*Lepidoptera: Noctuidae*) and Tobacco Budworm Larvae. *J. Econ. Entomol.* **2019**, *112*, 434–439. [[CrossRef](#)]
99. Kottaipalayam-Somasundaram, S.R.; Jacob, J.P.; Aiyar, B.; Merzendorfer, H.; Nambiar-Veetil, M. Chitin Metabolism as a Potential Target for RNAi-Based Control of the Forestry Pest *Hyblaea puera* Cramer (*Lepidoptera: Hyblaeidae*). *Pest Manag. Sci.* **2022**, *78*, 296–303. [[CrossRef](#)]
100. Martinez, Z.; De Schutter, K.; Van Damme, E.J.M.; Vogel, E.; Wynant, N.; Vanden Broeck, J.; Christiaens, O.; Smagghe, G. Accelerated Delivery of DsRNA in Lepidopteran Midgut Cells by a *Galanthus nivalis* Lectin (GNA)-DsRNA-Binding Domain Fusion Protein. *Pestic. Biochem. Physiol.* **2021**, *175*, 104853. [[CrossRef](#)]
101. Jin, H.; Abouzaid, M.; Lin, Y.; Hull, J.J.; Ma, W. Cloning and RNAi-Mediated Three Lethal Genes That Can Be Potentially Used for *Chilo suppressalis* (*Lepidoptera: Crambidae*) Management. *Pestic. Biochem. Physiol.* **2021**, *174*, 104828. [[CrossRef](#)]
102. Ramkumar, G.; Asokan, R.; Prasannakumar, N.R.; Kariyanna, B.; Karthi, S.; Alwahibi, M.S.; Elshikh, M.S.; Abdel-Megeed, A.; Ghaith, A.; Senthil-Nathan, S.; et al. RNA Interference Suppression of V-ATPase B and Juvenile Hormone Binding Protein Genes Through Topically Applied DsRNA on Tomato Leaves: Developing Biopesticides to Control the South American Pinworm, *Tuta absoluta* (*Lepidoptera: Gelechiidae*). *Front. Physiol.* **2021**, *12*, 742871. [[CrossRef](#)]

103. Kiselev, K.V.; Suprun, A.R.; Aleynova, O.A.; Ogneva, Z.V.; Dubrovina, A.S. Physiological Conditions and DsRNA Application Approaches for Exogenously Induced RNA Interference in *Arabidopsis thaliana*. *Plants* **2021**, *10*, 264. [[CrossRef](#)]
104. Hewezi, T.; Alibert, G.; Kallerhoff, J. Local Infiltration of High- and Low-Molecular-Weight RNA from Silenced Sunflower (*Helianthus annuus* L.) Plants Triggers Post-Transcriptional Gene Silencing in Non-Silenced Plants. *Plant Biotechnol. J.* **2005**, *3*, 81–89. [[CrossRef](#)]
105. Riga, M.; Denecke, S.; Livadaras, I.; Geibel, S.; Nauen, R.; Vontas, J. Development of Efficient RNAi in *Nezara viridula* for Use in Insecticide Target Discovery. *Arch. Insect Biochem. Physiol.* **2020**, *103*, e21650. [[CrossRef](#)]
106. Sharma, R.; Christiaens, O.; Taning, C.N.; Smagghe, G. RNAi-Mediated Mortality in Southern Green Stinkbug *Nezara viridula* by Oral Delivery of DsRNA. *Pest Manag. Sci.* **2021**, *77*, 77–84. [[CrossRef](#)]
107. Yu, X.; Gowda, S.; Killiny, N. Double-Stranded RNA Delivery through Soaking Mediates Silencing of the Muscle Protein 20 and Increases Mortality to the Asian Citrus Psyllid, *Diaphorina citri*. *Pest Manag. Sci.* **2017**, *73*, 1846–1853. [[CrossRef](#)]
108. De Schutter, K.; Christiaens, O.; Taning, C.N.T.; Smagghe, G. Boosting DsRNA Delivery in Plant and Insect Cells with Peptide- and Polymer-Based Carriers: Case-Based Current Status and Future Perspectives. In *RNAi for Plant Improvement and Protection*; CABI Books: Wallingford, UK, 2021; pp. 102–116. [[CrossRef](#)]
