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RNA Interference – Natural Gene-Based Technology for Highly Specific Pest Control (HiSPeC)

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Abstract

RNAi technologies are more environmentally friendly, as the technology provides greater specificity in pest targeting, while reducing the potential negative effects on ecosystems and leaving beneficial insects and other organisms unharmed in crop ecosystems. Consequently, the increase in native fauna improves the efficacy of biological control agents against pests and pathogens. A growing understanding of the ubiquitous nature of RNAi, along with evidence for efficient, non-transgenic, topical applications has already begun to garner support among organic and industry producers. Designing solutions to agricultural problems based upon the same mechanisms used in nature provides newer, safer solutions to pests and pathogens for all agricultural industries.

Keywords: Future, Crops, Organic, Non-transgenic, RNAi

1. Introduction

A phenomenon initially reported in plants [1] called the attention of the scientific community, leading to the discovery of a sophisticated mechanism of gene regulation and protection against invasive nucleic acids [2–4]. Furthermore, the mechanism described in plants, referred to as post-transcriptional gene silencing (PTGS), or virus-induced gene silencing (VIGS), had been described in the 1990s [5] and was often referred to as pathogen-derived resistance [6].

RNAi is a natural process of gene regulation and antiviral defense system of eukaryotic cells. RNAi is a mechanism that functions as a “gene silencer” by targeting specific RNA sequences. RNAi results in degradation, and in some situations, translation inhibition, resulting in a reduction or complete elimination of the expression of a targeted RNA [7]. RNAi is also linked to suppressing gene expression at transcriptional level by directing epigenetic alterations on chromatin [8].

The basic RNAi process consists of the trigger molecule, a long endogenous or exogenous double-strand RNA (dsRNA) molecule that is expressed in, or introduced into, the cell, which is processed by Dicer, a ribonuclease III (RNase III) enzyme into small RNA duplexes of 21–23 nucleotides in length. These duplexes are separated with one strand (the guide strand), producing a protein complex known as the RNA-induced silencing complex (RISC). The RISC complex uses the specific sequence of the guide strand to determine potential target messenger RNAs (mRNA). Once bound to the mRNA, the guide strand directs a RISC-bound endonuclease [called “slicer”, an Argonaute (AGO) protein] to cleave the mRNA which has homology to the guide strand [9]. Thus, the RISC complex can target the messenger RNA (mRNA), an invasive virus RNA, or a transposable element transcript (Please note that RNAi cannot eliminate transposons itself, but its transcripts.) [10]. These components appear to be cosmopolitan in their distribution across the RNAi spectrum of the eukaryotic phyla. This implies that a common ancestor had a functional RNAi pathway [11], estimated to have occurred over a billion years or more ago [12].

The generation of virus-resistant transgenic plants, by expressing fragments of viral genomes, was the first demonstration of the beneficial use of the RNAi [13,14]. However, with the demonstration that ingestion of dsRNAs can robustly silence genes in *Caenorhabditis elegans* [7], RNAi became not only an important tool in genetic studies to identify gene function but also opened a new field of application in plant protection against insects, arthropods, and pathogens.

In this chapter, we outlined some aspects on the development of RNAi-based strategies to control insects, presenting some considerations and research steps that are important to be addressed.

2. RNAi-based products for agricultural management of insect pest

Advances in genomics and initiatives to sequence genomes of agriculturally important organisms created big breakthroughs within the entomology fields of study, including taxonomy, insect physiology, toxicology, immunology, pest management, and microbe–host interactions. This new paradigm affected how research could be conducted, to make discoveries and increase the speed by which these could be accomplished. With these breakthroughs, entomologists, pathologists, and biologists are rapidly advancing toward better, safer, and more specific pest and pathogen management. Development of gene-based methods was dependent upon having the knowledge to understand how the cells in a living organism respond to the threats from viral pathogens, how they regulate their own gene expression, and how they maintain these natural complex systems throughout their lives.

