



RNAi in termites (Isoptera): current status and prospects for pest management

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With 1 figure, 2 tables, and 1 box

Abstract: Termites are keystone species and play an essential role in the ecosystem by decomposing plants and wood materials with adapted endogenous and symbiotic cellulase systems to obtain food and energy. However, some termites are also pests that have significant economic impacts directly or indirectly on the agricultural system, such as pastures, orchards, nurseries, and eucalyptus forests, and globally the estimated loss is \$40 billion annually. Recently, scientists have focused on RNAi technology to protect plants against insect pests, and the utilization of RNAi against termites is confounded because of their social nature and habitats. Many termite species transcriptomes and genome sequencing projects are currently underway, and the outputs open new avenues for the species-specific design of RNAi-based termiticides. With these genomes, symbiotic biological agents such as fungi, bacteria, viruses, and nematodes can be screened and evaluated to express RNAi bioactive molecules (dsRNA, siRNA, and shRNA). In parallel, dsRNA/siRNA can also be delivered through nanocarriers against termites. In this perspective, we highlight existing RNAi-based functional genomic studies against eusocial termite pests and further discuss the prospects for RNAi-termiticides, considering unique challenges in terms of dsRNA delivery, target efficiency, environmental risks and termite eusociality.

Keywords: Termites, RNA interference, symbiont-mediated transfer, nanoparticles, RNAi-termiticides, termite pest management, eusociality

1 Introduction

Termites are the primary decomposers of dead organic matter in tropical and subtropical regions, increasing ecosystem productivity by >10% of animal biomass and 95% of soil insect biomass (Khan & Ahmad 2018). Wood-feeding termites are classified into three categories: subterranean, dry wood, and wet wood termites, of which most of the damage (~80%) is caused by subterranean termites (Scharf 2015). Termites have also been considered pests resulting in economic losses worldwide (Rust & Su 2012). There is a requirement for robust and eco-friendly management strategies to control termites and minimize economic loss. Molecular tools such as RNA interference (RNAi) can be helpful in termite management. RNAi is a cellular process in which mRNA degradation and interruption of protein synthesis turn off gene function, and it is conserved in eukaryotes, including insects (Zhu &

Palli 2020). RNAi can be exploited through its sequence-specific mode of action to target essential genes in insects, including termites, leading to pest control. Using transgenic RNAi plants designed to express pest-specific dsRNA, scientists have successfully controlled plant pests (Cagliari et al. 2019; Mezzetti et al. 2020). The transgenic maize SmartStax PRO was recently approved for commercialization in North America to limit the damage caused by the corn rootworm *Diabrotica* spp. (Head et al. 2017), further confirming the potential of RNAi-based pest control. Alternatively, various exogenous application strategies for pest-specific dsRNAs have been theorized, developed, and tested (Taning et al. 2020). The prospect of RNAi against other wood-feeding forest pests was meticulously summarized by Joga et al. (2021), and the future market, regulatory, and biosafety of RNAi-based pest control products by Christiaens et al. (2022) and De Schutter et al. (2022).

So far, researchers have not focused on developing RNAi-based termiticides, probably due to the limited availability of transcriptome and genome data. Many termite species genome, metagenome, and transcriptome sequencing projects are underway. Researchers should utilize the rapidly expanding sequence information to develop species-specific RNAi-based termiticides. There are already quite a few notable omics studies published. For instance, a wood-feeding termite *Coptotermes formosanus* transcriptomic analysis revealed the unique mechanism for effective biomass degradation (Geng et al. 2018). Bucek et al. (2019) used transcriptome-based phylogenies to evolve termite symbiosis. Ye et al. (2019) conducted a transcriptome study to elucidate the genetic mechanism underlying the reproductive plasticity of lower termite workers. Rasheed et al. (2019) performed a comparative transcriptomic study of *Reticulitermes aculabialis* and identified the endocuticular protein genes of alate adults, workers, and soldiers. Recently, genomic and transcriptomic studies showed that gene duplication facilitates social evolution in *Reticulitermes speratus*, a subterranean termite (Shigenobu et al. 2022). However, there is little initiative for using sequence information from those generated datasets for RNAi studies, indicating the putative challenges of performing RNAi against eusocial termites. Many researchers believe that eusociality jeopardizes RNAi efficacy in termite colonies. In this perspective, we first confirmed the presence of key RNAi machinery core genes in a termite transcriptome through *in silico* analysis and then further discussed innovative strategies in developing and applying RNAi-based termiticides, considering unique challenges for deployment against eusocial termites and environmental risks.

2 Termites as pests

Termites are often considered pests causing damage globally estimated to be \$40 billion (Rust & Su 2012). Based on feeding habits and preferences of undecayed living and dead plant material, 10% of termites are reported as pests out of ~2700 described species (Scharf 2015). They can damage cultivated plants, buildings, pastures, forests, and non-cellulosic materials like electric cables (Scharf 2015). The lower and higher termites have different symbiotic partners, assisting them in utilizing wood materials. Lower termites have protists, bacteria, and fungi, but the higher termites lack protists. Protists perform essential functions like cellulose degradation, but the higher termites solely depend on the symbiotic bacteria for such a function. There are four families of lower termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, and Rhinotermitidae) and three subfamilies of higher termites (Termitinae, Nasutitermitinae, and

Macrotermitinae) that cause the most damage to tropical agriculture. A wide range of crops is affected by termites, such as orchards and plantation trees, coconuts, palms, sugar cane, rice, maize, wheat, sorghum, groundnuts, coffee, tea, cocoa, yam, and cassava (Rouland-Lefèvre 2010). Some termites are also considered pests for forest ecosystems; for example, *Coptotermes* (Rhinotermitidae) causes severe damage to mature trees (e.g., *Eucalyptus grandis*) in Malaysia and Australia (Cowie et al. 1989). Macrotermitinae (Termitidae), such as *Macrotermes*, *Odontotermes* and *Microtermes*, attack young, exotic trees such as Eucalyptus in India and Africa, causing up to 100% damage (Cowie et al. 1989). Although termites can be controlled using cultural management practices (Verma et al. 2009), chemicals [insecticides (Wood and Pearce 1991; Logan 1992; Sharma et al. 2008), chitin synthesis inhibitors (Stansly et al. 2001), and fungicides (Rouland-Lefèvre and Mora 2002)], and biological agents [bacteria (Devi et al. 2007), viruses (Al Fazairy et al. 1988), fungi (Chouvenc et al. 2009), protists (Jafri et al. 1976), and nematodes (Benmoussa-Haichour et al. 1998)], but they have some limitations and biosafety issues. Therefore, sustainable integrated pest management (IPM) strategies, including the use of alternative control products such as RNAi-based termiticides, are urgently needed for termite management.

3 RNAi against termites: current status

Three types of RNAi mechanisms have been identified in insects, i.e., small interfering RNA (siRNA), microRNA (miRNA), and piwiRNA (piRNA) (Zhu & Palli, 2020). In the context of RNAi-based control, the siRNA mechanism has been the most explored (Zhu & Palli, 2020). In brief, the successful delivery of dsRNA into the cell triggers the siRNA machinery, whereby the enzyme *Dicer-2* (*Dcr-2*) processes the dsRNA into siRNAs, which are in turn incorporated into the RNA-induced silencing complex (RISC). Subsequently, *Argonaute2* (*Ago2*) cleaves and removes one of the siRNA strands (sense strand), and the remaining antisense strand guides the RISC to the complementary mRNA strand in a sequence-specific manner. AGO2 in the RISC then degrades the mRNA strand, leading to post-transcriptional gene silencing (Zhu & Palli, 2020). The identification of RNAi machinery core genes has been explicitly reviewed for several insects, except for termites. For the first time in termites, we searched and confirmed the presence of RNAi core machinery and associated genes using the publicly available *Coptotermes formosanus* transcriptome data (Table 1; <http://v2.insect-genome.com/Pcg>). Most existing studies on RNAi against termites are so far for validating gene function. RNAi studies on termites in a species-specific manner are summarized in Box 1 and Table 2.

Table 1. RNAi machinery genes[#] identified in termites.