109. Rebijith, K.B.; Asokan, R.; Hande, H.R.; Kumar, N.K.K. The First Report of MiRNAs from a Thysanopteran Insect, Thrips Palmi Karny Using High-Throughput Sequencing. *PLoS ONE* **2016**, *11*, e0163635. [[CrossRef](#)]
110. Christiaens, O.; Swevers, L.; Smagghe, G. DsRNA Degradation in the Pea Aphid (*Acyrtosiphon pisum*) Associated with Lack of Response in RNAi Feeding and Injection Assay. *Peptides* **2014**, *53*, 307–314. [[CrossRef](#)]
111. Dow, J.A. Extremely High PH in Biological Systems: A Model for Carbonate Transport. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* **1984**, *246*, R633–R636. [[CrossRef](#)] [[PubMed](#)]
112. Bachman, P.; Fischer, J.; Song, Z.; Urbanczyk-Wochniak, E.; Watson, G. Environmental Fate and Dissipation of Applied DsRNA in Soil, Aquatic Systems, and Plants. *Front. Plant Sci.* **2020**, *11*, 21. [[CrossRef](#)] [[PubMed](#)]
113. Parker, K.M.; Barragán Borrero, V.; van Leeuwen, D.M.; Lever, M.A.; Mateescu, B.; Sander, M. Environmental Fate of RNA Interference Pesticides: Adsorption and Degradation of Double-Stranded RNA Molecules in Agricultural Soils. *Environ. Sci. Technol.* **2019**, *53*, 3027–3036. [[CrossRef](#)] [[PubMed](#)]
114. Lim, F.-H.; Rasid, O.A.; As'wad, A.W.M.; Vadamalai, G.; Parveez, G.K.A.; Wong, M.-Y. The Future Is Now: Revolution of RNA-Mediated Gene Silencing in Plant Protection against Insect Pests and Diseases. *Plant Biotechnol. Rep.* **2020**, *14*, 643–662. [[CrossRef](#)]
115. Gillet, F.-X.; Garcia, R.A.; Macedo, L.L.P.; Albuquerque, E.V.S.; Silva, M.C.M.; Grossi-de-Sa, M.F. Investigating Engineered Ribonucleoprotein Particles to Improve Oral RNAi Delivery in Crop Insect Pests. *Front. Physiol.* **2017**, *8*, 256. [[CrossRef](#)]
116. Yang, W.; Wang, B.; Lei, G.; Chen, G.; Liu, D. Advances in Nanocarriers to Improve the Stability of DsRNA in the Environment. *Front. Bioeng. Biotechnol.* **2022**, *10*, 1401. [[CrossRef](#)]
117. Avila, L.A.; Chandrasekar, R.; Wilkinson, K.E.; Balthazor, J.; Heerman, M.; Bechard, J.; Brown, S.; Park, Y.; Dhar, S.; Reeck, G.R.; et al. Delivery of Lethal DsRNAs in Insect Diets by Branched Amphiphilic Peptide Capsules. *J. Control Release* **2018**, *273*, 139–146. [[CrossRef](#)]
118. Castellanos, N.L.; Smagghe, G.; Sharma, R.; Oliveira, E.E.; Christiaens, O. Liposome Encapsulation and EDTA Formulation of DsRNA Targeting Essential Genes Increase Oral RNAi-Caused Mortality in the Neotropical Stink Bug *Euschistus Heros*. *Pest Manag. Sci.* **2019**, *75*, 537–548. [[CrossRef](#)]
119. Gurusamy, D.; Mogilicherla, K.; Shukla, J.N.; Palli, S.R. Lipids Help Double-Stranded RNA in Endosomal Escape and Improve RNA Interference in the Fall Armyworm, *Spodoptera frugiperda*. *Arch. Insect Biochem. Physiol.* **2020**, *104*, e21678. [[CrossRef](#)]
120. Linyu, W.; Lianjun, Z.; Ning, L.; Xiwu, G.; Xiaoning, L. Effect of RNAi Targeting CYP6CY3 on the Growth, Development and Insecticide Susceptibility of *Aphis Gossypii* by Using Nanocarrier-Based Transdermal DsRNA Delivery System. *Pestic. Biochem. Physiol.* **2021**, *177*, 104878. [[CrossRef](#)]
121. Zhang, Y.-H.; Ma, Z.-Z.; Zhou, H.; Chao, Z.-J.; Yan, S.; Shen, J. Nanocarrier-Delivered DsRNA Suppresses Wing Development of Green Peach Aphids. *Insect Sci.* **2022**, *29*, 669–682. [[CrossRef](#)] [[PubMed](#)]
