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Does RNAi-Based Technology Fit within EU Sustainability Goals?

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European Union (EU) and global sustainability policies emphasize the need to replace contentious pesticides with safe, efficient, and cost-effective alternatives to ensure sustainable food production. However, R&D for alternatives to contentious pesticides are lagging behind and need to be broadened. Here, we discuss how RNAi-based technology can contribute to pesticide risk reduction.

Future Agriculture: Ensuring Sustainable Food Production in the EU

The Farm-to-Fork (F2F) strategy[i], one of the pillars underneath the European Commission's new Green Deal, aims to ensure a more sustainable and food-secure society. Its aims include a reduction in agrochemical inputs, such as pesticides, fertilizers, and antimicrobials, to achieve greater sustainability and health, and reduce loss of biodiversity while ensuring continued crop protection. It envisages various practices that promote lesser pesticide usage, such as integrated pest management (IPM), and the use of precision agriculture and artificial intelligence. It also recommends imposing maximum levels (tolerances) for pesticide residues in imported commodities to enforce sustainable production and pesticide use in countries exporting to the EU. The F2F strategy's pesticide reduction measures are also cited by the Commission's concurrent Biodiversity Strategy 2030[ii], as a means towards reversing the alarming decline in farmland birds and insects (especially pollinating ones). It also proposes IPM and the establishment of variable landscapes hospitable to natural pest regulators. Furthermore, it envisages organic farming to cover 25% of agricultural land in 2030, and it suggests establishing forests that are resilient towards pests, and the banning of chemical pesticides from use in urban green spaces.

At the same time, the Commission also published a report on the experience gained with its policy towards more sustainable use of pesticides under the so-called 'Sustainable Use Directive' (SUD) of 2009[iii]. Under the SUD, EU member states had to develop and implement national action plans for the reduction of pesticide volumes and risk. The report concluded that one prevalent shortcoming across the board was the lack of assessment of the actual implementation of IPM practices, while there was an upward trend in non-chemical, low-risk, and basic active substances, and it also concluded that the R&D basis for such alternatives should be broadened. Notably, the European pesticide and biopesticide producers' organization (European Crop Protection Association; ECPA) recently pledged to support these with various commitments focused on innovation, sustainability, and health, including a €14 billion investment in the development of precision agricultural techniques for the more targeted (hence reduced) application of pesticides, and of natural biopesticides with favorable IPM characteristics, complementing other pesticides[iv]. Concurrent with the EU's policy towards less risky pesticides, the United Nations Environmental Program (UNEP), other international and national organizations, and companies are proactively pursuing risk reduction for 'highly hazardous pesticides'.

As already noted in the review of the SUD implementation, R&D for alternatives to chemical pesticides has been lagging behind and needs to be broadened. The new EU policies make the case for innovative, enabling technologies using versatile platforms for the development of agents that are widely applicable to a host of different crop pests and diseases, yielding products with high specificity for the targeted pest or disease, a benign environmental and health profile, and requiring a short development time and affordable in costs.

RNAi-Based Technology Enables Pesticide Risks Reduction Goals

RNAi is a well-known natural biological process in most eukaryotes, where double-stranded RNA (dsRNA) molecules regulate gene expression by targeting specific endogenous mRNA molecules in a sequence-specific manner (Figure 1). By exploiting this sequence-dependent mode of action, RNAi-based products with higher selectivity and better safety profiles (less mobile through the soil, less persistent, and less toxic) compared with contentious chemical pesticides are being developed [1–4]. RNAi-based control has several unique features that offer additional opportunities compared with contentious chemical pesticides. The dsRNA active molecules can be designed to target the expression of different genes without the need to change the sequence-dependent mode of action and, depending on the gene targeted in the pest, various outcomes ranging from sublethal to lethal effects can be achieved. Although selecting effective RNAi targets can be a challenging step, a combination of the availability of *in silico* tools and an increase in the availability of genome databases for various species has made it possible to design species-selective and efficient dsRNA molecules with zero to negligible off-target effects in non-target species [5,6]. This presents an advantage over current contentious chemical pesticides with broad action spectra, usually affecting non-target species. Additionally, dsRNA is a natural

