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Review

RNA-Interference-Mediated Aphid Control in Crop Plants: A Review

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Abstract: Crop plants suffer severe yield losses due to the significant damages caused by aphids. RNA interference (RNAi) technology is a versatile and environmentally friendly method for pest management in crop protection. Transgenic plants expressing siRNA/dsRNA and non-transformative methods such as spraying, microinjection, feeding, and a nanocarrier-delivery-mediated RNAi approach have been successfully applied for agricultural insect pest management. In this review, we summarize the application of host-induced gene silencing (HIGS)-mediated RNAi, spray-induced gene silencing (SIGS)-mediated RNAi, and other delivery-method-mediated RNAi methods for aphid control. We further discuss the challenges in RNAi application and propose potential solutions to enhance RNAi efficiency.

Keywords: RNA interference (RNAi); host-induced gene silencing (HIGS); spray-induced gene silencing (SIGS); aphid control; RNAi efficiency

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1. Introduction

Cereal plants are frequently attacked sequentially or simultaneously by different aphid species, significantly reducing the quality and quantity of grain. Although chemical control could successfully suppress aphid populations, it has accelerated insecticide resistance development and led to pest resurgence. The overuse of chemical pesticides has led to severe environmental problems and threatens human health [1]. Therefore, to guarantee food safety and security, it is important and imperative to develop effective pest management approaches to control aphid damage to cereals. Extensive research in recent decades has typically concentrated on further understanding crop–aphid interactions, which has significantly facilitated the development of sustainable aphid management strategies [2].

RNA interference (RNAi) is a biological process that can be triggered by endogenously expressed or exogenously applied double-stranded RNAs (dsRNAs). In this process, transcriptional silencing is induced by directing inhibitory chromatin modifications, and post-transcriptional silencing is induced by decreasing the stability or translation capability of the targeted mRNA [3–8]. The RNAi technique has enormous potential applications in agricultural practices, extending to viruses, bacteria, fungi, nematodes, insects, and plants. RNAi-mediated control has been exploited for several phloem-feeding aphids via targeting essential genes involved in ingestion, molting, development, and fecundity [9]. With applications in crop protection and production, host-induced gene silencing (HIGS), which employs transgenic plants that have been precisely engineered to produce dsRNA, and spray-induced gene silencing (SIGS), which uses topically applied dsRNA molecules, are being exploited. Here, we summarize the RNAi-based protection against different aphid species in crop plants, discuss the challenges associated with RNAi application, and propose potential solutions to improve RNAi efficiency.

2. RNA-Interference-Based Aphid Control in Crop Plants

The first evidence of RNA-induced gene silencing was described in pigmented petunia petals when they attempted to overexpress a key gene involved in flavonoid biosynthesis named chalcone synthase (CHS) but blocked anthocyanin biosynthesis via a post-transcriptional mechanism [10]. A subsequent investigation demonstrated that dsRNA resulted in the decreased or eliminated expression of a target transcript in *Caenorhabditis elegans*. This discovery established that dsRNA was more effective than single-stranded RNA (ssRNA), which represented an extraordinary milestone in the RNAi revolution [11]. Since the discovery that dsRNA induces effective target gene silencing, a variety of techniques have been investigated to deliver dsRNA in insect species. In laboratory or agricultural practice, exogenous RNAs are applied through surface treatments or invasive methods such as spraying, soaking, injection, infiltration, soil/root drenching, and petiole absorption [12–16]. Plant-mediated and insect-mediated RNAi have been exploited as promising alternative strategies for pest management [17–19] (Figure 1). The application of RNAi through expressing dsRNA in transgenic crop plants or utilizing dsRNA directly as an insecticide appears promising for agricultural pest control, which can be achieved by host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) [20,21].

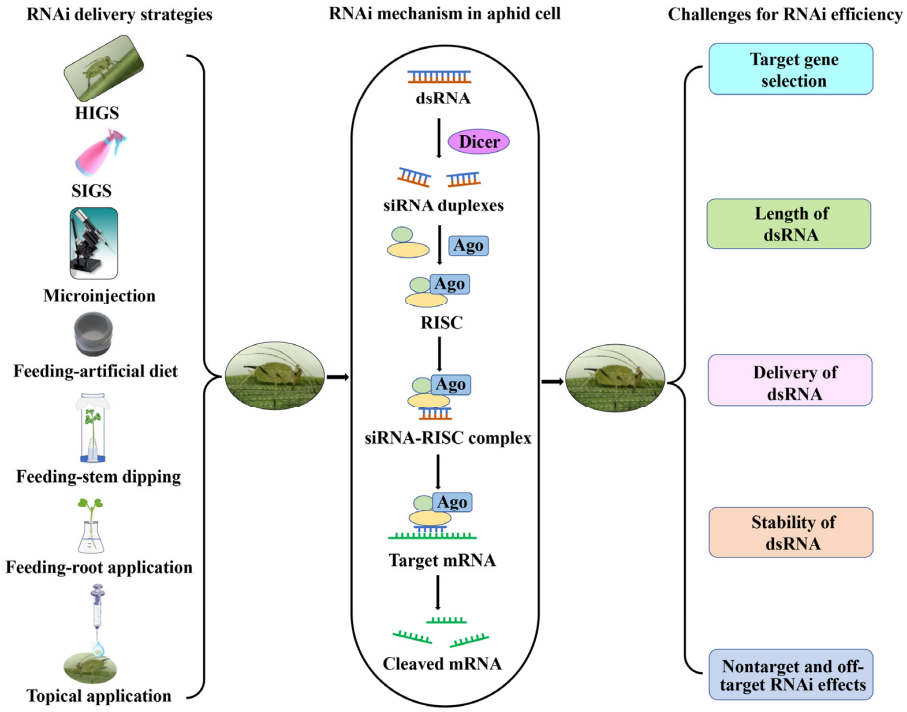

Figure 1. Schematic of RNA interference (RNAi) delivery strategies, the RNAi mechanism in aphid cells, and challenges affecting RNAi efficiency in aphids. HIGS: host-induced gene silencing, SIGS: spray-induced gene silencing, dsRNA: double-stranded RNA, Dicer: Dice-like, siRNA: short interfering RNA, Ago: Argonaute, RISC: RNA-induced silencing complex.

2.1. Host-Induced Gene Silencing

Host-induced gene silencing is known as a plant-mediated transgenic strategy in which plants are genetically engineered to produce pest- or pathogen-gene-targeting sRNAs or dsRNAs. Subsequently, these RNAs are transported into the pest or pathogen to silence target genes [22,23].

The HIGS molecular mechanisms in insects may differ from those in fungi. In herbivorous insects, long dsRNAs (including hpRNAs) appear to be absorbed directly from the host. Then, gene silencing is induced via RNAi machinery. In fungi, the existing evidence indicates that gene silencing is induced through taking up siRNAs and microRNAs (miRNAs) produced by the host plant [24].

Host-induced gene silencing (HIGS) was first reported in *Arabidopsis thaliana.* With an expressed hpRNA of a nematode *16D10* gene, transgenic plants exhibited significant resistance against four main root-knot nematode species [25]. The first proof-of-concept research on plant-mediated dsRNA delivery for insect pest management was reported in western corn rootworm (WCR). In a growth chamber assay, transgenic maize plants expressing WCR dsRNAs significantly reduced the damage caused by WCR feeding [26]. Subsequently, numerous studies have been reported using HIGS in crop plants to protect against various plant pathogens and pests, including fungi [27,28], oomycetes [29,30], and insects [31,32].

2.2. Host-Induced Gene Silencing Based Protection of Crop Plants from Aphids

HIGS has great potential to manage insects from the order Hemiptera that feed on plants, especially aphids. The application of HIGS has been exploited in different aphid species, including the peach aphid (*Myzus persicae*) and grain aphid (*Sitobion avenae*) (Table 1).

Table 1. Summary of RNAi application for aphid control.

Types of Gene Silencing	Aphid Species	Plant Species	Delivery Strategy	Target Genes	Molecule	Size	Main Effects	Reference
HIGS	*Myzus persicae*	*Nicotiana benthamiana* and *A. thaliana*	Transgenic *N. benthamiana* and *A. thaliana*	*MpC002, Rack1*	dsRNA	710 bp, 309 bp	Knockdown of target genes.	[17]
	Myzus persicae	*N. benthamiana* and *A. thaliana*	Transgenic *N. benthamiana* and *A. thaliana*	*MpC002, MpPIntO1 (Mp1), MpPIntO2 (Mp2)*	dsRNA	710 bp, 263 bp, 254 bp	Silencing of *MpC002* and *MpPIntO2* reduced nymph production.	[33]
	Myzus persicae	*N. tabacum, A. thaliana,* and *N. benthamiana*	Transgenic *N. tabacum, A. thaliana*, and *N. benthamiana*	*Mp55*	dsRNA	>900 bp	Reduced aphid reproduction.	[34]
	Myzus persicae	*A. thaliana*	Transgenic *A. thaliana*	*Rack1*, *MpC002, MpPIntO2 (Mp2)*	dsRNA	309 bp, 710 bp, 254 bp	Reduced aphid reproduction.	[35]
	Myzus persicae	*A. thaliana*	Transgenic *A. thaliana*	Cuticular protein *MyCP*	dsRNA	327 bp	Attenuation of fecundity in aphids.	[43]
	Myzus persicae	Tomato	Agrobacterium-mediated transformation and transgenic tomato	Acetylcholinesterase 1 (*Ace 1*)	dsRNA	571 bp	Silenced the target gene (*Ace 1*) and inhibited fecundity.	[36]
	Myzus persicae	Tobacco	Injection and feeding on transgenic tobacco	Cysteine protease Cathepsin B3 (*CathB3*)	dsRNA	230 bp	Improved the performance of non-tobacco-adapted lineages on tobacco.	[37]
	Myzus persicae	Tobacco	Plastid-mediated RNA interference and transgenic tobacco	*MpDhc64C*	dsRNA	269 bp	Reduced insect survival, impaired fecundity, and decreased weight of survivors.	[38]
HIGS	*Sitobion avenae*	Wheat	Particle bombardment method and transgenic wheat	Carboxylesterase (*CbE E4*)	dsRNA	350 bp	Suppressed *CbE E4* expression impaired *S. avenae* larval tolerance of phoxim insecticides.	[18]
	Sitobion avenae	Wheat	Particle bombardment method and transgenic wheat	Lipase maturation factor 2-like gene, *lmf2-like*	dsRNA	543 bp	Reductions in molting number, survival, and reproduction.	[39]
	Sitobion avenae	Wheat	Particle bombardment method and transgenic wheat	Chitin synthase 1 (*CHS1*)	dsRNA	550 bp	Decreased *CHS1* expression level and reduced total and molting aphid numbers.	[40]
	Sitobion avenae	Wheat	Particle bombardment method and transgenic wheat	Gq protein alpha subunit (*Gqα*)	dsRNA	517 bp	Reduced reproduction and molting in aphids.	[41]
	Sitobion avenae	Wheat	Particle bombardment method and transgenic wheat	Zinc finger protein (*SaZFP*)	dsRNA	198 bp	High mortality and decreased fecundity.	[42]