Gene group	Gene name		Gene symbol
Core RNAi machinery	siRNA	Protein argonaute-2	AGO2
		Endoribonuclease ZC3H12A	Zc3h12a
		Pre-mRNA-splicing factor CWC22 homolog	ncm
	miRNA	Probable RNA-binding protein EIF1AD	eif1ad
		Microprocessor complex subunit DGCR8	DGCR8
		Interferon-inducible double-stranded RNA-dependent protein kinase activator A	Prkra
		RISC-loading complex subunit tarbp2	tarbp2
		Endoribonuclease Dicer	DICER1
		Double-stranded RNA-specific editase Adar	Adar
		Probable ribonuclease ZC3H12C	Zc3h12c
	piRNA	Piwi-like protein Siwi	Siwi
		Tudor domain-containing protein 1	tdrd1
		Piwi-like protein Ago3	AGO3
dsRNA uptake	Clathrin-mediated transport	Epsin-2	Epn2
		Clathrin heavy chain	Chc
		Clathrin interactor 1	CLINT1
		Clathrin light chain	Clc
		AP2-associated protein kinase 1	AAK1
		Polyubiquitin-C	UBC
		Polyubiquitin-A	ubq-1
		Ankyrin repeat domain-containing protein 50	ANKRD50
		Protein unc-50 homolog	Unc50
	Receptor-mediated transport	Transferrin	TF
		Scavenger receptor class B member 1	Scarb1
		Sortilin-related receptor	Sorl1
		Innexin inx1	inx1
		Innexin inx2	inx2
		Innexin inx3	inx3
		Innexin inx1	inx7
		Scavenger receptor class B member 1	Scarb1
		Low-density lipoprotein receptor	LDLR
	<i>C. elegans</i>	SID1 transmembrane family member 1	SIDT1
		Spermatogenesis-defective protein 39 homolog	VIPAS39
Intracellular transport	Proton transport	V-type proton ATPase catalytic subunit A	Vha68-2
		V-type proton ATPase subunit H	VhaSFD
		V-type proton ATPase subunit B	Vha55
		V-type proton ATPase subunit S1	VhaAC45
		V-type proton ATPase subunit E	VHA26
		V-type proton ATPase 21 kDa proteolipid subunit	ATP6V0B
		V-type proton ATPase subunit F	VHA14
	Endosome	Ras-related protein rab7	RAB30
		ADP-ribosylation factor 1	Arf79F
		ADP-ribosylation factor 2	Arf102F
		ADP-ribosylation factor 6	ARF6
		VPS35 endosomal protein sorting factor-like	VPS35L
		Ras-related GTP-binding protein A	RRAGA
		Ras-related GTP-binding protein C	Rragc

Table 1. continued.

Gene group	Gene name		Gene symbol
	Lysosome	Protein lava lamp	lva
		Lysosomal-trafficking regulator	LYST
		Endosome/lysosome-associated apoptosis and autophagy regulator family member 2	Elapor2
	Exosome	Regulating synaptic membrane exocytosis protein 2	RIMS2
		Regulating synaptic membrane exocytosis protein 2	RIMS2
		DEAD-box helicase Dbp80	Dbp80
Lipid metabolism	SAP domain-containing ribonucleoprotein		Sarnp
	Sphingolipid delta (4)-desaturase DES1		DEGS1
	Prosaposin		PSAP
Nucleases	Small RNA 2'-O-methyltransferase		Hen1
	U3 small nucleolar RNA-interacting protein 2		Rrp9
	Ribonuclease H1		RNASEH1
	Ribonuclease H2 subunit A		RNASEH2 A
	Ribonuclease H2 subunit B		RNASEH2 B
	Ribonuclease H2 subunit C		RNASEH2 C
	Telomerase RNA component interacting RNase		TRIR
	Serine/threonine-protein kinase/endoribonuclease IRE1		ERN1
	Serine/threonine-protein kinase SIK2		SIK2
	Endoribonuclease ZC3H12A		Zc3h12a
Antiviral RNAi	Retinoid-inducible serine carboxypeptidase		Scepep1
	Multidrug resistance-associated protein 1		ABCC1
	Beta-1,4-mannosyltransferase egh		egh
RISC factors	F-box only protein 11		Fbxo11
	E3 ubiquitin-protein transferase MAEA		maea
	Hermansky-Pudlak syndrome 4 protein		HPS4
	Probable RNA helicase armi		armi
	Double-stranded RNA-binding protein Staufen homolog		Stau
	Protein dead ringer		retn
	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1 homolog		Etl1
	ATP-dependent RNA helicase DHX30		Dhx30
	ATP-dependent RNA helicase p62		Rm62
	Protein Gawky		gw
	Protein maelstrom homolog		mael
	RNA-binding protein Musashi homolog 1		MSI1
	Protein arginine N-methyltransferase 1		PRMT1
	Staphylococcal nuclease domain-containing protein 1		Tudor-SN
	Protein CLP1 homolog		cbc
	Pre-mRNA cleavage complex 2 protein Pcf11		PCF11
	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX16		DHX16
Others	Poly(A) RNA polymerase, mitochondrial		MTPAP
	Poly(A) RNA polymerase gld-2 homolog A		Gld2
	Poly(A) polymerase alpha		PAPOLA

#For more details on RNAi machinery genes, please refer to the following: Yoon et al. 2016; Zhu & Palli 2020; Joga et al. 2021.

Box 1: Functional genomic studies in termites using RNAi

***Reticulitermes flavipes*:** The first RNAi study was done in the *R. flavipes* by injecting short interfering RNAs (siRNAs) (Zhou et al. 2006a; 2006b), creating siRNA against the two *hexamerin* genes (*Hex-1* and *Hex-2*) that are involved in the control of caste polyphenism, and administered to the worker termites. Based on RNAi knockdown studies, it was discovered that the two hexamerins participate in the regulation of caste differentiation by modulating JH availability, and the elevated ratios of *Hex-2* to *Hex-1* expression are associated with caste phenotypes that differentiate in response to rising JH titers (i.e., workers, presoldiers, and soldiers) (Zhou et al. 2006a; 2006b). The same team also examined the expression of 17 genes linked to morphogenesis after the Hexamerin genes were silenced and proved that most target genes are components of a genomic network that responds to JH (Zhou et al. 2007). Using a high-dose dsRNA feeding strategy, two more termite genes were silenced. One of the genes encodes an endogenous digestive cellulase enzyme called *endoglucanase*, while the other encodes a caste-regulatory storage protein called *hexamerin*. Silencing genes through high-dose dsRNA feeding resulted in significantly lower group fitness and mortality (Zhou et al. 2008). A novel gene “*deviate*” that encoded a ligand-binding protein was identified in the takeout-homologous family and characterized in *R. flavipes* using the RNAi approach (Schwinghammer et al. 2011). The unique insect cytochrome P450 gene *Cyp15F1* was functionally characterized using RNAi, demonstrating its significance in termite caste regulation in the environment. These results significantly advance our understanding of termite caste homeostatic processes by providing the second instance of a termite caste regulating gene discovered using RNAi (Tarver et al. 2012). In *R. flavipes* termites, the injection of dsRNAs targeting *Termicin* and *GNBP2* resulted in a considerable decrease in gene expression and increased mortality after the termites were exposed to the entomopathogenic fungi *Metarhizium anisopliae*. This indicated that *Termicin* and *GNBP2* play an essential role in defence by limiting fungal establishment on the cuticle of *R. flavipes* (Hamilton & Bulmer, 2012). More recently, in *R. flavipes*, sixty-two dsRNAs were synthesized from thirty-one target genes and used for immersion or feeding assays, and three lead termiticidal dsRNAs were identified, i.e., 3' *Hexamerin-2*, 3' *Glycosyl Hydrolase Family (GHF) 9-2 cellulase*, and 5' *GHF9-2 cellulase* (Raje et al. 2018).

***Reticulitermes speratus*:** In 2015, Saiki et al. examined the *Methoprene-tolerant* gene function by RNAi and showed that the juvenile hormone (JH) titres of neotenic were significantly higher than those of nymphs and workers and suggested that abdominal activation of JH signalling may induce the reproduction of termite neotenic (Saiki et al. 2015).

***Reticulitermes chinensis*:** Active immunization can shield individuals from infectious diseases in social insects. To prove the *isocitrate dehydrogenase (IDH)* active immunization regulating mechanism against fungi, Liu et al. (2020) injected dsIDH into *R. chinensis* termites (Liu et al. 2020). In 2020, Zhao et al. investigated the active immunization function of *selenoprotein T (SELT)* gene in *R. chinensis* termites. The knockdown of *SELT* and *TG* demonstrated that both the *SELT* and *TG* play essential roles in driving active immunization against the entomopathogenic fungus *M. anisopliae* in *R. chinensis* (Zhao et al. 2020). Recently, Liu et al. (2022) showed that *Dicer-1*-silenced and *miR-71-5* stimulant-treated termite groups exhibited a high level of mortality during fungal contamination, suggesting that miRNAs play a role in shaping social immunity in termite colonies (Liu et al. 2022).

***Odontotermes formosanus*:** In termites, trail pheromones play significant roles in their ability to form tunnels and nests and engage in foraging behaviour, although it is practically unclear how termites perceive trail pheromones. Gao et al. (2020) discovered that *Orco* knockdown made it difficult for termites to follow pheromone trails and hindered their perception of trail pheromones, indicating the role of *Orco* in detecting their trail pheromones (Gao et al. 2020). Antimicrobial peptides (AMPs) are essential components of the innate immune system in insects. However, there is still a lack of precise knowledge about AMPs in termites. The *aftermicin* gene knockdown in *O. formosanus*, treated with *Serratia marcescens* Bizio (*SMI*), showed significantly higher mortality, suggesting that *aftermicin* genes are potent immunosuppressants in termites (Feng et al. 2022).