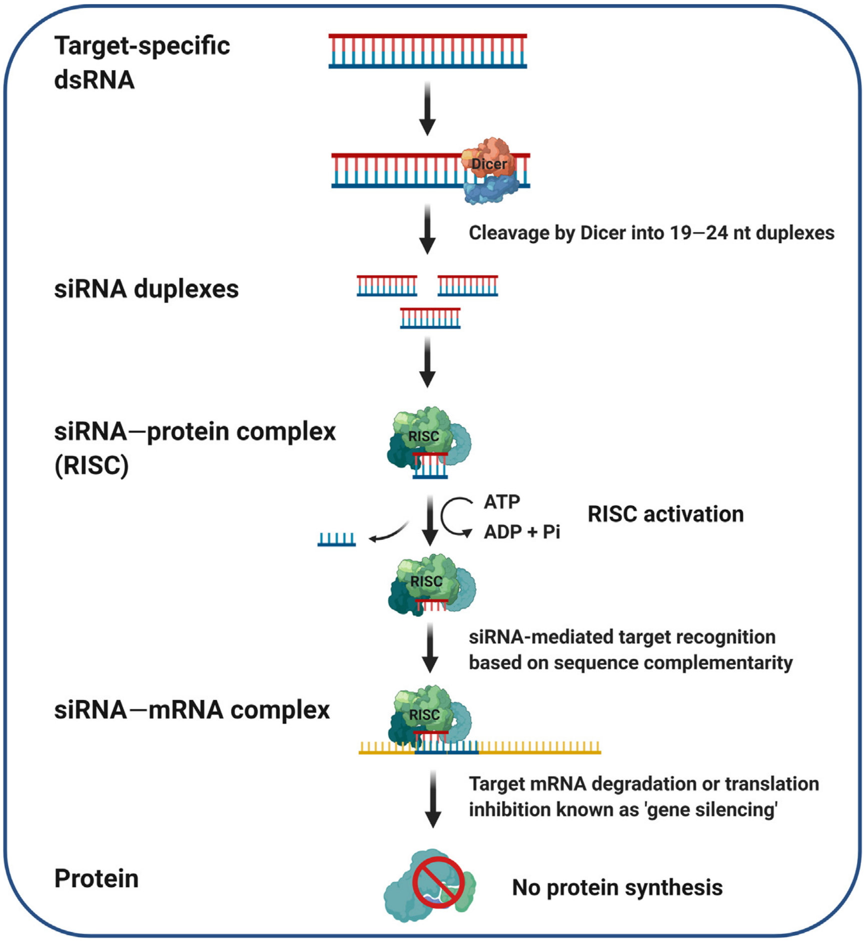

Figure 1. Illustration of RNAi-Mediated Gene Silencing. RNAi is a natural cellular process that is typically initiated by the endogenous production or exogenous introduction of long double-stranded RNAs (dsRNAs) into the eukaryotic cell. These long dsRNA molecules can originate from different sources, such as direct introduction of exogenously produced dsRNA; dsRNA-viral intermediates; or hybridization of complementary RNA transcripts present in the same cell, or from single-stranded RNAs (ssRNA) that contain near-complementary or complementary inverted repeats, or that are separated by a short spacer sequence capable of folding back onto themselves to form a hairpin structure (hpRNA). Once present in the cell, the dsRNA molecule triggers the RNAi mechanism by recruiting an RNase-III-like enzyme. known as Dicer. and its cofactors, which leads to the cleavage of the dsRNA molecule into siRNA duplexes (19–24 bp). These siRNAs are then loaded into a protein complex, forming an RNA-induced silencing complex (RISC). The activation of RISC involves the retention of one strand of the siRNA (guide) while the other strand (passenger) is released and degraded in the cell. The RISC is then guided in a sequence-specific manner by the loaded guide stand of the siRNA to target and bind to target mRNAs that are nearly perfectly complementary. The formation of the siRNA–mRNA complex ultimately leads to degradation or translational inhibition of the target mRNA, preventing protein synthesis and leading to post-transcriptional gene silencing.

molecule that is rapidly degraded by nucleases and UV radiation [7,8], in contrast to some chemical pesticides with longer persistence in the environment.

