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RNA-based biocontrol compounds: current status and perspectives to reach the market

Clauvis Nji Tizi Taning[1]*, Salvatore Arpaia[2], Olivier Christiaens[1], Antje Dietz-Pfeilstetter[3], Huw Jones[4], Bruno Mezzetti[5], Silvia Sabbadini[5], Hilde-Gunn Sorteberg[6], Jeremy Sweet[7], Vera Ventura[8], Guy Smagghe[1]*

[1] Laboratory of Agrozoology, Department of Plants and Crops, Faculty Bioscience Engineering, Ghent University, Ghent, Belgium

[2] Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), DTE-BBC, Rotondella, Italy

[3] Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Biosafety in Plant Biotechnology, Braunschweig, Germany

[4] IBERS, Aberystwyth University, Aberystwyth, Wales, UK

[5] Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche (UPM), Ancona, Italy

[6] Norwegian University of Life Sciences, Norway

[7] JT Environmental Consultants Ltd, Cambridge, UK

[8] Department of Environmental Science and Policy, Università degli Studi di Milano, Milan, Italy

***Corresponding authors:** Clauvis Nji Tizi Taning (tiziclauvistan.taningnji@ugent.be); Guy Smagghe (Guy.Smagghe@UGent.be)

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Abstract

Facing current climate challenges and drastically reduced chemical options for plant protection, the exploitation of RNA interference (RNAi) as an agricultural biotechnology tool has unveiled possible new solutions to the global problems of agricultural losses caused by pests and other biotic and abiotic stresses. While the use of RNAi as a tool in agriculture is still limited to a few transgenic crops, and only adopted in restricted parts of the world, scientists and industry are already seeking innovations in leveraging and exploiting the potential of RNAi in the form of RNA-based biocontrol compounds for external applications. Here, we highlight the expanding research and development pipeline, commercial landscape and regulatory environment surrounding the pursuit of RNA-based biocontrol compounds with improved environmental profiles. The commitments of well-established agrochemical companies to invest in research endeavours and the role of start-up companies are crucial for the successful development of practical applications for these compounds. Additionally, the availability of standardized guidelines to tackle regulatory ambiguities surrounding RNA-based biocontrol compounds will help to facilitate the entire commercialization process. Finally, communication to create awareness and public acceptance will be key to the deployment of these compounds.

Keywords: RNA interference, biocontrol, biosafety, biotechnology, dsRNA, regulatory

1. Introduction to RNAi-based technology

RNA interference (RNAi)-based technology has proven to be a powerful and precise strategy that can be exploited to improve crop production and protection. RNAi is based on natural sequence-specific and evolutionarily conserved mechanisms in eukaryotes regulating gene expression at the transcriptional or post-transcriptional level, thus providing a natural defence system that can target invading nucleic acids of hostile organisms and viruses. Deciphering the RNAi mechanisms has provided scientists with the ability to specifically silence target genes post-transcriptionally, which could be endogenous plant genes, genes of plant pathogens (viruses and fungi) or genes of other plant pests (insects, mites, nematodes and weeds).[1] By using different types of RNAi triggers, such as hairpin-structured RNAs (hpRNAs), artificial microRNAs (amiRNAs) and/or double-stranded RNAs (dsRNAs), the function of these targets can be blocked. Post-transcriptional RNAi mechanisms explore a sequence-dependent mode of action at the mRNA level to prevent the translation of the targeted mRNA into proteins by mRNA degradation or translational repression, hence leading to target gene silencing and subsequently the desired effect.[2,3] This sequence-dependent mode of action makes RNAi unique in selectivity and efficiency compared to other conventional agrochemicals. Products using the RNAi mode of action can be designed to selectively target the expression of specific genes or groups of similar gene sequences, in a targeted species for which they are developed while leaving other non-target organisms unaffected. As such, RNAi has gained remarkable prominence among researchers as a strategy of choice for improving crop yield, for generating plants with novel traits (PNTs), for post-harvest protection and for managing weeds, other pests and diseases caused by bacteria, fungi and viruses.[4]