***Zootermopsis nevadensis*:** In social insects, all castes exhibit distinctive morphologies suited to their functions and participate in social interactions. A significant development in the evolution of social insects was the acquisition of caste-specific characteristics. Nevertheless, knowledge of the genetic underpinnings and the developmental processes that give rise to these characteristics is still quite limited. A comparative study between termites (*Z. nevadensis*) and cockroaches showed that caste-specific cuticular pigmentation resulted from different expression patterns of “key genes” in the

Box 1. continued.

tyrosine metabolic pathway (*dopamine decarboxylase*, *NBAD synthase*, *acetyltransferase*, and *laccase 2*) using RNAi (Masuoka & Maekawa, 2016). In 2018, Masuoka et al. further investigated the function of JH and its signalling route in the *Z. nevadensis* termites and compared it with its sister group of termites *C. punctulatus* wood roach, using RNAi. They detected the inhibition of the presoldier molt in *Z. nevadensis* and the nymphal molt in *C. punctulatus* after silencing the JH receptor *Methoprene tolerant* (*Met*). *20-hydroxyecdysone* production genes were suppressed by *Met* knockdown in both species. However, *Met* RNAi particularly suppressed several *20E* signalling genes in *Z. nevadensis* (Masuoka et al. 2018a). Further using RNAi, the unique role of controlling the balance between JH and *20E* signalling during soldier differentiation in termites was thoroughly investigated (Masuoka et al. 2018b). In *Z. nevadensis*, larval gene expression revealed that a unique gene called *Neural Lazarillo* (*NLaz1*) expressed differently in these soldier-destined larvae compared to worker-destined larvae. Soldier differentiation rates were reduced, and trophallactic interactions with the queen were severely hindered by RNAi-induced *NLaz1* knockdown, demonstrating the significance of high larval *NLaz1* expression for termite soldier determination through social communication (Yaguchi et al. 2018).

***Coptotermes formosanus*:** Liu et al. (2017) used RNAi to functionally analyze the *exoglucanase* gene *cellobiohydrolase* (*CBH*), which was highly expressed in *Pseudotriconympha grassii*, a flagellate found in the hindgut of *C. formosanus*, in order to ascertain the function of *glycosyl hydrolase family 7* (*GHF7*) members in vivo. They showed that the *CBH* gene is crucial for host survival and the lignocellulolytic process in the protist (Liu et al. 2017). Termites use endogenous and symbiotic cellulases to digest wood and other wood-related materials for food and energy. Wu et al. (2019) investigated the impact of RNAi on *C. formosanus* termites by focusing on a conserved area of five *endoglucanase* genes (*CfEG1a*, *CfEG1b*, *CfEG2*, *CfEG3*, and *CfEG4*) (Wu et al. 2019).

***Cryptotermes secundus*:** Using cDNA representational difference analysis (cDNA-RDA), Karb et al. (2009) discovered the *Neofem2* gene and proposed that *Neofem2* is essential for queen-worker communication. They conducted behavioural experiments after using RNAi to silence *Neofem2* in queens in eight colonies of queenright, and they concluded that *Neofem2* is required for the queen to prevent worker reproduction by making workers act as though the colony is queenless. It might have evolved from an ancestral role of wood digestion to pheromonal communication within termites (Korb et al. 2009).

***Hodotermopsis sjostedti*:** In the damp wood termite *H. sjostedti*, *insulin/insulin-like growth factor signalling* (*IIS*) factors were studied by Hattori et al. (2013) throughout the soldier differentiation process. *Insulin receptor* (*InR*) was significantly expressed in the mandibular epithelial tissues by in situ hybridization, and RNAi against *InR* interfered with soldier-specific morphogenesis, including mandibular elongation. These results suggested that the *IIS* pathway is required for soldier-specific morphogenesis (Hattori et al. 2013). Sugime et al. (2019) performed 18 candidate gene expression analyses in *H. sjostedti* to determine the factors causing termite mandibular enlargement and discovered that *dachshund* (*dac*) and *distal-less* (*Dll*) are mandible-specific, and RNAi knockdown of *dac* and *Dll* decreased mandibular length and changed its morphology (Sugime et al. 2019).

***Nasutitermes takasagoensis*:** Toga et al. (2012) investigated the homeobox gene *Distal-less* (*Dll*) function in *N. takasagoensis* during nasus development to comprehend the developmental basis of the evolutionarily new form of termites. *Dll* RNAi inhibited nasus growth, not the formation of frontal-gland. The co-option of *Dll* is proposed to contribute to acquiring a novel defensive structure in a termite lineage and adaptive defensive behaviours (Toga et al. 2012). Later Toga et al. (2013) investigated *Dfd* roles in nasus/frontal gland development and mandibular regression. Mandibular regression during presoldier differentiation was prevented by *Dfd* RNAi, although nasus and frontal gland development were unaffected. These findings indicate that *Dfd* functions during presoldier differentiation in *N. takasagoensis* to determine mandibular positioning information and its specific modification (Toga et al. 2013).

Table 2. RNAi studies on termites.

Species	target gene	Gene function	dsRNA/ siRNA/miRNA	Delivery method	Reference
<i>Reticulitermes flavipes</i>	<i>Hexamerin</i>	<i>Hexamerins</i> have well-defined roles as JH-binding proteins and are involved in termite caste differentiation.	siRNA	Injection	Zhou et al. 2006a
<i>Reticulitermes flavipes</i>	<i>Hexamerin-1 (Hex-1)</i> and <i>Hexamerin-2 (Hex-2)</i>	<i>Hexamerins</i> have well-defined roles as JH-binding proteins and are involved in termite caste differentiation.	siRNA	Injection	Zhou et al. 2006b
<i>Reticulitermes flavipes</i>	<i>Hexamerin-1 (Hex-1)</i> and <i>Hexamerin-2 (Hex-2)</i>	<i>Hexamerins</i> have well-defined roles as JH-binding proteins and are involved in termite caste differentiation.	siRNA	Injection	Zhou et al. 2007
<i>Reticulitermes flavipes</i>	<i>Endoglucanase</i> and <i>hexamerin</i>	<i>Endoglucanases</i> cleave internal β -glycosidic bonds in the cellulose chain, making chain ends accessible to cellobiohydrolase. <i>Hexamerins</i> serve as storage proteins for gonad development, egg production and support foraging activity.	dsRNA	Injection and feeding	Zhou et al. 2008
<i>Cryptotermes secundus</i>	<i>Neofem2</i>	<i>Neofem2</i> produces a beta-glycosidase protein and allows termites to digest cellulose and large plant molecules. Also, pheromones production will enable termites to communicate with one another and allow the queen to suppress worker's sexual activities.	dsRNA	Feeding	Korb et al. 2009
<i>Reticulitermes flavipes</i>	<i>Deviate</i>	<i>Deviate</i> encodes an apparent ligand-binding protein and is involved in termite trail-following behaviour.	siRNA	Injection	Schwinghammer et al. 2011
<i>Reticulitermes flavipes</i>	<i>Termicin</i> and <i>GNBP2</i>	<i>Termicin</i> is a cysteine-rich antifungal peptide also exhibiting a weak antibacterial activity. <i>GNBP2</i> is involved in recognizing invading microorganisms and activating innate immune response and receptor signalling pathways. It binds specifically to beta-1,3-glucan and triggers the phenoloxidase cascade.	dsRNA	Injection	Hamilton & Bulmer 2012
<i>Reticulitermes flavipes</i>	<i>Cyp15 family gene (Cyp15F1)</i>	<i>Cyp15F1</i> is involved in juvenile hormone-dependent caste differentiation in the termites.	dsRNA	Feeding	Tarver et al. 2012
<i>Nasutitermes takasagoensis</i>	<i>Distal-less (Dll)</i>	<i>Distal-less (Dll)</i> is involved in forming a defensive structure in the termite lineage, aiding in acquiring adaptive defensive behaviours.	siRNA	Injection	Toga et al. 2012
<i>Hodotermopsis sjostedti</i>	<i>HsjInR</i>	<i>HsjInR</i> is involved in soldier-specific morphogenesis, including mandibular elongation.	dsRNA	Injection	Hattori et al. 2013
<i>Nasutitermes takasagoensis</i>	<i>Deformed (Dfd)</i>	<i>Deformed (Dfd)</i> is involved in determining mandibular positional information and specific modification during pre-soldier differentiation in termites.	siRNA	Injection	Toga et al. 2013
<i>Reticulitermes speratus</i>	<i>Methoprene-tolerant (Met)</i>	<i>Methoprene-Tolerant (Met)</i> is a JH-mediated intranuclear receptor required for larval metamorphosis and female reproduction.	dsRNA	Injection	Saiki et al. 2015
<i>Zootermopsis nevadensis</i>	<i>Dopamine decarboxylase (DDC)</i> , <i>NBAD synthase (ebony)</i> , <i>arylalkylamine N-acetyltransferase (aaNAT)</i> , <i>laccase2 (Lac2)</i>	<i>Dopamine decarboxylase</i> synthesizes dopamine and serotonin from L-DOPA and L-5-hydroxytryptophan, respectively. <i>NBAD synthase</i> catalyzes the synthesis of NBAD, the main sclerotization and pigmentation precursor of insects with brown cuticles, but also plays a role in brain neurotransmitter metabolism. <i>Arylalkylamine N-acetyltransferase</i> is involved in the day/night rhythmic melatonin production by modifying serotonin. <i>Lac2</i> has involved in insect cuticular pigmentation and hardening as well as melanization immune response.	dsRNA	Injection	Masuoka & Maekawa, 2016

Table 2. continued.