RNAi-based biocontrol can be applied using two main approaches: *in planta* delivery through genetically modified (GM) crops or exogenous application of formulated RNAi-based products (Figure 2). RNAi-based GM plants differ from most other GM plants expressing new proteins because the introduced gene sequences give rise to the stable expression of small dsRNA molecules with very high target specificity as active compounds. Moreover, the expressed short (s)RNAs are highly mobile in the plant vascular system, allowing horticultural crops to acquire resistance when grafted onto transgenic rootstocks [9]. This type of dsRNA application is of particular interest to seed and nursery industries, which are mainly interested in propagating and marketing new resistant high-quality varieties. Nevertheless, the trend toward the development of RNAi-based products for exogenous application against crop pests is favored because plants treated with dsRNAs are not considered GM organisms (GMOs) [10]. These products can also be directly applied using current agricultural practices, such as spray application, trunk injection for tree species, seed soaking, or root drenching through hydroponic systems in greenhouses. The possibility of developing exogenously applicable RNAi-based products with high target pest selectivity and a better safety profile has also stimulated the creation of various start-up companies (albeit at the moment mostly non-European) that exploit available biotechnology tools to support the development of these products for field-scale applications[v,vi].

As a spin-off of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) pandemic, the existing production capacity for dsRNA molecules may be ramped up exponentially in the near future to provide the global community with RNA-based vaccines, owing to substantive private investments running into the hundreds of millions of dollars[vii]. The same platforms could be converted to dsRNA production (with less stringent quality requirements than for biomedicines) for agricultural purposes. This would further promote the cost-effective manufacture of sizable volumes of dsRNA needed for large-scale pesticide applications. Furthermore, significant improvements are being made in the development of appropriate formulation technologies for