Table 1. *Cont.*

Types of Gene Silencing	Aphid Species	Plant Species	Delivery Strategy	Target Genes	Molecule	Size	Main Effects	Reference
SIGS	*Aphis glycines*		Aerosolized siRNA-nanoparticle delivery method	Carotene dehydrogenase (*tor*), branched-chain amino acid transaminase (*bcat*)	siRNA	25 nt	Knockdown of target genes.	[44]
	Aphis glycines		Nanocarrier-based dsRNA delivery system	*TREH*, *ATPD*, *ATPE*, and *CHS1*	dsRNA	431 bp, 504 bp, 536 bp, 429 bp	Silenced target gene expression and led to high mortality.	[45]
	Acyrthosiphon pisum		Aerosolized siRNA-nanoparticle delivery method	Carotene dehydrogenase (*tor*), branched-chain amino acid transaminase (*bcat*)	siRNA	25 nt	Knockdown of target genes.	[44]
	Sitobion avenae	Barley	Spraying	Structural sheath protein (*SHP*)	dsRNA	491 bp	Reduced *shp* expression level.	[46]
	Sitobion avenae	Barley	Spraying and feeding	Macrophage migration inhibitory factors, *SaMIF1*, *SaMIF2*, and *SaMIF3*	dsRNA	223 bp, 323 bp, 212 bp	Feeding on artificial diet led to high mortality rates; feeding from barley seedlings sprayed with naked *SaMIF*-dsRNAs did not alter nymph survival.	[47]
	Schizaphis graminum		Aerosolized siRNA-nanoparticle delivery method	Carotene dehydrogenase (*tor*) and branched-chain amino acid transaminase (*bcat*)	siRNA	25 nt	Knockdown of target genes.	[44]
SIGS	*Schizaphis graminum*	Wheat	Nanocarrier-mediated transdermal dsRNA delivery system	*Sg2204*	dsRNA	/	Induced a stronger wheat defense response and resulted in negative impacts on aphid feeding behavior, survival, and fecundity.	[48]
Other delivery method	*Aphis citricidus*		Feeding and citrus stem dipping	Insulin receptor genes *AcInR1* and *AcInR2*	dsRNA	511 bp, 609 bp	Developmental defects and co-silencing of *AcInR1* and *AcInR2* resulted in high mortality.	[49]
	Aphis citricidus		Feeding and citrus stem dipping	Acetylcholinesterase, *TcAChE1*, and *TcAChE2*	dsRNA	435 bp, 421 bp	High mortality and increased the susceptibility of *A. citricidus* to malathion and carbaryl.	[50]
	Aphis citricidus		Feeding and citrus stem dipping	Vitellogenin (*AcVg*), Vitellogenin receptor (*AcVgR*)	dsRNA	557 bp, 577 bp	Slower embryonic development and fewer newborn nymphs.	[51]
	Aphis citricidus		Feeding and citrus stem dipping	*AcCP19*	dsRNA	183 bp	Induced target gene silencing and high mortality.	[52]
	Aphis citricidus		Feeding and citrus stem dipping	*AcGNBP1*	dsRNA	431 bp	Decreased the activity of immune-related phenoloxidase.	[53]
	Aphis glycines		Topical application, nanocarrier, and detergent-mediated transdermal delivery system	Hemocytin, *Hem*	dsRNA	555 bp	Reduced the target gene expression and aphid population density.	[54]
	Aphis gossypii		Feeding	Carboxylesterase *CarE*	dsRNA	686 bp	Decreased resistance to organophosphorus insecticides.	[55]

Table 1. *Cont.*

Types of Gene Silencing	Aphid Species	Plant Species	Delivery Strategy	Target Genes	Molecule	Size	Main Effects	Reference
	Aphis gossypii		Feeding	Cytochrome P450 monooxygenase gene *CYP6A2*	dsRNA	773 bp	Increased sensitivity to spirotetramat and alpha-cypermethrin.	[56]
	Aphis gossypii		Feeding	Odorant-binding proteins *AgOBP2*	dsRNA	434 bp	Interfered with the odorant perception of aphids.	[57]
	Aphis gossypii		Feeding	*CYP6CY14*	dsRNA	459 bp	Increased the resistant aphid's susceptibility to thiamethoxam.	[58]
	Aphis gossypii		Feeding	*CYP380C6*	dsRNA	436 bp	Increased the sensitivity of the resistant adults and nymphs to spirotetramat.	[59]
	Aphis gossypii		Feeding	*dsCYP6DC1*, *dsCYP6CY14*, and *dsCYP6CZ1*	dsRNA	494 bp, 499 bp, 499 bp	Increased the *Ace-R* strain's sensitivity to acetamiprid.	[60]
	Aphis gossypii		Feeding	Ecdysone receptor (*EcR*)	dsRNA	486 bp	Increased mortality rates and decreased longevity and fecundity.	[61]
	Aphis gossypii		Injection	Crustacean cardioactive peptide (*ApCCAP*), crustacean cardioactive peptide receptor (*ApCCAPR*)	dsRNA	339 bp, 519 bp	Developmental failure during nymph–adult ecdysis.	[62]
	Acyrthosiphon Pisum		Injection	*C002*	siRNA	21-23 nt	Decreased *C002* transcript level.	[63]
	Acyrthosiphon Pisum		Injection	Calreticulin, *cathepsin-L*	dsRNA	434 bp, 353 bp	Induced target gene silencing.	[64]
	Acyrthosiphon Pisum		Feeding	*ApAQP1*	dsRNA	451 bp	Knocked down the *ApAQP1* expression level, resulting in elevated hemolymph osmotic pressure.	[65]
Other delivery method	*Acyrthosiphon Pisum*		injection	*vATPase*	dsRNA	185 bp	Induced high levels of mortality.	[66]
	Acyrthosiphon Pisum		Feeding	*Hunchback*	dsRNA	524 bp, 497 bp	Reduced *Aphb* transcripts and increased insect lethality.	[67]
	Acyrthosiphon pisum		Injection and feeding	Enzyme *Cathepsin-L*	dsRNA	357 bp	Induced lethal effects.	[68]
	Acyrthosiphon pisum		Injection	*ACYPI39568*	dsRNA	246 bp	Reduced *ACYPI39568* expression level but did not affect the survival rate.	[69]
	Acyrthosiphon Pisum		Injection	Angiotensin-converting enzymes *ACE1*, *ACE2*	dsRNA	313 bp, 468 bp	Knockdown of *ACE1* and *ACE2* caused a higher mortality rate.	[70]
	Acyrthosiphon Pisum		Injection	*ApMIF1*	dsRNA	213 bp	Disturbed their ability to feed from phloem sap.	[71]
	Acyrthosiphon Pisum		Injection	*Armet*	dsRNA	286 bp	Disturbed feeding behavior and led to a shortened life span.	[72]
	Acyrthosiphon Pisum		Injection	Structural sheath protein (*SHP*)	dsRNA	491 bp	Disrupted sheath formation, prevented efficient long-term feeding from sieve tubes, and had a silencing effect on reproduction but not survival.	[73]

Table 1. *Cont.*

Types of Gene Silencing	Aphid Species	Plant Species	Delivery Strategy	Target Genes	Molecule	Size	Main Effects	Reference
Other delivery method	*Acyrthosiphon Pisum*		Injection	Peroxiredoxins, *ApPrx1*	dsRNA	206 bp	Decreased survival rate.	[74]
	Acyrthosiphon Pisum		Injection and ingestion	Cytochrome P450 gene, *CYP4G51*	dsRNA	310 bp, 325 bp	Reduced *CYP4G51* expression, caused reductions in internal and external long-chain hydrocarbons (HCs), and increased mortality.	[75]
	Acyrthosiphon Pisum		Injection	Odorant receptors, *ApisOR5*, odorant-binding proteins, *ApisOBP3*, and *ApisOBP7*	dsRNA	/	The repellent behavior of *A. pisum* to EBF disappeared.	[76]
	Acyrthosiphon Pisum		Feeding	Cuticular protein, *Stylin-01*, *Stylin-02*	siRNA	19 bp	Silencing *stylin-01* decreased the efficiency of cauliflower mosaic virus transmission by *M. persicae*.	[77]
	Acyrthosiphon Pisum		Injection	Neuropeptide F (*NPF*), NPF receptor (*NPFR*)	dsRNA	232 bp, 354 bp	Reduced aphid food intake and indicated a lower appetite for food after *NPF* silencing.	[78]
	Acyrthosiphon Pisum		Feeding	*amiD*, *ldcA1*	dsRNA	311 bp, 353 bp	Reduction in *Buchnera* abundance and activity was accompanied by depressed aphid growth rates.	[19]
	Acyrthosiphon Pisum		Injection	Gap gene *Hunchback*	dsRNA	448 bp	Knockdown of target gene.	[79]
	Acyrthosiphon Pisum		Injection and ingestion	Chitin synthase, *CHS*	dsRNA	364 bp	Induced mortality and development deformity.	[80]
	Acyrthosiphon Pisum		Injection	*ApHRC*	dsRNA	263 bp	Serratia-infected aphids displayed shorter phloem-feeding durations and caused Ca^{2+} elevation and ROS accumulation in plants.	[81]
	Acyrthosiphon pisum		Feeding and bean stem dipping	Cuticle protein gene, *ApCP19*	dsRNA	216 bp	Induced target gene silencing and high mortality.	[52]
Other delivery method	*Acyrthosiphon pisum*		Feeding and bean stem dipping	Carotenoid desaturase, *CdeB*	dsRNA	431 bp	Reduced aphid performance and altered the age structure of the population.	[82]
	Acyrthosiphon pisum		Feeding and bean stem dipping	Gram-negative binding proteins, *ApGNBP1*, *ApGNBP2*	dsRNA	550 bp, 518 bp	Decreased the activity of immune-related phenoloxidase.	[53]
	Acyrthosiphon Pisum		Injection	CCHamide-2 receptor (*CCHa2-R*)	dsRNA	478 bp	Reduced *CCHa2-R* expression, food intake in adult aphids, and reproduction but not survival.	[83]
	Acyrthosiphon Pisum		Injection	Fatty acid synthase 1 (*FASN1*) and diacylglycerol-o-acyltransferase 2 (*DGAT2*)	dsRNA	609 bp, 388 bp	Prolonged the nymphal growth period and decreased the aphid body weight.	[84]
	Acyrthosiphon pisum		Injection and nanocarrier delivery	*flightin*	dsRNA	374 bp	Malformed wings, deformed dorsal longitudinal muscle (*DLM*) shapes, and wider and looser dorsoventral flight muscles (*DVMs*) were observed.	[85]

Table 1. *Cont.*

Types of Gene Silencing	Aphid Species	Plant Species	Delivery Strategy	Target Genes	Molecule	Size	Main Effects	Reference
	Eriosoma lanigerum Hausmann		Topical application and nanocarrier-mediated transdermal dsRNA delivery system	V-ATPase subunit D (*ATPD*)	dsRNA	/	Induced target gene silencing and led to high mortality.	[86]
	Myzus nicotianae		Feeding	*TRV-ALY*, *TRV-Eph*	dsRNA	182 bp, 249 bp	Inhibition of target genes.	[87]
	Myzus persicae		Injection	*MpMIF1*	dsRNA	205 bp	Disturbed their ability to feed from phloem sap.	[71]
	Myzus persicae		Foliar application	*ZYMV HC-Pro*	dsRNA	588 bp	Insects successfully took up dsRNA; the dsRNA was processed into siRNA by the insect RNAi machinery.	[88]
	Myzus persicae		Feeding	Cuticular protein, *Stylin-01*, *Stylin-02*	siRNA	19 bp	Silencing *stylin-01* decreased the efficiency of cauliflower mosaic virus transmission by *Myzus persicae*.	[77]
Other delivery method	*Myzus persicae*		Feeding	Voltage-gated sodium channel *MpNav*	dsRNA	289 bp	Induced high mortality and lower fecundity and longevity.	[89]
	Myzus persicae		Feeding and *Brassica* stem dipping	*MpCP19*	dsRNA	139 bp	Induced target gene silencing and high mortality.	[52]
	Myzus persicae		Feeding and *Brassica* stem dipping	*MpGNBP1*	dsRNA	450 bp	Decreased the activity of immune-related phenoloxidase.	[53]
	Myzus persicae		Feeding	*Mp58*, *OBP2*	dsRNA	423 bp, 428 bp	Induced high mortality.	[90]
	Myzus persicae		Topical and root applications and nanocarrier-mediated delivery system	Vestigial (*vg*), Ultrabithorax (*Ubx*)	dsRNA	489 bp, 359 bp	Downregulated target genes and caused wing aberration.	[91]
	Myzus persicae		Injection	ATP-binding cassette transporter gene (*ABCG4*), DnaJ homolog subfamily C member 1 (*DnaJC1*)	dsRNA	~400 bp	Increased mortality rate.	[92]
	Megoura viciae		Injection	Tyrosine hydroxylase *MV-TH*	dsRNA	400 bp	Reduced the *L-DOPA* level in aphids and a slight decrease in exuvia tanning.	[93]
	Rhopalosiphum padi		Injection	Acetylcholinesterase gene *RpAce1*	dsRNA	383 bp	Increased susceptibilities to pirimicarb and malathion in *R. padi* and reduced fecundity.	[94]
	Sitobion avenae		Feeding	Catalase *CAT*	dsRNA	471 bp	Reduced survival rate and ecdysis index.	[95]
	Sitobion avenae		Feeding	Unigenes *DSR8*, *DSR32*, *DSR33*, *DSR48*	dsRNA	162 bp, 411 bp, 439 bp, 397 bp	Downregulation of target genes and aphid mortality.	[96]
	Sitobion avenae		Injection	Acetylcholinesterase gene *SaAce1*	dsRNA	400 bp	Increased susceptibility to pirimicarb in *S. avenae* and reduced fecundity.	[94]
	Sitobion avenae		Feeding	Ecdysone receptor (*SaEcR*), ultraspiracle protein (*SaUSP*)	dsRNA	469 bp, 411 bp	Significantly decreased the survival of aphids.	[97]