122. Sun, Y.; Wang, P.; Abouzaid, M.; Zhou, H.; Liu, H.; Yang, P.; Lin, Y.; Hull, J.J.; Ma, W. Nanomaterial-Wrapped DsCYP15C1, a Potential RNAi-Based Strategy for Pest Control against *Chilo suppressalis*. *Pest Manag. Sci.* **2020**, *76*, 2483–2489. [[CrossRef](#)] [[PubMed](#)]
123. Ma, Z.; Zheng, Y.; Chao, Z.; Chen, H.; Zhang, Y.; Yin, M.; Shen, J.; Yan, S. Visualization of the Process of a Nanocarrier-Mediated Gene Delivery: Stabilization, Endocytosis and Endosomal Escape of Genes for Intracellular Spreading. *J. Nanobiotechnol.* **2022**, *20*, 124. [[CrossRef](#)] [[PubMed](#)]
124. Christiaens, O.; Tardajos, M.G.; Martinez Reyna, Z.L.; Dash, M.; Dubruel, P.; Smagghe, G. Increased RNAi Efficacy in *Spodoptera Exigua* via the Formulation of DsRNA with Guanlylated Polymers. *Front. Physiol.* **2018**, *9*, 316. [[CrossRef](#)] [[PubMed](#)]
125. Mujtaba, M.; Khawar, K.M.; Camara, M.C.; Carvalho, L.B.; Fraceto, L.F.; Morsi, R.E.; Elsabee, M.Z.; Kaya, M.; Labidi, J.; Ullah, H.; et al. Chitosan-Based Delivery Systems for Plants: A Brief Overview of Recent Advances and Future Directions. *Int. J. Biol. Macromol.* **2020**, *154*, 683–697. [[CrossRef](#)]
126. Kolge, H.; Kadam, K.; Galande, S.; Lanjekar, V.; Ghormade, V. New Frontiers in Pest Control: Chitosan Nanoparticles-Shielded DsRNA as an Effective Topical RNAi Spray for Gram Podborer Biocontrol. *ACS Appl. Bio Mater.* **2021**, *4*, 5145–5157. [[CrossRef](#)]

127. Dhandapani, R.K.; Gurusamy, D.; Howell, J.L.; Palli, S.R. Development of CS-TPP-DsRNA Nanoparticles to Enhance RNAi Efficiency in the Yellow Fever Mosquito, *Aedes Aegypti*. *Sci. Rep.* **2019**, *9*, 8775. [[CrossRef](#)]
128. Mitter, N.; Worrall, E.A.; Robinson, K.E.; Li, P.; Jain, R.G.; Taochy, C.; Fletcher, S.J.; Carroll, B.J.; Lu, G.Q.; Xu, Z.P. Clay Nanosheets for Topical Delivery of RNAi for Sustained Protection against Plant Viruses. *Nat. Plants* **2017**, *3*, 16207. [[CrossRef](#)]
129. Wang, K.; Peng, Y.; Chen, J.; Peng, Y.; Wang, X.; Shen, Z.; Han, Z. Comparison of Efficacy of RNAi Mediated by Various Nanoparticles in the Rice Striped Stem Borer (*Chilo suppressalis*). *Pestic. Biochem. Physiol.* **2020**, *165*, 104467. [[CrossRef](#)]
130. Caccia, S.; Astarita, F.; Barra, E.; Di Lelio, I.; Varricchio, P.; Pennacchio, F. Enhancement of *Bacillus Thuringiensis* Toxicity by Feeding *Spodoptera littoralis* Larvae with Bacteria Expressing Immune Suppressive DsRNA. *J. Pest Sci.* **2020**, *93*, 303–314. [[CrossRef](#)]
131. Chen, X.; Li, L.; Hu, Q.; Zhang, B.; Wu, W.; Jin, F.; Jiang, J. Expression of DsRNA in Recombinant *Isaria fumosorosea* Strain Targets the TLR7 Gene in *Bemisia tabaci*. *BMC Biotechnol.* **2015**, *15*, 64. [[CrossRef](#)]
132. Drinnenberg, I.A.; Weinberg, D.E.; Xie, K.T.; Mower, J.P.; Wolfe, K.H.; Fink, G.R.; Bartel, D.P. RNAi in Budding Yeast. *Science* **2009**, *326*, 544–550. [[CrossRef](#)]
