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Review

The dsRNA Delivery, Targeting and Application in Pest Control

Yuzhen Lu †, Xinyue Deng †, Qijun Zhu, Denghui Wu, Jielai Zhong, Liang Wen and Xiaoqiang Yu *

Guangdong Provincial Key Laboratory of Insect Developmental Biology and Applied Technology, Guangzhou Key Laboratory of Insect Development Regulation and Application Research, Institute of Insect Science and Technology, School of Life Sciences, South China Normal University, Guangzhou 510631, China

* Correspondence: xqyu@m.scnu.edu.cn

† These authors contributed equally to this work.

Abstract: RNA interference (RNAi) is a simple and rapid method for silencing genes in various organisms, and it is widely used in gene function and genetics research. RNAi has been developed as a novel pest management strategy. Double-stranded RNAs (dsRNAs) delivered via microinjection, ingestion, or soaking are effective for silencing genes in insect pests, whereas oral and topical delivery methods are feasible for field applications. Here, we summarize oral and topical delivery, in pests, of dsRNA target genes, including those involved in energy metabolism, synthesis of essential cellular components, hormone homeostasis, chitin metabolism, the digestive system, immunity, detoxification, insecticide resistance, and other processes. RNAi pesticides have been developed in the form of genetically modified (GM) crops expressing dsRNAs as well as applied as foliar sprays. In this review, RNAi-based products are also summarized.

Keywords: RNA interference; target genes; dsRNA delivery; pest control

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1. Introduction

RNA interference (RNAi) is a conserved biological response to double-stranded RNA (dsRNA) that induces sequence-specific gene silencing by targeting mRNA for degradation, thus preventing protein translation [1]. RNAi has proven to be a powerful reverse genetics tool for studying gene function, regulation, and interaction at the cellular and organismal levels [1]. RNAi of genes in pest insects is typically regulated via the small interfering RNA (siRNA) pathway, in which long dsRNAs are cleaved by Dicer-2 associated with R2D2 into siRNAs. Then, siRNAs are loaded into Argonaute-2 to assemble the RNA-induced silencing complex (RISC) which guides cleavage of the complementary target RNA [2].

RNAi technology is an emerging new tool for pest control and integrated pest management (IPM) [3]. In general, the efficiency of RNAi varies across insect orders as well as species within the same order due to dynamic dsRNA degradation, uptake, transport, or processing in vivo [1,4,5]. RNAi is more effective in Coleopterans than in Hemipterans and Lepidopterans, especially in Tenebrionidae (e.g., *Tribolium castaneum*) and Chrysomelidae (e.g., *Diabrotica virgifera virgifera*) [6]. Within Hemiptera, RNAi has been shown to be effective in *Cimex*, *Halyomorpha*, and *Oncopeltus*, but is not highly effective in *Acyrthosiphon* [1]. Other factors to consider in developing RNAi approaches include target gene selection, the efficiency of dsRNA delivery, the amount and length of dsRNA delivered, and the presence of RNAi machinery within the pests [7]. In this review, we describe dsRNA delivery methods, RNAi target genes for pest control, and RNAi-based pesticides.

2. Double-Stranded RNA Delivery Methods

An efficient dsRNA delivery system is critical for screening and/or validating the efficacy of gene silencing. Various methods of delivering dsRNA to pests have been developed based on species and purposes, and these methods are primarily classified as microinjection, oral ingestion, and topical application (Figure 1) [8]. Nanoparticles protect

dsRNA/siRNA molecules from degradation and increase the cellular uptake of intact molecules; thus, dsRNA mixed with nanoparticles can enhance RNAi efficacy [9].

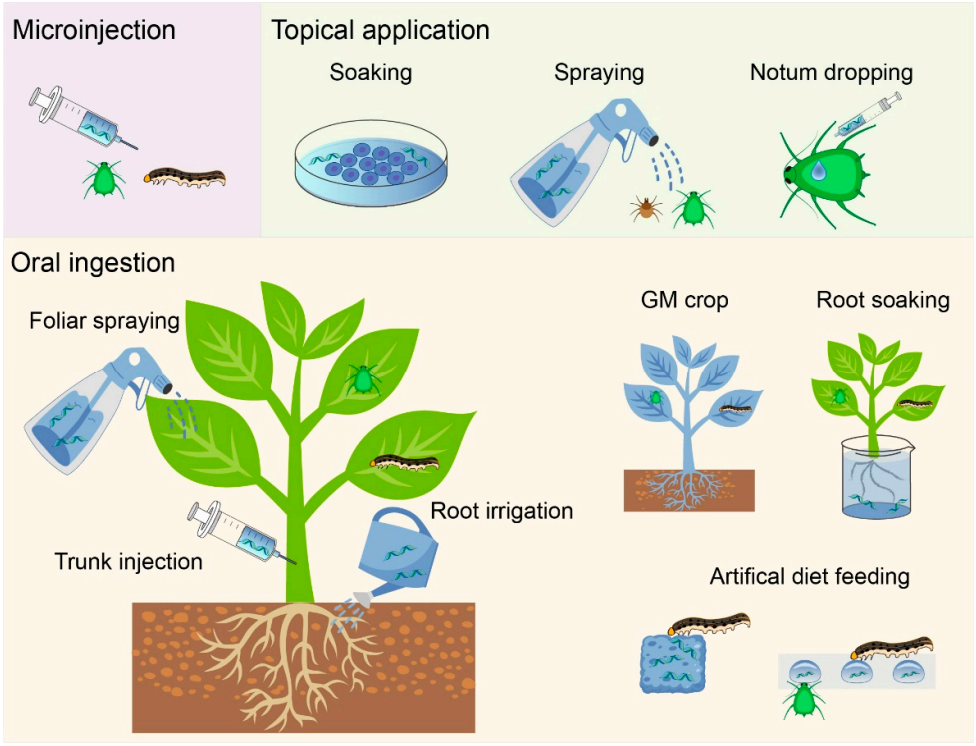

Figure 1. The schematic diagram of dsRNA delivery methods.

2.1. Microinjection

Double-strand RNA can be delivered into target tissues or the hemolymph via microinjection, therefore, bypassing physical barriers such as the integument or gut epithelium. dsRNAs for microinjection are frequently synthesized in vitro via a T7 RNA polymerase [8,10,11]. The first microinjection experiment was performed on *Drosophila melanogaster* embryos [12], and experiments on many other insect species, including *T. castaneum*, *Apis mellifera*, *Acyrthosiphon pisum*, *Bombyx mori*, *Manduca sexta*, *Nilaparvata lugens*, and *Blattella germanica*, were conducted later [8,9]. The microinjection method for delivering dsRNA is labor intensive, experience and instrument dependent, and cannot be used in the field [8]. However, a precise amount of dsRNA can be delivered to the target position immediately by microinjection, resulting in significantly higher RNAi efficiency than other methods [13]. It is a very effective research tool for gene functional analysis in most pests.

2.2. Ingestion

Although orally delivered dsRNA can be degraded by midgut endonucleases, it can also be uptaken by midgut cells, and some dsRNA can be transported to other tissues [14–16]. RNAi induced by oral ingestion is more effective for genes expressed in the midgut rather than in other tissues [16]. An artificial diet mixed/sprayed/coated with dsRNA and plant leaves immersed/coated/soaked with dsRNA are typical non-transformative oral dsRNA delivery methods. Non-transformative delivered dsRNAs can be synthesized in vitro or by microbes such as *Escherichia coli*, yeast, and entomopathogenic fungi [11,17]. *E. coli* HT115 (DE3) is commonly used for expressing dsRNA with the L4440 vector carrying the target gene segment [1,3]. The pET28-BL21 (DE3) RNaseIII system has been found to be more suitable for efficient and mass production of dsRNA, producing more than three times that of the L4440-HT115 (DE3) system [18].

Transgenic plants that express dsRNA via nuclear or chloroplast transformation are effective at silencing pest genes [6,19]. The ability of nuclear-transformed plants expressing

dsRNA to trigger RNAi was first tested on cotton bollworm (*Helicoverpa armigera*) and western corn rootworm (*D. v. virgifera*) [6,20]. The plant's RNAi machinery converted dsRNA expressed in the nuclei into siRNA, reducing its effect when fed to insects. Plant chloroplasts lack RNAi machinery and, therefore, allow stable dsRNA to accumulate, thus increasing RNAi efficacy in insects [21,22]. Further research is needed to broaden the range of transplastomic plants, particularly in food crops such as rice, wheat, and maize [23].

RNAi via ingestion is efficient and feasible in field applications [24]. Non-transformative delivery strategies are less expensive, available for all crops, and have a short development time [25]. Transgenic plants expressing dsRNA can reduce the need for pesticides, whereas developing transgenic plant is a time-consuming and expensive process, and it is not practical to every target plant due to a lack of genetic transformation technology [23]. Endonucleases in the midgut may degrade orally delivered dsRNA, thus resulting in reduced RNAi efficiency [14,15]. Ingestion has a lower RNAi efficiency than microinjection due to midgut endonuclease degradation, and the target genes should be expressed in the midgut due to the inefficient transport of orally delivered dsRNA across the midgut epithelium [16,26].