Table 1. *Cont.*

Types of Gene Silencing	Aphid Species	Plant Species	Delivery Strategy	Target Genes	Molecule	Size	Main Effects	Reference
Other delivery method	*Sitobion avenae*		Feeding	*Laccase 1*, *SaLac 1*	dsRNA	613 bp	Inhibited the transcript levels of *SaLac 1* and decreased the survival rate.	[98]
	Sitobion avenae		Feeding	Odorant-binding protein (*SaveOBP9*)	dsRNA	501 bp	Reduced *SaveOBP9* expression and induced a nonsignificant response in *S. avenae* to tetradecane, octanal, decanal, and hexadecane.	[99]
	Sitobion avenae		Feeding	Odorant-binding protein (*SaveOBP10*)	dsRNA	432 bp	Aphids exhibited nonattraction towards β-caryophyllene and a nonsignificant behavioral response to pentadecane, butylated hydroxytoluene, and tetradecane.	[100]
	Schizaphis graminum		Feeding	*SgC002*	siRNA	476 bp	Feeding on artificial diet for 3 days followed by transfer to aphid-susceptible wheat suppressed *SgC002* expression and led to lethality.	[101]
	Schizaphis graminum		Feeding	*MRA*, *GAT*, *TLP*	dsRNA	376 bp, 433 bp, 422 bp	Increased susceptibility to imidacloprid.	[102]
	Sitobion miscanthi		Topical application and nanocarrier-mediated transdermal dsRNA delivery system	*Sm9723*	dsRNA	/	Decreased fecundity and survival and negatively affected the feeding behavior.	[103]

Note: HIGS: host-induced gene silencing. SIGS: spray-induced gene silencing.

Many studies of HIGS focused on *M. persicae* through various transgenic plants, for example, *Arabidopsis thaliana*, *Nicotiana benthamiana*, and *Solanum lycopersicon*. Some salivary effectors have been identified in aphids, such as MpC002, MpPIntO1 (Mp1), MpPIntO2 (Mp2), and Mp55. The knockdown of these genes reduced the reproduction of aphids, which indicated that these effectors could be selected as potential RNAi targets [17,33,34]. Rack-1 is a conserved multifunctional scaffold protein that was identified as a luteovirus-binding protein in peach aphids. The knockdown of *Rack-1* reduced the fecundity of peach aphids [17]. Based on previous studies of *Rack1*, *MpC002*, and *MpPIntO2*, the persistence and transgenerational effects of plant-mediated RNAi were also investigated through transgenic *Arabidopsis* [35]. Transgenic tomato plant mediated RNAi has been shown to effectively silence the Acetylcholinesterase 1 (*Ace1*) gene and reduce the fecundity of peach aphids when fed transgenic plants [36]. A study reported that the knockdown of the cysteine protease Cathepsin B3 (*CathB3*) gene improved the performance of a non-tobacco-adapted (NTA) aphid lineage on tobacco. *CathB3* elicited host defenses to suppress phloem sap ingestion by the aphid [37]. Plastid-mediated RNA interference (RNAi) was successfully employed to silence *MpDhc64C*. Both transgenic and transplastomic tobacco plants exhibited significant resistance to peach aphids, as demonstrated by decreased survival fecundity and survivor weight [38].

Most of the studies on *S. avanae* were applied by wheat-mediated HIGS. A particle-bombardment-mediated wheat transformation method was used to obtain stable transgenic wheat plants. Feeding on transgenic wheat expressing the carboxylesterase (*CbE E4*) gene

could suppress the expression level of *CbE E4* in grain aphids and impair larval tolerance to phoxim insecticides [18]. Silencing the lipase maturation factor 2-like (*lmf2-like*) gene reduced the molting number and decreased the survival and reproduction of aphids [39]. Similarly, the knockdown of the Chitin synthase 1 (*CHS1*) gene reduced the molting and survival of aphids [40]. Silencing the G protein (*Gqα*) gene could also reduce reproduction and molting in grain aphids [41]. Silencing the zinc finger protein (*SaZFP*) gene led to high mortality and decreased fecundity of grain aphids. The transgenerational silencing effect was investigated in the successive first to fourth generations [42].

2.3. Spray-Induced Gene Silencing

Although transgenes are convenient, they are not required for ectopic gene silencing activation in pathogens or pests. According to some research, eukaryotic pests and pathogens, including fungi and nematodes, are able to take up RNAs from the environment [104–106]. This phenomenon was defined as 'Environmental RNAi', in which the transferred RNAs complemented to the sequence of target genes in the organism can induce highly effective target gene silencing [104,107]. These studies prompted the development of spray-induced gene silencing (SIGS). In spray-induced gene silencing, dsRNAs or sRNAs that target pathogen or pest genes are sprayed directly onto plants. Then, these RNAs move into the pest or pathogen cells and silence target genes [106,108].

The first evidence of the exogenous application of dsRNA for pest control was in citrus and grapevine trees, in which dsRNA targeting the arginine kinase gene was used to control psyllids and sharpshooter pests [109]. *Fusarium graminearum* development in barley leaves was suppressed by spraying dsRNA to target the fungal cytochrome P450, establishing the feasibility of spray-induced gene silencing (SIGS) [110]. Moreover, the potential non-transgenic, spray-based exogenous dsRNA or sRNA (SIGS) application has been widely used to decrease disease in crop plants [111–114].

2.4. Spray-Induced Gene Silencing Based Aphid Control

The delivery of siRNA and dsRNA via nanoparticle carriers is a novel strategy that has been successfully applied in some insect systems [115–117]. The majority of SIGS-based studies employed nanocarrier delivery systems for aphid control (Table 1).

tor is a carotene dehydrogenase gene that plays an important role in pigmentation in *A. pisum*. The branched-chain amino acid transaminase (*bcat*) gene is important in branched-chain amino acid metabolism in aphids. An aerosolized siRNA-nanoparticle delivery strategy induced a modest *tor* gene knockdown in *A. pisum* and a *bcat* gene knockdown in *Aphis glycines* as well as the associated phenotype. These results indicated that the aerosolized siRNA-nanoparticle method was an effective RNAi delivery system [44].

According to previous studies, Yan et al. [45] selected the *soluble trehalase* (*TREH*), *V-type proton ATPase subunit D* (*ATPD*), *V-type proton ATPase subunit E* (*ATPE*), and *chitin synthase 1* (*CHS1*) genes as RNAi target genes to test the silencing effect in *A. glycines* [66,115,118,119]. This study indicated that *A. glycines* exhibited higher mortality when it fed on soybean seedlings sprayed with a dsATPD + dsCHS1 nanoparticle formulation. They also demonstrated that a water-soluble cationic dendrimer (nanocarrier) was an efficient gene carrier [45].

Biedenkopf et al. [46] reported that the application of dsRNA to detached barley leaves resulted in the effective SIGS of the sheath protein (*Shp*) gene in grain aphids. Systemic RNAi was also observed in *Hordeum vulgare* after a spray treatment in which sprayed dsRNA moved from barley leaves to stems and root tissues. This research contributed significantly to understanding the mechanism of RNA spray technology, especially for SIGS. However, another study in barley suggested that grain aphids fed barley seedlings sprayed with naked *SaMIF*-dsRNAs did not affect the survival of nymphs, which indicated that aphids were unable to absorb dsRNA from these plants [47]. A recently published paper reported that the SIGS-based nanocarrier-mediated dsRNA delivery system effectively silenced the putative salivary effector Sg2204 in *Schizaphis graminum* and its homologs from four other aphid species. Aphids with silenced *Sg2204* exhibited a stronger defense

response, and the treatment induced a negative impact on aphid survival, fecundity, and feeding behavior [48].

2.5. Other Delivery-Method-Mediated Gene Silencing for Aphid Control

Microinjection is an efficient and widely used research method for delivering dsRNAs. The first evidence of successful dsRNA microinjection was applied to silence the *frizzled* and *frizzled 2* genes in *Drosophila melanogaster* embryos by injecting their corresponding dsRNAs [120]. Since then, microinjection has become a potential method for delivering dsRNA into various insect species. This method was reported to apply in many aphid species, namely *A. gossypii*, *A. pisum*, *M. persicae*, and *S. avenae* (Table 1). The injection of siRNA-*C002* into pea aphids decreased the transcription level of *C002* [63]. Injections of dsRNAs of different aphid genes that play important roles in aphid sheath formation (*SHP)* [73], cuticular waterproofing (*CYP4G51)* [75], (E)-b-farnesene (EβF) reception (*ApisOR5*, *ApisOBP3,* and *ApisOBP7)* [76], chitin biosynthesis (*CHS)* [80], molting (*ApCCAP* and *ApCCAPR)* [62], flight musculature formation, and wing extension (*flightin)* [85] induced effective target gene silencing.

Feeding was another basic delivery method for aphids because of its less laborious and easier operation. Aphids fed a diet containing synthetic dsRNA were more appliable for target gene knockdown. It was first reported that feeding on *E. coli* bacteria expressing dsRNA in *C. elegans* conferred silencing effects on the nematode larvae [121]. In *Aphis citricidus*, RNAi was performed by feeding dsRNAs of target genes with citrus leaf through stem dipping. Acetylcholinesterase (*AChE*) is an important gene targeted by insecticides based on organophosphates and carbamates. The silencing of two aphid *AChE* genes, *Tcace1* and *Tcace2*, increased susceptibility to malathion and carbaryl insecticides. Furthermore, *Tcace1* silencing resulted in higher aphid mortality than *Tcace2* silencing, which indicated that *TcAChE1* was essential for *A. citricidus* postsynaptic neurotransmission [50]. A knockdown of Vitellogenin (*Vg*) and its receptor (*VgR*) had a negative impact on embryonic and postembryonic development, which led to nymph–adult transition delay, a longer pre-reproductive period, and a shorter reproductive period [51]. Cuticle protein is a primary target in insect development and molting. The silencing of the cuticle protein 19 (*CP19*) gene in *A. citricidus* led to aphid mortality [52]. Similarly, aphids fed dsRNA of a Gram-negative binding protein gene (*AcGNBP1*) caused target gene silencing and high mortality [53]. The same delivery strategy was applied in *A. pisum*. Different dsRNAs were fed with bean leaves through stem dipping. The silencing of the *CP19* gene in pea aphids also led to high mortality [52]. Parental silencing of the carotenoid desaturase gene (*CdeB*) reduced the intensity of the body color in vivo in the treated aphids and subsequent generations and negatively affected aphid performance [82]. The silencing of *ApGNBP1* but not *ApGNBP2* in *A. pisum* decreased immune-related phenoloxidase activity [53]. Feeding on *Brassica* leaves inserted into a solution containing *MpCP19* and *MpGNBP1* dsRNAs also induced effective target gene silencing [52,53]. With the aim of decreasing insecticide use and eliminating pesticide-resistant evolved populations, RNAi has also been used to increase the susceptibility of aphids to insecticides. A study reported that *RpAce1* suppression increased the susceptibility to pirimicarb and malathion in *Rhopalosiphum padi*. Silencing *SaAce1* also increased *S. avenae* susceptibility to pirimicarb [94].