133. Kang, S.; Sun, D.; Qin, J.; Guo, L.; Zhu, L.; Bai, Y.; Wu, Q.; Wang, S.; Zhou, X.; Guo, Z.; et al. Fused: A Promising Molecular Target for an RNAi-Based Strategy to Manage Bt Resistance in *Plutella xylostella* (L.). *J. Pest Sci.* **2022**, *95*, 101–114. [[CrossRef](#)]
134. Timmons, L.; Court, D.L.; Fire, A. Ingestion of Bacterially Expressed DsRNAs Can Produce Specific and Potent Genetic Interference in *Caenorhabditis elegans*. *Gene* **2001**, *263*, 103–112. [[CrossRef](#)]
135. Hashiro, S.; Yasueda, H. RNA Interference-Based Pesticides and Antiviral Agents: Microbial Overproduction Systems for Double-Stranded RNA for Applications in Agriculture and Aquaculture. *Appl. Sci.* **2022**, *12*, 2954. [[CrossRef](#)]
136. Timmons, L.; Fire, A. Specific Interference by Ingested DsRNA. *Nature* **1998**, *395*, 854. [[CrossRef](#)]
137. Tenllado, F.; Martínez-García, B.; Vargas, M.; Díaz-Ruiz, J.R. Crude Extracts of Bacterially Expressed DsRNA Can Be Used to Protect Plants against Virus Infections. *BMC Biotechnol.* **2003**, *3*, 3. [[CrossRef](#)]
138. Verdonck, T.-W.; Vanden Broeck, J. Methods for the Cost-Effective Production of Bacteria-Derived Double-Stranded RNA for in Vitro Knockdown Studies. *Front. Physiol.* **2022**, *13*, 559. [[CrossRef](#)]
139. Voloudakis, A.E.; Holeva, M.C.; Sarin, L.P.; Bamford, D.H.; Vargas, M.; Poranen, M.M.; Tenllado, F. Efficient Double-Stranded RNA Production Methods for Utilization in Plant Virus Control. In *Plant Virology Protocols: New Approaches to Detect Viruses and Host Responses*; Methods in Molecular Biology; Uyeda, I., Masuta, C., Eds.; Springer: New York, NY, USA, 2015; pp. 255–274. ISBN 978-1-4939-1743-3.
140. Park, M.G.; Kim, W.J.; Choi, J.Y.; Kim, J.H.; Park, D.H.; Kim, J.Y.; Wang, M.; Je, Y.H. Development of a *Bacillus Thuringiensis* Based DsRNA Production Platform to Control Sacbrood Virus in *Apis Cerana*. *Pest Manag. Sci.* **2020**, *76*, 1699–1704. [[CrossRef](#)] [[PubMed](#)]
141. Lezzerini, M.; van de Ven, K.; Veerman, M.; Brul, S.; Budovskaya, Y.V. Specific RNA Interference in *Caenorhabditis elegans* by Ingested DsRNA Expressed in *Bacillus subtilis*. *PLoS ONE* **2015**, *10*, e0124508. [[CrossRef](#)]
142. Saelim, H.; Loprasert, S.; Phongdara, A. *Bacillus subtilis* Expressing DsVP28 Improved Shrimp Survival from WSSV Challenge. *ScienceAsia* **2020**, *46S*, 19. [[CrossRef](#)]
143. Asghari, S.; Harighi, B.; Ashengroph, M.; Clement, C.; Aziz, A.; Esmaeel, Q.; Ait Barka, E. Induction of Systemic Resistance to *Agrobacterium tumefaciens* by *Endophytic bacteria* in Grapevine. *Plant Pathol.* **2020**, *69*, 827–837. [[CrossRef](#)]
144. Taracena, M.L.; Oliveira, P.L.; Almendares, O.; Umaña, C.; Lowenberger, C.; Dotson, E.M.; Paiva-Silva, G.O.; Pennington, P.M. Genetically Modifying the Insect Gut Microbiota to Control Chagas Disease Vectors through Systemic RNAi. *PLoS Negl. Trop. Dis.* **2015**, *9*, e0003358. [[CrossRef](#)] [[PubMed](#)]
145. Whitten, M.; Dyson, P. Gene Silencing in Non-Model Insects: Overcoming Hurdles Using Symbiotic Bacteria for Trauma-Free Sustainable Delivery of RNA Interference: Sustained RNA Interference in Insects Mediated by Symbiotic Bacteria: Applications as a Genetic Tool and as a Biocide. *Bioessays* **2017**, *39*, 1600247. [[CrossRef](#)] [[PubMed](#)]
