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Review

Leveraging advances in RNAi and CRISPR for improved biological pest control

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The limitations of chemical pesticides and their associated risks highlight the need for more sustainable pest management strategies. Biological control using natural enemies offers an eco-friendly alternative but is sometimes constrained by efficiency and scalability. Emerging molecular tools—RNA interference (RNAi) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based gene editing—present novel opportunities to enhance existing biological control or to control pests directly. RNAi induces targeted gene knockdown via a non-heritable, transient response. CRISPR enables precise genetic modifications and could improve traits in beneficial insects or disrupt essential genes in pests, optionally including a gene drive for increased power. Although limitations remain for several species, these technologies could be valuable tools for integrated pest management. Their future implementation raises biosafety and regulatory considerations, particularly for self-propagating systems like gene drives. This review showcases developments in RNAi and CRISPR-based pest control, and calls for risk-based, adaptive governance to enable their responsible use in sustainable agriculture.

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Introduction

There is an urgent need to develop new approaches for pest control, which largely still relies on the use of broad-spectrum chemical pesticides. The increased legislation to ban the use of chemical pesticides, the continuous evolution of insecticide resistance in insect pests, and the large concerns regarding the use of chemical insecticides for human health (including farmers and consumers), non-target organisms, and the environment require the development of more sustainable alternatives. The use of natural enemies (predators or parasitoids) to control pest species has been implemented in various forms, from natural and conservation biological control to importation and augmentative release of natural enemies. These strategies have been achieving many important successes, including the complete eradication of chemical pesticide usage in several crop systems across the globe (see van Lenteren et al. [1], Mason [2], and van Lenteren et al. [3] for recent reviews).

Despite the successes, biological control does not always provide effective pest management and developing these approaches is a time-consuming and labour-intensive process [2]. Since recent years, novel molecular technologies offer new opportunities to enhance the efficiency and safety of biological control—specifically,

by using RNA interference (RNAi) to knock down gene expression or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based genome editing to knock in or out certain genes in pests or their natural enemies.

In insects, the RNAi pathway is a naturally occurring, sequence-specific gene-silencing mechanism elicited by small RNA molecules, often functioning in antiviral defence [4] (Figure 1a). Pest management can benefit from RNAi-based biopesticides that silence essential genes in insects, while minimising toxicity to the environment. This application involves inducing the exogenous (meaning, experimentally activated) small interfering RNA (siRNA) pathway by introducing double stranded RNA (dsRNA) to knock down specific genes, thereby reducing pest fitness or causing mortality (Figure 1c). Due to its sequence specificity, RNAi poses minimal risks for off-target effects and is not considered a genetic modification as its effects are not heritable. Besides direct pest control, dsRNA can also be used to silence genes in natural enemies to enhance their effectiveness as biological control agents (Figure 1e).

CRISPR is a powerful genome-editing technology that allows precise modification of DNA sequences in a wide range of organisms [5] (Figure 1b). It can be used to disrupt key genes in pest species (Figure 1d) or enhance the efficacy of beneficial insects in biological control (Figure 1e). Furthermore, CRISPR has enabled the development of synthetic gene drive systems, which bias their own inheritance pattern to rapidly spread desired traits through a population, even deleterious ones like female sterility or a male-biased sex ratio for pest control [6] (Figure 1d).

In this article, we discuss the use of RNAi and CRISPR for developing and optimising current pest control strategies. These tools offer alternative solutions for managing pests and invasive species that threaten food security, human health and biodiversity. Importantly, we also discuss the ecological and regulatory concerns these technologies raise due to their potential for irreversible environmental impacts, and thus the need for a balanced and flexible governance thereof [7].