It has also been demonstrated that mechanical inoculation can help deliver dsRNA and induce RNAi by spreading dsRNA with soft sterile brushes and gentle rubbing inoculation [122,123]. The molecules were rapidly absorbed by tomato plants and were ingested by peach aphids (*M. persicae*) when the tomato leaves were gently rubbed with dsRNA solution [88]. With the use of a nanocarrier and detergent, a novel dsRNA formulation was exploited, which can quickly penetrate through the body wall of *A. glycines* and effectively suppress gene expression. This suggests that transdermal dsRNA delivery could be developed as a potential SIGS-based aphid control strategy. Hemocytin (*Hem*) is an important factor in the hemocytes and fat bodies of insects, which might regulate aphid population density. When spreading a dsRNA-*HEM* nanocarrier/detergent formulation on *A. glycines*,

the expression level of *hemocytin* was efficiently silenced, which impaired the survival and fecundity of aphids and suppressed aphid population growth [54]. Another study also investigated the RNAi efficacy of the *ATPD* gene in woolly apple aphids (*Eriosoma lanigerum*) via a nanocarrier-mediated transdermal dsRNA delivery system. Their results suggested that the interference efficiency was greatly increased using nanocarriers and induced high aphid mortality [86]. The nanocarrier-delivered RNAi method was also used to silence the *flightin, vestigial* (*vg*), and *Ultrabithorax* (*Ubx*) genes, which suppressed the wing development in *M. persicae* [85,91]. In *Sitobion miscanthi*, the putative salivary effector Sm9723 was effectively silenced via a nanocarrier-mediated transdermal dsRNA delivery system. The fecundity and survival of *S. miscanthi* dramatically decreased after *Sm9723* silencing, and the aphid feeding behavior was also impaired [103].

3. Challenges for Enhancing RNA Interference Efficiency

3.1. Target Gene Selection

The selection of the target gene is essential to the successful application of RNAi-based insect control. RNAi efficiency varies considerably among different insect species for the same transcripts [26,124]. The efficiency can vary in the same species with different transcripts, genotypes, and tissues, even among the same transcript from different areas [26,125–129]. The ideal RNAi gene target must be essential for insect survival and highly expressed and should not have functional redundancy [130,131]. Therefore, potential target genes should be thoroughly investigated for the capacity to suppress specific transcripts and the ability to cause mortality to enhance the efficiency of RNAi-based pest control.

RNAi targets are initially selected based on the discovery of key genes in other organisms or by cDNA library screening. Numerous studies have indicated that genome-wide screens of high-sensitivity target genes are effective in RNAi. Other high-throughput approaches, such as RNA-seq and digital gene expression tag profiles (DGE-tag), were used in the Asian corn borer (ACB; *Ostrinia furnacalis*) to identify potential RNAi targets [132]. The expression profiling and transcriptome reconstruction of an increasing number of insects have been made possible by second-generation sequencing. High-throughput screens such as feeding assays [66] and the topical application of dsRNA [44,54,132] are also powerful tools to identify potential RNAi targets. With the available databases growing, tissue-specific and developmental-stage-specific expression profiles of insects may narrow down candidate pools for target gene selection. After identifying candidate genes, screening for dsRNA-induced mortality is necessary to evaluate the capacity of specific dsRNAs to induce the desirable phenotype. The potential for the candidate dsRNA sequences to cause mortality at various stages of life can be examined in further experiments. Targeting multiple genes, dsRNA concatemerization, or using different dsRNA structures can all be performed to improve the efficiency of RNAi [133–136].

3.2. Length of dsRNA

In some insect species, the uptake and silencing efficiency of RNAi are determined by the length of the expressed dsRNA. Different insect species require different minimum lengths of dsRNA to achieve maximal RNAi silencing [137]. In *Tribolium castaneum*, an analysis revealed that the dsRNA length had a significant impact on the effectiveness of the RNAi response. Longer dsRNA is proving to be more effective at suppressing gene expression. The desired interference requires a minimum length of 70 nucleotides [138]. The length of dsRNA sequences between 139 bp and 773 bp was used in the majority of the aphid feeding experiments to obtain successful RNAi (Table 1).

As we described above, siRNA injections were able to suppress the target gene (*C002*) expression in pea aphids, which dramatically reduced aphid survival [63]. In grain aphids, RNAi targeting the sheath protein (*SHP*) gene with transgenic barley plants expressing a 491 bp *shp*-dsRNA strongly inhibited the feeding and reproductive behavior of grain aphids and negatively impacted their survival [31]. Gq proteins play critical roles in insect cellular signal transduction. The downregulation of the *Gqα* gene with a 540 bp fragment

of dsRNA resulted in decreases in the fecundity and molting rate [41]. A 198 bp *dsSaZFP* fragment could induce target gene silencing in grain aphids when feeding on transgenic wheat plants, resulting in decreased reproduction and survival rates [42].

Therefore, both short and long dsRNAs effectively induce gene silencing, depending on the target pests and genes. Longer dsRNAs may increase the possibility of off-target effects on beneficial organisms due to the generation of potentially large siRNA pools. Accordingly, RNAi efficiency will be improved by selecting the optimal lengths of target-specific RNAi targets combined with effective siRNA analysis [9].

3.3. Delivery of dsRNA

Various dsRNA delivery methods, including microinjection, feeding, soaking, HIGS mediated by transgenic plants, and SIGS mediated by spraying, have been applied in pest management. As we discussed before, microinjection and feeding are the two basic delivery methods. The soaking delivery method was usually applied in insect cell lines via adding dsRNA directly into the cell culture medium [139,140], and some studies have investigated topically applied dsRNA/siRNA formulations penetrating into the insect cuticles to induce mortality [13,141–145]. Transgenic plants expressing dsRNA or siRNA have lots of advantages for pest control [146]. The SIGS-mediated delivery method does not require plant genetic engineering. dsRNAs/siRNAs are applied topically to the plant surface via spraying in this silencing type [106].

To improve dsRNA delivery efficiency, various new technologies have been exploited, such as cationic-liposome-assisted and nanoparticle-enabled methods. The application of RNAi in conjunction with nanotechnology may develop as a more environmentally friendly approach to pest control. In the first investigation of nanoparticle-mediated dsRNA delivery, chitosan was used to silence the chitin synthase genes in *Anopheles gambiae*, and the RNAi effectiveness was found to be enhanced [115]. Short interfering RNA (siRNA)–nanoparticle complexes, peptide nanomaterial branched amphiphilic peptide capsules (BAPCs), and nanocarrier-based transdermal dsRNA delivery systems were demonstrated to be successful for aphid RNAi, which could efficiently silence gene expression [44,45,54,147].

3.4. The Stability of dsRNA

RNAi stability and efficiency vary drastically depending on the length and concentration of the dsRNA, the delivery method and technique, plant-organ-specific processes, insect life stage, target gene selection, and adverse environmental conditions [145,148,149]. Environmental microorganisms can degrade dsRNA before it is consumed by pathogens or pests. Nucleases in pest saliva, the gut lumen, and hemolymph may also rapidly degrade dsRNA [19,127,150–153].

The stability of dsRNA in the insect gut is critical for a successful RNAi response, and increased nuclease expression can result in dsRNA degradation and subsequent RNAi failure [154]. The activity of gut nucleases can be impacted by the high or low pH present in the gut lumena of particular pests, which can directly or indirectly decrease dsRNA stability [155]. Some strategies have already been exploited to improve the stability of dsRNA. For example, the nanoparticle-mediated dsRNA delivery system was demonstrated to be efficient in increasing dsRNA stability and efficacy, and has been applied to improve the penetration and persistence of dsRNA into plants or insects [9,149,156,157].

3.5. Nontarget and Off-Target RNAi Effects

Silencing nontarget genes in the same or nontarget organisms has resulted in off-target effects [158–160]. To improve RNAi efficiency and minimize off-target effects, species-specific or tissue-specific dsRNA could be selected. A study reported that the silencing of *V-ATPase* genes in *A. pisum*, *D. melanogaster*, *M. sexta*, and *T. castaneum* was observed without affecting nontarget species using species-specific dsRNA [66].

To design efficient and potent RNAi targets, various web-based computational design approaches have been exploited to minimize potential off-target effects. For example, pssRNAit was developed to design specific and effective siRNAs [161]. Further assessments were applied to selected sequences using software, for example, ERNAi [162], dsCheck [163], and basic local alignment search tool (BLAST) [164] analysis against the transcriptomic datasets of human and beneficial insects [9].

4. Conclusions and Perspectives

During the past few years, RNAi has developed as a promising, valuable, and effective technique for functional genomic studies. Various RNAi-based approaches have been applied in crop protection for species-specific and ecofriendly pest management. In this review, we summarize the present studies on numerous strategies exploited against different aphid species.

Growing evidence suggests that HIGS-based and SIGS-based crop protection against pests is effective. Transgenic plants appear to be a more beneficial approach to enhancing RNAi effects. Nevertheless, a lack of transformation technology in several crop species has restricted the widespread application of HIGS. Furthermore, they are still regarded as genetically modified (GM) products in many countries, requiring a thorough assessment of the plants before being licensed. The development of transplastomic technology was also restricted by the extensive regulatory process. Global applications of HIGS are limited by public concern over the biosafety of genetically modified organisms (GMOs) [165,166]. Using optimized target gene and fragment selection strategies, more effective transformation constructs, and stable transgenic systems, the major challenges for the HIGS strategy will be overcome [167]. SIGS, in comparison to HIGS, does not produce GMOs. However, it has become clear that the instability of naked dsRNA is a significant limitation of SIGS, resulting in a relatively short period of protection. In order to address this issue and improve the insecticidal activity of non-transformative RNAi products, SIGS-based dsRNAs affiliated with different types of nanoparticles would be an efficient technique [168–174]. These prospective strategies may decrease the cost and improve the dependability of the present delivery techniques. They may also create new opportunities to study the roles of important genes. Another consideration for RNAi application is to exclude potential off-target effects and effects on nontarget organisms. To support the biosafety claims of RNAi applications, a combination of bioinformatics and ecological bioassays using selected target species is essential.

With the development of new technology, clustered regularly interspaced short palindromic repeat/CRISPR-associated endonuclease Cas9 (CRISPR/Cas9)-based genome editing had been reported in *Spodoptera exigua* [175], *Helicoverpa armigera* [176–178], *S. litura* [179,180], and *Nilaparvata lugens* [181,182]. However, many of these studies have focused on insect genomic functions. Further study is needed to exploit genome editing as a viable strategy to create resistant varieties against numerous insect pests and enhance pest resistance in crops [183].

Overall, by obtaining a deep understanding of the RNAi machinery and the development of various dsRNA delivery strategies, RNAi will be more effectively used in aphid control for crop protection.