Species	target gene	Gene function	dsRNA/ siRNA/miRNA	Delivery method	Reference
<i>C. formosanus</i>	<i>Protistan gene (PgCBH)</i>	<i>PgCBH</i> gene plays a role in the protist lignocellulolytic process and is essential for host survival.	siRNA	Feeding	Liu et al. 2017
<i>Reticulitermes flavipes</i>	3' Hexamerin-2, 3' Glycosyl Hydrolase Family 9-2 cellulase, and 5' GHF9-2 cellulase	<i>Hexamerin 2</i> may influence larval and ovary development. Members of family GH9 are mainly cellulases, including endo-glucanases, that hydrolyze the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety.	dsRNA	Feeding	Raje et al. 2018
<i>Zootermopsis nevadensis</i>	<i>Methoprene tolerant (Met)</i> , 20E signalling and nuclear receptor; <i>Hormone receptor 39 (HR39/FTZ-F1)</i> genes	<i>Methoprene-tolerant (Met)</i> is an intranuclear receptor that mediates JH actions. 20E signalling regulates egg production and egg hatching. <i>Hormone receptor 39</i> plays multiple roles in reproductive gland development.	dsRNA	Injection	Masuoka et al. 2018a
<i>Zootermopsis nevadensis</i>	<i>Transcription factor SOX-11-like isoform X3 (ZnSox-11)</i> and <i>uncharacterized protein gene (Znev_01548)</i>	<i>Transcription factor SOX-11-like isoform X3 (ZnSox-11)</i> and <i>uncharacterized protein gene (Znev_01548)</i> are involved in TGFβ signalling and play a role in regulating between JH and 20E signalling during soldier differentiation in termites.	dsRNA	Injection	Masuoka et al. 2018b
<i>Zootermopsis nevadensis</i>	<i>Neural Lazarillo (NLaz1)</i>	<i>NLaz1</i> is involved in soldier determination through social communication in termites.	siRNA	Injection	Yaguchi et al. 2018
<i>Coptotermes formosanus</i>	<i>β-glucosidase genes (CjBG-Ia and CjBG-Ib)</i>	<i>β-Glucosidase</i> genes are rate-limiting enzymes in the cellulose degradation process.	dsRNA	Injection	Wu et al. 2019
<i>Hodotermopsis sjostedti holmgren</i>	<i>dachshund (dac)</i> and <i>Distal-less (Dll)</i>	<i>Dachshund (dac)</i> is involved in eye and leg development, whereas <i>Distalless (Dll)</i> is engaged in leg development in insects.	dsRNA	Injection	Sugime et al. 2019
<i>Reticulitermes chinensis</i>	<i>Isocitrate dehydrogenase (IDH)</i>	<i>Isocitrate dehydrogenase (IDH)</i> is a Krebs cycle enzyme that catalyzes the oxidative decarboxylation of isocitrate, resulting in alpha-ketoglutarate and carbon dioxide. Also involved in immunization against fungal pathogens in termites.	dsRNA	Injection	Liu et al. 2020
<i>Reticulitermes chinensis Snyder and Odontotermes formosanus Shiraki</i>	<i>Olfactory co-receptor (Orco)</i>	<i>The olfactory co-receptor</i> is necessary for the localization of ORs to dendritic membranes and, thus, is essential for odorant detection.	dsRNA	Injection	Gao et al. 2020
<i>Reticulitermes chinensis</i>	<i>Selenoprotein T (SELT)</i> and <i>Transglutaminase (TG)</i>	<i>Selenoprotein T (SELT)</i> is encoded by the UGA codon that usually signals translation termination. <i>Transglutaminase (TG)</i> plays a crucial role in cuticular morphogenesis, hemolymph coagulation, and entrapment against invading pathogens and is involved in maintaining homeostasis in gut microbiota.	dsRNA	Injection	Zhao et al. 2020
<i>Reticulitermes chinensis</i>	<i>Dicer-1</i> and <i>miR-71-5</i>	<i>Dicer-1</i> , also known as endoribonuclease, plays a central role in short dsRNA-mediated post-transcriptional gene silencing and cleaves double-stranded RNA and pre-microRNA into short double-stranded RNA fragments called small interfering RNA and microRNA, respectively. <i>miR-71-5</i> is involved in developmental regulation and antifungal activity.	dsRNA and miRNA	Feeding	Liu et al. 2022
<i>Odontotermes formosanus</i>	<i>Termicin</i>	<i>Termicin</i> has antifungal activity in insects.	dsRNA	Feeding	Feng et al. 2022

4 RNAi-based termiticides: barriers and innovative solutions

The social lifestyle, habitat, and feeding habits of termites (e.g., soil contains many microbes that produce dsRNA-degrading nucleases, and insects have also been reported to produce these nucleases in their guts) present complex challenges in the application of RNAi-based termiticides. Therefore, the stability and delivery strategy of dsRNA are likely to be the key factors that could affect the effectiveness of this control strategy. Carefully designed dsRNA packaging strategies could help in protecting dsRNA from environmental degradation and facilitate cellular uptake. The dsRNA can be encapsulated within a few hundred nanometres size organic and inorganic materials to form nano-complexes. For controlling many insect species, several types of nano-complexes have shown great potential (Christiaens et al. 2020b; Zhu & Palli, 2020; Gurusamy et al. 2020; Kolge et al. 2021), and these can be used against termite pests via GMO-free approaches such as spraying on nests and barks, trunk injection, root absorption, etc. (Hunter et al. 2012; Dalakouras et al. 2018; Máximo et al. 2020). Moreover, some recent studies have shown that some dsRNA-nanocarrier complexes can enable successful transdermal delivery of dsRNA into insects (Zheng et al. 2019; Yan et al. 2020a; Wei et al. 2022). Some nanoparticles that improve RNAi efficacy in insects include chitosan, guanylated polymers, core-shell nanoparticles, liposomes, and branched amphiphilic peptide capsules (BAPCs) (Christiaens et al. 2020a). Researchers demonstrated that non-toxic and biodegradable chitosan binds to dsRNA via electrostatic interactions, protecting the chitosan-dsRNA complexes from nucleases and successfully knocking down the target genes in RNAi refractory pest *Spodoptera frugiperda* (Gurusamy et al. 2020a).

Recently, in our lab, for in vitro studies, we used chitosan nanoparticles to protect dsRNA from enzymatic degradation in the gut of termites, and the initial results seem very promising but need further experimental corroboration (unpublished data). The synthesis of chitosan-dsRNA nano-complexes is a simple and cost-effective technology that might be used in termite pest management. Polyethylenimine glycol (PEI) and Polyethylene Glycol 200 (PEG200) have been successfully used to make carbon quantum dot (CQD) nanoparticles, where PEI with a positively charged surface could efficiently bind with negatively charged dsRNA to form a CQD-dsRNA nano-complex. The study demonstrated that the CQD-dsRNA nanocomplexes could protect against degradation by dsRNA-degrading nucleases and successfully shut down genes in moderate RNAi-resistant pests such as mosquitoes and whiteflies (Das et al. 2015, Kaur et al. 2020). These CQD-dsRNA nanoparticles can also be specifically designed to target termites used for termite pest control. Researchers found that dsRNA interacts with the cationic surface of branched amphiphilic peptide capsules (BAPCs) to generate dsRNA-BAPCs pills, which protect dsRNA-

BAPCs from nucleases and improve RNAi in two distinct insect species, the red flour beetle *Tribolium castaneum* and the pea aphid *Acyrtosiphon pisum* (Avila et al. 2018). Termite-specific dsRNA-BAPCs capsules could be developed, combined with wood powder and fed to termites in the context of RNAi-based termite pest management in the field. The delivery of dsRNA via liposomes would be a promising technique because liposomes are made up of natural lipids and are biodegradable. Through endocytosis, liposomes fuse with the plasma membrane and facilitate the transport of liposome-encapsulated dsRNAs into the cell's cytoplasm. Several studies have demonstrated that liposome-encapsulated dsRNAs can be protected from the degrading action of nucleases and can considerably improve RNAi efficiency in RNAi recalcitrant pests (Dalby et al. 2004; Taning et al. 2016; Zhang et al. 2018; Gurusamy et al. 2020b). However, lipid-based nanoparticles are expensive, and there have been no reports of dsRNA administration using liposomes in the field to suppress insect pests. However, with constant improvements in the field of nanotechnology, this could be a feasible option for insect pest control in the future.