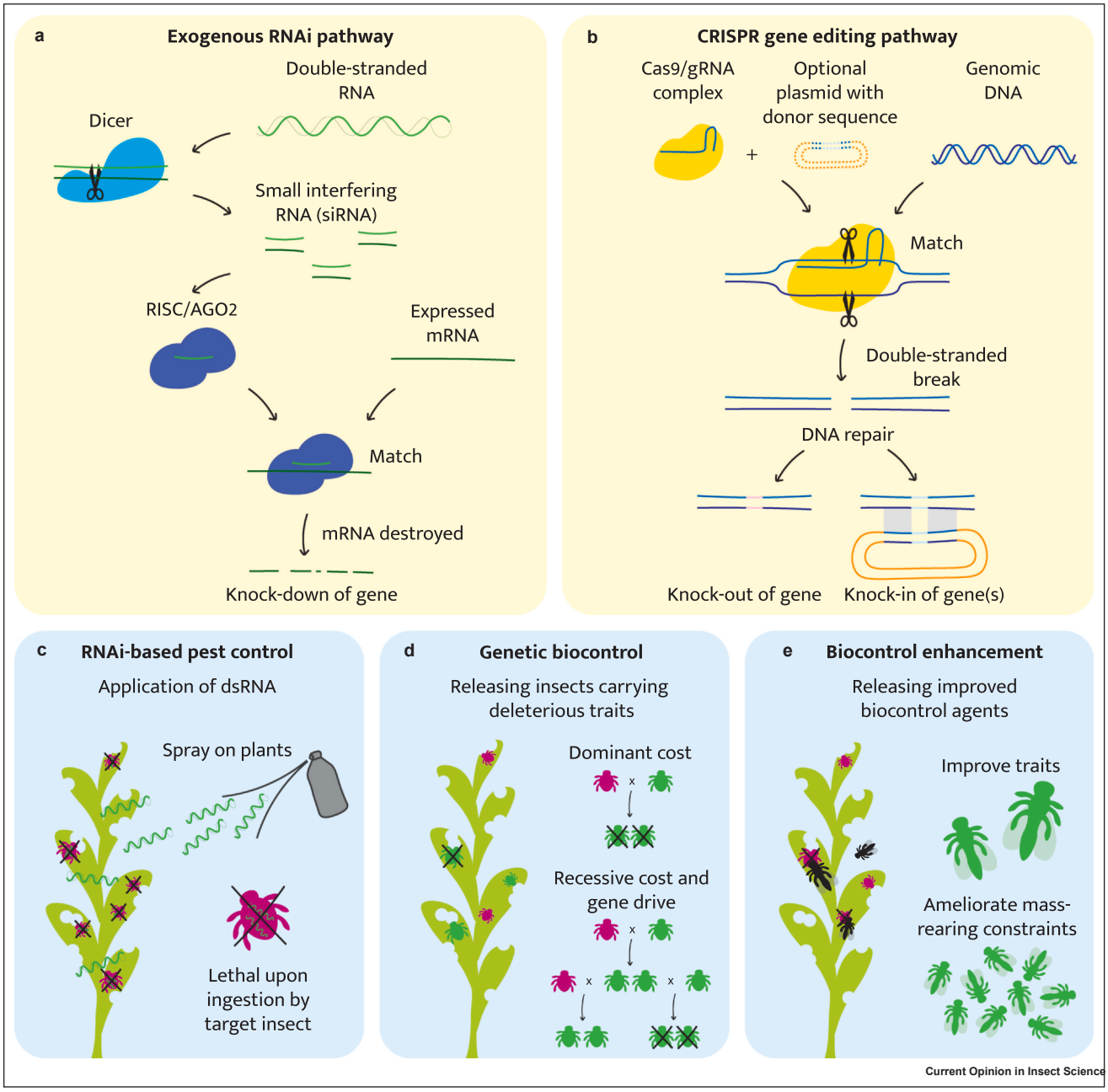
RNAi-based pest control

Research on RNAi for pest control has surged in the last 28 years, advancing our understanding of biological processes in many insects [8]. Two extensively studied species are the red flour beetle, *Tribolium castaneum*, and the western corn rootworm, *Diabrotica virgifera virgifera*—both coleopterans for which RNAi is generally highly effective. Scientists have also attempted to control populations of other crop pests, mosquitoes, and invasive social species by delivering dsRNA via injection, feeding, or submersion, but results have been mixed. The first commercial RNAi product was a transgenic maize expressing dsRNA *in planta* to protect against western corn rootworm damage. A sprayable form of dsRNA biopesticide against the Colorado potato beetle, *Leptinotarsa decemlineata*, is now available [9], and a feeding solution to protect honey bees from *Varroa destructor* mites will be on the market soon [10].