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References

1. Budzinski, H.; Couderchet, M. Environmental and human health issues related to pesticides: From usage and environmental fate to impact. *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 14277–14279. [CrossRef] [PubMed]
2. Luo, K.; Zhao, H.; Wang, X.; Kang, Z. Prevalent pest management strategies for grain aphids: Opportunities and challenges. *Front. Plant Sci.* **2021**, *12*, 790919. [CrossRef] [PubMed]
3. Dang, Y.; Yang, Q.; Xue, Z.; Liu, Y. RNA interference in fungi: Pathways, functions, and applications. *Eukaryot. Cell* **2011**, *10*, 1148–1155. [CrossRef]
4. Li, Y.; Lu, J.; Han, Y.; Fan, X.; Ding, S.-W. RNA interference functions as an antiviral immunity mechanism in mammals. *Science* **2013**, *342*, 231–234. [CrossRef]
5. Nicolás, F.E.; Ruiz-Vázquez, R.M. Functional diversity of RNAi-associated sRNAs in fungi. *Int. J. Mol. Sci.* **2013**, *14*, 15348–15360. [CrossRef]
6. Bologna, N.G.; Voinnet, O. The diversity, biogenesis, and activities of endogenous silencing small RNAs in *Arabidopsis*. *Annu. Rev. Plant Biol.* **2014**, *65*, 473–503. [CrossRef]
7. Guo, Z.; Li, Y.; Ding, S.-W. Small RNA-based antimicrobial immunity. *Nat. Rev. Immunol.* **2019**, *19*, 31–44. [CrossRef]
8. Song, L.; Fang, Y.; Chen, L.; Wang, J.; Chen, X. Role of non-coding RNAs in plant immunity. *Plant Commun.* **2021**, *2*, 100180. [CrossRef]
9. Jain, R.G.; Robinson, K.E.; Asgari, S.; Mitter, N. Current scenario of RNAi-based hemipteran control. *Pest Manag. Sci.* **2021**, *77*, 2188–2196. [CrossRef]
10. Napoli, C.; Lemieux, C.; Jorgensen, R. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell* **1990**, *2*, 279–289. [CrossRef]
11. Fire, A.; Xu, S.; Montgomery, M.K.; Kostas, S.A.; Driver, S.E.; Mello, C.C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **1998**, *391*, 806–811. [CrossRef] [PubMed]
12. Dalakouras, A.; Wassenegger, M.; McMillan, J.N.; Cardoza, V.; Maegele, I.; Dadami, E.; Runne, M.; Krczal, G.; Wassenegger, M. Induction of silencing in plants by high-pressure spraying of in vitro-synthesized small RNAs. *Front. Plant Sci.* **2016**, *7*, 1327. [CrossRef] [PubMed]
13. 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]
14. Numata, K.; Ohtani, M.; Yoshizumi, T.; Demura, T.; Kodama, Y. Local gene silencing in plants via synthetic ds RNA and carrier peptide. *Plant Biotechnol. J.* **2014**, *12*, 1027–1034. [CrossRef]
15. Ghosh, S.K.B.; Hunter, W.B.; Park, A.L.; Gundersen-Rindal, D.E. Double strand RNA delivery system for plant-sap-feeding insects. *PLoS ONE* **2017**, *12*, e0171861. [CrossRef]
16. Dalakouras, A.; Jarausch, W.; Buchholz, G.; Bassler, A.; Braun, M.; Manthey, T.; Krczal, G.; Wassenegger, M. Delivery of hairpin RNAs and small RNAs into woody and herbaceous plants by trunk injection and petiole absorption. *Front. Plant Sci.* **2018**, *9*, 1253. [CrossRef]
17. Pitino, M.; Coleman, A.D.; Maffei, M.E.; Ridout, C.J.; Hogenhout, S.A. Silencing of aphid genes by dsRNA feeding from plants. *PLoS ONE* **2011**, *6*, e25709. [CrossRef]
18. Xu, L.; Duan, X.; Lv, Y.; Zhang, X.; Nie, Z.; Xie, C.; Ni, Z.; Liang, R. Silencing of an aphid carboxylesterase gene by use of plant-mediated RNAi impairs *Sitobion avenae* tolerance of Phoxim insecticides. *Transgenic Res.* **2014**, *23*, 389–396. [CrossRef]
19. Chung, S.H.; Jing, X.; Luo, Y.; Douglas, A.E. Targeting symbiosis-related insect genes by RNAi in the pea aphid-*Buchnera* symbiosis. *Insect Biochem. Mol. Biol.* **2018**, *95*, 55–63. [CrossRef]
20. Koch, A.; Kogel, K.H. New wind in the sails: Improving the agronomic value of crop plants through RNAi-mediated gene silencing. *Plant Biotechnol. J.* **2014**, *12*, 821–831. [CrossRef]
21. Christiaens, O.; Niu, J.; Taning, C.N.T. RNAi in insects: A revolution in fundamental research and pest control applications. *Insects* **2020**, *11*, 415. [CrossRef]
22. Santala, J.; Valkonen, J.P. Sensitivity of small RNA-based detection of plant viruses. *Front. Microbiol.* **2018**, *9*, 939. [CrossRef]
23. Sang, H.; Kim, J.-I. Advanced strategies to control plant pathogenic fungi by host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS). *Plant Biotechnol. Rep.* **2020**, *14*, 1–8. [CrossRef]
24. Zand Karimi, H.; Innes, R.W. Molecular mechanisms underlying host-induced gene silencing. *Plant Cell* **2022**, *34*, 3183–3199. [CrossRef] [PubMed]
25. Huang, G.; Allen, R.; Davis, E.L.; Baum, T.J.; Hussey, R.S. Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 14302–14306. [CrossRef] [PubMed]
26. Baum, J.A.; Bogaert, T.; Clinton, W.; Heck, G.R.; Feldmann, P.; Ilagan, O.; Johnson, S.; Plaetinck, G.; Munyikwa, T.; Pleau, M. Control of coleopteran insect pests through RNA interference. *Nat. Biotechnol.* **2007**, *25*, 1322–1326. [CrossRef]

27. Nowara, D.; Gay, A.; Lacomme, C.; Shaw, J.; Ridout, C.; Douchkov, D.; Hensel, G.; Kumlehn, J.; Schweizer, P. HIGS: Host-induced gene silencing in the obligate biotrophic fungal pathogen *Blumeria graminis*. *Plant Cell* **2010**, *22*, 3130–3141. [CrossRef]
28. Koch, A.; Kumar, N.; Weber, L.; Keller, H.; Imani, J.; Kogel, K.-H. Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase–encoding genes confers strong resistance to *Fusarium* species. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 19324–19329. [CrossRef]
29. Govindarajulu, M.; Epstein, L.; Wroblewski, T.; Michelmore, R.W. Host-induced gene silencing inhibits the biotrophic pathogen causing downy mildew of lettuce. *Plant Biotechnol. J.* **2015**, *13*, 875–883. [CrossRef]
30. Jahan, S.N.; Åsman, A.K.; Corcoran, P.; Fogelqvist, J.; Vetukuri, R.R.; Dixelius, C. Plant-mediated gene silencing restricts growth of the potato late blight pathogen *Phytophthora infestans*. *J. Exp. Bot.* **2015**, *66*, 2785–2794. [CrossRef]
31. Abdellatef, E.; Will, T.; Koch, A.; Imani, J.; Vilcinskas, A.; Kogel, K.H. Silencing the expression of the salivary sheath protein causes transgenerational feeding suppression in the aphid *Sitobion avenae*. *Plant Biotechnol. J.* **2015**, *13*, 849–857. [CrossRef] [PubMed]
32. 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] [PubMed]
33. Pitino, M.; Hogenhout, S.A. Aphid protein effectors promote aphid colonization in a plant species-specific manner. *Mol. Plant Microbe Interact.* **2013**, *26*, 130–139. [CrossRef] [PubMed]
34. Elzinga, D.A.; De Vos, M.; Jander, G. Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. *Mol. Plant Microbe Interact.* **2014**, *27*, 747–756. [CrossRef] [PubMed]
35. Coleman, A.D.; Wouters, R.H.; Mugford, S.T.; Hogenhout, S.A. Persistence and transgenerational effect of plant-mediated RNAi in aphids. *J. Exp. Bot.* **2015**, *66*, 541–548. [CrossRef]
36. Faisal, M.; Abdel-Salam, E.M.; Alatar, A.A.; Saquib, Q.; Alwathnani, H.A.; Canto, T. Genetic transformation and siRNA-mediated gene silencing for aphid resistance in tomato. *Agronomy* **2019**, *9*, 893. [CrossRef]
37. Guo, H.; Zhang, Y.; Tong, J.; Ge, P.; Wang, Q.; Zhao, Z.; Zhu-Salzman, K.; Hogenhout, S.A.; Ge, F.; Sun, Y. An aphid-secreted salivary protease activates plant defense in phloem. *Curr. Biol.* **2020**, *30*, 4826–4836. [CrossRef]
38. Dong, Y.; Wu, M.; Zhang, Q.; Fu, J.; Loiacono, F.V.; Yang, Y.; Wang, Z.; Li, S.; Chang, L.; Bock, R.; et al. Control of a sap-sucking insect pest by plastid-mediated RNA interference. *Mol. Plant* **2022**, *15*, 1176–1191. [CrossRef]
39. Xu, L.; Hou, Q.; Zhao, Y.; Lu, L.; Li, B.; Ni, Z.; Liang, R. Silencing of a lipase maturation factor 2-like gene by wheat-mediated RNAi reduces the survivability and reproductive capacity of the grain aphid, *Sitobion avenae*. *Arch. Insect Biochem. Physiol.* **2017**, *95*, e21392. [CrossRef]
40. Zhao, Y.; Sui, X.; Xu, L.; Liu, G.; Lu, L.; You, M.; Xie, C.; Li, B.; Ni, Z.; Liang, R. Plant-mediated RNAi of grain aphid CHS1 gene confers common wheat resistance against aphids. *Pest Manag. Sci.* **2018**, *74*, 2754–2760. [CrossRef]
41. Hou, Q.; Xu, L.; Liu, G.; Pang, X.; Wang, X.; Zhang, Y.; You, M.; Ni, Z.; Zhao, Z.; Liang, R. Plant-mediated gene silencing of an essential olfactory-related Gqalpha gene enhances resistance to grain aphid in common wheat in greenhouse and field. *Pest Manag. Sci.* **2019**, *75*, 1718–1725. [CrossRef] [PubMed]
42. Sun, Y.; Sparks, C.; Jones, H.; Riley, M.; Francis, F.; Du, W.; Xia, L. Silencing an essential gene involved in infestation and digestion in grain aphid through plant-mediated RNA interference generates aphid-resistant wheat plants. *Plant Biotechnol. J.* **2019**, *17*, 852–854. [CrossRef] [PubMed]
43. Bhatia, V.; Bhattacharya, R. Host-mediated RNA interference targeting a cuticular protein gene impaired fecundity in the green peach aphid *Myzus persicae*. *Pest Manag. Sci.* **2018**, *74*, 2059–2068. [CrossRef] [PubMed]
44. Thairu, M.W.; Skidmore, I.H.; Bansal, R.; Novakova, E.; Hansen, T.E.; Li-Byarlay, H.; Wickline, S.A.; Hansen, A.K. Efficacy of RNA interference knockdown using aerosolized short interfering RNAs bound to nanoparticles in three diverse aphid species. *Insect Mol. Biol.* **2017**, *26*, 356–368. [CrossRef]
45. 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]
46. Biedenkopf, D.; Will, T.; Knauer, T.; Jelonek, L.; Furch, A.C.U.; Busche, T.; Koch, A. Systemic spreading of exogenous applied RNA biopesticides in the crop plant *Hordeum vulgare*. *ExRNA* **2020**, *2*, 12. [CrossRef]
47. Liu, S.; Ladera-Carmona, M.J.; Poranen, M.M.; van Bel, A.J.E.; Kogel, K.-H.; Imani, J. Evaluation of dsRNA delivery methods for targeting macrophage migration inhibitory factor MIF in RNAi-based aphid control. *J. Plant Dis. Prot.* **2021**, *128*, 1201–1212. [CrossRef]
48. Zhang, Y.; Liu, X.; Francis, F.; Xie, H.; Fan, J.; Wang, Q.; Liu, H.; Sun, Y.; Chen, J. The salivary effector protein Sg2204 in the greenbug *Schizaphis graminum* suppresses wheat defence and is essential for enabling aphid feeding on host plants. *Plant Biotechnol. J.* **2022**, *20*, 2187–2201. [CrossRef]
49. Ding, B.Y.; Shang, F.; Zhang, Q.; Xiong, Y.; Yang, Q.; Niu, J.Z.; Smagghe, G.; Wang, J.J. Silencing of two insulin receptor genes disrupts nymph-adult transition of alate brown citrus aphid. *Int. J. Mol. Sci.* **2017**, *18*, 357. [CrossRef]
50. Mou, X.; Yuan, G.R.; Jiang, H.B.; Liu, Z.; Wang, J.J. Functional characterization of two acetylcholinesterase genes in the brown citrus aphid, *Aphis* (Toxoptera) *citricidus* (Kirkaldy), using heterologous expression and RNA interference. *Pestic. Biochem. Physiol.* **2017**, *138*, 76–83. [CrossRef]
51. Shang, F.; Niu, J.Z.; Ding, B.Y.; Zhang, Q.; Ye, C.; Zhang, W.; Smagghe, G.; Wang, J.J. Vitellogenin and its receptor play essential roles in the development and reproduction of the brown citrus aphid, *Aphis* (Toxoptera) *citricidus*. *Insect Mol. Biol.* **2018**, *27*, 221–233. [CrossRef] [PubMed]