2.3. Soaking/Topical Application

Soaking experiments have typically been performed in insect cell lines by adding dsRNA into cell culture media, and many insect cells, such as S2, Bm5, Sf21, and CiE1, have been established to induce RNAi [8,27,28]. Despite the cuticle barriers, topical application of dsRNA to insects is possible. Sprayed/topically applied dsRNA/siRNA formulations have been shown to penetrate insect cuticles and to cause lethal effects in several insects, such as *Aphis glycines* [29], *Aedes aegypti* [30], *Leptinotarsa decemlineata* [31], *Anastrepha fraterculus* [32], and *Spodoptera frugiperda* [33]. Topical application is easy to operate; however, RNAi efficiency is low due to the limited penetration ability of dsRNA across the cuticles. It is not suitable for RNAi in many stages of pests that have a thicker cuticle even with the help of nanoparticles, such as the late instar larvae, pupae and adult moths [33].

2.4. Nanoparticle-Mediated dsRNA Delivery

Nanoparticles such as chitosan, liposomes, and cationic dendrimers protect dsRNA/siRNA molecules from degradation and promote their translocation across cell membranes, thus increasing RNAi efficacy by improving dsRNA stability and uptake [9]. Nanoparticles loaded dsRNAs have been shown to improve RNAi efficacy in many pests, such as aphids, mosquitos, mites, the German cockroach, and the Asian corn borer [34–38]. For example, a facile-synthesized star polycation (SPc)-based RNAi system with topical application, oral feeding, and injection methods is shown to be efficient in RNAi insensitive *S. frugiperda* [33]. Soaking the eggs with ds*ATP-d* alone (100 ng/μL) or dropping ds*ATP-d* alone (0.5 μg) on the notum of the 2nd instar larvae could not down-regulate *SfATP-d* expression, while the expression of *SfATP-d* was decreased more than one half via facile-synthesized star polycation (SPc)-delivered ds*ATP-d* treatment. The *SfATP-d* expression in the 4th and 6th instar larvae could be significantly decreased through oral feeding of 0.1 μg and 3 μg SPc-loaded ds*ATP-d*, respectively. The *SfATP-d* expression in pupae and adults could be successfully inhibited through injection with 10 μg SPc-loaded ds*ATP-d* [33]. Although nanoparticles can improve RNAi efficiency, production costs must be reduced, and the potential risk of nanoparticle-mediated dsRNA delivery must be evaluated in the future [9].

3. Target Genes for RNAi in Pest Control

The ideal target genes for RNAi are those that are essential for insect development or survival, have no functional redundancy, and can be knocked down by large-scale delivery of dsRNA in the field [39]. Here, we summarize RNAi target genes identified for pest control via oral ingestion and topical uptake assays (Tables 1 and S1).

Table 1. RNAi target genes that were identified through oral or topical RNAi assays for pest control.

Process	Target	References
energy metabolism (ATPase)	*V-ATPase A*, *V-ATPase B*, *V-ATPase D*, *V-ATPase E*, *V-ATPase G*, *ATPase*	[6,11,13,17,26,36,40–49]
energy metabolism (others)	arginine kinase, ADP/ATP translocase, adenylate kinase 2, NADH dehydrogenase	[6,41,43,50–54]
cytoskeleton related protein	tubulin, actin, myosin, *shibire*	[6,11,21,26,38,43,44,46,55,56]
ribosome	*RPL9*, *RPS13*, *rpL19*, *rpL9*, *rps-14*, *rpS4*, *rps10*	[6,13,43,57]
proteasome	*Rpn7*, *Rpn3*, *rpn6*, *rpn11*, *Prosα2*, *pat3*, *protb*, *PSMB5*, *PSMD7*, E2 enzyme	[6,13,45,57]
SCRT	*Snf7*, *vps2*, *vps28*,	[6,13,44,58,59]
COPI	*COPβ*, *αCOP*	[6,45,46,50,60]
protein trafficking/vesicular transport	SNAP, *rab1*, *rab11*, GTPase activator, ADP-ribosylation factor, *Rop*	[45,56]
hormone homeostasis	*EcR*, *ultraspiracle*, *shadow*, *HR3*, *JHE*, *JHBP*, *PBAN*, *bursicon*	[47–49,61–68]
chitin metabolism	trehalase, hexokinase, *GFAT*, chitin synthase, chitinase, chitin deacetylase 1, cuticular protein, *laccase2*, *knk*	[6,33–37,63,69–75]
digestive system	*nitrophorin 2*, *MIF1*, *SSK*, *DvSSJ1*, *DvSSJ2*, *AMY48*, *AMY49*, *KTIs*	[57,58,67,76–78]
immunity	*hemocytin*, *GNBP1*, *PGRP-LB*, *Toll*, *terminicin*, *serpin2*, *Ap15782*, *Ap20844*, *chaoptin*, IAP	[30,58,79–85]
detoxify or pesticide resistance	P450s, GSTs, *Ces*, *EstFE4*, acetylcholinesterase, *synapsin*, *transferrin*	[20,47,78,86–96]
transcription	mRNA capping enzyme, helicase-DNA-binding protein, RNA polymerase II, transcription factor IIB, activating transcription factor, *Inr-α*, *dre4*	[6,45,97]
others	*Tssk1*, *Tektin1*, *MSL3*, *SRP54*, *GDPH3*, *calmodulin*, *aquaporin*, heat shock protein, *ZC3H10*, *apple* (ATPase), *integrin β1*, *hexamerin II*, *GHF9-2*	[6,45,51,54–57,59,98–101]

Detail information was listed in Table S1.

3.1. Targeting Energy Metabolism

Energy metabolism is essential for cell survival and proliferation, as well as cellular regulation, stem cell applications, and pathogenic infection [102]. Silencing genes involved in energy metabolism, such as vacuolar ATPase (V-ATPase) and arginine kinase, have been shown to cause significant mortality in pests (Table S1). V-ATPase is an ATP-driven proton pump that generates energy gradients across membranes; it is one of the most important enzymes in eukaryotic cells, and it is also a well-studied target for RNAi [22]. RNAi suppression of V-ATPase subunit A, B, D, E, or G caused significant mortalities in Coleopteran, Hemipteran, Lepidopteran, Dipteran, and Orthopteran insects and mites (Table S1) [6,10,17,40–42]. Arginine kinase (AK) plays a fundamental role in energy homeostasis. Compared to controls, the mortality of dsAK-treated larvae increased by 15% in *Nylanderia fulva* [50], 26% in *Tuta absoluta* [41], 26% in *Plutella xylostella* [51], and 18% in *H. armigera* [52]. ADP/ATP translocase exchanges free ATP with free ADP across the inner mitochondrial membrane. *D. v. virgifera* larvae were stunted and died after ingesting dsRNA against ADP/ATP translocase [6]. Silencing the ADP/ATP translocase gene resulted in increased mortality in whiteflies (*Bemisia tabaci*) as compared with a control [43]. Adenylate kinase 2 (AK2) regulates cellular energy homeostasis and cellular adenine nucleotide metabolism. RNAi knockdown of *AK2* in *H. armigera* larvae caused growth defects [53]. A topical foliar application targeting NADH dehydrogenase, which is a key metabolic enzyme in the electron transport chain, increased mortality by 30% in *Chilo suppressalis* larvae [54].

3.2. Targeting Essential Cellular Components

Defects in essential cellular components, such as cytoskeletal and ribosomal proteins, are frequently fatal or cause severe diseases in organisms, and therefore, essential cellular components are ideal targets for RNAi [103,104]. Silencing genes that encode essential cellular components, such as cytoskeleton, ribosome, proteasome, and complexes required for membrane fusion or intracellular trafficking, resulted in stunting and mortality [6].

Tubulin and actin are cytoskeletal proteins that are frequently targeted by RNAi. Oral administration of dsRNA targeting α-tubulin resulted in significantly increased mortality in *D. v. virgifera* [6], *Blattella germanica* [38], *B. tabac*i [43], and *Frankliniella occidentalis* [44]. Silencing the β- and γ-tubulin genes also resulted in death in flies, beetles, aphids, and tobacco hornworms [6,10]. RNAi of actin genes caused lethal effects in several insects, such as beetles [6], thrips [44], whiteflies [43], and stink bugs [26]. Actin-based motility is controlled by myosins. RNAi of the putative myosin heavy chain in *D. v. virgifera* led to increased mortality [6]. Knockdown of *Shibire*, a microtubule-associated force-producing protein, resulted in more than 80% mortality rate in *Dendroctonus frontalis* [55]. Ingestion of dsRNAs against mRNA encoding ribosomal proteins L9, L19, S4, S10, S14, and S13 resulted in larval death in *D. v. virgifera*, *B. tabaci,* or *Myllocerus undecimpustulatus undatus* (Table S1) [6,13,43,57]. RNAi of genes encoding proteasome regulatory particles rpn3, rpn6, and rpn11 and proteasome subunits Prosα2, pat3, protb, PSMD5, and PSMD7 and an E2 ubiquitin-conjugating enzyme caused death in beetles and mites through ingestion or soaking (Table S1) [6,45,57].