The initial genomes which were sequenced and annotated to elucidate these very complicated interactions from: the nematode – *C. elegans*, the fruit fly – *Drosophila melanogaster*, the red flour beetle – *Tribolium castaneum*, the silkworm – *Bombyx mori*, the pea aphid – *Acyrtosiphon pisum*, the honey bee – *Apis mellifera*, to bumble bee – *Bombus terrestris* and including a few more examples from the world of agriculture [15]. Current genome initiatives, like the i5K

Arthropod initiative [15] and the Beijing Institute of Genomics, BGI, China, plan to sequence thousands of arthropod and plant genomes. The enormous amount of new information produced will also increase the understanding of the genetic basis of the mechanisms nature uses to solve problems at the cellular and organismal levels.

The number of arthropod species which have had successful RNAi reports continues to increase, and this trend will carry agriculture into the future, covering five Classes, in four Subphyla of the Arthropoda phylum, which includes eight insect orders and over 30 insect species [16–17].

3. Pitfalls and solutions – Relevant considerations for development of RNAi in insects

Though the use of RNAi strategies to control a desired insect pest seems to be very straightforward; however, some issues should be taken into considerations: (1) for oral treatments the dsRNA must survive ingestion, then be absorbed by the epithelial cells, and depending upon the target translocated through the hemolymph to reach other tissues. In insects, the dsRNA is mainly introduced through feeding, but dermal application has already been reported to possibly bypass gut issues [18–21]; (2) once inside the body, the dsRNA must enter into the cell to activate the RNAi mechanism. In insects, cell uptake of dsRNA varies widely between species, because there are different mechanisms of systemic absorption and translocation of dsRNAs within and between cells, respectively, leading to differences in response and influencing the efficiency in silencing the target gene [17,22]; (3) once the RNAi mechanisms are triggered in a group of cells, it has been demonstrated that a systemic spread of silencing may also occur, so that other tissues / cells are also affected, which may increase the RNA effect. Successful studies have shown that dsRNA can circulate in the hemolymph, and cause a suppression of genes in tissues distant from initial entry sites in the insect gut, affecting cuticle formation, the nervous system, or ovaries[23–26]. However, in insects results can be highly variable and research efforts continue to elucidate the effects of systemic signaling.

In theory, any cellular mRNA can be inactivated in a precise and controlled manner. With this in mind, the use of the RNAi mechanism to manage an insect pest relies on the capacity to design the dsRNA. The sequence of the dsRNA provides specificity and the researcher must determine the active concentration needed to obtain the desired RNAi outcome; thus, proper design and evaluation of the dsRNA becomes critical.

Identification of vitally important (i.e., with high mortality) target genes of a particular insect is a crucial step toward development of RNAi-based control strategy. Thanks to world science development and increasing efforts of the research community, the identification of an essential target can be achieved by an extended literature search and analyses of available DNA/RNA sequence databases [27]. Once identified, “candidate” target sequences are used to design potent “RNAi causing structures”. Then, the dsRNAs must be experimentally validated for functionality, specificity, and stability, toward the specific RNAi target of interest. Furthermore, the development of an efficient delivery system for “RNAi causing structures”

is another key step. There are several methods available that include, but are not limited to: microinjection [28], soaking (for mosquito larvae, and nematodes) [29,30], and feeding (chewing and piercing-sucking insects) [17,17,27,31,32].

One approach to identify potential target genes which will function under field conditions is to perform bioassays that closely mimic the conditions in the field, for example, using a bioassay that mimics the feeding of a hemipteran insect acquiring the dsRNA during the natural feeding process performed on the crop plant. One problem with delivering dsRNA through feeding is that, depending on the bioassay, it may be difficult to measure the dose of dsRNA ingested, from the dose absorbed by the gut cells and the target cells [33].

Oral delivery of dsRNA through feeding can be performed by using artificial diet, detached plant parts (leaves, buds, roots), or intact plants [17,24,34,35]. Delivering dsRNA through the diet provides an easy procedure to screen large numbers of dsRNAs in insect larvae and adults [23,24,34,37]. In addition, it allows addressing different issues, such as effective length of dsRNA, determine regions of the gene to be target which may provide better suppression, and to determine the effective lethal concentration (LC50) [23,38].