52. 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] [PubMed]
53. Ye, C.; Wang, Z.-W.; Sheng, Y.-L.; Wang, Z.-G.; Smagghe, G.; Christiaens, O.; Niu, J.; Wang, J. GNBP1 as a potential RNAi target to enhance the virulence of *Beauveria bassiana* for aphid control. *J. Pest Sci.* **2021**, *95*, 87–100. [CrossRef]
54. 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]
55. Gong, Y.H.; Yu, X.R.; Shang, Q.L.; Shi, X.Y.; Gao, X.W. Oral delivery mediated RNA interference of a carboxylesterase gene results in reduced resistance to organophosphorus insecticides in the cotton aphid, *Aphis gossypii* Glover. *PLoS ONE* **2014**, *9*, e102823. [CrossRef] [PubMed]
56. Peng, T.; Pan, Y.; Yang, C.; Gao, X.; Xi, J.; Wu, Y.; Huang, X.; Zhu, E.; Xin, X.; Zhan, C.; et al. Over-expression of CYP6A2 is associated with spirotetramat resistance and cross-resistance in the resistant strain of *Aphis gossypii* Glover. *Pestic. Biochem. Physiol.* **2016**, *126*, 64–69. [CrossRef] [PubMed]
57. Rebijith, K.B.; Asokan, R.; Hande, H.R.; Kumar, N.K.; Krishna, V.; Vinutha, J.; Bakthavatsalam, N. RNA interference of odorant-binding protein 2 (OBP2) of the cotton aphid, *Aphis gossypii* (Glover), resulted in altered electrophysiological responses. *Appl. Biochem. Biotechnol.* **2016**, *178*, 251–266. [CrossRef]
58. Wu, Y.; Xu, H.; Pan, Y.; Gao, X.; Xi, J.; Zhang, J.; Shang, Q. Expression profile changes of cytochrome P450 genes between thiamethoxam susceptible and resistant strains of *Aphis gossypii* Glover. *Pestic. Biochem. Physiol.* **2018**, *149*, 1–7. [CrossRef]
59. Pan, Y.; Chai, P.; Zheng, C.; Xu, H.; Wu, Y.; Gao, X.; Xi, J.; Shang, Q. Contribution of cytochrome P450 monooxygenase CYP380C6 to spirotetramat resistance in *Aphis gossypii* Glover. *Pestic. Biochem. Physiol.* **2018**, *148*, 182–189. [CrossRef]
60. Ullah, F.; Gul, H.; Tariq, K.; Desneux, N.; Gao, X.; Song, D. Functional analysis of cytochrome P450 genes linked with acetamiprid resistance in melon aphid, *Aphis gossypii*. *Pestic. Biochem. Physiol.* **2020**, *170*, 104687. [CrossRef]
61. Ullah, F.; Gul, H.; Tariq, K.; Hafeez, M.; Desneux, N.; Gao, X.; Song, D. RNA interference-mediated silencing of ecdysone receptor (EcR) gene causes lethal and sublethal effects on melon aphid, *Aphis gossypii*. *Entomol. Gen.* **2022**, *42*, 791–797. [CrossRef]
62. Shi, Y.; Liu, T.Y.; Ding, B.Y.; Niu, J.; Jiang, H.B.; Liu, T.X.; Wang, J.J. Crustacean cardioactive peptide and its receptor modulate the ecdysis behavior in the pea aphid, *Acyrthosiphon pisum*. *J. Insect Physiol.* **2022**, *137*, 104364. [CrossRef] [PubMed]
63. Mutti, N.S.; Park, Y.; Reese, J.C.; Reeck, G.R. RNAi knockdown of a salivary transcript leading to lethality in the pea aphid, *Acyrthosiphon pisum*. *J. Insect Sci.* **2006**, *6*, 38. [CrossRef] [PubMed]
64. Jaubert-Possamai, S.; Le Trionnaire, G.; Bonhomme, J.; Christophides, G.K.; Rispe, C.; Tagu, D. Gene knockdown by RNAi in the pea aphid *Acyrthosiphon pisum*. *BMC Biotechnol.* **2007**, *7*, 63. [CrossRef] [PubMed]
65. Shakesby, A.J.; Wallace, I.S.; Isaacs, H.V.; Pritchard, J.; Roberts, D.M.; Douglas, A.E. A water-specific aquaporin involved in aphid osmoregulation. *Insect Biochem. Mol. Biol.* **2009**, *39*, 1–10. [CrossRef]
66. 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]
67. Mao, J.; Zeng, F. Feeding-based RNA interference of a gap gene is lethal to the pea aphid, *Acyrthosiphon pisum*. *PLoS ONE* **2012**, *7*, e48718. [CrossRef]
68. Sapountzis, P.; Duport, G.; Balmand, S.; Gaget, K.; Jaubert-Possamai, S.; Febvay, G.; Charles, H.; Rahbe, Y.; Colella, S.; Calevro, F. New insight into the RNA interference response against cathepsin-L gene in the pea aphid, *Acyrthosiphon pisum*: Molting or gut phenotypes specifically induced by injection or feeding treatments. *Insect Biochem. Mol. Biol.* **2014**, *51*, 20–32. [CrossRef]
69. Guo, K.; Wang, W.; Luo, L.; Chen, J.; Guo, Y.; Cui, F. Characterization of an aphid-specific, cysteine-rich protein enriched in salivary glands. *Biophys. Chem.* **2014**, *189*, 25–32. [CrossRef]
70. Wang, W.; Luo, L.; Lu, H.; Chen, S.; Kang, L.; Cui, F. Angiotensin-converting enzymes modulate aphid-plant interactions. *Sci. Rep.* **2015**, *5*, 8885. [CrossRef]
71. Naessens, E.; Dubreuil, G.; Giordanengo, P.; Baron, O.L.; Minet-Kebdani, N.; Keller, H.; Coustau, C. A secreted MIF cytokine enables aphid feeding and represses plant immune responses. *Curr. Biol.* **2015**, *25*, 1898–1903. [CrossRef] [PubMed]
72. Wang, W.; Dai, H.; Zhang, Y.; Chandrasekar, R.; Luo, L.; Hiromasa, Y.; Sheng, C.; Peng, G.; Chen, S.; Tomich, J.M.; et al. Armet is an effector protein mediating aphid-plant interactions. *FASEB J.* **2015**, *29*, 2032–2045. [CrossRef] [PubMed]
73. Will, T.; Vilcinskas, A. The structural sheath protein of aphids is required for phloem feeding. *Insect Biochem. Mol. Biol.* **2015**, *57*, 34–40. [CrossRef]
74. Zhang, Y.; Lu, Z. Peroxiredoxin 1 protects the pea aphid *Acyrthosiphon pisum* from oxidative stress induced by *Micrococcus luteus* infection. *J. Invertebr. Pathol.* **2015**, *127*, 115–121. [CrossRef] [PubMed]
75. Chen, N.; Fan, Y.L.; Bai, Y.; Li, X.D.; Zhang, Z.F.; Liu, T.X. Cytochrome P450 gene, CYP4G51, modulates hydrocarbon production in the pea aphid, *Acyrthosiphon pisum*. *Insect Biochem. Mol. Biol.* **2016**, *76*, 84–94. [CrossRef] [PubMed]
76. Zhang, R.; Wang, B.; Grossi, G.; Falabella, P.; Liu, Y.; Yan, S.; Lu, J.; Xi, J.; Wang, G. Molecular basis of alarm pheromone detection in aphids. *Curr. Biol.* **2017**, *27*, 55–61. [CrossRef]
77. Webster, C.G.; Pichon, E.; van Munster, M.; Monsion, B.; Deshoux, M.; Gargani, D.; Calevro, F.; Jimenez, J.; Moreno, A.; Krenz, B.; et al. Identification of plant virus receptor candidates in the stylets of their aphid vectors. *J. Virol.* **2018**, *92*, e00432-18. [CrossRef]
78. Li, X.; Qu, M.J.; Zhang, Y.; Li, J.W.; Liu, T.X. Expression of neuropeptide F gene and its regulation of feeding behavior in the pea aphid, *Acyrthosiphon pisum*. *Front. Physiol.* **2018**, *9*, 87. [CrossRef]