2. RNA-based biocontrol compounds for external application

In the context of field application, the RNAi approach for crop protection can be applied *in planta* via the production of a genetically modified (GM) crop in which host-induced gene silencing (HIGS) can be accomplished by stably integrating RNAi-based constructs designed against one or multiple target sequences. Alternatively, an RNAi strategy can be employed via the exogenous application of a formulated product with dsRNA as the active ingredient by itself, through a modified virus (virus induced gene silencing), modified bacteria (live or inactivated) or through modified fungi.[4] An increasing number of *in planta* RNAi-based events have been developed, risk-assessed, and have received approval by international regulatory agencies in different countries.[5] These events encompass a wide variety of plant species from maize to potato as well as a range of traits including virus resistance, pest resistance and plant composition modification. However, the difficulty in genetic transformation of some crop species, expensive capital requirements and political/public concerns surrounding the cultivation and use of GM crops[6] has favoured the need to develop sprayable dsRNA-containing end- use products (dsRNA -EPs). Spray-induced gene silencing (SIGS) and other exogenous applications (such as root or seed soaking, trunk injection, petiole absorption and mechanical inoculation)[1,4] as alternatives to the GM plant approach, silence target genes in a target organism without introducing heritable changes in the genome, hence do not fall within the restrictions currently defined by the EU regulation on GMOs. This of course excludes the scenario where the final product containing the dsRNA active ingredient is a GMO, as is the case when microbes are engineered to produce specific dsRNAs.

Several studies have demonstrated that dsRNAs applied exogenously on plant tissues can induce RNAi-mediated silencing of targeted pest or pathogen genes. Examples include the induction of plant resistance to fungi, insects and viruses, following external application of bacterially produced or *in vitro* synthesized long dsRNAs, hpRNAs, or small interfering RNAs (siRNAs), designed to selectively target essential genes of pests and pathogens.[7,8] Some studies have also shown that topically applied dsRNAs can act on both the treated plant area as well as on distal non-treated parts by systemic translocation of dsRNAs via the plant vascular system, following application via mechanical wounding.[9,10,11] These results demonstrate the possibility of active uptake of exogenously applied dsRNAs by plant cells and their processing into siRNAs, which should extend the time of control/prevention against some plant diseases. However, specific features of the target organism (i.e., RNAi machinery, structural characteristics, host-pathogen interaction mechanisms) can influence the efficacy of such RNAi-based strategies. It has been demonstrated for example that the absence of secondary siRNA amplification or the lack of dsRNA uptake by the target pathogen, limits the application of exogenous RNAi approaches against specific fungi.[12,13] Furthermore, the delivery method chosen for dsRNA molecules (i.e. by mechanical inoculation or leaf spraying) represents another crucial point that influences the effectiveness of exogenous RNAi-based strategies, and that should be selected on the basis of the plant type and on the type of pest species targeted.[14] Efficient delivery methods have been described in several reports on the down-regulation of plant endogenes and transgenes by the external application of either naked dsRNAs, dsRNAs protected by liposome, artificial extracellular vesicles (EV), or complexed with nanoparticles or a protein carrier.[15-19] Nanocarriers are increasingly being used as effective translational tools in achieving commercial viability of sprayable RNA-based biocontrol compounds.[20] As such, the combination of RNAi technology and nanotechnology is perceived as a game changer in the crop protection industry.[21] An example is the use of “layered double hydroxide clay nanoparticles” (BioClay) to deliver dsRNA as a stable application with increased longevity, highlighting the significance of nanoparticles as delivery vehicles, and the progress towards translation from the lab to the field.[22] This will however be dependent on the cost of production and efficiency of the developed nanocarriers. Recently, the use of DNA nanostructures serving as RNA carriers through Watson-Crick base pairing has been reported to be an efficient tool for delivery of siRNA into plant cells.[23] However, parameters such as the compactness, stiffness, size, shape and location of the siRNA attachment locus on the nanostructure will have to be taken into consideration during formulation in order to assure proper internalization of the nanostructure into plant cells for a corresponding good gene silencing efficiency. Although RNA-based biocontrol compounds are still in the R&D pipeline, they are broadly anticipated to fall under the following categories; direct control agents, resistance factor repressors, developmental disruptors and growth enhancers.[24] Additionally, these biocontrol products will likely be applied both outdoor on field crops and indoors in green houses using already available delivery systems in agriculture.