The endosomal complexes required for transport (ESCRT) complexes are involved in membrane budding or bending events. RNAi targeting genes encoding ESCRT subunits, for example *Snf7*, *Vps2*, and *Vps28*, caused mortality in several insect species including beetles [6] and thrips [44]. Coatomer complexes mediate ER-Golgi transport; RNAi of coat genes *αCOP*, *βCOP*, or *sec23* increased mortality in beetles [46,60], ants [50], and mites [45] by 15–72%. SNAP participates in intracellular membrane fusion and vesicular trafficking [56]; RNAi of the *SNAP* gene resulted in >80% mortality rate in the mite *Tetranychus urticae* [45] and 75% mortality in *Henosepilachna vigintioctopunctata* [56]. Ras opposite (Rop) regulates vesicle trafficking; RNAi of *TuRop* caused >80% mortality rate in *T. urtica*e [45]. RNAi of the GTPase genes *rab1* and *rab11*, *pMON97111* (a GTPase activator involved in intracellular protein transport), and *pIC16006* (an ADP-ribosylation factor that regulates vesicular traffic and actin remodeling) caused death in western corn rootworm (Table S1) [6].

3.3. Targeting Hormone Homeostasis

Hormones play a crucial role in the life cycle of insects, and therefore, they are ideal targets for pest control. Insect development, metamorphosis, and reproduction are controlled by two vital hormones, 20-hydroxyecdysone (20E) and juvenile hormone (JH). RNAi by feeding dsRNA that targeted the ecdysone receptor (*EcR*) in *B. tabaci*, *Hyblaea puera*; and *H. armigera* [47,61–63], *ultraspiracle* (USP, the partner of EcR) in *H. armigera*; *shadow* (P450 involved in ecdysteroidogenesis) in *Laodelphax striatellus* resulted in a significant increase in mortality rate (Table S1) [64]. *HR3* is a molt-regulating transcription factor that is up-regulated by 20E and plays a key role during metamorphosis [105]. Silencing *HaHR3* by feeding transgenic plants increased the mortality rate of *H. armigera* by 17–24% [65], and ingestion of ds *CiHR3* disrupted the growth of *C. infuscatellus* larvae [66].

Juvenile hormone binding protein (JHBP) is the carrier partner protein of JH and is required for JH titer balance [106]. Topically applied RNAi against *T. absoluta JHBP* increased the mortality by approximately 55% [48]. Juvenile hormone esterase (JHE) is the primary catabolic enzyme that specifically degrades JH, feeding dsRNA that targeted Ha-JHE resulted in 41% mortality in *H. armigera* larvae [67]. However, ingestion of dsRNA against juvenile hormone esterase (pIC16007) in *D. v. virgifera* had no effect on survival [6].

Pheromone biosynthesis activating neuropeptide (PBAN) is a neurohormone that activates pheromone biosynthesis [107]. RNAi of *PBAN* increased mortality in *Solenopsis invicta*,

Heliothis virescens, and *H. zea* [68,107]. Bursicon is a neuropeptide hormone that regulates cuticle tanning and wing expansion, silencing *bursicon* caused 28–37% mortality in the mealybug *Phenacoccus solenopsis* [49].

3.4. Targeting Chitin Metabolism

Chitin is a major component of insect cuticle and an essential component of the insect peritrophic matrix (PM), a lining in insects' midgut that is essential for nutrition and antimicrobial defense [108]. Many enzymes are involved in chitin synthesis, including trehalase (Tre), hexokinase (HK), glucose-6-phosphate isomerase (G6PI), glutamine:fructose-6-phosphate aminotransferase (GFAT), glutamine:glucosamine-6-phosphate N-acetyl-transferase (GNAT), phosphoglucosamine mutase (PGM), UDP-N-acetylglucosamine pyrophosphorylase (UAP), and chitin synthase (CHS) [108]. The mortality of *Aphis glycines* was increased by 51% after silencing a trehalase gene (*TREH*) during an infiltration assay [36]. The mortality of *Diaphorina citri* larvae was increased by 10% after feeding dsRNA targeting hexokinase [69]. Knockdown of the *H. cunea GFAT* gene by feeding dsRNA repressed larval growth [70]. A significant increase in mortality was observed following topical application or transgenic plant-mediated RNAi of the chitin synthase gene in *Hyblaea puera*, *H. armigera*, *A. glycines*, and *Liriomyza trifolii* [36,63,71,72]. *Anopheles gambiae* larvae fed *AgCHS1* or *AgCHS2* dsRNA were more sensitive to diflubenzuron and calcofluor white, or dithiothreitol [34]. Chitin polymer is degraded by chitinases and N-acetylglucosaminidase, silencing the chitinase genes increased mortality in *H. puera*, *D. v. virgifera*, and *Ostrinia furnacalis* (Table S1) [6,37,63]. Chitin deacetylases (CDAs) are chitin degradation enzymes that regulate insect metamorphosis and development [109]. Knockdown of *SfCAD1* with SPc-loaded dsCDA1 via topical application for 4 days and then oral feeding for 3 days caused growth retardation and mortality in *S. frugiperda* larvae [33].

Cuticular proteins (CPs) are the primary structural components of the exocuticle and endocuticle layers that comprise the procuticle. RNAi of the cuticle protein gene *DcCP64* in *Diaphorina citri* and *CP19* in three aphids increased mortality [73,74]. Knockdown of *CPR* increased the cuticle permeability and the sensitivity to acaricides in the mite *T. cinnabarinus* [35]. Laccase2 is a phenol oxidase involved in cuticle formation and pigmentation [75]. Knk is a GPI-anchored protein that is required for chitin organization in the cuticle and the tracheal system [6]. RNAi against *BtLac2* in *B. tabaci* and *knk* in *D. v. virgifera* reduced larval survival [6,75].

3.5. Targeting the Digestive System

Salivary glands and gut comprise the insect digestive system, and genes encoding digestive enzymes have been studied for pest control via oral dsRNA delivery. Nitrophorins (NPs) are multifunctional proteins found in saliva. RNAi of *NP2* via ingestion of dsRNA resulted in a low level of mortality in *Rhodnius prolixus* [76]. Macrophage inhibitory factor (MIF1) is expressed in the salivary glands of aphids and mediates the inhibition of plant innate immunity [77]. RNAi of *MIF1* mediated by transgenic potato in *Myzus persicae* resulted in a significant increase in mortality [77].

Snakeskin (SSK) and mesh form a complex to take part in the development of the midgut [57]. Oral delivery of dsRNA targeting *SSK* gene led to 80% mortality in *Anoplophora glabripennis*, and silencing the *SSK* ortholog gene *DvSSJ1* (smooth septate junction protein 1) led to 100% mortality in *D. v. virgifera* [57,58]. Silencing *DvSSJ2* (an ortholog of mesh) resulted in 100% mortality in *D. v. virgifera* [57]. Carbohydrases and proteases are important digestive enzymes. Alpha-amylase is a carbohydrase that catalyzes the first step of starch hydrolysis [110]. *H. armigera* larvae fed ds*AMY48* and ds*AMY49* showed an increase in mortality by 17.7% and 20.6%, respectively [67]. Silencing of Kunitz-type trypsin inhibitors (*KTIs*) increased mortality in *O. furnacalis* by 19.5% [67].

3.6. Targeting Immunity, Detoxification, and Pesticide Resistance Molecules

The immune system of insects serves as the first defense line against various infectious conditions. Hemocytin (humoral lectin) is distributed in steady-state hemocytes and is involved in immune responses, especially in encapsulation [79]. RNAi of *hemocytin* increased the mortality of green peach aphids *M. persicae*, and co-delivery of *hemocytin* dsRNA and botanical pesticide matrine increased the lethal effect even more [79]. Gram-negative binding proteins (GNBPs) are pattern recognition receptors that recognize fungi; knockdown of *ApGNBP1* enhanced the virulence of *Beauveria bassiana* against two aphids [80]. Silencing the pattern recognition receptors *LgPGRP-LB* and *LgPGRP-LB2b* increased mortality in *Leguminivora glycinivorella* by 19–25% as compared with a dsGFP-fed control [81]. Knockdown of Toll-like receptors *LgToll5-1* and *LgTLR-7* also increased the mortality of *L. glycinivorella* [81]. RNAi of *B. tabaci* Toll gene (*BtToll*) increased mortality after larvae were challenged with destruxin A and *Isaria fumosorosea* [82,83]. RNAi of *Odontotermes formosanus* antimicrobial peptide gene (*Termicin*) enhanced the toxicity of *Serratia marcescens* against larvae [111]. Clip domain trypsin and serpin are involved in polyphenol oxidase activity via serine protease cascade [81,112]. RNAi of *LgSerpin2* increased mortality in *L. glycinivorella* by 19–25% [81], and silencing of *A. pisum Ap15782* (Clip domain trypsin) enhanced the virulence of *B. bassiana* [112]. Furthermore, silencing of the immune gene *LgChaoptin* in *L. glycinivorella* and *Ap20844* (zinc-finger domain) in *A. pisum* also increased larval mortality [81,112].

Apoptosis is a form of programmed cell death that is critical to development, homeostasis, and immune defense; inhibitors of apoptosis proteins (IAPs) are endogenous inhibitors for apoptosis [113]. RNAi of *IAP* caused 14–42% mortality in *A. aegypti* [30] via topical application, and 33% and 90% mortality in *Agrilus planipennis* and *A. glabripennis*, respectively, through dsRNA ingestion [58]. However, ingestion of dsIAP in *Dendroctonus frontalis* and *Lygus lineolaris* had no effect on survival [55,86].