For field application, a large amount of dsRNA is required. *E. coli* strains such as HT115(DE3) and Pet28-BL21(DE3), which have T7 expression vectors, can be used for large-scale dsRNA production at a low cost (Ma et al. 2020). The biotechnology company “GENOLUTION” has developed a platform under the name AgroRNA, which exploits a bacterial system for large-scale manufacturing of dsRNA with potential for field application. In the Asian long-horned beetle, Colorado potato beetle, South American tomato pin worm, *Plagioderma versicolora*, and *Henosepilachna vigintioctopunctata*, bacterially-expressed dsRNA showed higher RNAi efficiency (Leelesh & Rieske 2020; Zhang et al. 2019). Likewise, bacteria symbionts have been identified in the kissing bug and the western flower thrips, which can be used for dsRNA expression and delivery (Rotenberg et al. 2020). The symbiont-mediated dsRNA delivery method is intriguing because it carries species-specific dsRNA by species-specific symbionts, making it less costly and efficient for termite pest management. Furthermore, many promising studies have already been done to understand symbiotic associations in termites, which may facilitate identifying suitable candidates for symbiont-mediated dsRNA transfer (SMT) (Auer et al. 2017; Tokuda et al. 2018; Noda et al. 2018).

Termite-specific viruses can be exploited through a process known as virus-induced gene silencing (VIGS) to develop RNAi-based termiticides. In this approach, a termite-specific virus that can replicate in termites without causing significant mortality is modified to express an mRNA fragment of a termite-specific essential gene. During viral replication, the production of complementary viral strands leads to the formation of termite-specific dsRNAs, which then target a specific endogenous mRNA in the insect cell, ultimately leading to lethal effects. Proof of concept of VIGS has been successfully reported for several insect species, confirm-

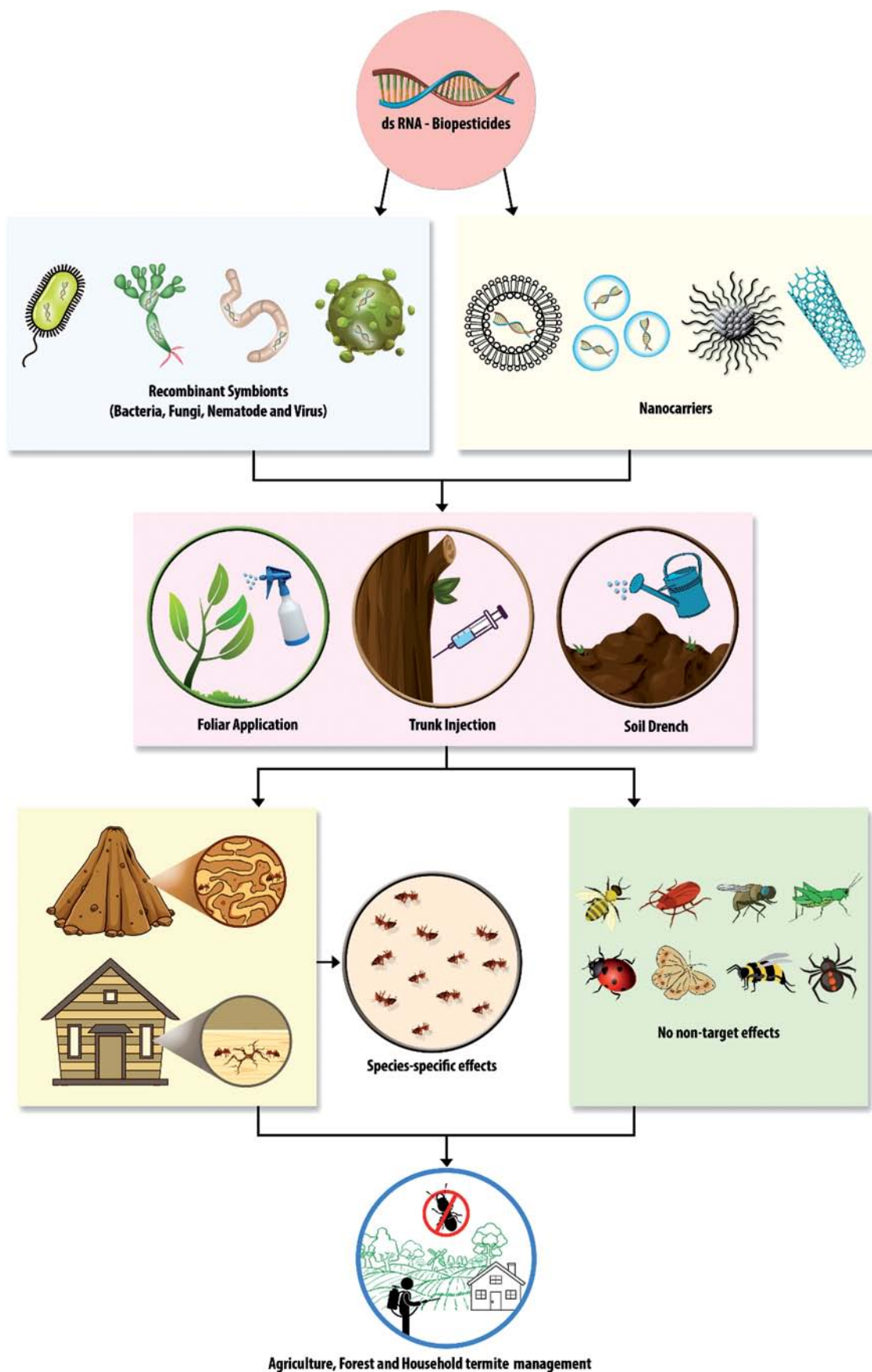


Fig. 1. Scheme illustrating the method and goals of RNAi-based termite management.

ing its feasibility (Kolliopoulou et al. 2017). However, the application of recombinant viruses in the environment can be challenging due to strict regulations and social concerns. Alternatively, a fungal-mediated gene silencing (FMGS) approach could be adopted for termite pest management. Here, entomopathogenic fungi or fungal symbionts from termites could be modified to produce termite-specific dsRNA to trigger RNAi in termites. The feasibility of FMGS has been confirmed in other insect species (Niu et al. 2018; Hu & Xia 2019). Nevertheless, the increasing amount of omics data for termites and identifying the true symbionts will significantly contribute to developing these innovative delivery strategies for dsRNA, facilitating termite pest control.

5 Biosafety considerations for RNAi-termiticides

The consideration of potential ecological, environmental, and human risk assessment is essential in developing RNAi-based termiticides. RNAi-based termiticides will likely also be used indoors to protect wooden structures, implying that the definition of non-target organisms (NTOs) in safety evaluations should be extended to include house pets. In the field, safety assessment for NTOs should include soil organisms (including beneficial microbes) and other beneficial organisms (i.e., pollinators such as bees and natural enemies such as predators and parasitic wasps) that share a common habitat with termite pests. As more omics data for termites and NTOs becomes available, the ability of scientists to design termite pest-specific dsRNAs will improve, thereby contributing to limiting potential risks. Fortunately, in collaboration with colleagues from Japan (Okinawa Institute of Science and Technology), researchers from our university (CZU, Prague) have recently sequenced ~50 termite genomes (unpublished), which will significantly enhance sequence information on termites and facilitate future species-specific RNAi-based termiticide development. The development of novel delivery methods (i.e., BioClay spray) also raises high hopes for deploying RNAi against termites (Jain et al. 2022).

6 Summary

RNAi in insects is obtained by the siRNA, miRNA, and piRNA pathways. However, lepidopterans are less sensitive, and coleopterans are more sensitive to RNAi, whereas other insect orders, including termites, are moderately sensitive (Zhu & Palli 2020). This variation may result from the choice of the target gene, cellular uptake and intracellular transit of dsRNA, stability of dsRNA, RNAi core machinery gene expression, and dsRNA processing to siRNA. Researchers have relied on simple dsRNA delivery methods like microinjection and feeding but have not seen any notable outcomes (Table 2). For superior transport of dsRNA, combining more modern development technologies (such

as nanoparticle-enabled and symbiont-mediated) into feeding methods is necessary. These technologies may also aid dsRNA stability, endosomal escape, and dsRNA processing. The effectiveness of RNAi, the absence of dependable dsRNA delivery techniques, off-target and non-target effects, and potential resistance development are the main obstacles to the widespread use of RNAi for termite pest management. Obtaining high effectiveness of RNAi in eusocial termites will be more challenging due to their social behaviour and immunity to defend against RNAi activity. In this perspective, we revisited such problems and searched for putative mitigation strategies (Fig. 1). We first identified complete RNAi machinery genes in termites and cross-checked them with RNAi-sensitive coleopteran RNAi machinery genes (Joga et al. 2021) and concluded that termites have the same type of RNAi machinery and the potential to be sensitive to RNAi. Finding suitable target genes and dsRNA delivery methods concerning dsRNA stability and cellular transport will be the key to success. Impediments to RNAi efficacy in eusocial termite colonies must be resolved using superior technology. All facets of termite biology as holobiont, from eusocial behaviour to symbiosis, await further illumination to develop RNAi-based termite control products.