While RNAi is a promising technology, difficulties linger before wide commercialisation. Firstly, selecting the best target gene for RNAi-mediated pest control is paramount, but information on gene function and expression is limited for most species; the best RNAi lethal targets for one species might not be the best targets for another species [11]. Secondly, efficiency of RNAi varies; while moths and butterflies appear recalcitrant to its effects [12], target genes in many beetles are successfully knocked down [13]. Thirdly, RNAi sensitivity differs among species due to tissue-specific activation of double-stranded ribonucleases (dsRNases), which results in dsRNA digestion. A series of formulations to shield dsRNA constructs from these dsRNases has been developed. In *Drosophila suzukii* larvae and *Polistes dominula* adults, gene silencing was achieved when dsRNA was combined with a transfection reagent opposed to when dsRNA was ingested naked [11,14]. BioClay-based dsRNA spray enhances dsRNA uptake and subsequent control of *Bemisia tabaci*, outperforming naked dsRNA in field conditions [15]. Fourthly, even if dsRNA reaches the cytoplasm, it can be trapped in endosomes. In *D. suzukii*, effective protection from endosomal entrapment and degradation was achieved when encapsulating dsRNA into virus-like particles [16]. Finally, the functioning and composition of the core RNAi machinery also determine whether dsRNA is processed to siRNA. Large-scale manufacturing and field deployment of RNAi insecticides are in the early stages and require species-specific optimisation, as there is no 'one size fits all' for RNAi development [17]. Overall, advances in production and delivery of dsRNA will facilitate the widespread use of this sustainable method of pest management in the future.

In addition to efficacy of RNAi, biosafety towards the environment and non-target organisms like parasitoids, predators and pollinators should be evaluated before deployment. The stability and persistence of dsRNA in the environment depend on UV light, temperature and pH. Although dsRNA does not appear to accumulate for long in aquatic or terrestrial environments [18], studies remain scarce. Potential off-target effects include unintended silencing of non-target genes or silencing orthologous genes in non-target organisms. The likelihood of these effects correlates with the mismatch rate between the dsRNA sequence and non-target messenger RNA (mRNA) [19], gene expression level, and renewing rate

Figure 1



RNAi and CRISPR technologies and how they can be applied to improve biological control of pests. **(a)** The exogenous RNAi pathway involves the capacity of cells to degrade target mRNA with sequence homology to the administered dsRNA. When dsRNAs reach the cell cytoplasm, they are cleaved by Dicer enzymes into siRNA that are loaded into RNA-Induced Silencing Complexes (RISC). The siRNA fragments guide argonaute-2 (AGO2)/RISC to target complementary mRNA for degradation, thus impeding replication, transcription and translation of the targeted gene. **(b)** CRISPR technology can make precisely targeted changes in genomic DNA. To generate genetically modified insects, Cas9 (mRNA or protein) and gRNAs are delivered into an embryo, where they find the target match in the genome and generate double-stranded breaks. These breaks are usually repaired via end-joining repair, which often generates small insertions or deletions in the sequence, thus effectively knocking out that gene. If a donor DNA sequence homologous to the target locus is supplied along with the CRISPR components, potentially containing extra sequences in between, the double-stranded break can also be repaired via homologous recombination. One or multiple genes (such as a gene drive) can be knocked in this way. **(c)** Direct pest control can be achieved using RNAi. **(d)** Direct genetic biocontrol can be achieved using CRISPR, potentially also through gene drive. **(e)** Biocontrol can be enhanced based on RNAi or CRISPR.

of gene expression products. Fortunately, bioinformatic tools that help design dsRNAs and test for off-target effects on other species' genomes are available [20,21]. Besides off-target effects, dsRNA may induce immune response activation or saturation of the RNAi-machinery (reviewed by Chen and De Schutter [18]). Importantly, parasitoids, pollinators, decomposers and predators of a pest will also be exposed to the dsRNA intended for the target insect. One study found no evidence of dsRNA harming parasitoid wasps unintentionally [22], and it is hypothesised that although some off-target down-regulation might happen, rigorously designed dsRNAs are not lethal to natural enemies, parasitic wasps, and soil decomposers [18]. Further research and thorough risk assessments are still paramount to ascertain how potential detrimental effects can be mitigated.

CRISPR-based genetic biocontrol and gene drives

Since 2013, a myriad of CRISPR-based genetic biocontrol strategies have been developed [23] to control pest populations directly or to modify them to remove undesirable traits like the ability to transmit disease [24]. Implementing any CRISPR-based biocontrol strategy presents considerable technical hurdles, because CRISPR genome editing must first be established and optimised for each new species. Transformation efficiencies vary among species and may require extensive optimisation, as seen in *Nasonia vitripennis*, a model hymenopteran that has been recalcitrant to transformation in the past [25]. However, in many species, successful CRISPR-based genome editing has been achieved (reviewed by Sun et al. [26] for arthropod species). Innovations like DIPA-CRISPR [27], ReMOT/BAPC-mediated delivery [28], and a delivery formulation called SYNCAS [29] have recently further enabled gene editing in many arthropods. The development of viable population suppression strategies also requires detailed genetic knowledge for identifying gene targets, similar to RNAi-based approaches. To obtain this fundamental genetic knowledge, having functional CRISPR-based genome editing in a species is critical.