146. Whitten, M.M. Novel RNAi Delivery Systems in the Control of Medical and Veterinary Pests. *Curr. Opin. Insect Sci.* **2019**, *34*, 1–6. [[CrossRef](#)] [[PubMed](#)]
147. Whitten, M.M.A.; Facey, P.D.; Del Sol, R.; Fernández-Martínez, L.T.; Evans, M.C.; Mitchell, J.J.; Bodger, O.G.; Dyson, P.J. Symbiont-Mediated RNA Interference in Insects. *Proc. R. Soc. B Biol. Sci.* **2016**, *283*, 20160042. [[CrossRef](#)]
148. Zhong, C.; Smith, N.A.; Zhang, D.; Goodfellow, S.; Zhang, R.; Shan, W.; Wang, M.-B. Full-Length Hairpin RNA Accumulates at High Levels in Yeast but Not in Bacteria and Plants. *Genes* **2019**, *10*, 458. [[CrossRef](#)]
149. Dubreuil, G.; Magliano, M.; Dubrana, M.P.; Lozano, J.; Lecomte, P.; Favery, B.; Abad, P.; Rosso, M.N. Tobacco Rattle Virus Mediates Gene Silencing in a Plant Parasitic Root-Knot Nematode. *J. Exp. Bot.* **2009**, *60*, 4041–4050. [[CrossRef](#)]
150. Kumar, P.; Pandit, S.S.; Baldwin, I.T. Tobacco Rattle Virus Vector: A Rapid and Transient Means of Silencing *Manduca sexta* Genes by Plant Mediated RNA Interference. *PLoS ONE* **2012**, *7*, e31347. [[CrossRef](#)]
151. Kanwal, F.; Chen, T.; Zhang, Y.; Simair, A.; Rujie, C.; Sadaf Zaidi, N.S.; Guo, X.; Wei, X.; Siegel, G.; Lu, C. Large-Scale in Vitro Transcription, RNA Purification and Chemical Probing Analysis. *CPB* **2018**, *48*, 1915–1927. [[CrossRef](#)]
152. Li, T.; Huang, L.; Yang, M. Lipid-Based Vehicles for siRNA Delivery in Biomedical Field. *Curr. Pharm. Biotechnol.* **2020**, *21*, 3–22. [[CrossRef](#)]
153. Li, J.; Qian, J.; Xu, Y.; Yan, S.; Shen, J.; Yin, M. A Facile-Synthesized Star Polycation Constructed as a Highly Efficient Gene Vector in Pest Management. *ACS Sustain. Chem. Eng.* **2019**, *7*, 6316–6322. [[CrossRef](#)]

154. Li, W.; O'Neill, K.R.; Haft, D.H.; DiCuccio, M.; Chetvernin, V.; Badretdin, A.; Coulouris, G.; Chitsaz, F.; Derbyshire, M.K.; Durkin, A.S.; et al. RefSeq: Expanding the Prokaryotic Genome Annotation Pipeline Reach with Protein Family Model Curation. *Nucleic Acids Res.* **2021**, *49*, D1020–D1028. [[CrossRef](#)]
155. Mendiola, S.Y.; Civitello, D.J.; Gerardo, N.M. An Integrative Approach to Symbiont-Mediated Vector Control for Agricultural Pathogens. *Curr. Opin. Insect Sci.* **2020**, *39*, 57–62. [[CrossRef](#)]
156. Zotti, M.; dos Santos, E.A.; Cagliari, D.; Christiaens, O.; Taning, C.N.T.; Smagghe, G. RNA Interference Technology in Crop Protection against Arthropod Pests, Pathogens and Nematodes. *Pest Manag. Sci.* **2018**, *74*, 1239–1250. [[CrossRef](#)]
157. Guan, R.; Chu, D.; Han, X.; Miao, X.; Li, H. Advances in the Development of Microbial Double-Stranded RNA Production Systems for Application of RNA Interference in Agricultural Pest Control. *Front. Bioeng. Biotechnol.* **2021**, *9*, 753790. [[CrossRef](#)]
158. Suhag, A.; Yadav, H.; Chaudhary, D.; Subramanian, S.; Jaiwal, R.; Jaiwal, P.K. Biotechnological Interventions for the Sustainable Management of a Global Pest, Whitefly (*Bemisia tabaci*). *Insect Sci.* **2021**, *28*, 1228–1252. [[CrossRef](#)]