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References

- Al Fazairy, A. L., & Hassan, F. A. (1988). Infection of termites by *Spodoptera littoralis* nuclear polyhedrosis virus. *International Journal of Tropical Insect Science*, 9(1), 37–39. <https://doi.org/10.1017/S1742758400009991>
- Auer, L., Lazuka, A., Sillam-Dussès, D., Miambi, E., O'Donohue, M., & Hernandez-Raquet, G. (2017). Uncovering the potential of termite gut microbiome for lignocellulose bioconversion in anaerobic batch bioreactors. *Frontiers in Microbiology*, 8, 2623. <https://doi.org/10.3389/fmicb.2017.02623>
- Avila, L. A., Chandrasekar, R., Wilkinson, K. E., Balthazor, J., Heerman, M., Bechard, J., ... Tomich, J. M. (2018). Delivery of lethal dsRNAs in insect diets by branched amphiphilic peptide capsules. *Journal of Controlled Release*, 273, 139–146. <https://doi.org/10.1016/j.jconrel.2018.01.010>
- Benmoussa-Haïchour, D., Reversat, G., & Rouland, C. (1998). Sensitivity of the different castes of higher termites to an infection with entomopathogenic nematodes-role of the lipids composition. *Actes des Colloques Insectes Sociaux*, 11(11), 45–52.
- Bucek, A., Šobotník, J., He, S., Shi, M., McMahon, D. P., Holmes, E. C., ... Bourgui, T. (2019). Evolution of termite symbiosis informed by transcriptome-based phylogenies. *Current Biology*, 29(21), 3728–3734. <https://doi.org/10.1016/j.cub.2019.08.076>
- Cagliari, D., Dias, N. P., Galdeano, D., dos Santos, E. Á., Smaghe, G., & Zotti, M. J. (2019). Management of pest insects and plant diseases by non-transformative RNAi. *Frontiers in Plant Science*, 10, 1319. <https://doi.org/10.3389/fpls.2019.01319>

- Chouvenc, T., Su, N.-Y., & Robert, A. (2009). Susceptibility of seven termite species (Isoptera) to the entomopathogenic fungus *Metarhizium anisopliae*. *Sociobiology*, 54, 723–748.
- Christiaens, O., Petek, M., Smagghe, G., & Taning, C. N. T. (2020a). The use of nanocarriers to improve the efficiency of RNAi-based pesticides in agriculture. In L. F. Fraceto, V. L. S. S. de Castro, R. Grillo, D. Avila, H. C. Oliveira, & R. Lima (Eds.), *Nanopesticides* (pp. 49–68). Springer. https://doi.org/10.1007/978-3-030-44873-8_3
- Christiaens, O., Whyard, S., Velez, A. M., & Smagghe, G. (2020b). Double-stranded RNA technology to control insect pests: Current status and challenges. *Frontiers in Plant Science*, 11, 451. <https://doi.org/10.3389/fpls.2020.00451>
- Christiaens, O., Sweet, J., Dzhambova, T., Urru, I., Smagghe, G., Kostov, K., & Arpaia, S. (2022). Implementation of RNAi-based arthropod pest control: Environmental risks, potential for resistance and regulatory considerations. *Journal of Pest Science*, 95(1), 1–15. <https://doi.org/10.1007/s10340-021-01439-3>
- Cowie, R., Logan, J., & Wood, T. (1989). Termite (Isoptera) damage and control in tropical forestry with special reference to Africa and Indo-Malaysia: A review. *Bulletin of Entomological Research*, 79(2), 173–184. <https://doi.org/10.1017/S0007485300018150>
- Dalakouras, A., Jarausch, W., Buchholz, G., Bassler, A., Braun, M., Manthey, T., ... Wassenegger, M. (2018). Delivery of hairpin RNAs and small RNAs into woody and herbaceous plants by trunk injection and petiole absorption. *Frontiers in Plant Science*, 9, 1253. <https://doi.org/10.3389/fpls.2018.01253>
- Dalby, B., Cates, S., Harris, A., Ohki, E. C., Tilkins, M. L., Price, P. J., & Ciccarone, V. C. (2004). Advanced transfection with Lipofectamine 2000 reagent: Primary neurons, siRNA, and high-throughput applications. *Methods (San Diego, Calif.)*, 33(2), 95–103. <https://doi.org/10.1016/j.ymeth.2003.11.023>
- Das, S., Debnath, N., Cui, Y., Unrine, J., & Palli, S. R. (2015). Chitosan, carbon quantum dot, and silica nanoparticle mediated dsRNA delivery for gene silencing in *Aedes aegypti*: A comparative analysis. *ACS Applied Materials & Interfaces*, 7(35), 19530–19535. <https://doi.org/10.1021/acsami.5b05232>
- De Schutter, K., Taning, C. N. T., Van Daele, L., Van Damme, E. J. M., Dubruiel, P., & Smagghe, G. (2022). RNAi-based bio-control products: Current status on market, regulatory aspects and risk assessment. *Frontiers in Insect Science*, 1, 818037. <https://doi.org/10.3389/finsc.2021.818037>
- Devi, K. K., Seth, N., Kothamasi, S., & Kothamasi, D. (2007). Hydrogen cyanide-producing rhizobacteria kill subterranean termite *Odontotermes obesus* (Rambur) by cyanide poisoning under in vitro conditions. *Current Microbiology*, 54(1), 74–78. <https://doi.org/10.1007/s00284-006-0473-z>
- Feng, K., Li, W., Tang, X., Luo, J., & Tang, F. (2022). Termicin silencing enhances the toxicity of *Serratia marcescens* Bizio (SM1) to *Odontotermes formosanus* (Shiraki). *Pesticide Biochemistry and Physiology*, 185, 105120. <https://doi.org/10.1016/j.pestbp.2022.105120>
- Gao, Y., Huang, Q., & Xu, H. (2020). Silencing Orco impaired the ability to perceive trail pheromones and affected locomotion behavior in two termite species. *Journal of Economic Entomology*, 113(6), 2941–2949. <https://doi.org/10.1093/jee/toaa248>
- Geng, A., Cheng, Y., Wang, Y., Zhu, D., Le, Y., Wu, J., ... Sun, J. (2018). Transcriptional analysis of the digestive system of a wood-feeding termite (*Coptotermes formosanus*) revealed a unique mechanism for effective biomass degradation. *Biotechnology for Biofuels*, 11(1), 1–14. <https://doi.org/10.1186/s13068-018-1015-1>
- Gurusamy, D., Mogilicherla, K., & Palli, S. R. (2020a). Chitosan nanoparticles help double-stranded RNA escape from endosomes and improve RNA interference in the fall armyworm, *Spodoptera frugiperda*. *Archives of Insect Biochemistry and Physiology*, 104(4), e21677. <https://doi.org/10.1002/arch.21677>
- Gurusamy, D., Mogilicherla, K., Shukla, J. N., & Palli, S. R. (2020b). Lipids help double-stranded RNA in endosomal escape and improve RNA interference in the fall armyworm, *Spodoptera frugiperda*. *Archives of Insect Biochemistry and Physiology*, 104(4), e21678. <https://doi.org/10.1002/arch.21678>
- Hamilton, C., & Bulmer, M. S. (2012). Molecular antifungal defenses in subterranean termites: RNA interference reveals in vivo roles of termicins and GNBPs against a naturally encountered pathogen. *Developmental and Comparative Immunology*, 36(2), 372–377. <https://doi.org/10.1016/j.dci.2011.07.008>
- Hattori, A., Sugime, Y., Sasa, C., Miyakawa, H., Ishikawa, Y., Miyazaki, S., ... Miura, T. (2013). Soldier morphogenesis in the damp-wood termite is regulated by the insulin signaling pathway. [Molecular and Developmental Evolution]. *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution*, 320(5), 295–306. <https://doi.org/10.1002/jez.b.22501>
- Head, G. P., Carroll, M. W., Evans, S. P., Rule, D. M., Willse, A. R., Clark, T. L., ... Meinke, L. J. (2017). Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: Efficacy and resistance management. *Pest Management Science*, 73(9), 1883–1899. <https://doi.org/10.1002/ps.4554>
- Hu, J., & Xia, Y. (2019). Increased virulence in the locust-specific fungal pathogen *Metarhizium acridum* expressing dsRNAs targeting the host F1F0-ATPase subunit genes. *Pest Management Science*, 75(1), 180–186. <https://doi.org/10.1002/ps.5085>
- Hunter, W. B., Glick, E., Paldi, N., & Bextine, B. R. (2012). Advances in RNA interference: dsRNA treatment in trees and grapevines for insect pest suppression. *Southwestern Entomologist*, 37(1), 85–87. <https://doi.org/10.3958/059.037.0110>
- Jafri, R. H., Ahmad, M., & Idrees, K. (1976). Microsporidian infection in the workers of termite *Microcerotermes championi*. *Pakistan Journal of Zoology*, 8, 234–236.
- Jain, R., Fletcher, S., & Mitter, N. (2022). Effective RNAi-mediated control of the crop pest whitefly. *Nature Plants*, 8(5), 461–462. <https://doi.org/10.1038/s41477-022-01160-8>
- Joga, M. R., Mogilicherla, K., Smagghe, G., & Roy, A. (2021). RNA interference-based forest protection products (FPPs) against wood-boring Coleopterans: Hope or hype? *Frontiers in Plant Science*, 12, 733608.
- Kaur, R., Gupta, M., Singh, S., Joshi, N., & Sharma, A. (2020). Enhancing RNAi efficiency to decipher the functional response of potential genes in *Bemisia tabaci* AsiaII-1(Gennadius) through dsRNA feeding assays. *Frontiers in Physiology*, 11, 123. <https://doi.org/10.3389/fphys.2020.00123>
- Khan, M. A., & Ahmad, W. (2018). Termites: An Overview. In M. A. Khan, & W. Ahmad (Eds.), *Termites and sustainable management* (pp. 1–25). Springer.
- Kolge, H., Kadam, K., Galande, S., Lanjekar, V., & Ghormade, V. (2021). New frontiers in pest control: Chitosan nanoparticles-shielded dsRNA as an effective topical RNAi spray for Gram Podborer biocontrol. *ACS Applied Bio Materials*, 4(6), 5145–5157. <https://doi.org/10.1021/acsabm.1c00349>