Genetic biocontrol strategies span a spectrum primarily defined by a trade-off between controllability and power. On one end are strategies that require the continuous release of modified insects carrying deleterious traits (usually male sterility), such as CRISPR-based versions of Sterile Insect Technique (SIT) called precision-guided SIT (pgSIT) [30]. pgSIT provides benefits over conventional SIT because it avoids fitness costs from radiation and can aid in producing male insects through sex-biasing genetics. However, mass-rearing is still a major limiting factor on efficiency [31]. Field trials are ongoing for *D. suzukii* [32], but have not yet been reported on. Companies are already running (contained) field trials with similar, non-CRISPR-based genetic biocontrol strategies, for example, in *Aedes aegypti* [33], the Mediterranean fruit fly (*Ceratitis capitata*) [34], and the diamondback moth (*Plutella xylostella*) [35]. On the other end of the genetic biocontrol spectrum lie CRISPR-based homing gene drives, which are capable of spreading through a population over multiple generations by biasing inheritance in their own favour [36]. Concurrently, they are designed to induce recessive female sterility, thus leading to large-scale control of a pest species [37,38]. Gene drives pose containment challenges due to their ability to spread autonomously, even from a single, small release, which necessitates stringent regulation and confinement strategies. Although this technology is drastic and comes with obvious ethical challenges [39], it is under consideration for insect pests with large, global impacts, such as malaria-transmitting mosquitoes (*Anopheles* spp.) and the new world screwworm (*Cochliomyia hominivorax*). Interestingly, to leverage the strengths of both sides of the spectrum, many hybrid systems combining features of pgSIT and gene drives have been proposed to impose spatially or temporally limited population control, thus creating a distinction, besides the one between non-gene drive and gene drive strategies, between local and global gene drive technologies [40]. For gene drives, no field trials have been approved yet; only the fitness of an inactive non-CRISPR-based gene drive in *Anopheles coluzzii* has been tested, which revealed moderate fitness costs in drive-bearing individuals [41].

The efficacy of CRISPR-based genetic biocontrol strategies will ultimately depend on two factors: 1) the molecular design and efficiency of the construct, and 2) the ecological characteristics of the target species [42]. For pgSIT and similar genetic biocontrol strategies, the identification of female-expressed or sex-determining genes is vital [30]. Gene drives, however, additionally demand highly precise control of molecular components, as they must be active specifically during meiosis in the germline, while avoiding expression at other times or in different tissues [43]. In *Anopheles* mosquitoes, extremely high gene drive efficiencies have been achieved [37], but these same designs have proved entirely inefficient in *P. xylostella* [44] and shown reduced efficiency in *Aedes* mosquitoes [45] and the fruit fly *Drosophila melanogaster* [46]. Increasingly, it seems that 'the wheel' may need to be reinvented for every species in which a CRISPR-based genetic biocontrol technology is developed [43,47]. In terms of ecological characteristics of the target species, SIT-like strategies are most suitable for insects that can mate only once and are easily mass-reared [48]. Gene drives are likely more suitable for long-term control over large areas [38], such as the eradication of an invasive species like *D. suzukii* over (part of) its invasive range [49]. There, both the escape of gene drive individuals to the native range and re-invasion of the

species to the invasive range are significant risks, so controlling migration is key [50]. Ultimately, life history traits of some insects may be obstructive to genetic biocontrol, such as inbreeding in *V. destructor* [51]. Factors like spatial population structure, density dependence, mate choice, and polyandry can also interfere with the success of both pgSIT and gene drives [42]. All in all, much remains unknown about CRISPR-based genetic biocontrol, and the technology likely has a long way to go until practical application becomes feasible.