159. Taning, C.N.; Arpaia, S.; Christiaens, O.; Dietz-Pfeilstetter, A.; Jones, H.; Mezzetti, B.; Sabbadini, S.; Sorteberg, H.-G.; Sweet, J.; Ventura, V.; et al. RNA-Based Biocontrol Compounds: Current Status and Perspectives to Reach the Market. *Pest Manag. Sci.* **2020**, *76*, 841–845. [[CrossRef](#)]
160. Saksmerprome, V.; Charonnart, P.; Gangnonngiw, W.; Withyachumnarnkul, B. A Novel and Inexpensive Application of RNAi Technology to Protect Shrimp from Viral Disease. *J. Virol. Methods* **2009**, *162*, 213–217. [[CrossRef](#)]
161. Hoang, B.T.L.; Fletcher, S.J.; Brosnan, C.A.; Ghodke, A.B.; Manzie, N.; Mitter, N. RNAi as a Foliar Spray: Efficiency and Challenges to Field Applications. *Int. J. Mol. Sci.* **2022**, *23*, 6639. [[CrossRef](#)] [[PubMed](#)]
162. Mezzetti, B.; Smagghe, G.; Arpaia, S.; Christiaens, O.; Dietz-Pfeilstetter, A.; Jones, H.; Kostov, K.; Sabbadini, S.; Opsahl-Sorteberg, H.-G.; Ventura, V.; et al. RNAi: What Is Its Position in Agriculture? *J. Pest Sci.* **2020**, *93*, 1125–1130. [[CrossRef](#)]
163. Rodrigues, T.B.; Petrick, J.S. Safety Considerations for Humans and Other Vertebrates Regarding Agricultural Uses of Externally Applied RNA Molecules. *Front. Plant Sci.* **2020**, *11*, 407. [[CrossRef](#)]
164. Christiaens, O.; Whyard, S.; Vélez, A.M.; Smagghe, G. Double-Stranded RNA Technology to Control Insect Pests: Current Status and Challenges. *Front. Plant Sci.* **2020**, *11*, 451. [[CrossRef](#)] [[PubMed](#)]
165. San Miguel, K.; Scott, J.G. The next Generation of Insecticides: DsRNA Is Stable as a Foliar-Applied Insecticide. *Pest Manag. Sci.* **2016**, *72*, 801–809. [[CrossRef](#)] [[PubMed](#)]
166. Zhu, F.; Xu, J.; Palli, R.; Ferguson, J.; Palli, S.R. Ingested RNA Interference for Managing the Populations of the Colorado Potato Beetle, *Leptinotarsa decemlineata*. *Pest Manag. Sci.* **2011**, *67*, 175–182. [[CrossRef](#)]
167. Tian, H.; Peng, H.; Yao, Q.; Chen, H.; Xie, Q.; Tang, B.; Zhang, W. Developmental Control of a Lepidopteran Pest Spodoptera Exigua by Ingestion of Bacteria Expressing DsRNA of a Non-Midgut Gene. *PLoS ONE* **2009**, *4*, e6225. [[CrossRef](#)]
168. Vatanparast, M.; Kim, Y. Optimization of Recombinant Bacteria Expressing DsRNA to Enhance Insecticidal Activity against a Lepidopteran Insect, Spodoptera Exigua. *PLoS ONE* **2017**, *12*, e0183054. [[CrossRef](#)]
169. Bento, F.M.; Marques, R.N.; Campana, F.B.; Demétrio, C.G.; Leandro, R.A.; Parra, J.R.P.; Figueira, A. Gene Silencing by RNAi via Oral Delivery of DsRNA by Bacteria in the South American Tomato Pinworm, Tuta Absoluta. *Pest Manag. Sci.* **2020**, *76*, 287–295. [[CrossRef](#)]
170. Xu, J.; Wang, X.-F.; Chen, P.; Liu, F.-T.; Zheng, S.-C.; Ye, H.; Mo, M.-H. RNA Interference in Moths: Mechanisms, Applications, and Progress. *Genes* **2016**, *7*, 88. [[CrossRef](#)]
171. Yan, S.; Ren, B.; Zeng, B.; Shen, J. Improving RNAi Efficiency for Pest Control in Crop Species. *BioTechniques* **2020**, *68*, 283–290. [[CrossRef](#)]
172. De Andrade, E.C.; Hunter, W.B. *RNA Interference—Natural Gene-Based Technology for Highly Specific Pest Control (HiSPeC)*; IntechOpen: London, UK, 2016; ISBN 978-953-51-2272-2.