Insects have evolved a detoxification system to protect themselves from xenobiotics [87]. Insecticide resistance is associated with increased levels of detoxification enzymes such as cytochrome P450 monooxygenases (P450s) in phase I, and glutathione S-transferases (GSTs) and carboxy/cholinesterases (CCEs) in phase II reactions [87]. RNAi-mediated inhibition of P450 genes, such as *CYP321A7* and *CYP6AE43* in *S. frugipreda* [88], *CYP6AE14* in *H. armigera* [20], and *CYP6CM1* in *B. tabaci* [84], increased the larval susceptibility to xenobiotics. RNAi of several P450 genes, including *CYP6CM1* in *B. tabaci* [84], *CYP6B6* in *H. armigera* [78], and *Cyp18A1* in *N. lugens,* resulted in lethality [89]. GSTs catalyze the conversion of endogenous and xenobiotic compounds into less toxic products by conjugating glutathione (GSH). Ingestion of dsBtGST led to increased mortality in *B. tabaci* [90]. Silencing seven GSTs in the brown planthopper *N. lugens* increased its sensitivity to gramine [114]; RNAi of four GSTs (*NlGSTs1*, *NlGSTs2*, *NlGSTe1*, and *NlGSTd1*) increased sensitivity to fipronil [91]; and knockdown of two GSTs (*DcGSTe2* and *DcGSTd1*) in *D. citri* increased their pesticide sensitivity [92]. Silencing *HaGST* and *GST16* retarded larval growth in *H. armigera* [20,93]. RNAi of the carboxylesterase in *N. lugens* (*Ces*) and *D. citri* (*EstFE4*) increased mortality by 37% and 43%, respectively [89,115].

Acetylcholinesterase (AChE) is involved in the hydrolysis of the neurotransmitter acetylcholine at the synaptic cleft to acetate and choline, which cease nerve impulse transmission between neurons [94,115]. *AChE* is the primary target for organophosphate and carbamate insecticides, and silencing *AchE* caused mortality in *D citri* [62], *B. tabaci* [115], *H. armigera* [94], and *P. xylostella* [95]. *Synapsin*, a gene regulating synaptic vesicle clustering, was upregulated when aphids were exposed to neonicotinoids [85]. Knockdown of *synapsin* led to 93.3% mortality within four days in *A. gossypii* [85]. Co-delivery of syn-dsRNA and thiamethoxam (TMX) increased the susceptibility of *A. gossypii* to low-dose TMX, suggesting that *synapsin* could be a potential RNAi target for neonicotinoid resistance management [85]. In addition to these targets, transferrins aid in insect adaptation to a variety of stresses. RNAi of *HaTrf* significantly increased the susceptibility of *H. armigera* to 2-tridecanone, a xenobiotic plant secondary chemical [96].

3.7. Other Target Genes

RNAi target genes also include those involved in transcription, DNA repair, male fertility, and male lethality. Several studies have found that dsRNA delivery that targeted essential genes, such as signal recognition particle protein 54 (*SRP54*), glycerol 3-phosphate dehydrogenase (GPDH3), aquaporin, heat shock protein, and calmodulin, increased larval mortality (Tables 1 and S1).

RNAi of several genes involved in transcription, including the putative mRNA capping enzyme, chromodomain helicase DNA-binding protein, RNA polymerase II, transcription factor IIB, and activating transcription factor, caused mortality in the western corn rootworm during the survey of RNAi targets [6]. RNAi of inverse regulator α (*Inr-α*), an RNA cleavage and polyadenylation factor in the transcription process, caused 80% mortality in the mite *T. urticae* [45]. Dre4, a Spt16/FACT homolog, plays a critical role in transcription and DNA repair; silencing of *dre4* contributed to mortality of *Phyllotreta striolata* [97].

Tssk1 and Tektin1 are involved in male fertility; RNAi knockdown of *Tssk1* and *Tektin1* resulted in male sterility up to 59% and 64.5%, respectively [98]. Male-specific lethal 3 (MSL3) plays a critical role in *Drosophila* dosage compensation pathways, and loss of function in ML3 led to male-specific lethality [116]. The mortality rate in *C. suppressalis* larvae fed ds*MSL3* was also higher [54].

RNAi of *RP54*, an essential component of eukaryotic signal recognition particle (SRP), resulted in at least 80% mortality in *T. urticae* [45] and *Henosepilachna vigintioctopunctata* [56]. GPDH3 plays an essential role in glycerolipid metabolism; suppressed expression of *GPDH3* by RNAi caused larval mortality in *C. suppressalis* [54] and *D. v. virgifera* [6]. Calmodulin (CaM) is an essential calcium-binding protein that regulates multiple protein targets; a *calmodulin*-based dsRNA was effective to control Varroa mites (*Varroa destructor*) within honey bees [99]. RNAi of water channel aquaporin in *T. tabaci* [59], heat shock protein (HSP) in *D. frontalis* [55], a CCCH-type ZFP gene (ZC3H10) in *M. persicae* [100], a putative ATPase in *D. v. virgifera*, and integrin β1 in *P. xylostella* [51] caused mortality. In a screening of dsRNA for insecticidal activity against termites, RNAi of *Hexamerin II* and *GHF9-2* cellulase caused 86.7% and 60% mortality, respectively [101].

4. RNAi-Based Products for Pest Management

RNAi-based products are species-specific, low in toxicity, and environmentally friendly as compared with conventional chemical pesticides. Current RNAi applications include genetically modified (GM) crops and topically applied RNAi sprays (Table 2), both of which have advantages and drawbacks. GM crops are efficient for RNAi responses in a systematic manner, while RNAi sprays are most effective for large-scale protection in a variety of plants in terms of cost, time consumption, and labor intensity [117]. GM crops face complex challenges, such as public concerns about the safety of GM plants, a lengthy and costly process, and a shortage of genetic transformation technology in some crops [23]. Sprayed dsRNA on the leaves resulted in a strong response from some chewing insects, but other target pests, such as sucking insects, require plant uptake and systemic movement of dsRNA. The instability of dsRNA in the environment is a challenge for the development of topically applied products. Environmental factors such as microorganisms, UV, wash-off, heat, and pH can have a significant impact on the persistence and stability of dsRNAs [117]. Double-strand RNA displayed a half-life of 15 to 28 h in soil and a half-life of less than 3 d in sediment water [118,119]; the degradation of dsRNA is primarily driven by microbial degradation or abiotic processes [119].

Monsanto/Bayer's genetically modified corn MON87411 was approved by the U. S. Environmental Protection Agency (EPA) in 2015 as the first GM crop based on RNAi technology (Table 2). MON87411 was engineered to produce dsRNAs against *DvSnf7*, the CRW-active Cry3Bb1, and the herbicide-tolerant associated CP4 EPSPS protein and resulted in Coleopteran pest resistance and glyphosate tolerance. Later, the EPA approved more hybrid products derived from MON87411, such as SmartStax Pro (DAS-59122-7 × MON87411 × MON89034 × TC1507), Vorceed™ Enlist® (DP4114 × MON87411 × MON89034 × DAS-

40278-9), VT4PRO™ (MON89034 × MIR162 × MON87411), and DP4114 × MON87411 Maize. Food Standards Australia New Zealand (FSANZ) approved food from the DP23211 GM line provided by Dow AgroSciences (DAS) (Table 2). DP23211 maize is genetically modified to express dsRNAs to *DvSSJ1*, an insecticidal protein IPD072Aa, the herbicide-tolerant associated phosphinothricin acetyltransferase (PAT), and the selection marker phosphomannose isomerase (PMI) protein, resulting in resistance to *D. v. virgifera* and tolerance to glufosinate-ammonium herbicide [120].

Table 2. RNAi-based products for pest control.

RNAi Event	Products	Company	RNAi Gene	Target Pest	Approval
MON87411 (GM corn)	SmartStax Pro, Vorceed™ Enlist®, VT4PRO™ . . .	Bayer, Corteva, Pioneer Hi-Bred	*DvSnf7*	*D. v. virgifera D. v. zeae D. barberi*	2015–2017
DP23211 (GM corn)	- [1]	DAS	*DvSSJ1*	*D. v. virgifera*	2021
Ledprona	Calantha	GreenLight	*PSMB5*	*L. decemlineata*	2021 [2]

[1] -: unknown. [2] Registration.

Calantha, a foliar-applied dsRNA spray developed by GreenLight to protect against the Colorado potato beetle, has been submitted, in 2021, to the EPA for approval (Table 2). Calantha's active ingredient, Ledprona, is a 490-bp dsRNA designed to target *L. decemlineata PSMB5* mRNA [121]. In 2019, Bayer submitted a spray RNAi biopesticide developed with BioDirect technology to the EPA for registration; this product was developed to control varroa mites using dsRNA. Other Biodirect products are in the early stages of development, including a spray that effectively targets canola flea beetles. RNAi pesticides against diamond month, varroa, and Colorado potato beetle are under development, while more RNAi products against a broader range of insect species are needed in the future.

5. Conclusions and Future Perspectives

RNAi is a powerful tool for gene functional analysis and pest control, and several dsRNA delivery approaches have been developed based on different species and purposes. Microinjection is efficient for gene functional research in most insects and other pests; soaking and non-transformative feeding methods are less expensive and easier to use; ingestion is suitable for high-throughput gene screening and has potential field applications; soaking is mostly used in cell lines and mites. The nanoparticle-mediated dsRNA delivery system is a promising approach for improving dsRNA stability and promoting dsRNA uptake.