- Kolliopoulou, A., Taning, C. N., Smagghe, G., & Swevers, L. (2017). Viral delivery of dsRNA for control of insect agricultural pests and vectors of human disease: Prospects and challenges. *Frontiers in Physiology*, 8, 399. <https://doi.org/10.3389/fphys.2017.00399>
- Korb, J., Weil, T., Hoffmann, K., Foster, K. R., & Rehli, M. (2009). A gene necessary for reproductive suppression in termites. *Science*, 324(5928), 758. <https://doi.org/10.1126/science.1170660>
- Leelesh, R. S., & Rieske, L. K. (2020). Oral ingestion of bacterially expressed dsRNA can silence genes and cause mortality in a highly invasive, tree-killing pest, the emerald ash borer. *Insects*, 11(7), 440. <https://doi.org/10.3390/insects11070440>
- Liu, L., Wang, C. C., Zhao, X. Y., Guan, J. X., Lei, C. L., & Huang, Q. Y. (2020). Isocitrate dehydrogenase-mediated metabolic disorders disrupt active immunization against fungal pathogens in eusocial termites. *Journal of Pest Science*, 93(1), 291–301. <https://doi.org/10.1007/s10340-019-01164-y>
- Liu, L., Yan, F. M., Zhao, C. C., Su, L. J., Huang, Q. Y., & Tang, Q. B. (2022). microRNAs shape social immunity: A potential target for biological control of the termite *Reticulitermes chinensis*. *Journal of Pest Science*. <https://doi.org/10.1007/s10340-022-01495-3>
- Liu, X. J., Xie, L., Liu, N., Zhan, S., Zhou, X. G., & Wang, Q. (2017). RNA interference unveils the importance of Pseudotriconympha grassii cellobiohydrolase, a protozoan exoglucanase, in termite cellulose degradation. *Insect Molecular Biology*, 26(2), 233–242. <https://doi.org/10.1111/imb.12287>
- Logan, J. W. M. (1992). Termites (Isoptera): A pest or resource for small farmers in Africa? *Tropical Science*, 32, 71–79.
- Ma, Z. Z., Zhou, H., Wei, Y. L., Yan, S., & Shen, J. (2020). A novel plasmid–*Escherichia coli* system produces large batch dsRNAs for insect gene silencing. *Pest Management Science*, 76(7), 2505–2512. <https://doi.org/10.1002/ps.5792>
- Masuoka, Y., & Maekawa, K. (2016). Gene expression changes in the tyrosine metabolic pathway regulate caste-specific cuticular pigmentation of termites. *Insect Biochemistry and Molecular Biology*, 74, 21–31. <https://doi.org/10.1016/j.ibmb.2016.04.006>
- Masuoka, Y., Toga, K., Nalepa, C. A., & Maekawa, K. (2018a). A crucial caste regulation gene detected by comparing termites and sister group cockroaches. *Genetics*, 209(4), 1225–1234. <https://doi.org/10.1534/genetics.118.301038>
- Masuoka, Y., Yaguchi, H., Toga, K., Shigenobu, S., & Maekawa, K. (2018b). TGF β signaling related genes are involved in hormonal mediation during termite soldier differentiation. *PLOS Genetics*, 14(4), e1007338. <https://doi.org/10.1371/journal.pgen.1007338>
- Máximo, W. P., Howell, J. L., Mogilicherla, K., Basij, M., Chereddy, S. C., & Palli, S. R. (2020). Inhibitor of apoptosis is an effective target gene for RNAi-mediated control of Colorado potato beetle, *Leptinotarsa decemlineata*. *Archives of Insect Biochemistry and Physiology*, 104(4), e21685. <https://doi.org/10.1002/arch.21685>
- Mezzetti, B., Smagghe, G., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., ... Sweet, J. (2020). RNAi: What is its position in agriculture? *Journal of Pest Science*, 93, 1125–1130. <https://doi.org/10.1007/s10340-020-01238-2>
- Niu, J. Z., Taning, C. N. T., Christiaens, O., Smagghe, G., & Wang, J. J. (2018). Rethink RNAi in insect pest control: Challenges and perspectives. *Advances in Insect Physiology*, 55, 1–17. <https://doi.org/10.1016/bs.aiip.2018.07.003>
- Noda, S., Shimizu, D., Yuki, M., Kitade, O., & Ohkuma, M. (2018). Host-symbiont cospeciation of termite-gut cellulolytic protists of the genera *Teranympha* and *Eucomonympha* and their Treponema endosymbionts. *Microbes and Environments*, 33(1), 26–33. <https://doi.org/10.1264/jsme2.ME17096>
- Raje, K. R., Peterson, B. F., & Scharf, M. E. (2018). Screening of 57 candidate double-stranded RNAs for insecticidal activity against the pest termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Journal of Economic Entomology*, 111(6), 2782–2787. <https://doi.org/10.1093/jee/toy294>
- Rasheed, H., Ye, C., Meng, Y., Ran, Y., Li, J., & Su, X. (2019). Comparative transcriptomic analysis and endocuticular protein gene expression of alate adults, workers and soldiers of the termite *Reticulitermes aculabialis*. *BMC Genomics*, 20(1), 1–12. <https://doi.org/10.1186/s12864-019-6149-4>
- Rotenberg, D., Baumann, A. A., Ben-Mahmoud, S., Christiaens, O., Dermauw, W., Ioannidis, P., ... Richards, S. (2020). Genome-enabled insights into the biology of thrips as crop pests. *BMC Biology*, 18(1), 1–37. <https://doi.org/10.1186/s12915-020-00862-9>
- Roulant-Lefèvre, C. (2010). Termites as pests of agriculture. In D. E. Bignell, Y. Roisin, & N. Lo (Eds.), *Biology of termites: a modern synthesis* (pp. 499–517). Springer. https://doi.org/10.1007/978-90-481-3977-4_18
- Roulant-Lefèvre, C., & Mora, P. (2002). Control of *Ancistrotermes guineensis* Silvestri (Termitidae: Macrotermitinae), a pest of sugarcane in Chad. *International Journal of Pest Management*, 48(1), 81–86. <https://doi.org/10.1080/09670870110094387>
- Rust, M. K., & Su, N. Y. (2012). Managing social insects of urban importance. *Annual Review of Entomology*, 57(1), 355–375. <https://doi.org/10.1146/annurev-ento-120710-100634>
- Saiki, R., Gotoh, H., Toga, K., Miura, T., & Maekawa, K. (2015). High juvenile hormone titre and abdominal activation of JH signalling may induce reproduction of termite neotronics. *Insect Molecular Biology*, 24(4), 432–441. <https://doi.org/10.1111/imb.12169>
- Scharf, M. E. (2015). Termites as targets and models for biotechnology. *Annual Review of Entomology*, 60(1), 77–102. <https://doi.org/10.1146/annurev-ento-010814-020902>
- Schwinghammer, M. A., Zhou, X., Kambhampati, S., Bennett, G. W., & Scharf, M. E. (2011). A novel gene from the takeout family involved in termite trail-following behavior. *Gene*, 474(1–2), 12–21. <https://doi.org/10.1016/j.gene.2010.11.012>
- Sharma, K., Sharma, V., Gupta, P., Jaya, M., Kumar, A., & Singh, B. (2008). Persistence and vertical distribution of termiticide fipronil in modified ground board test. *Environmental Monitoring and Assessment*, 137, 179–184. <https://doi.org/10.1007/s10661-007-9738-7>
- Shigenobu, S., Hayashi, Y., Watanabe, D., Tokuda, G., Hojo, M. Y., Toga, K., ... Maekawa, K. (2022). Genomic and transcriptomic analyses of the subterranean termite *Reticulitermes speratus*: gene duplication facilitates social evolution. *Proceedings of the National Academy of Sciences of the USA*, 119(3), e2110361119.
- Stansly, P. A., Su, N. Y., & Conner, J. M. (2001). Management of subterranean termites, *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a citrus orchard with hexaflumuron bait. *Crop Protection (Guildford, Surrey)*, 20(3), 199–206. [https://doi.org/10.1016/S0261-2194\(00\)00127-7](https://doi.org/10.1016/S0261-2194(00)00127-7)
- Sugime, Y., Oguchi, K., Gotoh, H., Hayashi, Y., Matsunami, M., Shigenobu, S., ... Miura, T. (2019). Termite soldier mandibles are elongated by dachshund under hormonal and Hox gene con-