173. Werrie, P.-Y.; Burgeon, C.; Le Goff, G.J.; Hance, T.; Fauconnier, M.-L. Biopesticide Trunk Injection into Apple Trees: A Proof of Concept for the Systemic Movement of Mint and Cinnamon Essential Oils. *Front. Plant Sci.* **2021**, *12*, 650132. [[CrossRef](#)]
174. Baum, J.A.; Bogaert, T.; Clinton, W.; Heck, G.R.; Feldmann, P.; Ilagan, O.; Johnson, S.; Plaetinck, G.; Munyikwa, T.; Pleau, M.; et al. Control of Coleopteran Insect Pests through RNA Interference. *Nat. Biotechnol.* **2007**, *25*, 1322–1326. [[CrossRef](#)]
175. Mao, Y.-B.; Cai, W.-J.; Wang, J.-W.; Hong, G.-J.; Tao, X.-Y.; Wang, L.-J.; Huang, Y.-P.; Chen, X.-Y. Silencing a Cotton Bollworm P450 Monooxygenase Gene by Plant-Mediated RNAi Impairs Larval Tolerance of Gossypol. *Nat. Biotechnol.* **2007**, *25*, 1307–1313. [[CrossRef](#)]
176. Garbutt, J.S.; Bellés, X.; Richards, E.H.; Reynolds, S.E. Persistence of Double-Stranded RNA in Insect Hemolymph as a Potential Determiner of RNA Interference Success: Evidence from Manduca Sexta and Blattella Germanica. *J. Insect Physiol.* **2013**, *59*, 171–178. [[CrossRef](#)]
177. Guan, R.-B.; Li, H.-C.; Fan, Y.-J.; Hu, S.-R.; Christiaens, O.; Smagghe, G.; Miao, X.-X. A Nuclease Specific to Lepidopteran Insects Suppresses RNAi. *J. Biol. Chem.* **2018**, *293*, 6011–6021. [[CrossRef](#)]
178. Arora, A.K.; Chung, S.H.; Douglas, A.E. Non-Target Effects of DsRNA Molecules in Hemipteran Insects. *Genes* **2021**, *12*, 407. [[CrossRef](#)]
179. Christiaens, O.; Dzhambazova, T.; Kostov, K.; Arpaia, S.; Joga, M.R.; Urru, I.; Sweet, J.; Smagghe, G. Literature Review of Baseline Information on RNAi to Support the Environmental Risk Assessment of RNAi-Based GM Plants. *EFSA Support. Publ.* **2018**, *15*, 1424E. [[CrossRef](#)]

-
180. Arpaia, S.; Christiaens, O.; Giddings, K.; Jones, H.; Mezzetti, B.; Moronta-Barrios, F.; Perry, J.N.; Sweet, J.B.; Taning, C.N.T.; Smaghe, G.; et al. Biosafety of GM Crop Plants Expressing DsRNA: Data Requirements and EU Regulatory Considerations. *Front. Plant Sci.* **2020**, *11*, 940. [[CrossRef](#)]
 181. Dietz-Pfeilstetter, A.; Mendelsohn, M.; Gathmann, A.; Klinkenbuß, D. Considerations and Regulatory Approaches in the USA and in the EU for DsRNA-Based Externally Applied Pesticides for Plant Protection. *Front. Plant Sci.* **2021**, *12*, 974. [[CrossRef](#)]