Biocontrol with RNAi- or CRISPR-enhanced natural enemies

Recent advances in RNAi and CRISPR could improve existing biological control strategies by enabling targeted genome manipulation in natural enemies. Generally, mass rearing of biological control agents poses key challenges that influence pest management efficiency [52]. A major constraint is the endosymbiont *Wolbachia*, which alters reproductive traits, leading to sex ratio distortion via feminisation, male killing, or parthenogenesis [53,54]. Such imbalances hinder efficient mass rearing, where maintaining optimal female-biased progeny is critical in biological control programs, as females are responsible for host parasitisation [55]. RNAi studies in *N. vitripennis* provide functional insights into sex determination genes, potentially enabling the correction of such sex ratio anomalies in the future [56]. Similarly, in *Trichogramma brassicae*, if *Wolbachia* infection can be suppressed, this alters clock gene expression, in turn suppressing diapause and boosting fecundity, a phenomenon tied to RNAi-regulated pathways [57]. This regulatory mechanism influences host–symbiont interactions, ultimately affecting the parasitoid's reproductive timing and host-searching efficiency, a key trait for effective biological control. Thus, if feasible at a large scale, incorporating RNAi into biocontrol strategies may overcome rearing constraints like *Wolbachia*-induced effects, supporting the development of robust and sustainable pest control agents [58,59].

Likewise, CRISPR can be used for the direct improvement of traits relevant to natural enemy performance. Recent studies have demonstrated the applicability of CRISPR in beneficial insects relevant to biological control. For instance, in *Harmonia axyridis*, editing of genes associated with diapause and reproduction enhanced its adaptability and predation efficiency under varying climates [60]. Likewise, disruption of the *nAChR-α* subunit in *Coccinella septempunctata* conferred insecticide tolerance without compromising fitness [61]. Importantly, successful heritable gene knockouts in parasitoid wasps such as *N. vitripennis* [62] and *Habrobracon hebetor* [59] highlight the potential of CRISPR tools to improve parasitoid performance through manipulation of host-finding ability, reproductive traits, or stress tolerance, thereby strengthening their efficacy in sustainable pest management. Together, RNAi and CRISPR present complementary tools that could enhance biocontrol agents' efficacy while minimising ecological impact.

Considerations for governance and biosafety

RNAi and CRISPR enable precise, species-specific insect control, offering eco-friendly alternatives to pesticides while minimising off-target impacts [18,23]. However, their implementation requires a robust regulatory framework to ensure biosafety, efficacy, and public confidence [7,63]. Therefore, these technologies must undergo comprehensive environmental and human health risk assessments, focusing on off-target effects, environmental persistence, and impact on non-target organisms [64]. CRISPR-based gene drive systems, due to their capacity for self-propagation and potential transboundary spread, raise complex ecological and ethical concerns, necessitating a precautionary regulatory approach and alignment with international frameworks like the Cartagena Protocol on Biosafety [65,66]. However, a regulatory distinction between local and global gene drives is important to avoid unnecessarily delaying the deployment of a wide range of localised CRISPR-based pest control strategies. The current absence of dedicated regulatory guidelines for RNAi and gene-editing tools in pest control underscores the need for technology-specific, case-by-case evaluations, stakeholder engagement, and inter-agency coordination [67]. Adopting global best practices, such as those from the United States' Environmental Protection Agency and European Food Safety Authority, will enhance regulatory readiness while fostering innovation and ecological integrity [68,69]. Harmonised and transparent policies will facilitate the responsible integration of RNAi and CRISPR into Integrated Pest Management, ensuring agricultural resilience and environmental sustainability.

Noteworthy, current legislation and governmental bureaucracy already pose challenges to the implementation of biological control, even without the added complexity of RNAi and CRISPR [70]. One major hurdle is the implementation of Access and Benefit Sharing agreements, which give countries sovereign rights to genetic resources within their borders. While this legislation rightly governs the collection and exchange of species for research or commercial use, it also complicates efforts to develop biological control strategies for invasive pests and limits access to new potential biocontrol agents [71]. Meanwhile, global trade continues to accelerate the unintentional spread of invasive species.

Another barrier is the stringent registration process for new biological control agents or genetic control methodologies, which mirrors the regulatory standards for chemical pesticides, including extensive risk assessments. Although these precautions are warranted, given

potential risks such as non-target effects, disruption of native natural enemy populations, or disease transmission [72,73], the number of documented harmful outcomes remains low. The aforementioned stability, persistence and off-target effects of dsRNA constructs can be assessed experimentally before field application. These risks must be weighed against the ecological damage caused by invasive pests and the often-greater harms associated with chemical control methods [72]. Thus, while regulatory safeguards are essential, a balanced and flexible framework is needed to facilitate the development of responsible biological control. Failing this, we risk delaying sustainable solutions to the growing threat of invasive pests.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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