Although oral feeding provides a more natural screening system, it is important to take into consideration that for some insect species from across all taxonomic orders, they may not provide an effective RNAi response when conducting oral feeding bioassays regardless of the dosage of dsRNA, as the dsRNA may not enter, or be detected in the insect's body [22]. In contrast, in these same insects when dsRNA was injected directly into the insect's body, a potent RNAi response was observed [23,39,40]. Indeed, lack of positive results using feeding bioassays does not necessarily indicate that the insect is insensitive to RNAi, but in a majority of the situations, insects have nucleases in the saliva, in the midgut, or even in the hemolymph that degrades dsRNA before it can be absorbed by the cells [40–42].

Wynant et al. [40] discussed the interactions of enzymes and RNAi-causing dsRNAs in the alimentary tract and hemolymph of insects and other arthropods using oral delivery. The elucidation of the roles of microbes and host enzymes on RNAi efficacy across arthropod species continues to be a challenging field of research [43].

Where information about absorption of dsRNA or presence of nucleases is not available for a particular insect species, the use of reporter dsRNAs molecules are useful to clarify possible issues. It is essential that the dsRNAs sequence should not match with any insect's transcript sequence. The dsRNA "movement" can be monitored (detected), or quantified in the insect's body by RT-qPCR, showing that the insect has acquired the potent, fully functional, systemically spreading dsRNA during feeding (plant, diet, drop of water, etc.) (Figure 1).

When conducting a RT-PCR detection of reporter dsRNA after insect feeding, it is important to sample a tissue other than the gut such as the hemolymph, fat body, or ovary. Careful collection of tissues which are not in direct contact with the gut provides evidence that the dsRNA was truly absorbed by the cells, and you are not just detecting dsRNA just in the digestive tract.

With small insects, as the Asian Citrus Psyllid (ACP) *Diaphorina citri* (whitefly or aphids), collection of material can be difficult without bringing in gut tissues, so another option is to