79. Ye, C.; An, X.; Jiang, Y.D.; Ding, B.Y.; Shang, F.; Christiaens, O.; Taning, C.N.T.; Smagghe, G.; Niu, J.; Wang, J.J. Induction of RNAi core machinery's gene expression by exogenous dsRNA and the effects of pre-exposure to dsRNA on the gene silencing efficiency in the pea aphid (*Acyrthosiphon pisum*). *Front. Physiol.* **2018**, *9*, 1906. [CrossRef]
80. Ye, C.; Jiang, Y.D.; An, X.; Yang, L.; Shang, F.; Niu, J.; Wang, J.J. Effects of RNAi-based silencing of chitin synthase gene on moulting and fecundity in pea aphids (*Acyrthosiphon pisum*). *Sci. Rep.* **2019**, *9*, 3694. [CrossRef]
81. Wang, Q.; Yuan, E.; Ling, X.; Zhu-Salzman, K.; Guo, H.; Ge, F.; Sun, Y. An aphid facultative symbiont suppresses plant defence by manipulating aphid gene expression in salivary glands. *Plant Cell Environ.* **2020**, *43*, 2311–2322. [CrossRef] [PubMed]
82. Ding, B.Y.; Niu, J.; Shang, F.; Yang, L.; Zhang, W.; Smagghe, G.; Wang, J.J. Parental silencing of a horizontally transferred carotenoid desaturase gene causes a reduction of red pigment and fitness in the pea aphid. *Pest Manag. Sci.* **2020**, *76*, 2423–2433. [CrossRef] [PubMed]
83. Shahid, S.; Shi, Y.; Yang, C.; Li, J.; Ali, M.Y.; Smagghe, G.; Liu, T.X. CCHamide2-receptor regulates feeding behavior in the pea aphid, *Acyrthosiphon pisum*. *Peptides* **2021**, *143*, 170596. [CrossRef]
84. Zhou, X.; Ling, X.; Guo, H.; Zhu-Salzman, K.; Ge, F.; Sun, Y. Serratia symbiotica enhances fatty acid metabolism of pea aphid to promote host development. *Int. J. Mol. Sci.* **2021**, *22*, 5951. [CrossRef]
85. Chang, M.; Cheng, H.; Cai, Z.; Qian, Y.; Zhang, K.; Yang, L.; Ma, N.; Li, D. miR-92a-1-p5 modulated expression of the flightin gene regulates flight muscle formation and wing extension in the pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphidoidea). *J. Insect Sci.* **2022**, *22*, 14. [CrossRef] [PubMed]
86. Guo, Y.; Fan, Y.; Teng, Z.; Wang, L.; Tan, X.; Wan, F.; Zhou, H. Efficacy of RNA interference using nanocarrier-based transdermal dsRNA delivery system in the woolly apple aphid, *Eriosoma lanigerum*. *Arch. Insect Biochem. Physiol.* **2022**, *110*, e21888. [CrossRef]
87. Mulot, M.; Boissinot, S.; Monsion, B.; Rastegar, M.; Clavijo, G.; Halter, D.; Bochet, N.; Erdinger, M.; Brault, V. Comparative analysis of RNAi-based methods to down-regulate expression of two genes expressed at different levels in *Myzus persicae*. *Viruses* **2016**, *8*, 316. [CrossRef]
88. Gogoi, A.; Sarmah, N.; Kaldis, A.; Perdikis, D.; Voloudakis, A. Plant insects and mites uptake double-stranded RNA upon its exogenous application on tomato leaves. *Planta* **2017**, *246*, 1233–1241. [CrossRef]
89. Tariq, K.; Ali, A.; Davies, T.G.E.; Naz, E.; Naz, L.; Sohail, S.; Hou, M.; Ullah, F. RNA interference-mediated knockdown of voltage-gated sodium channel (MpNav) gene causes mortality in peach-potato aphid, *Myzus persicae*. *Sci. Rep.* **2019**, *9*, 5291. [CrossRef]
90. Mahmood, I.; Afroz, A.; Malik, M.F.; Zeeshan, N.; Khan, M.R.; Rashid, U.; Khan, M.A.U.; Ashraf, N.M.; Alam, S. RNA interference-mediated knockdown of odorant-binding protein 2 and MP58 gene causes mortality in *Myzus persicae*. *Int. J. Trop. Insect Sci.* **2021**, *42*, 315–326. [CrossRef]
91. 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]
92. Ding, C.Y.; Ma, Y.M.; Li, B.; Wang, Y.; Zhao, L.; Peng, J.N.; Li, M.Y.; Liu, S.; Li, S.G. Identification and functional analysis of differentially expressed genes in *Myzus persicae* (Hemiptera: Aphididae) in response to trans-anethole. *J. Insect Sci.* **2022**, *22*, 3. [CrossRef] [PubMed]
93. Wang, X.X.; Feng, Z.J.; Chen, Z.S.; Zhang, Z.F.; Zhang, Y.; Liu, T.X. Use of tyrosine hydroxylase RNAi to study *Megoura viciae* (Hemiptera: Aphididae) sequestration of its host's l-DOPA for body melanism. *J. Insect Physiol.* **2019**, *114*, 136–144. [CrossRef] [PubMed]
94. Xiao, D.; Lu, Y.H.; Shang, Q.L.; Song, D.L.; Gao, X.W. Gene silencing of two acetylcholinesterases reveals their cholinergic and non-cholinergic functions in *Rhopalosiphum padi* and *Sitobion avenae*. *Pest Manag. Sci.* **2015**, *71*, 523–530. [CrossRef]
95. Deng, F.; Zhao, Z. Influence of catalase gene silencing on the survivability of *Sitobion avenae*. *Arch. Insect Biochem. Physiol.* **2014**, *86*, 46–57.
96. Wang, D.; Liu, Q.; Li, X.; Sun, Y.; Wang, H.; Xia, L. Double-stranded RNA in the biological control of grain aphid (*Sitobion avenae* F.). *Funct. Integr. Genom.* **2015**, *15*, 211–223. [CrossRef]
97. Yan, T.; Chen, H.; Sun, Y.; Yu, X.; Xia, L. RNA interference of the ecdysone receptor genes EcR and USP in grain aphid (*Sitobion avenae* F.) affects its survival and fecundity upon feeding on wheat plants. *Int. J. Mol. Sci.* **2016**, *17*, 2098. [CrossRef]
98. Zhang, Y.; Fan, J.; Francis, F.; Chen, J. Molecular characterization and gene silencing of Laccase 1 in the grain aphid, *Sitobion avenae*. *Arch. Insect Biochem. Physiol.* **2018**, *97*, e21446. [CrossRef]
99. Ullah, R.M.K.; Quershi, S.R.; Adeel, M.M.; Abdelnabby, H.; Waris, M.I.; Duan, S.G.; Wang, M.Q. An odorant binding protein (SaveOBP9) involved in chemoreception of the wheat aphid *Sitobion avenae*. *Int. J. Mol. Sci.* **2020**, *21*, 8331. [CrossRef]
100. Ullah, R.M.K.; Waris, M.I.; Qureshi, S.R.; Rasool, F.; Duan, S.G.; Zaka, S.M.; Atiq, M.N.; Wang, M.Q. Silencing of an odorant binding protein (SaveOBP10) involved in the behavioural shift of the wheat aphid *Sitobion avenae* (Fabricius). *Insect Mol. Biol.* **2022**, *31*, 568–584. [CrossRef]
101. Zhang, Y.; Fan, J.; Sun, J.-R.; Chen, J.-L. Cloning and RNA interference analysis of the salivary protein C002 gene in *Schizaphis graminum*. *J. Integr. Agric.* **2015**, *14*, 698–705. [CrossRef]
102. Zhang, B.Z.; Ma, K.S.; Liu, J.J.; Lu, L.Y.; Chen, X.L.; Zhang, S.P.; Gao, X.W. Differential expression of genes in greenbug (*Schizaphis graminum* Rondani) treated by imidacloprid and RNA interference. *Pest Manag. Sci.* **2019**, *75*, 1726–1733. [CrossRef] [PubMed]
103. Zhang, Y.; Liu, X.; Fu, Y.; Crespo-Herrera, L.; Liu, H.; Wang, Q.; Zhang, Y.; Chen, J. Salivary effector Sm9723 of grain aphid *Sitobion miscanthi* suppresses plant defense and is essential for aphid survival on wheat. *Int. J. Mol. Sci.* **2022**, *23*, 6909. [CrossRef]

104. Whangbo, J.S.; Hunter, C.P. Environmental RNA interference. *Trends Genet.* **2008**, *24*, 297–305. [CrossRef] [PubMed]
105. Wang, M.; Weiberg, A.; Lin, F.-M.; Thomma, B.P.; Huang, H.-D.; Jin, H. Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. *Nat. Plants* **2016**, *2*, 16151. [CrossRef] [PubMed]
106. Qiao, L.; Lan, C.; Capriotti, L.; Ah-Fong, A.; Nino Sanchez, J.; Hamby, R.; Heller, J.; Zhao, H.; Glass, N.L.; Judelson, H.S.; et al. Spray-induced gene silencing for disease control is dependent on the efficiency of pathogen RNA uptake. *Plant Biotechnol. J.* **2021**, *19*, 1756–1768. [CrossRef]
107. Ghosh, S.K.B.; Hunter, W.B.; Park, A.L.; Gundersen-Rindal, D.E. Double-stranded RNA oral delivery methods to induce RNA interference in phloem and plant-sap-feeding hemipteran insects. *J. Vis. Exp.* **2018**, *135*, e57390. [CrossRef]
108. Wang, M.; Jin, H. Spray-induced gene silencing: A powerful innovative strategy for crop protection. *Trends Microbiol.* **2017**, *25*, 4–6. [CrossRef]
109. Hunter, W.B.; Glick, E.; Paldi, N.; Bextine, B.R. Advances in RNA interference: dsRNA treatment in trees and grapevines for insect pest suppression. *Southwest Entomol.* **2012**, *37*, 85–87. [CrossRef]
110. Koch, A.; Biedenkopf, D.; Furch, A.; Weber, L.; Rossbach, O.; Abdellatef, E.; Linicus, L.; Johannsmeier, J.; Jelonek, L.; Goesmann, A. An RNAi-based control of Fusarium graminearum infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. *PLoS Pathog.* **2016**, *12*, e1005901. [CrossRef]
111. 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] [PubMed]
112. Dalakouras, A.; Wassenegger, M.; Dadami, E.; Ganopoulos, I.; Pappas, M.L.; Papadopoulou, K. Genetically modified organism-free RNA interference: Exogenous application of RNA molecules in plants. *Plant Physiol.* **2020**, *182*, 38–50. [CrossRef]
113. Vetukuri, R.R.; Dubey, M.; Kalyandurg, P.B.; Carlsson, A.S.; Whisson, S.C.; Ortiz, R. Spray-induced gene silencing: An innovative strategy for plant trait improvement and disease control. *Crop Breed. Appl. Biotechnol.* **2021**, *21*, e387921S387911. [CrossRef]
114. Bilir, Ö.; Göl, D.; Hong, Y.; McDowell, J.M.; Tör, M. Small RNA-based plant protection against diseases. *Front. Plant Sci.* **2022**, *13*, 2973. [CrossRef] [PubMed]
115. Zhang, X.; Zhang, J.; Zhu, K. 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]
116. Li-Byarlay, H.; Li, Y.; Stroud, H.; Feng, S.; Newman, T.C.; Kaneda, M.; Hou, K.K.; Worley, K.C.; Elsik, C.G.; Wickline, S.A. RNA interference knockdown of DNA methyl-transferase 3 affects gene alternative splicing in the honey bee. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 12750–12755. [CrossRef]
117. Das, S.; Debnath, N.; Cui, Y.; Unrine, J.; Palli, S.R. Chitosan, carbon quantum dot, and silica nanoparticle mediated dsRNA delivery for gene silencing in *Aedes aegypti*: A comparative analysis. *ACS Appl. Mater Interfaces* **2015**, *7*, 19530–19535. [CrossRef]
118. Chen, J.; Tang, B.; Chen, H.; Yao, Q.; Huang, X.; Chen, J.; Zhang, D.; Zhang, W. Different functions of the insect soluble and membrane-bound trehalase genes in chitin biosynthesis revealed by RNA interference. *PLoS ONE* **2010**, *5*, e10133. [CrossRef]
119. 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]
120. 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]
121. Timmons, L.; Fire, A. Specific interference by ingested dsRNA. *Nature* **1998**, *395*, 854. [CrossRef] [PubMed]
122. Dubrovina, A.S.; Kiselev, K.V. Exogenous RNAs for gene regulation and plant resistance. *Int. J. Mol. Sci.* **2019**, *20*, 2282. [CrossRef] [PubMed]
123. Lau, S.-E.; Schwarzacher, T.; Othman, R.Y.; Harikrishna, J.A. dsRNA silencing of an R2R3-MYB transcription factor affects flower cell shape in a *Dendrobium hybrid*. *BMC Plant Biol.* **2015**, *15*, 194. [CrossRef] [PubMed]
124. Ulrich, J.; Dao, V.A.; Majumdar, U.; Schmitt-Engel, C.; Schwirz, J.; Schultheis, D.; Ströhlein, N.; Troelenberg, N.; Grossmann, D.; Richter, T. Large scale RNAi screen in *Tribolium* reveals novel target genes for pest control and the proteasome as prime target. *BMC Genom.* **2015**, *16*, 674. [CrossRef]
125. 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]
126. Zhang, M.; Zhou, Y.; Wang, H.; Jones, H.D.; Gao, Q.; Wang, D.; Ma, Y.; Xia, L. Identifying potential RNAi targets in grain aphid (*Sitobion avenae* F.) based on transcriptome profiling of its alimentary canal after feeding on wheat plants. *BMC Genom.* **2013**, *14*, 560. [CrossRef]
127. Luo, Y.; Wang, X.; Wang, X.; Yu, D.; Chen, B.; Kang, L. Differential responses of migratory locusts to systemic RNA interference via double-stranded RNA injection and feeding. *Insect Mol. Biol.* **2013**, *22*, 574–583. [CrossRef]
128. Camargo, R.D.A.; Herai, R.H.; Santos, L.N.; Bento, F.M.; Lima, J.E.; Marques-Souza, H.; Figueira, A. De novo transcriptome assembly and analysis to identify potential gene targets for RNAi-mediated control of the tomato leafminer (*Tuta absoluta*). *BMC Genom.* **2015**, *16*, 635. [CrossRef]
129. Bona, A.C.D.; Chitolina, R.F.; Fermino, M.L.; de Castro Poncio, L.; Weiss, A.; Lima, J.B.P.; Paldi, N.; Bernardes, E.S.; Henen, J.; Maori, E. Larval application of sodium channel homologous dsRNA restores pyrethroid insecticide susceptibility in a resistant adult mosquito population. *Parasites Vectors* **2016**, *9*, 397. [CrossRef]