3. Commercial environment for RNA-based biocontrol compounds

The analysis of this specific innovation sector reveals that RNAi-based biocontrol is an emerging technology endowed with a positive patenting trend over time and promising future applications and development.[25] As for the type of applicant involved in this specific innovation cluster, it can be said that globally almost 50% of the total patent activity derives from public R&D, principally from US and Chinese applicants. As for the private sector, agbiotech companies are still the major players in patent production, but the sector was characterized by a greater concentration in the early years of development, while recently a larger number of small-medium players have become involved in this research area.[26] It is evident that commercial interest towards sprayable RNA-based compounds in agriculture has significantly increased considering recent investments in R&D for RNA co-formulants. The initiated pipeline branded "BioDirect" by Monsanto is developing RNA-based compounds to control insect pests (Colorado potato beetle, Brassica flea beetle and Varroa mites), pathogens (Tospovirus) and glyphosate resistant weeds.[27,28] Bayer CropScience (who acquired Monsanto)[29] is also joining the race towards the development of the first RNA-based compounds in agriculture. Another multinational company, Syngenta (ChemChina), who acquired the Belgian firm Devgen brought in the latter's technical expertise to develop RNA-based applications against various above and below ground insect pests.[30] The commercial interest for RNA applications by these large companies has stimulated the development of start-up companies that exploit existing or emerging biotechnology tools to create the needed platforms and technologies for the agribusiness. Examples include technologies for mass production of dsRNA at low cost by companies such as GreenLight Biosciences, RNAgri (former APSE) and AgroRNA (Genolution). GreenLight Biosciences has successfully developed a distinctive cell-free bioprocessing platform capable of producing RNA sequences in a fully-scalable fashion at a low cost of $0.5/g compared to fermentation ($1/g), *in vitro* transcription ($1000/g) and chemical synthesis ($100,000/g) platforms.[31] Considering that for field application, dsRNA should be available in large quantities and in a cost-effective manner, it does not necessarily have to be as pure as for medical application and the source of the dsRNA should entail no GMO concerns (if produced using a GMO), AgroRNA has developed an effective procedure for synthesizing dsRNA (200 to 800 bp) of mg to kg scale through a fermentation platform (http://www.agrorna.com/). A significant decrease in the cost price of dsRNA over the last decade (from $12,500/g in 2008) has significantly enhanced the feasibility of applying RNA-based compounds at field scales in a cost-effective manner. However, it is worth noting that naked dsRNA will have to be formulated for protection against degradation and for improved delivery to the target prior to application in crop protection. To achieve this, the start-up company AgroSpheres has developed a proprietary bioparticle platform consisting of small, spherical cells lacking chromosomes, that can encapsulate dsRNA thereby enhancing its delivery for crop protection (https://www.agrospheres.com/). In a recent research collaboration with GreenLight, AgroSpheres intends to study the delivery of dsRNA produced by GreenLight using its proprietary bioparticle platform. This bioparticle can shield the dsRNA active molecule from nucleolytic enzymes (RNAses) and from UV radiation in the environment that would otherwise destroy the dsRNA. The enormous potential of AgroSpheres' Minicell platform has recently led to the company raising $4 million in a Series A financing round led by the venture capital firm Ospraie Ag Science. This Series A financing will fund the development and commercialization of AgroSpheres' core Minicell technology. The biotech company RNAagri has also developed a technology that uses a protein to bind the RNA as it is produced thereby protecting it from degradation, allowing subsequent isolation and purification (https://www.rnagri.com/). Using large scale fermentation processes these protein-protected and ready-to-spray dsRNAs are also produced at costs near $1/g and there is interest in exploiting the protein bound to the dsRNA to improve delivery. In pursuit of achieving dsRNA stability against degradation and improved delivery to their targets, the biotech start-up Nanosur has developed a proprietary platform to produce modified RNA (MdsRNA) formulations with improved translocation across cellular membranes, preventing rapid degradation and hence improving the efficacy of the RNA-based biopesticide (http://www.nanosur.com/). Similarly, the start-up company TrilliumAg has developed a novel biological platform for agriculture known as Agrisome, where modified RNA molecules known as MV-RNA are self-assembled into protein-based nanoparticles to improve delivery and stability (http://www.trilliumag.com/).