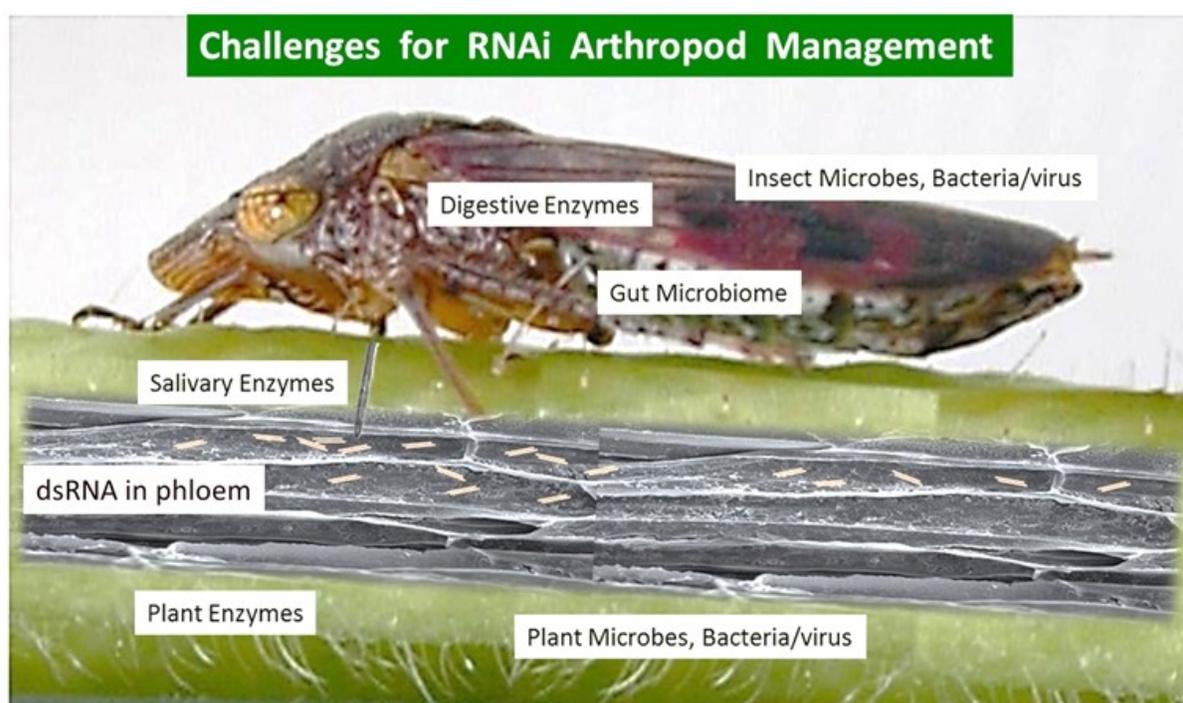


Figure 1. Typically applied RNA, provides long dsRNAs for insect ingestion. If absorbed into the plant, then long dsRNA persists in plant vascular tissues, xylem, and phloem, for several weeks due to lower metabolic activity, fewer enzymes, and microbes. Once the dsRNA enters a plant or insect cell, the RNAi mechanism is triggered, and the dsRNA processed [Image: Hunter, W.B., USDA-ARS, 2015].

let the insects feed on the source of the dsRNA (plant, diet, etc.) for a period of 24–48 h, then transfer them to an untreated food source. After feeding for 36 h, or more, the insect should excrete any food residue from the treated food source, which contains dsRNA. After this period, proceed with sample collection for dsRNA detection.

The reporter dsRNA is designed so that the sequence does not match with any known mRNA transcript in your insect. This is to avoid off target of other transcripts in the insect. Some commonly used dsRNAs which are used as negative controls in RNAi experiments are: green fluorescent protein (GFP), β -glucuronidase (GUS), and enhanced yellow fluorescent protein (EYFP) [44].

When designing RNAi experiments, important questions arise regarding the design of dsRNA, including: the length of the molecule and the region targeted within the mRNA. The minimal required length to achieve an RNAi effect will vary depending on insect species [45]. For example, in *Tribolium castaneum*, analysis showed that the dsRNA length had a strong influence on the efficacy of the RNAi response, with longer dsRNA proven to be more effective on curtailing gene expression [46]. In *T. castaneum* dsRNA, it was observed that length was crucial for cellular uptake; a minimum of 70 nucleotides were necessary to achieve the desired interference. However, other studies in the potato/tomato psyllid, *Bactericera cockerelli* [47], the pea aphid *Acyrtosiphon pisum* [48], and the lepidopteran *Manduca sexta* [49] have reported gene suppression using shorter dsRNAs, between 21 and 27 nucleotides in length. These molecules are called small interfering RNAs (siRNA). Overall, the majority of studies dem-

onstrate success with dsRNA ranging from 140 to 520 nucleotides in length. Interestingly, Huvenne and Smagghe [31] reported success using a dsRNA 1,842 nucleotides in length. dsRNAs longer than 200 nucleotides provide the advantage of resulting in many siRNAs, post-cleavage against the targeted mRNA, thus maximizing the RNAi response and preventing the development of individuals with “resistance” due to the natural genetic variation.

There is no consensus on the mRNA region that the dsRNA should match to (e.g., 5′ or 3′). For example, in the pea aphid, *A. pisum*, no difference in mortality was observed in groups of insects that received dsRNA matching the 5′ or 3′ end of the *hunchback* (*hb*) gene [27]. In the mosquito, *Aedes aegypti* greater RNAi effects were achieved when insect larvae ingested dsRNA targeting the 3′ end versus 5′ end of an apoptosis gene, *AaeIAP1* [18]. These different results highlight the need to screen several dsRNA molecules across the entire mRNA [50].

In the context of field applications of RNAi for insect management, dsRNAs can be designed to be highly specific to both the target gene and the insect species. If desired, the RNAs can be designed to have a broader spectrum to affect several pest species. For example, RNAi strategies can be designed to remove one aphid species from a cropping system, or be designed to remove multiple aphid species from that same ecosystem [24,38].

4. Bioassays for dsRNA screening

For RNAi research attempting the development of a viable pest management product. It is of utmost importance to identify the best delivery mechanisms (i.e., topical sprays, baits, or transgenic plants) as early as possible; this will expedite the entire process and can cut years off of the development and commercialization timeline.

The example outlined below highlights RNAi bioassays directed toward two citrus insect pests, each one with different feeding behaviors: piercing-sucking plant-feeding (the Asian citrus psyllid *Diaphorina citri*, Hemiptera) and a chewing beetle pest (the weevil *Diaprepes abbreviatus*, Coleoptera). In both situations, the bioassays were designed to evaluate the efficacy of oral ingestion of dsRNAs under “natural feeding conditions” which mimic conditions the insects will encounter in the field.