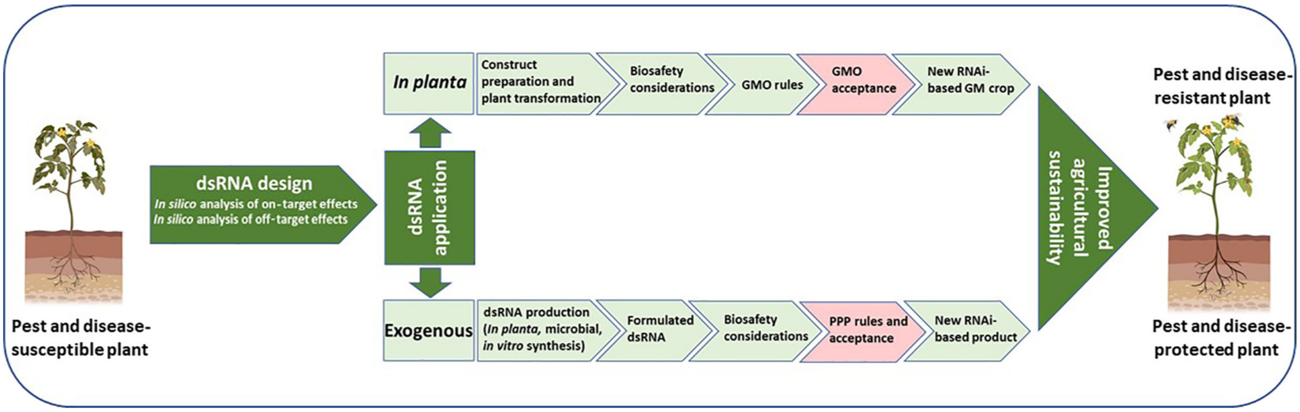

Figure 2. Two Main Delivery Approaches for RNAi-Based Control of Pests and Diseases. RNAi-based control typically starts with designing the double-stranded (ds)RNA active molecule to be specific to an essential gene in the pest or pathogen, while remaining safe for non-target organisms. The increase in availability of genome/transcriptome databases for more organisms and the development of sophisticated high-throughput approaches, such as RNA-seq and digital gene expression tag profile (DGE-tag) technologies, has significantly improved the selection of potential RNAi targets. Although *in silico* tools can help in the design of pest or pathogen-specific dsRNA molecules, empirical evidence from bioassays is required to support effectiveness against the pest/pathogen and zero to negligible adverse effects in non-target organisms. There are two main dsRNA application approaches for RNAi-based control; one approach involves the *in planta* expression of RNAi constructs to target genes of pests/pathogens, while the second approach involves the exogenous application of dsRNA. The choice of the application method depends on the interaction between the crop and the pest/pathogen, and on the feasibility of developing an efficient application method against the pest/pathogen. These delivery approaches follow two separate paths during the product development pipeline, with the *in planta* approach falling under genetically modified organism (GMO) regulation, while the exogenous approach is expected to fall under the plant protection product (PPP) regulation. The genetically modified (GM) approach can be challenged by a lack of technological tools to modify some plant species, high cost of production to registration of the GMO, and some public opposition to GMOs. The exogenous approach is challenged by the high cost of producing dsRNA for large-scale field application and the lack of appropriate formulation technologies to improve RNAi efficiency against recalcitrant pests/pathogens. Despite these challenges, both delivery approaches have successfully led to the development of safe and effective RNAi-based products, which can significantly contribute to agricultural sustainability by reducing the use of contentious pesticides.

the delivery of dsRNA molecules to target crop pests or pathogens [3]. Formulations are generally designed to either improve dsRNA stability and/or ensure effective delivery of dsRNA to pests or pathogens, thereby improving RNAi efficiency. Developing the right formulation can be challenging and depends on the interaction between plants and pests or pathogens. As such, risk assessment for formulated dsRNA should be performed on a case-by-case basis. At a time when the EU strategy is directed towards sustainability with significant reduction in contentious pesticides, the interests of agribusinesses to invest into the development of RNAi-based products with a better biosafety profile [11], coupled with the stimulation of start-up companies to support product development, indicate that RNAi-based pest control can contribute to the EU pesticide reduction goal in the F2F strategy.

Societal Perception and Acceptance of RNAi-Based Biocontrol

Alternatives to pesticides address policy needs towards increased sustainability, an acknowledged field of science [12] and a term widely used in media but still a complex concept for the main public. Consumer perceptions of pesticides have often been studied as risk perception linked with adverse effects of pesticides [13]. It is important to explicitly include the perception of both risks and benefits to capture also trade-offs that consumers are willing to make to accept new generations of pesticides. Perceived benefits alone might not be the decisive factor in societal acceptance of RNAi-based control; other relevant factors, such as the extent to which the application is perceived to be important or necessary, or unimportant, could shape societal acceptance [14]. Therefore, it is of fundamental importance to develop dialog with stakeholders to ensure that ethical and social concerns are addressed early on in the development process of RNAi-based control strategies. If the attitude of stakeholders towards RNAi-based control remains uncrystallized, a constant re-evaluation of what the stakeholders think is advised [15], because such an attitude is unlikely to be fixed but rather influenced by external events, including the order of entry of RNAi-based products into the market. Findings from such evaluations would further shape communication strategies that could drive societal acceptance of RNAi-based control strategies.

Concluding Remarks and Future Perspectives

The sequence-specific mode of action of RNAi-based products makes them unique in selectivity and efficiency compared with other conventional agrochemicals and suggests them as a promising solution to substitute contentious pesticides or reduce reliance in general. However, to enable society to accept RNAi-based products, several key tasks will have to be accomplished. The co-creation of new, effective, and safe RNAi-based products in collaboration with stakeholders under the responsible research and innovation paradigm promoted by the EU, will foster greater knowledge and acceptance of technology. Furthermore, an advancement in understanding consumers' perception will facilitate the successful market introduction of RNAi-based, sustainable products for crop protection.

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Resources

[i]https://ec.europa.eu/food/sites/food/files/safety/docs/f2f_action-plan_2020_strategy-info_en.pdf

[ii]https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52011DC0244

[iii]https://ec.europa.eu/food/plant/pesticides/sustainable_use_pesticides_en

[iv]www.ecpa.eu/commitments/2030-commitments/innovation-investment

[v]www.agrorna.com/sub_05.html

[vi]www.greenlightbiosciences.com/markets/plant-sciences/

[vii]www.greenlightbiosciences.com/covid19/

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