Although these RNA-based biocontrol compounds will probably be more expensive than conventional products, their adoption by farmers will likely be linked to their selective mode of action and better biosafety profiles compared to conventional products. Furthermore, these biocontrol compounds are expected to fit synergistically into existing integrated pest management (IPM) strategies designed to protect agricultural crops from economic damage by plant pathogens, weeds, pests and other harmful organisms, while reducing reliance on hazardous conventional control products. Organic agriculture could also benefit from these new natural molecules, particularly in horticultural production, where the permitted biopesticides have strong limitations in pest/disease control. Additionally, seminal studies have suggested that the exploitation of exogenous RNAi-based compounds for biocontrol could have increased public acceptance, given the fact that this technology does not require the genetic modification of crops.[32,33]

4. Regulatory guidelines for RNA-based biocontrol compounds

As with any new emerging technology, developed RNA-based biocontrol compounds will have to be approved by the regulatory frameworks existing in different countries. Biosafety studies, prior to the approval of these products, especially tailored to confirm the predicted selectivity of dsRNA, can reliably be conducted according to the existing risk assessment approaches, taking into the account the novel mode of action of these products.[34] For RNA-based biocontrol compounds where additional research might be necessary for risk assessments, it will be necessary to first envisage a plausible pathway to harm whereby non-target organisms (NTOs) might be exposed to the compound, eventually leading to an adverse effect to the environment. The pathway to harm should explain how the deployment of the RNA-based biocontrol product could lead to adverse impacts on NTOs through a chain of events that take into account both hazard and exposure. In the context of crop protection, the NTOs must first be exposed to the dsRNA active molecule and first exposure will most often occur through the oral route (directly or indirectly via food chains). This will be dependent on the persistence of dsRNA in the environment (ability to resist degradation) at high enough quantities to be taken up by the NTO and at sufficient amounts to activate the endogenous RNAi machinery of the NTO. Once the RNAi machinery of the NTO is activated, it must lead to the translational suppression or degradation of the corresponding mRNA in a sequence-dependent manner. The loss of the targeted transcript should eventually have an adverse effect on the NTO. If the likelihood of any of the steps in the pathway to harm is experimentally proven to be impossible or unlikely, then the risk of the RNA-based biocontrol compound to the NTO can be considered negligible. Sequence-independent effects on the NTO following exposure to non-specific dsRNA has also been hypothesized to potentially affect the NTO.[35] This includes immune stimulation or saturation of the enzymes involved in the RNAi machinery, which could in turn negatively affect the fitness of the NTO. The likelihood of this occurring in the NTO will probably be low considering the multiple barriers in the pathway to harm. Nevertheless, this is also taken into account during risk assessment when the probability of lethal and sub lethal effects in the NTOs is evaluated. Another layer of complexity to be taken into account during the risk assessment of RNA-based biocontrol compounds will be the type of formulations used to improve persistence and delivery to targets. Protecting the dsRNA from degradation in the environment and further enhancing uptake into cells might increase risks to NTOs by overcoming natural barriers such as the presence of nucleases and difficult cellular uptake and release mechanisms which could have otherwise protected the NTO. Additionally, the dose of dsRNA taken up by the NTO is expected to be higher when exposed to the formulated dsRNA compared with exposure to the naked dsRNA, considering the longer persistence of the protected dsRNA molecules in the environment. Thus, the level of exposure of NTOs will be influenced by the persistence of the dsRNA as well as the routes of exposure.