4.1. Bioassays for piercing-sucking insects

The artificial feeding bioassay is being widely used for studies on insect nutrition, pathogen acquisition, toxicity, and RNAi (Figure 2A) [51–53].

It is notable that liquid feeding bioassays (dsRNAs mixed in a liquid diet or a sucrose solution) frequently result in high mortality levels in the controls, and increased degradation of dsRNA in the solution due to bacterial or fungal contaminations [42,57]. In addition, these bioassays require significantly high dsRNA concentrations to achieve insect mortality. Concentrations up to 1 μg/μL [58–60] cannot be reproduced inside plant vascular tissues.

Hemipteran pests in citrus (psyllids, leafhoppers, aphids, whiteflies) have piercing-sucking mouthparts that are inserted into the plant vascular system to feed. The development of an

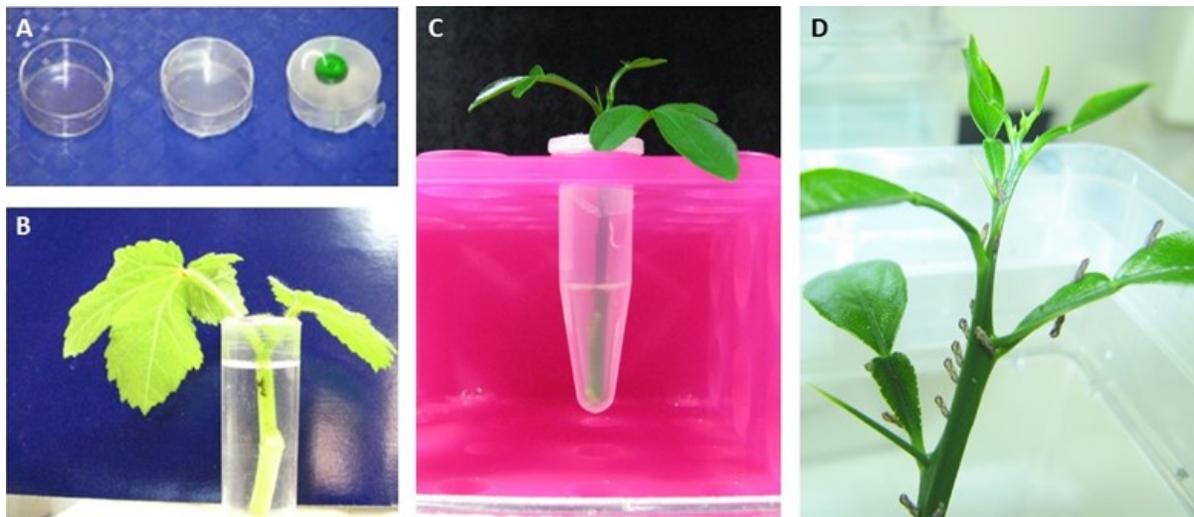


Figure 2. RNAi feeding assays used at the USDA lab, ARS, Ft. Pierce, FL (2007-2014), to successfully screen dsRNA constructs rapidly and under controlled conditions: (A) sucrose solutions within artificial membranes (5 ng dsRNA/ μ L) [54], (B) rooted okra plant cuttings, (C) citrus flush (5 ng to 100 ng dsRNA/ 0.25 g plant tissue). Plant cuttings absorb dsRNA directly providing systemic movement of dsRNA, (D) insects are given a feeding access period to ingest the dsRNA across 10 days to observe mortality (Hunter and Andrade, USDA-ARS 2015) [54–56].

RNAi control strategy against these insects relies on effective delivery of the dsRNA through the vascular tissues.

Demonstration of the first dsRNA delivery into full-sized citrus trees and grapevines, without a delivery vector, expression vector, or transformation event was performed in 2008 [56]. These results showed that two hemipteran insects, the xylem-feeding leafhopper (*H. vitripennis*), and the phloem-feeding Asian citrus psyllid (*D. citri*), tested positive for ingested dsRNA after feeding from host plants treated with dsRNA as either a foliar spray or root drench. These results along with other studies that demonstrated successful RNAi through oral ingestion in insects [24,34,36,61,62] support the potential for exogenously delivered RNAi control strategies.