Many target genes have been identified in Coleoptera, Hemiptera, Lepidoptera, and other species; these genes are involved in energy metabolism, synthesis of core cellular components, hormone homeostasis, chitin metabolism, digestive system, and other essential biological processes. Some target genes have been well studied in multiple species, such as V-ATPase, tubulin, actin, Snf7, COPI, ecdysone receptor, chitin synthase, ribosome subunit, and proteasome subunit. Furthermore, using dsRNAs that target multiple genes or combining RNAi with other pest control methods (such as entomopathogen, toxins, phytoecdysteroids, and pesticide) can produce synergistic lethal effects.

dsRNA-induced gene silencing is sequence-specific; however, dsRNAs containing more than 19-nucleotide perfect matches to unintended targets lead to false positives in *Drosophia* [122]. Non-target organisms (NTOs) could be exposed to dsRNA in several ways, such as consuming plant material containing dsRNA, being in contact with soil and water, or feeding on exposed arthropods. Therefore, dsRNAs should be carefully designed to avoid the off-target effects in target species and species-specifically designed to avoid the non-target effects in non-target organisms (NTOs) through in silico examination [122]. dsRNA may also affect the fitness of non-target arthropod species in a sequence-unspecific way, such as dsRNA-triggered immune responses [123]. Environmental risk assessment

should also be conducted to assess the hazardous potential of RNAi pesticides to NTOs, especially for different valued NTO groups [124].

RNAi-based biocontrol is an excellent alternative to chemical pesticides, and several RNAi pesticides have been approved and will be on the market soon. However, RNAi applications are limited due to multi-layered challenges which are yet to be overcome in the future. Some of the challenges are:

(1) Selecting efficient RNAi target genes and delivery methods according to different pests;
(2) Enhancing the persistence and stability of dsRNA in the environment and its subsequent effects;
(3) Facilitating plant uptake and systemic movement of dsRNA spray;
(4) Solving the shortage of genetic transformation technology in some crops and expanding the range of transplastomic plants expressing dsRNA;
(5) Developing more RNAi products against a wide range of insect species;
(6) Searching for synergistic effects, for example, dsRNAs targeting multiple genes, and combing of RNAi with other pest control methods;
(7) Establishing a consensus regulatory framework for the commercialization of RNAi pesticides;
(8) Reducing the costs for large-scale application of RNAi biopesticides.

Although some of the described aspects still need to be thoroughly researched and rigorously conducted, RNAi-based pesticides have undeniable potential for pest management.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13030714/s1, Table S1. Potential RNAi target genes for pest control and their effects after silencing.

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References

1. Zhu, K.Y.; Palli, S.R. Mechanisms, Applications, and Challenges of Insect RNA Interference. *Annu. Rev. Entomol.* **2020**, *65*, 293–311. [CrossRef] [PubMed]
2. Preall, J.B.; Sontheimer, E.J. RNAi: RISC gets loaded. *Cell* **2005**, *123*, 543–545. [CrossRef] [PubMed]
3. Nandety, R.S.; Kuo, Y.W.; Nouri, S.; Falk, B.W. Emerging strategies for RNA interference (RNAi) applications in insects. *Bioengineered* **2015**, *6*, 8–19. [CrossRef] [PubMed]
4. Wang, K.; Peng, Y.; Pu, J.; Fu, W.; Wang, J.; Han, Z. Variation in RNAi efficacy among insect species is attributable to dsRNA degradation in vivo. *Insect Biochem. Mol. Biol.* **2016**, *77*, 1–9. [CrossRef] [PubMed]
5. Singh, I.K.; Singh, S.; Mogilicherla, K.; Shukla, J.N.; Palli, S.R. Comparative analysis of double-stranded RNA degradation and processing in insects. *Sci. Rep.* **2017**, *7*, 17059. [CrossRef] [PubMed]
6. 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]
7. 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]
8. Yu, N.; Christiaens, O.; Liu, J.; Niu, J.; Cappelle, K.; Caccia, S.; Huvenne, H.; Smagghe, G. Delivery of dsRNA for RNAi in insects: An overview and future directions. *Insect Sci.* **2013**, *20*, 4–14. [CrossRef]
9. Yan, S.; Ren, B.-Y.; Shen, J. Nanoparticle-mediated double-stranded RNA delivery system: A promising approach for sustainable pest management. *Insect Sci.* **2021**, *28*, 21–34. [CrossRef]
10. Garbatti Factor, B.; de Moura Manoel Bento, F.; Figueira, A. Methods for Delivery of dsRNAs for Agricultural Pest Control: The Case of Lepidopteran Pests. *Methods Mol. Biol.* **2022**, *2360*, 317–345.
11. Whyard, S.; Singh, A.D.; Wong, S. Ingested double-stranded RNAs can act as species-specific insecticides. *Insect Biochem. Mol. Biol.* **2009**, *39*, 824–832. [CrossRef]
12. 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] [PubMed]

13. 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, *Myllocerus undecimpustulatus undatus* Marshall. *Pest Manag. Sci.* **2020**, *76*, 936–943. [CrossRef] [PubMed]
14. Song, H.; Fan, Y.; Zhang, J.; Cooper, A.M.; Silver, K.; Li, D.; Li, T.; Ma, E.; Zhu, K.Y.; Zhang, J. Contributions of dsRNases to differential RNAi efficiencies between the injection and oral delivery of dsRNA in *Locusta migratoria*. *Pest Manag. Sci.* **2019**, *75*, 1707–1717. [CrossRef]
15. Prentice, K.; Smagghe, G.; Gheysen, G.; Christiaens, O. Nuclease activity decreases the RNAi response in the sweetpotato weevil *Cylas puncticollis*. *Insect Biochem. Mol. Biol.* **2019**, *110*, 80–89. [CrossRef] [PubMed]
16. Gurusamy, D.; Howell, J.L.; Chereddy, S.; Koo, J.; Palli, S.R. Transport of orally delivered dsRNA in southern green stink bug, *Nezara viridula*. *Arch. Insect Biochem. Physiol.* **2020**, *104*, e21692. [CrossRef]
17. Hu, J.; Xia, Y. Increased virulence in the locust-specific fungal pathogen *Metarhizium acridum* expressing dsRNAs targeting the host F(1) F(0)-ATPase subunit genes. *Pest Manag. Sci.* **2019**, *75*, 180–186. [CrossRef]
18. Ma, Z.Z.; Zhou, H.; Wei, Y.L.; Yan, S.; Shen, J. A novel plasmid-*Escherichia coli* system produces large batch dsRNAs for insect gene silencing. *Pest Manag. Sci.* **2020**, *76*, 2505–2512. [CrossRef]
19. Wu, M.; Zhang, Q.; Dong, Y.; Wang, Z.; Zhan, W.; Ke, Z.; Li, S.; He, L.; Ruf, S.; Bock, R.; et al. Transplastomic tomatoes expressing double-stranded RNA against a conserved gene are efficiently protected from multiple spider mites. *New Phytol.* **2023**, *237*, 1363–1373. [CrossRef]
20. 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]
21. Zhang, J.; Khan, S.A.; Hasse, C.; Ruf, S.; Heckel, D.G.; Bock, R. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. *Science* **2015**, *347*, 991–994. [CrossRef]
22. Jin, S.; Singh, N.D.; Li, L.; Zhang, X.; Daniell, H. Engineered chloroplast dsRNA silences cytochrome p450 monooxygenase, V-ATPase and chitin synthase genes in the insect gut and disrupts *Helicoverpa zea* larval development and pupation. *Plant Biotechnol. J.* **2015**, *13*, 435–446. [CrossRef] [PubMed]
23. Zhang, J.; Khan, S.A.; Heckel, D.G.; Bock, R. Next-Generation Insect-Resistant Plants: RNAi-Mediated Crop Protection. *Trends Biotechnol.* **2017**, *35*, 871–882. [CrossRef]
24. Fletcher, S.J.; Reeves, P.T.; Hoang, B.T.; Mitter, N. A Perspective on RNAi-Based Biopesticides. *Front. Plant Sci.* **2020**, *11*, 51. [CrossRef] [PubMed]
25. Cagliari, D.; Dias, N.P.; Galdeano, D.M.; Dos Santos, E.; Smagghe, G.; Zotti, M.J. Management of Pest Insects and Plant Diseases by Non-Transformative RNAi. *Front. Plant Sci.* **2019**, *10*, 1319. [CrossRef] [PubMed]
26. 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]
27. Clemens, J.C.; Worby, C.A.; Simonson-Leff, N.; Muda, M.; Maehama, T.; Hemmings, B.A.; Dixon, J.E. Use of double-stranded RNA interference in *Drosophila* cell lines to dissect signal transduction pathways. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6499–6503. [CrossRef]
28. Sivakumar, S.; Rajagopal, R.; Venkatesh, G.R.; Srivastava, A.; Bhatnagar, R.K. Knockdown of aminopeptidase-N from *Helicoverpa armigera* larvae and in transfected Sf21 cells by RNA interference reveals its functional interaction with *Bacillus thuringiensis* insecticidal protein Cry1Ac. *J. Biol. Chem.* **2007**, *282*, 7312–7319. [CrossRef]
29. Zheng, Y.; Hu, Y.; Yan, S.; Zhou, H.; Song, D.; Yin, M.; Shen, J. A polymer/detergent formulation improves dsRNA penetration through the body wall and RNAi-induced mortality in the soybean aphid *Aphis glycines*. *Pest Manag. Sci.* **2019**, *75*, 1993–1999. [CrossRef]
30. Pridgeon, J.W.; Zhao, L.; Becnel, J.J.; Strickman, D.A.; Clark, G.G.; Linthicum, K.J. Topically applied AaeIAP1 double-stranded RNA kills female adults of *Aedes aegypti*. *J. Med. Entomol.* **2008**, *45*, 414–420. [CrossRef]
31. 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]
32. Dias, N.; Cagliari, D.; Kremer, F.S.; Rickes, L.N.; Nava, D.E.; Smagghe, G.; Zotti, M. The South American Fruit Fly: An Important Pest Insect With RNAi-Sensitive Larval Stages. *Front. Physiol.* **2019**, *10*, 794. [CrossRef]
33. Chao, Z.; Zhongzheng, M.; Yunhui, Z.; Yan, S.; Shen, J. RNA interference cannot be operated in lepidopteran insect? A nanocarrier breaks bottlenecks at all developmental stages of *Spodoptera frugiperda*. *J. Insect Physiol.* **2022**. *preprint*. [CrossRef]
34. Zhang, X.; Zhang, J.; Zhu, K.Y. Chitosan/double-stranded RNA nanoparticle-mediated RNA interference to silence chitin synthase genes through larval feeding in the African malaria mosquito (*Anopheles gambiae*). *Insect Mol. Biol.* **2010**, *19*, 683–693. [CrossRef] [PubMed]
35. Zhou, H.; Liu, S.; Wan, F.; Jian, Y.; Guo, H.; Chen, J.; Ning, Y.; Ding, W. Graphene oxide-acaricide nanocomposites advance acaricidal activity of acaricides against *Tetranychus cinnabarinus* by directly inhibiting the transcription of a cuticle protein gene. *Environ. Sci. Nano* **2021**, *8*, 3122–3137. [CrossRef]
36. Yan, S.; Qian, J.; Cai, C.; Ma, Z.; Li, J.; Yin, M.; Ren, B.; Shen, J. Spray method application of transdermal dsRNA delivery system for efficient gene silencing and pest control on soybean aphid *Aphis glycines*. *J. Pest Sci.* **2020**, *93*, 449–459. [CrossRef]