130. Li, H.; Jiang, W.; Zhang, Z.; Xing, Y.; Li, F. Transcriptome analysis and screening for potential target genes for RNAi-mediated pest control of the beet armyworm, *Spodoptera exigua*. *PLoS ONE* **2013**, *8*, e65931. [CrossRef]
131. Lomazzo, E.; Hussmann, G.P.; Wolfe, B.B.; Yasuda, R.P.; Perry, D.C.; Kellar, K.J. Effects of chronic nicotine on heteromeric neuronal nicotinic receptors in rat primary cultured neurons. *J. Neurochem.* **2011**, *119*, 153–164. [CrossRef] [PubMed]
132. 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] [PubMed]
133. Abbasi, R.; Heschuk, D.; Kim, B.; Whyard, S. A novel paperclip double-stranded RNA structure demonstrates clathrin-independent uptake in the mosquito *Aedes aegypti*. *Insect Biochem. Mol. Biol.* **2020**, *127*, 103492. [CrossRef] [PubMed]
134. Kwon, D.H.; Park, J.H.; Ashok, P.A.; Lee, U.; Lee, S.H. Screening of target genes for RNAi in *Tetranychus urticae* and RNAi toxicity enhancement by chimeric genes. *Pestic. Biochem. Physiol.* **2016**, *130*, 1–7. [CrossRef]
135. 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]
136. Silver, K.; Cooper, A.M.; Zhu, K.Y. Strategies for enhancing the efficiency of RNA interference in insects. *Pest Manag. Sci.* **2021**, *77*, 2645–2658. [CrossRef]
137. Bolognesi, R.; Ramaseshadri, P.; Anderson, J.; Bachman, P.; Clinton, W.; Flannagan, R.; Ilagan, O.; Lawrence, C.; Levine, S.; Moar, W. Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *PLoS ONE* **2012**, *7*, e47534. [CrossRef]
138. Miller, S.C.; Miyata, K.; Brown, S.J.; Tomoyasu, Y. Dissecting systemic RNA interference in the red flour beetle *Tribolium castaneum*: Parameters affecting the efficiency of RNAi. *PLoS ONE* **2012**, *7*, e47431. [CrossRef]
139. 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]
140. Johnson, J.A.; Bitra, K.; Zhang, S.; Wang, L.; Lynn, D.E.; Strand, M.R. The UGA-CiE1 cell line from *Chrysodeixis includens* exhibits characteristics of granulocytes and is permissive to infection by two viruses. *Insect Biochem. Mol. Biol.* **2010**, *40*, 394–404. [CrossRef]
141. 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] [PubMed]
142. El-Shesheny, I.; Hajeri, S.; El-Hawary, I.; Gowda, S.; Killiny, N. Silencing abnormal wing disc gene of the Asian citrus psyllid, *Diaphorina citri* disrupts adult wing development and increases nymph mortality. *PLoS ONE* **2013**, *8*, e65392. [CrossRef]
143. Gong, L.; Chen, Y.; Hu, Z.; Hu, M. Testing insecticidal activity of novel chemically synthesized siRNA against *Plutella xylostella* under laboratory and field conditions. *PLoS ONE* **2013**, *8*, e62990. [CrossRef]
144. Zhang, H.; Li, H.; Guan, R.; Miao, X. Lepidopteran insect species-specific, broad-spectrum, and systemic RNA interference by spraying ds RNA on larvae. *Entomol. Exp. Appl.* **2015**, *155*, 218–228.
145. 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] [PubMed]
146. 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]
147. Avila, L.; Chandrasekar, R.; Wilkinson, K.; Balthazor, J.; Heerman, M.; Bechard, J.; Brown, S.; Park, Y.; Dhar, S.; Reeck, G. Delivery of lethal dsRNAs in insect diets by branched amphiphilic peptide capsules. *J. Control. Release* **2018**, *273*, 139–146. [CrossRef]
148. 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]
149. Marques, V.V.; Angelotti-Mendonça, J.; Roberto, S.R. Advances and challenges in RNA interference technology for citrus Huanglongbing vector control. *Horticulturae* **2021**, *7*, 277. [CrossRef]
150. Allen, M.L.; Walker, W.B., III. Saliva of *Lygus lineolaris* digests double stranded ribonucleic acids. *J. Insect Physiol.* **2012**, *58*, 391–396. [CrossRef]
151. 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] [PubMed]
152. Katoch, R.; Thakur, N. Insect gut nucleases: A challenge for RNA interference mediated insect control strategies. *Int. J. Biochem. Biotechnol.* **2012**, *1*, 198–203.
153. Kennedy, S.; Wang, D.; Ruvkun, G. A conserved siRNA-degrading RNase negatively regulates RNA interference in *C. elegans*. *Nature* **2004**, *427*, 645–649. [CrossRef] [PubMed]
154. Dias, N.; Cagliari, D.; Dos Santos, E.; Smagghe, G.; Jurat-Fuentes, J.; Mishra, S.; Nava, D.; Zotti, M. Insecticidal gene silencing by RNAi in the neotropical region. *Neotrop. Entomol.* **2020**, *49*, 1–11. [CrossRef]
155. 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]
156. Laisney, J.; Rose, V.L.; Watters, K.; Donohue, K.V.; Unrine, J.M. Delivery of short hairpin RNA in the neotropical brown stink bug, *Euschistus heros*, using a composite nanomaterial. *Pestic. Biochem. Physiol.* **2021**, *177*, 104906. [CrossRef]
157. Pugsley, C.E.; Isaac, R.E.; Warren, N.J.; Cayre, O.J. Recent advances in engineered nanoparticles for RNAi-mediated crop protection against insect pests. *Front. Agron.* **2021**, *3*, 652981. [CrossRef]

158. Xu, P.; Zhang, Y.; Kang, L.; Roossinck, M.J.; Mysore, K.S. Computational estimation and experimental verification of off-target silencing during posttranscriptional gene silencing in plants. *Plant Physiol.* **2006**, *142*, 429–440. [CrossRef]
159. Reddy, K.; Rajam, M. 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.
160. Senthil-Kumar, M.; Mysore, K.S. Virus-induced gene silencing can persist for more than 2 years and also be transmitted to progeny seedlings in *Nicotiana benthamiana* and tomato. *Plant Biotechnol. J.* **2011**, *9*, 797–806. [CrossRef]
161. Ahmed, F.; Senthil-Kumar, M.; Dai, X.; Ramu, V.S.; Lee, S.; Mysore, K.S.; Zhao, P.X. pssRNAit: A web server for designing effective and specific plant siRNAs with genome-wide off-target assessment. *Plant Physiol.* **2020**, *184*, 65–81. [CrossRef] [PubMed]
162. Horn, T.; Boutros, M. E-RNAi: A web application for the multi-species design of RNAi reagents-2010 update. *Nucleic Acids Res.* **2010**, *38*, W332–W339. [CrossRef] [PubMed]
163. Naito, Y.; Yamada, T.; Matsumiya, T.; Ui-Tei, K.; Saigo, K.; Morishita, S. dsCheck: Highly sensitive off-target search software for double-stranded RNA-mediated RNA interference. *Nucleic Acids Res.* **2005**, *33*, W589–W591. [CrossRef]
164. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef] [PubMed]
165. Zhao, J.H.; Zhang, T.; Liu, Q.Y.; Guo, H.S. Trans-kingdom RNAs and their fates in recipient cells: Advances, utilization, and perspectives. *Plant Commun.* **2021**, *2*, 100167. [CrossRef] [PubMed]
166. Yan, S.; Ren, B.; Zeng, B.; Shen, J. Improving RNAi efficiency for pest control in crop species. *BioTechniques* **2020**, *68*, 283–290. [CrossRef]
167. 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]
168. Mitter, N.; Worrall, E.A.; Robinson, K.E.; Li, P.; Jain, R.G.; Taochy, C.; Fletcher, S.J.; Carroll, B.J.; Lu, G.; Xu, Z.P. Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nat. Plants* **2017**, *3*, 16207. [CrossRef]
169. Mitter, N.; Worrall, E.A.; Robinson, K.E.; Xu, Z.P.; Carroll, B.J. Induction of virus resistance by exogenous application of double-stranded RNA. *Curr. Opin. Virol.* **2017**, *26*, 49–55. [CrossRef]
170. Huang, C.-Y.; Wang, H.; Hu, P.; Hamby, R.; Jin, H. Small RNAs-big players in plant-microbe interactions. *Cell Host Microbe* **2019**, *26*, 173–182. [CrossRef]
171. Kumar, S.; Nehra, M.; Dilbaghi, N.; Marrazza, G.; Hassan, A.A.; Kim, K.-H. Nano-based smart pesticide formulations: Emerging opportunities for agriculture. *J. Control. Release* **2019**, *294*, 131–153. [CrossRef] [PubMed]
172. 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]
173. Fletcher, S.J.; Reeves, P.T.; Hoang, B.T.; Mitter, N. A perspective on RNAi-based biopesticides. *Front. Plant Sci.* **2020**, *11*, 51. [CrossRef]
174. Cai, Q.; He, B.; Wang, S.; Fletcher, S.; Niu, D.; Mitter, N.; Birch, P.R.; Jin, H. Message in a bubble: Shuttling small RNAs and proteins between cells and interacting organisms using extracellular vesicles. *Annu. Rev. Plant Biol.* **2021**, *72*, 497. [CrossRef]
175. Zuo, Y.; Wang, H.; Xu, Y.; Huang, J.; Wu, S.; Wu, Y.; Yang, Y. CRISPR/Cas9 mediated G4946E substitution in the ryanodine receptor of *Spodoptera exigua* confers high levels of resistance to diamide insecticides. *Insect Biochem. Mol. Biol.* **2017**, *89*, 79–85. [CrossRef] [PubMed]
176. Wang, J.; Zhang, H.; Wang, H.; Zhao, S.; Zuo, Y.; Yang, Y.; Wu, Y. Functional validation of cadherin as a receptor of Bt toxin Cry1Ac in *Helicoverpa armigera* utilizing the CRISPR/Cas9 system. *Insect Biochem. Mol. Biol.* **2016**, *76*, 11–17. [CrossRef] [PubMed]
177. Ye, Z.-F.; Liu, X.-L.; Han, Q.; Liao, H.; Dong, X.-T.; Zhu, G.-H.; Dong, S.-L. Functional characterization of PBP1 gene in *Helicoverpa armigera* (Lepidoptera: Noctuidae) by using the CRISPR/Cas9 system. *Sci. Rep.* **2017**, *7*, 8470. [CrossRef]
178. Dong, X.T.; Liao, H.; Zhu, G.H.; Khuhro, S.A.; Ye, Z.F.; Yan, Q.; Dong, S.L. CRISPR/Cas9-mediated PBP1 and PBP3 mutagenesis induced significant reduction in electrophysiological response to sex pheromones in male *Chilo suppressalis*. *Insect Sci.* **2019**, *26*, 388–399. [CrossRef]
179. Zhu, G.-H.; Peng, Y.-C.; Zheng, M.-Y.; Zhang, X.-Q.; Sun, J.-B.; Huang, Y.; Dong, S.-L. CRISPR/Cas9 mediated BLOS2 knockout resulting in disappearance of yellow strips and white spots on the larval integument in *Spodoptera litura*. *J. Insect Physiol.* **2017**, *103*, 29–35. [CrossRef]
180. Bi, H.L.; Xu, J.; Tan, A.J.; Huang, Y.P. CRISPR/Cas9-mediated targeted gene mutagenesis in *Spodoptera litura*. *Insect Sci.* **2016**, *23*, 469–477. [CrossRef]
181. Zhao, Y.; Huang, G.; Zhang, W. Mutations in NlInR1 affect normal growth and lifespan in the brown planthopper *Nilaparvata lugens*. *Insect Biochem. Mol. Biol.* **2019**, *115*, 103246. [CrossRef] [PubMed]
182. Lu, H.-P.; Luo, T.; Fu, H.-W.; Wang, L.; Tan, Y.-Y.; Huang, J.-Z.; Wang, Q.; Ye, G.-Y.; Gatehouse, A.M.; Lou, Y.-G. Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. *Nat. Plants* **2018**, *4*, 338–344. [CrossRef] [PubMed]
183. Seni, A. Frontier insect pest management technologies for sustainable rice production. *J. Cereal Res.* **2021**, *13*, 136–148. [CrossRef]