Use of cut plant feeding bioassays for hemipteran pests enables the screening of a large number of dsRNAs molecules at a reduced cost of materials and time. The bioassay, can use leaf disks, whole leaf, new growth leaves and stem, or rooted cuttings, to absorb and deliver dsRNAs. In citrus, the “flush”, which are new growth foliar shoots, are collected from potted citrus seedlings grown in a glasshouse (USDA-ARS, Fort Pierce, FL). The leaves and stem material are about 7–8 cm long. The plant material is washed in 0.2% bleach water, for 10 min. Then the base of each stem is cut at a 45 degree angle while submerged in filtered water. The material is then placed into a 1.5 mL tube containing 0.5 mL water (Figure 2B and C). The dsRNA solution, 300 μ L, is added to the water, the tube top is wrapped with plastic or Parafilm™ and placed under artificial lighting to stimulate absorption of dsRNA solution. The next day the tube is filled with water using a 26 gauge syringe needle and syringed filtered (0.45 μ). The treated cuttings are then placed into a cage and adult insects provided feeding access for 10 days (Figure 2D). The plant material can remain viable for up to 40 days on average. While most bioassays may terminate after eight to 10 days of observations for mortality, having a

longer feeding access time enables observations on insect oviposition, egg viability, or nymph development.

Each dsRNA molecule has an optimal concentration. So each dsRNA molecule is evaluated across a range of total concentrations (i.e., 5, 20, 50, 100 nanograms/ tissue). The bioassay permits screening for synergistic effects of multiple dsRNAs and to screen a single dsRNA against multiple insect species. For example, the assay using citrus flush permits screening of dsRNAs designed against psyllids for off target effects in the citrus aphid (*Toxoptera citricidus*) and glassy-winged sharpshooter leafhopper (*Homalodisca vitripennis* (Germar)), two closely related hemipterans, which also use citrus trees as a host plant.

4.2. Bioassays for chewing insects

For insects which are foliage feeders, the delivery of dsRNA can be achieved as a foliar topical spray. In this scenario, the dsRNAs are evaluated similarly as topical insecticides. The dsRNA solution is sprayed on leaves, and then fed to the insects. An example of the effectiveness of this approach was reported by Bolognesi [45] working with the coleopteran *Diabrotica virgifera*, in which dsRNA administered through feeding, silenced genes in tissues far from the gut epithelium.

Similar results were obtained while developing an RNAi strategy against the Diaprepes root weevil (DRW), *Diaprepes abbreviatus* L., (Curculionidae: Coleoptera) (Andrade and Hunter). The adults feed and oviposition on mature citrus leaves. Topically applied dsRNA was sprayed on a bouquet of citrus leaves for delivery to DRW adults (Figure 3A). RNA's have been shown to move through the plant xylem and phloem [6].