Discussions on the risk assessment of RNA-based biopesticides are ongoing. In 2013, a framework for the risk assessment of RNA-based pesticides was presented by the USA EPA in a white paper submitted to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Science Advisory Panel.[24] FIFRA responded to this document by organising a meeting the following year that explicitly addressed several concerns pertaining to ecological risk considerations and human health considerations and provided various recommendations to address uncertainties related to these concerns.[36] A similar exercise was conducted by the European Food Safety Authority (EFSA) which triggered the collection of literature reviews based on scientific information of RNAi studies.[37-39] These reviews, intended to support the risk assessment of RNAi-based GM plants, provide a comprehensive overview of RNAi and will also be useful in the development of regulatory guidelines for externally applied RNA-based biocontrol compounds. With a similar objective, an OECD conference on RNA-based pesticides was recently organised in April 2019 in Paris, with the aim of providing an overview on the current status and future possibilities for the regulation of externally applied dsRNA-based compounds. This event provided both inputs and recommendations supporting the development of guidance document(s) by the OECD expert group on RNAi-based pesticides as practical tool(s) for policy makers and regulators of pesticides all over the world. Nevertheless, there remains a need for clear regulatory guidelines for RNA-based biocontrol compounds if the technology deployment is to be assured. By advocating for a biopesticide approach for RNA-based biocontrol compounds, substantial time and money will be saved in the registration of these new biomolecules. This takes into consideration the comparably low cost of about US$3–7 million to develop a biopesticide and the short time of approximately 4 years or less to get into market in the USA, in contrast to more than US$280 million and nearly 12 years required to develop one new synthetic pesticide.[40,41]

5. Importance of good communication for the acceptance of new biotech products

In matters relating to biotechnology and biosafety, particularly for biotech products such as

RNA-based biopesticides, a good and clear communication is not only important to reassure stakeholders but also to ensure and assist with regulatory compliance. Concerns by farmers, consumers and other players in the food production chain indicate that it is important to engage in dialogues with different stakeholders using a range of approaches and information. Lessons drawn from debates on the acceptance of GM crops indicate that while consumers can positively influence the acceptance and introduction of a biotech product, awareness and proper understanding are required, which is usually a neglected issue. Farmers on the other hand are more experienced with novel crop protection products and can influence the choice of new products that are to be used on their fields. Thus, both consumers and farmers need to be engaged with, by both the industry and researchers. Scientists can play a key role in the processes of dialogue and creating awareness amongst stakeholders. The engagement of different stakeholders to find a consensus which is realistic, practical and can meet high scientific standards, is important prior to meeting legislators. Such engagements could include discussions over environmental and food safety, and how to ensure that the development of these new technologies for crop protection are appropriately regulated. It should be emphasised that the goal is to develop scientific evidence-based and appropriate legislation to enable these new tools for crop protection to be introduced into sustainable farming systems.

6. Conclusions

Several factors such as reductions in farmland, climate change, population growth, strict regulations on current chemical pesticides and scepticism over the safety of GM plants has promoted the development of new environmentally friendly approaches to control/manage plant pests and diseases and to improve plant traits. The exogenous application of dsRNA for crop protection holds an alluring promise to address these societal concerns and needs. However, going forward, the commitment of agribusinesses to invest in research endeavours and the role of start-up companies will be crucial to the successful development of practical applications of RNA-based biocontrol compounds. Additionally, though the current regulations on pesticides can be applied to RNA-based biocontrol products, more appropriate and standardized regulatory guidelines are urgently needed to facilitate the entire commercialization process. Finally, communication to create awareness and public acceptance will be key to the deployment of these compounds.

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Conflict of interest

The authors declare no conflicts of interest in relation to this perspective and state that the opinions expressed are their own and should not be considered to reflect those of any other individuals or organisations.

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