- trols. *Development (Cambridge, England)*, 146(5), dev171942. <https://doi.org/10.1242/dev.171942>
- Taning, C. N. T., Christiaens, O., Berkvens, N., Casteels, H., Maes, M., & Smagghe, G. (2016). Oral RNAi to control *Drosophila suzukii*: Laboratory testing against larval and adult stages. *Journal of Pest Science*, 89(3), 803–814. <https://doi.org/10.1007/s10340-016-0736-9>
- Taning, C. N., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., Mezzetti, B., ... Smagghe, G. (2020). RNA-based biocontrol compounds: Current status and perspectives to reach the market. *Pest Management Science*, 76(3), 841–845. <https://doi.org/10.1002/ps.5686>
- Tarver, M. R., Coy, M. R., & Scharf, M. E. (2012). Cyp15F1: A novel cytochrome P450 gene linked to juvenile hormone-dependent caste differentiation in the termite *Reticulitermes flavipes*. *Archives of Insect Biochemistry and Physiology*, 80(2), 92–108. <https://doi.org/10.1002/arch.21030>
- Toga, K., Hojo, M., Miura, T., & Maekawa, K. (2012). Expression and function of a limb-patterning gene Distal-less in the soldier-specific morphogenesis in the nasute termite *Nasutitermes takasagoensis*. *Evolution & Development*, 14(3), 286–295. <https://doi.org/10.1111/j.1525-142X.2012.00545.x>
- Toga, K., Saiki, R., & Maekawa, K. (2013). Hox gene deformed is likely involved in mandibular regression during presoldier differentiation in the nasute termite *Nasutitermes takasagoensis*. *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution*, 320(6), 385–392. <https://doi.org/10.1002/jez.b.22512>
- Tokuda, G., Mikaelyan, A., Fukui, C., Matsuura, Y., Watanabe, H., Fujishima, M., & Brune, A. (2018). Fiber-associated spirochetes are major agents of hemicellulose degradation in the hindgut of wood-feeding higher termites. *Proceedings of the National Academy of Sciences of the United States of America*, 115(51), E11996–E12004. <https://doi.org/10.1073/pnas.1810550115>
- Verma, M., Sharma, S., & Prasad, R. (2009). Biological alternatives for termite control: A review. *International Biodeterioration & Biodegradation*, 63(8), 959–972. <https://doi.org/10.1016/j.ibiod.2009.05.009>
- Wei, H., Tan, S., Yan, S., Li, Z., Shen, J., & Liu, X. (2022). Nanocarrier-mediated transdermal dsRNA-NPF1 delivery system contributes to pest control via inhibiting feeding behavior in *Grapholita molesta*. *Journal of Pest Science*, 95(2), 983–995. <https://doi.org/10.1007/s10340-021-01422-y>
- Wood, T. G., & Pearce, M. J. (1991). Termites in Africa: The environmental impact of control measures and damage to crops, trees, rangeland and rural buildings. *Sociobiology*, 19, 221–234.
- Wu, W., Gu, D., Yan, S., & Li, Z. (2019). RNA interference of endoglucanases in the formosan subterranean termite *Coptotermes formosanus Shiraki* (Blattodea: Rhinotermitidae) by dsRNA injection or ingestion. *Journal of Insect Physiology*, 112, 15–22. <https://doi.org/10.1016/j.jinsphys.2018.11.007>
- Yaguchi, H., Shigenobu, S., Hayashi, Y., Miyazaki, S., Toga, K., Masuoka, Y., & Maekawa, K. (2018). A lipocalin protein, Neural Lazarillo, is key to social interactions that promote termite soldier differentiation. *Proceedings of the Royal Society B. Biological Sciences*, 285(1883), 20180707. <https://doi.org/10.1098/rspb.2018.0707>
- Yan, S., Qian, J., Cai, C., Ma, Z., Li, J., Yin, M., ... Shen, J. (2020a). Spray method application of transdermal dsRNA delivery system for efficient gene silencing and pest control on soybean aphid *Aphis glycines*. *Journal of Pest Science*, 93(1), 449–459. <https://doi.org/10.1007/s10340-019-01157-x>
- Ye, C., Rasheed, H., Ran, Y., Yang, X., Xing, L., & Su, X. (2019). Transcriptome changes reveal the genetic mechanisms of the reproductive plasticity of workers in lower termites. *BMC Genomics*, 20(1), 1–13. <https://doi.org/10.1186/s12864-019-6037-y>
- Yoon, J. S., Shukla, J. N., Gong, Z. J., Mogilicherla, K., & Palli, S. R. (2016). RNA interference in the Colorado potato beetle, *Leptinotarsa decemlineata*: Identification of key contributors. *Insect Biochemistry and Molecular Biology*, 78, 78–88. <https://doi.org/10.1016/j.ibmb.2016.09.002>
- Zhang, Y., Cui, J., Zhou, Y., Cao, J., Gong, H., Zhang, H., & Zhou, J. (2018). Liposome mediated double-stranded RNA delivery to silence ribosomal protein P0 in the tick *Rhipicephalus haemaphysaloides*. *Ticks and Tick-Borne Diseases*, 9(3), 638–644. <https://doi.org/10.1016/j.ttbdis.2018.01.015>
- Zhang, Y., Xu, L., Li, S., & Zhang, J. (2019). Bacteria-mediated RNA interference for management of *Plagioderma versicolora* (Coleoptera: Chrysomelidae). *Insects*, 10(12), 415. <https://doi.org/10.3390/insects10120415>
- Zhao, X., Liu, L., Zhou, W., Cai, Q., & Huang, Q. (2020). Roles of selenoprotein T and transglutaminase in active immunization against entomopathogenic fungi in the termite *Reticulitermes chinensis*. *Journal of Insect Physiology*, 125, 104085. <https://doi.org/10.1016/j.jinsphys.2020.104085>
- Zheng, Y., Hu, Y., Yan, S., Zhou, H., Song, D., Yin, M., & Shen, J. (2019). A polymer/detergent formulation improves dsRNA penetration through the body wall and RNAi-induced mortality in the soybean aphid *Aphis glycines*. *Pest Management Science*, 75(7), 1993–1999. <https://doi.org/10.1002/ps.5313>
- Zhou, X., Oi, F. M., & Scharf, M. E. (2006a). Social exploitation of hexamerin: RNAi reveals a major caste-regulatory factor in termites. *Proceedings of the National Academy of Sciences of the United States of America*, 103(12), 4499–4504. <https://doi.org/10.1073/pnas.0508866103>
- Zhou, X., Tarver, M. R., & Scharf, M. E. (2007). Hexamerin-based regulation of juvenile hormone-dependent gene expression underlies phenotypic plasticity in a social insect. *Development (Cambridge, England)*, 134(3), 601–610. <https://doi.org/10.1242/dev.02755>
- Zhou, X., Tarver, M. R., Bennett, G. W., Oi, F. M., & Scharf, M. E. (2006b). Two hexamerin genes from the termite *Reticulitermes flavipes*: Sequence, expression, and proposed functions in caste regulation. *Gene*, 376(1), 47–58. <https://doi.org/10.1016/j.gene.2006.02.002>
- Zhou, X., Wheeler, M. M., Oi, F. M., & Scharf, M. E. (2008). RNA interference in the termite *Reticulitermes flavipes* through ingestion of double-stranded RNA. *Insect Biochemistry and Molecular Biology*, 38(8), 805–815. <https://doi.org/10.1016/j.ibmb.2008.05.005>
- Zhu, K. Y., & Palli, S. R. (2020). Mechanisms, applications, and challenges of insect RNA interference. *Annual Review of Entomology*, 65(1), 293–311. <https://doi.org/10.1146/annurev-ento-011019-025224>

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