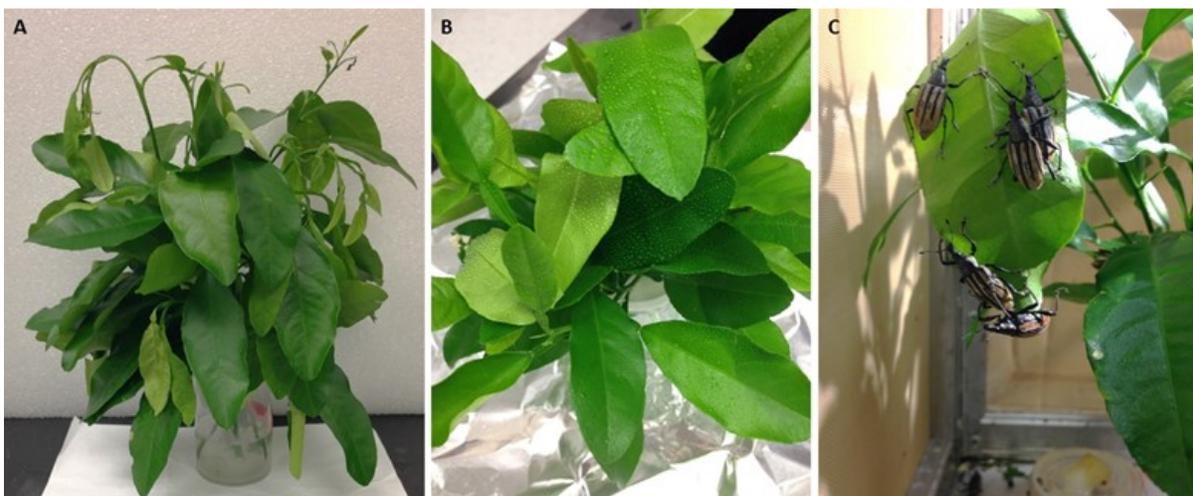


Figure 3. Citrus “leaf bouquet” feeding bioassay for *Diaprepes* root weevil. (A) Fresh stems with leaves are washed in 0.2% bleach water, rinsed with Nanopure™ filtered water, then the stems are freshly cut while submerged in water. The leaf bouquets are placed in water-filled containers and placed under artificial lighting for 24 h prior to use. (B) Topical foliar treatment: the dsRNA is mixed with water and applied using a low-volume aerosol sprayer. (C) After the leaves dry, the “bouquets” are caged with adult insects.

A test spray using only water established the volume needed to provide full coverage of leaf bouquets without excessive run off (Figure 3B). After the leaves have dried, they are caged with adult insects (Figure 3C). Freshly treated citrus leaf bouquets replaced previous bouquets every five to seven days for a 5-week period. The total amount of dsRNA to be sprayed over the leaf bouquet was determined by evaluating a range of concentrations in a pretest experiment for efficacy. The effects from RNAi in insects usually start to appear within 4 to 5 days post-ingestion, which suggests there may be a dose response [63]. Since foliage feeding insects tend to eat a lot of leaf material each day, a low-dose spray may be able to deliver a significant amount of dsRNA.

5. Final considerations on RNAi applied to agriculture

Efficient delivery and increased stability of dsRNA need to be developed if non-transgenic, topically delivered, RNAi strategies are to be established. Increased stability and superior delivery into some insects can be achieved using nanoparticle-mediated RNAi [63–65], traditional crop improvement strategies, in which plants express hairpin dsRNAs, will continue to be a mainstay of agricultural approaches [63,65,66].

Transgenic plants have successfully used RNAi strategies to produce crops with improved virus resistance, increased nutrition and fiber content [67]; biotechnology companies are trying to move towards a faster, more natural process of topically applied RNAi. dsRNA molecules are part of naturally occurring processes in all living organisms. They exist in our foods, and our bodies [66]. The short persistence time of dsRNA in the environment is demonstrated by the fact that analyses of soils and plant debris, treated with dsRNA have consistently shown rapid breakdown of dsRNAs within 2–3 days [68], also means less concerns about unintended contamination of water supplies, soils, or adverse air quality effects. Furthermore, since all living things have evolved to break down dsRNA and use the nucleic acids as cellular nutrients, this technology will be safer than conventional chemistries for those who apply RNAi products, or eat the produce [66–69].

RNAi technologies have greater specificity in pest targeting, which reduces negative impacts on crop ecosystems by leaving more insects and other organisms unharmed in the field. The increased fauna consequently improves the efficacy of pollination, and biological control agents that help suppress a broad range of pests. The increased understanding of the ubiquitous nature of RNAi, along with evidence of efficient topical application, has already begun to garner support for this technology among members of the organic grower's communities, which desperately need a truly natural, innovative breakthrough, to manage many of the pests and pathogens which plague the organic industries.