37. He, B.; Chu, Y.; Yin, M.; Mullen, K.; An, C.; Shen, J. Fluorescent nanoparticle delivered dsRNA toward genetic control of insect pests. *Adv. Mater.* **2013**, *25*, 4580–4584. [CrossRef] [PubMed]
38. Lin, Y.H.; Huang, J.H.; Liu, Y.; Belles, X.; Lee, H.J. Oral delivery of dsRNA lipoplexes to German cockroach protects dsRNA from degradation and induces RNAi response. *Pest Manag. Sci.* **2017**, *73*, 960–966. [CrossRef] [PubMed]
39. 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] [PubMed]
40. Haller, S.; Widmer, F.; Siegfried, B.D.; Zhuo, X.; Romeis, J. Responses of two ladybird beetle species (Coleoptera: Coccinellidae) to dietary RNAi. *Pest Manag. Sci.* **2019**, *75*, 2652–2662. [CrossRef]
41. Camargo, R.A.; Barbosa, G.O.; Possignolo, I.P.; Peres, L.E.; Lam, E.; Lima, J.E.; Figueira, A.; Marques-Souza, H. RNA interference as a gene silencing tool to control *Tuta absoluta* in tomato (*Solanum lycopersicum*). *PeerJ* **2016**, *4*, e2673. [CrossRef]
42. Ma, Z.; Zhang, Y.; Li, M.; Chao, Z.; Du, X.; Yan, S.; Shen, J. A first greenhouse application of bacteria-expressed and nanocarrier-delivered RNA pesticide for *Myzus persicae* control. *J. Pest Sci.* **2023**, *96*, 181–193. [CrossRef]
43. Upadhyay, S.K.; Chandrashekar, K.; Thakur, N.; Verma, P.C.; Borgio, J.F.; Singh, P.K.; Tuli, R. RNA interference for the control of whiteflies (*Bemisia tabaci*) by oral route. *J. Biosci.* **2011**, *36*, 153–161. [CrossRef]
44. Wu, M.; Dong, Y.; Zhang, Q.; Li, S.; Chang, L.; Loiacono, F.V.; Ruf, S.; Zhang, J.; Bock, R. Efficient control of western flower thrips by plastid-mediated RNA interference. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2120081119. [CrossRef] [PubMed]
45. Bensoussan, N.; Dixit, S.; Tabara, M.; Letwin, D.; Milojevic, M.; Antonacci, M.; Jin, P.; Arai, Y.; Bruinsma, K.; Suzuki, T.; et al. Environmental RNA interference in two-spotted spider mite, *Tetranychus urticae*, reveals dsRNA processing requirements for efficient RNAi response. *Sci. Rep.* **2020**, *10*, 19126. [CrossRef] [PubMed]
46. 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]
47. Sharif, M.N.; Iqbal, M.S.; Alam, R.; Awan, M.F.; Tariq, M.; Ali, Q.; Nasir, I.A. Silencing of multiple target genes via ingestion of dsRNA and PMRi affects development and survival in *Helicoverpa armigera*. *Sci. Rep.* **2022**, *12*, 10405. [CrossRef] [PubMed]
48. 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]
49. Khan, A.M.; Ashfaq, M.; Khan, A.A.; Naseem, M.T.; Mansoor, S. Evaluation of potential RNA-interference-target genes to control cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcuidae). *Insect Sci.* **2018**, *25*, 778–786. [CrossRef]
50. Meng, J.; Lei, J.; Davitt, A.; Holt, J.R.; Huang, J.; Gold, R.; Vargo, E.L.; Tarone, A.M.; Zhu-Salzman, K. Suppressing tawny crazy ant (*Nylanderia fulva*) by RNAi technology. *Insect Sci.* **2020**, *27*, 113–121. [CrossRef]
51. Fu, S.; Liu, Z.; Chen, J.; Sun, G.; Jiang, Y.; Li, M.; Xiong, L.; Chen, S.; Zhou, Y.; Asad, M.; et al. Silencing arginine kinase/integrin β(1) subunit by transgenic plant expressing dsRNA inhibits the development and survival of *Plutella xylostella*. *Pest Manag. Sci.* **2020**, *76*, 1761–1771. [CrossRef]
52. Liu, F.; Wang, X.D.; Zhao, Y.Y.; Li, Y.J.; Liu, Y.C.; Sun, J. Silencing the *HaAK* gene by transgenic plant-mediated RNAi impairs larval growth of *Helicoverpa armigera*. *Int. J. Biol. Sci.* **2015**, *11*, 67–74. [CrossRef]
53. Chen, R.P.; Liu, C.Y.; Shao, H.L.; Zheng, W.W.; Wang, J.X.; Zhao, X.F. Adenylate kinase 2 (AK2) promotes cell proliferation in insect development. *BMC Mol. Biol.* **2012**, *13*, 31. [CrossRef]
54. 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] [PubMed]
55. Kyre, B.R.; Rodrigues, T.B.; Rieske, L.K. RNA interference and validation of reference genes for gene expression analyses using qPCR in southern pine beetle, *Dendroctonus frontalis*. *Sci. Rep.* **2019**, *9*, 5640. [CrossRef] [PubMed]
56. Xu, W.; Zhang, M.; Li, Y.; He, W.; Li, S.; Zhang, J. Complete protection from *Henosepilachna vigintioctopunctata* by expressing long double-stranded RNAs in potato plastids. *J. Integr. Plant Biol.* **2022**, *0*, 1–9. [CrossRef] [PubMed]
57. Hu, X.; Richtman, N.M.; Zhao, J.Z.; Duncan, K.E.; Niu, X.; Procyk, L.A.; Oneal, M.A.; Kernodle, B.M.; Steimel, J.P.; Crane, V.C.; et al. Discovery of midgut genes for the RNA interference control of corn rootworm. *Sci. Rep.* **2016**, *6*, 30542. [CrossRef]
58. Dhandapani, R.K.; Duan, J.J.; Palli, S.R. 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]
59. Singh, S.; Gupta, M.; Pandher, S.; Kaur, G.; Goel, N.; Rathore, P.; Palli, S.R. RNA sequencing, selection of reference genes and demonstration of feeding RNAi in *Thrips tabaci* (Lind.) (Thysanoptera: Thripidae). *BMC Mol. Biol.* **2019**, *20*, 6. [CrossRef]
60. Velez, A.M.; Fishilevich, E.; Rangasamy, M.; Khajuria, C.; McCaskill, D.G.; Pereira, A.E.; Gandra, P.; Frey, M.L.; Worden, S.E.; Whitlock, S.L.; et al. Control of western corn rootworm via RNAi traits in maize: Lethal and sublethal effects of Sec23 dsRNA. *Pest Manag. Sci.* **2020**, *76*, 1500–1512. [CrossRef]
61. Zhu, J.Q.; Liu, S.; Ma, Y.; Zhang, J.Q.; Qi, H.S.; Wei, Z.J.; Yao, Q.; Zhang, W.Q.; Li, S. Improvement of pest resistance in transgenic tobacco plants expressing dsRNA of an insect-associated gene *EcR*. *PLoS ONE* **2012**, *7*, e38572. [CrossRef] [PubMed]
62. Malik, H.J.; Raza, A.; Amin, I.; Scheffler, J.A.; Scheffler, B.E.; Brown, J.K.; Mansoor, S. RNAi-mediated mortality of the whitefly through transgenic expression of double-stranded RNA homologous to acetylcholinesterase and ecdysone receptor in tobacco plants. *Sci. Rep.* **2016**, *6*, 38469. [CrossRef] [PubMed]