5.1. Cost-effective methods for the mass production and formulation of dsRNA

Cost-efficient methods for mass production of vast amounts of dsRNA are being developed, and include bacterial, plant, and synthetic production [65]. While small amounts of dsRNA can be easily produced in the laboratory for research purposes, commercially available kits are

not a viable, cost-effective method for the production of large quantities of dsRNA [65]. The costs associated with the commercialization and implementation of RNAi products are decreasing rapidly. The costs of dsRNA production have dropped from \$500,000 USD for 40 g in 2008 to less than \$4,000 USD for 40 g today. *For example, see [70].* As interests in commercialization of RNAi-based products increase, better production systems will be developed to meet the predicted demands of these growing markets [17,65,71]. One of the most cost-effective methods for production is in bacteria, since for most countries bacteria-produced dsRNA would provide an affordable production system which could advance RNAi as, “*The novel biological insecticide of the future!*” Most agricultural companies interested in the future of RNAi are working on developing their own technologies that will further reduce production costs predicted to be near \$4 USD per one gram by the end of 2015. *For example, see [72].*

5.2. Other applications

Future applications of RNAi and other gene-based targeting biotechnologies will add value to existing beneficial insects (pollinators, predators, parasitoids). A real-world example is a study conducted over several years in which an RNAi product designed to reduce Israeli acute paralysis virus replication was fed to honey bees. The treated bees had significantly greater survival and produced significantly more honey [73]. RNAi strategies have also reduced honey bee parasites, like *Varroa* mites [74,75], and internal microsporidian parasites [76] without deleterious effects to the bees. The highly specific nature of RNAi approaches can be exploited to reduce pests with no harmful effects on non-target species. The use of RNAi in combination with beneficial pollinators and natural enemies has the potential to raise the level of all pest management efforts [17].

Biotechnology has demonstrated the safe production of plants which are more nutritious, less toxic, more resistant to drought, and more efficient for biofuel production [66, 67]. RNAi has already been successfully used to produce crops which are virus- and drought-resistant [66]. However, plants expressing dsRNAs while stable and safe take years to develop and millions of dollars to commercialize [65]. Development of topically applied RNAi, which is a non-transgenic approach improves crop traits and provides a major step forward for environmentally sound crop management [66, 67].

6. Conclusions

As more insects and mites develop chemical resistance to one or more insecticides, now estimated to be over 500 species with resistance to one or more products [77], it is imperative that new types of pest control are developed. The public would like the world to be filled with environmentally friendly technologies, safe for human and animal consumption, technologies which are safe for use around animals and beneficial insects, safe for each type of ecosystem, forest, field, crop, or backyard, a technology that will not endanger water or food quality, a more natural solution, with a natural approach toward problem-solving. So enters RNA interference!

RNA interference, or gene silencing, is a way to reduce specific mRNAs so that a particular protein is either not made or it is reduced. The RNAi mechanism is a natural one which occurs in the cells of humans, animals, insects, and plants, and appears to have evolved as a primary defense system against virus replication [78]. Andrew Fire and Craig Mello, won the Nobel Prize in 2006 [79] for explaining how the RNAi mechanism is triggered, when a cell encounters double-stranded RNA, and how this could be used to benefit humanity. Humanity's greatest discoveries have come from observing the natural world; while RNAi will not solve every problem, it certainly can help improve plant health, reduce insect pests and pathogens [17, 66, 67, 80]. Some of the benefits from developing RNAi as topically applied products are: 1) The rapid degradation of the molecules ensures low environmental risks. All cells have the capacity to degrade dsRNA, and the salvage pathways to recycle these bases and nucleosides to form new nucleotides. Thus, cells constantly breaking down DNA and RNA into recycled nucleotides [81]. 2) Topical RNAi applications do not insert genes, so do not produce proteins. RNAi reduces the expression of the targeted proteins, a modulation effect of the natural system. 3) RNAi can be designed and tested faster (in about 2–3 years) than producing transgenic crops, which can take 10 to 20 years and cost hundreds of millions of dollars [65]. 4) Finally, RNAi strategies as topical sprays would, for the first time, be able to remove one or two closely related insect species, while leaving all the other insects unharmed [38]. The ability to design RNAi as highly specific pest control will finally provide relief to biological control agents and beneficial insects [17, 44, 73], significantly improving integrated pest management programs.

The advantages and promises from RNAi technology sound amazing. However, serious efforts in outreach and education are needed to better inform the different stake holders including the general public, and agricultural industry, leaders as well as decision makers in the regulatory and political communities to help expedite the release and adoption of RNAi products and technology.

7. Disclaimer

Mention of trade names or commercial products herein is solely for the purpose of providing specific information and does not imply recommendation or endorsement, to the exclusion of other similar products or services by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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