63. 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] [PubMed]
64. Yang, J.; Han, Z.-J. Efficiency of Different Methods for dsRNA Delivery in Cotton Bollworm (*Helicoverpa armigera*). *J. Integr. Agric.* **2014**, *13*, 115–123. [CrossRef]
65. Xiong, Y.; Zeng, H.; Zhang, Y.; Xu, D.; Qiu, D. Silencing the *HaHR3* gene by transgenic plant-mediated RNAi to disrupt *Helicoverpa armigera* development. *Int. J. Biol. Sci.* **2013**, *9*, 370–381. [CrossRef]
66. Zhang, Y.L.; Zhang, S.Z.; Kulye, M.; Wu, S.R.; Yu, N.T.; Wang, J.H.; Zeng, H.M.; Liu, Z.X. Silencing of molt-regulating transcription factor gene, *CiHR3*, affects growth and development of sugarcane stem borer, *Chilo infuscatellus*. *J. Insect Sci.* **2012**, *12*, 91. [CrossRef]
67. Vatanparast, M.; Kazzazi, M.; Mirzaie-Asl, A.; Hosseininaveh, V. RNA interference-mediated knockdown of some genes involved in digestion and development of *Helicoverpa armigera*. *Bull. Entomol. Res.* **2017**, *107*, 777–790. [CrossRef]
68. 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]
69. Yang, S.; Zou, Z.; Xin, T.; Cai, S.; Wang, X.; Zhang, H.; Zhong, L.; Xia, B. Knockdown of hexokinase in *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) by RNAi inhibits chitin synthesis and leads to abnormal phenotypes. *Pest Manag. Sci.* **2022**, *78*, 4303–4313. [CrossRef]
70. Zou, H.; Zhang, B.; Zou, C.; Ma, W.; Zhang, S.; Wang, Z.; Bi, B.; Li, S.; Gao, J.; Zhang, C.; et al. Knockdown of *GFAT* disrupts chitin synthesis in *Hyphantria cunea* larvae. *Pestic. Biochem. Physiol.* **2022**, *188*, 105245. [CrossRef]
71. Chang, Y.W.; Wang, Y.C.; Yan, Y.Q.; Xie, H.F.; Yuan, D.R.; Du, Y.Z. RNA Interference of Chitin Synthase 2 Gene in *Liriomyza trifolii* through Immersion in Double-Stranded RNA. *Insects* **2022**, *13*, 832. [CrossRef] [PubMed]
72. Reddy, M.K.R.K.; Rajam, M.V. Targeting chitinase gene of *Helicoverpa armigera* by host-induced RNA interference confers insect resistance in tobacco and tomato. *Plant Mol. Biol.* **2016**, *90*, 281–292.
73. Shang, F.; Ding, B.Y.; Ye, C.; Yang, L.; Chang, T.Y.; Xie, J.; Tang, L.D.; Niu, J.; Wang, J.J. Evaluation of a cuticle protein gene as a potential RNAi target in aphids. *Pest Manag. Sci.* **2020**, *76*, 134–140. [CrossRef]
74. 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] [PubMed]
75. Yang, C.H.; Zhang, Q.; Zhu, W.Q.; Shi, Y.; Cao, H.H.; Guo, L.; Chu, D.; Lu, Z.; Liu, T.X. Involvement of Laccase2 in Cuticle Sclerotization of the Whitefly, *Bemisia tabaci* Middle East-Asia Minor 1. *Insects* **2022**, *13*, 471. [CrossRef]
76. Araujo, R.N.; Santos, A.; Pinto, F.S.; Gontijo, N.F.; Lehane, M.J.; Pereira, M.H. RNA interference of the salivary gland nitrophorin 2 in the triatomine bug *Rhodnius prolixus* (Hemiptera: Reduviidae) by dsRNA ingestion or injection. *Insect Biochem. Mol. Biol.* **2006**, *36*, 683–693. [CrossRef]
77. Murtaza, S.; Tabassum, B.; Tariq, M.; Riaz, S.; Yousaf, I.; Jabbar, B.; Khan, A.; Samuel, A.O.; Zameer, M.; Nasir, I.A. Silencing a *Myzus persicae Macrophage* Inhibitory Factor by Plant-Mediated RNAi Induces Enhanced Aphid Mortality Coupled with Boosted RNAi Efficacy in Transgenic Potato Lines. *Mol. Biotechnol.* **2022**, *64*, 1152–1163. [CrossRef] [PubMed]
78. Zhao, J.; Liu, N.; Ma, J.; Huang, L.; Liu, X. Effect of Silencing CYP6B6 of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Its Growth, Development, and Insecticide Tolerance. *J. Econ. Entomol.* **2016**, *109*, 2506–2516. [CrossRef]
79. Li, M.; Ma, Z.; Peng, M.; Li, L.; Yin, M.; Yan, S.; Shen, J. A gene and drug co-delivery application helps to solve the short life disadvantage of RNA drug. *Nano Today* **2022**, *43*, 101452. [CrossRef]
80. Ye, C.; Wang, Z.-W.; Sheng, Y.-L.; Wang, Z.-G.; Smagghe, G.; Christiaens, O.; Niu, J.; Wang, J.-J. *GNBP1* as a potential RNAi target to enhance the virulence of *Beauveria bassiana* for aphid control. *J. Pest Sci.* **2022**, *95*, 87–100. [CrossRef]
81. Ran, R.; Li, T.; Liu, X.; Ni, H.; Li, W.; Meng, F. RNA interference-mediated silencing of genes involved in the immune responses of the soybean pod borer *Leguminivora glycinivorella* (Lepidoptera: Olethreutidae). *PeerJ* **2018**, *6*, e4931. [CrossRef]
82. Zhang, C.; Yan, S.Q.; Shen, B.B.; Ali, S.; Wang, X.M.; Jin, F.L.; Cuthbertson, A.G.S.; Qiu, B.L. RNAi knock-down of the *Bemisia tabaci* Toll gene (*BtToll*) increases mortality after challenge with destruxin A. *Mol. Immunol.* **2017**, *88*, 164–173. [CrossRef]
83. 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] [PubMed]
84. Li, J.; Li, X.; Bai, R.; Shi, Y.; Tang, Q.; An, S.; Song, Q.; Yan, F. RNA interference of the P450 CYP6CM1 gene has different efficacy in B and Q biotypes of *Bemisia tabaci*. *Pest Manag. Sci.* **2015**, *71*, 1175–1181. [CrossRef]
85. Qu, X.; Wang, S.; Lin, G.; Li, M.; Shen, J.; Wang, D. The Synergistic Effect of Thiamethoxam and Synapsin dsRNA Targets Neurotransmission to Induce Mortality in *Aphis gossypii*. *Int. J. Mol. Sci.* **2022**, *23*, 9388. [CrossRef]
86. Allen, M.L.; Walker, W.B., 3rd. Saliva of *Lygus lineolaris* digests double stranded ribonucleic acids. *J. Insect Physiol.* **2012**, *58*, 391–396. [CrossRef] [PubMed]
87. Lu, K.; Song, Y.; Zeng, R. The role of cytochrome P450-mediated detoxification in insect adaptation to xenobiotics. *Curr. Opin. Insect Sci.* **2021**, *43*, 103–107. [CrossRef] [PubMed]
88. Hafeez, M.; Li, X.; Ullah, F.; Zhang, Z.; Zhang, J.; Huang, J.; Fernández-Grandon, G.M.; Khan, M.M.; Siddiqui, J.A.; Chen, L.; et al. Down-Regulation of P450 Genes Enhances Susceptibility to Indoxacarb and Alters Physiology and Development of Fall Armyworm, *Spodoptera frugipreda* (Lepidoptera: Noctuidae). *Front. Physiol.* **2022**, *13*, 884447. [CrossRef]

89. Li, H.; Guan, R.; Guo, H.; Miao, X. New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests. *Plant Cell Environ.* **2015**, *38*, 2277–2285. [CrossRef]
90. Asokan, R.; Rebijith, K.B.; Roopa, H.K.; Kumar, N.K. Non-invasive delivery of dsGST is lethal to the sweet potato whitefly, *Bemisia tabaci* (G.) (Hemiptera: Aleyrodidae). *Appl. Biochem. Biotechnol.* **2015**, *175*, 2288–2299. [CrossRef]
91. Gao, H.; Lin, X.; Yang, B.; Liu, Z. The roles of GSTs in fipronil resistance in *Nilaparvata lugens*: Over-expression and expression induction. *Pestic. Biochem. Physiol.* **2021**, *177*, 104880. [CrossRef]
92. Yu, X.; Killiny, N. RNA interference of two glutathione S-transferase genes, *Diaphorina citri DcGSTe2* and *DcGSTd1*, increases the susceptibility of Asian citrus psyllid (Hemiptera: Liviidae) to the pesticides fenpropathrin and thiamethoxam. *Pest Manag. Sci.* **2018**, *74*, 638–647. [CrossRef] [PubMed]
93. Shabab, M.; Khan, S.A.; Vogel, H.; Heckel, D.G.; Boland, W. OPDA isomerase GST16 is involved in phytohormone detoxification and insect development. *FEBS J.* **2014**, *281*, 2769–2783. [CrossRef] [PubMed]
94. Kumar, M.; Gupta, G.P.; Rajam, M.V. Silencing of acetylcholinesterase gene of *Helicoverpa armigera* by siRNA affects larval growth and its life cycle. *J. Insect Physiol.* **2009**, *55*, 273–278. [CrossRef]
95. Sharath Chandra, G.; Asokan, R.; Manamohan, M.; Krishna Kumar, N. Enhancing RNAi by using concatemerized double-stranded RNA. *Pest Manag. Sci.* **2019**, *75*, 506–514. [CrossRef] [PubMed]
96. Zhang, L.; Shang, Q.; Lu, Y.; Zhao, Q.; Gao, X. A transferrin gene associated with development and 2-tridecanone tolerance in *Helicoverpa armigera*. *Insect Mol. Biol.* **2015**, *24*, 155–166. [CrossRef] [PubMed]
97. Chen, D.; Yan, R.; Xu, Z.; Qian, J.; Yu, Y.; Zhu, S.; Wu, H.; Zhu, G.; Chen, M. Silencing of *dre4* Contributes to Mortality of *Phyllotreta striolata*. *Insects* **2022**, *13*, 1072. [CrossRef]
98. 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]
99. Inberg, A.; Mahak, K. Monsanto Technology LLC and Beeologics. Inc. U.S. Patent 62156751, 3 May 2016.
100. Xie, X.; Shang, F.; Ding, B.Y.; Yang, L.; Wang, J.J. Assessment of a zinc finger protein gene (*MPZC3H10*) as potential RNAi target for green peach aphid *Myzus persicae* control. *Pest Manag. Sci.* **2022**, *78*, 4956–4962. [CrossRef] [PubMed]
101. Raje, K.R.; Peterson, B.F.; Scharf, M.E. Screening of 57 Candidate Double-Stranded RNAs for Insecticidal Activity Against the Pest Termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* **2018**, *111*, 2782–2787. [CrossRef]
102. Ayari, A.; Rosa-Calatrava, M.; Lancel, S.; Barthelemy, J.; Pizzorno, A.; Mayeuf-Louchart, A.; Baron, M.; Hot, D.; Deruyter, L.; Soulard, D.; et al. Influenza infection rewires energy metabolism and induces browning features in adipose cells and tissues. *Commun. Biol.* **2020**, *3*, 237. [CrossRef]
103. Read, E.B.; Okamura, H.H.; Drubin, D.G. Actin- and tubulin-dependent functions during Saccharomyces cerevisiae mating projection formation. *Mol. Biol. Cell* **1992**, *3*, 429–444. [CrossRef]
104. Kang, J.; Brajanovski, N.; Chan, K.T.; Xuan, J.; Pearson, R.B.; Sanij, E. Ribosomal proteins and human diseases: Molecular mechanisms and targeted therapy. *Signal Transduct.Target. Ther.* **2021**, *6*, 323. [CrossRef] [PubMed]
105. Zhao, X.; Qin, Z.; Liu, W.; Liu, X.; Moussian, B.; Ma, E.; Li, S.; Zhang, J. Nuclear receptor HR3 controls locust molt by regulating chitin synthesis and degradation genes of *Locusta migratoria*. *Insect Biochem. Mol. Biol.* **2018**, *92*, 1–11. [CrossRef]
106. Kramer, K.J.; Sanburg, L.L.; Kézdy, F.J.; Law, J.H. The Juvenile Hormone Binding Protein in the Hemolymph of *Manduca sexta* Johannson (Lepidoptera: Sphingidae). *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 493–497. [CrossRef] [PubMed]
107. Choi, M.Y.; Vander Meer, R.K.; Coy, M.; Scharf, M.E. Phenotypic impacts of PBAN RNA interference in an ant, *Solenopsis invicta*, and a moth, *Helicoverpa zea*. *J. Insect Physiol.* **2012**, *58*, 1159–1165. [CrossRef] [PubMed]
108. Merzendorfer, H.; Zimoch, L. Chitin metabolism in insects: Structure, function and regulation of chitin synthases and chitinases. *J. Exp. Biol.* **2003**, *206*, 4393–4412. [CrossRef] [PubMed]
109. Yang, W.J.; Xu, K.K.; Yan, X.; Chen, C.X.; Cao, Y.; Meng, Y.L.; Li, C. Functional characterization of chitin deacetylase 1 gene disrupting larval-pupal transition in the drugstore beetle using RNA interference. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2018**, *219–220*, 10–16. [CrossRef] [PubMed]
110. Da Lage, J.L. The Amylases of Insects. *Int. J. Insect Sci.* **2018**, *10*. [CrossRef]
111. Feng, K.; Li, W.; Tang, X.; Luo, J.; Tang, F. Termicin silencing enhances the toxicity of *Serratia marcescens* Bizio (SM1) to *Odontotermes formosanus* (Shiraki). *Pestic. Biochem. Physiol.* **2022**, *185*, 105120. [CrossRef]
112. Zhang, J.; Ye, C.; Wang, Z.-G.; Ding, B.-Y.; Smagghe, G.; Zhang, Y.; Niu, J.; Wang, J.-J. DsRNAs spray enhanced the virulence of entomopathogenic fungi *Beauveria bassiana* in aphid control. *J. Pest Sci.* **2023**, *96*, 241–251. [CrossRef]
113. Hay, B.A.; Wassarman, D.A.; Rubin, G.M. *Drosophila* homologs of baculovirus inhibitor of apoptosis proteins function to block cell death. *Cell* **1995**, *83*, 1253–1262. [CrossRef] [PubMed]
114. Yang, J.; Kong, X.D.; Zhu-Salzman, K.; Qin, Q.M.; Cai, Q.N. The Key Glutathione S-Transferase Family Genes Involved in the Detoxification of Rice Gramine in Brown Planthopper *Nilaparvata lugens*. *Insects* **2021**, *12*, 1055. [CrossRef]
115. Kishk, A.; Anber, H.A.; AbdEl-Raof, T.K.; El-Sherbeni, A.D.; Hamed, S.; Gowda, S.; Killiny, N. RNA interference of carboxyesterases causes nymph mortality in the Asian citrus psyllid, *Diaphorina citri*. *Arch. Insect Biochem. Physiol.* **2017**, *94*, e21377. [CrossRef]
116. Sural, T.H.; Peng, S.; Li, B.; Workman, J.L.; Park, P.J.; Kuroda, M.I. The MSL3 chromodomain directs a key targeting step for dosage compensation of the *Drosophila melanogaster* X chromosome. *Nat. Struct. Mol. Biol.* **2008**, *15*, 1318–1325. [CrossRef]

117. 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]
118. 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]
119. Fischer, J.R.; Zapata, F.; Dubelman, S.; Mueller, G.M.; Uffman, J.P.; Jiang, C.; Jensen, P.D.; Levine, S.L. Aquatic fate of a double-stranded RNA in a sediment—Water system following an over-water application. *Environ. Toxicol. Chem.* **2017**, *36*, 727–734. [CrossRef] [PubMed]
120. Anderson, J.A.; Mickelson, J.; Challender, M.; Moellring, E.; Sult, T.; TeRonde, S.; Walker, C.; Wang, Y.; Maxwell, C.A. Agronomic and compositional assessment of genetically modified DP23211 maize for corn rootworm control. *GM Crop. Food* **2020**, *11*, 206–214. [CrossRef]
121. Rodrigues, T.B.; Mishra, S.; Sridharan, K.; Barnes, E.; Alyokhin, A.; Tuttle, R.; Wimalanathan, K.; Garby, D.; Skizim, N.; Tang, Y.-W.; 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**, *12*, 728652. [CrossRef]
122. Kulkarni, M.M.; Booker, M.; Silver, S.J.; Friedman, A.; Hong, P.; Perrimon, N.; Mathey-Prevot, B. Evidence of off-target effects associated with long dsRNAs in *Drosophila melanogaster* cell-based assays. *Nat. Methods* **2006**, *3*, 833–838. [CrossRef] [PubMed]
123. Jarosch, A.; Moritz, R.F.A. RNA interference in honeybees: Off-target effects caused by dsRNA. *Apidologie* **2012**, *43*, 128–138. [CrossRef]
124. Boeckman, C.J.; Anderson, J.A.; Linderblood, C.; Olson, T.; Roper, J.; Sturtz, K.; Walker, C.; Woods, R. Environmental risk assessment of the DvSSJ1 dsRNA and the IPD072Aa protein to non-target organisms. *GM Crop. Food* **2021**, *12*, 459–478. [CrossRef] [PubMed]