

# COST ACTION CA 15223 - MODIFYING PLANTS TO PRODUCE INTERFERING RNA

## Working Group 3: Specific biosafety issues associated with RNAi

### Minutes of the Workshop on “Biosafety issues associated with RNAi” – Ghent (Belgium) 6 – 7 September 2017

The first day of the workshop organized by the WG3 consisted of 1) a scientific session in the framework of the workshop “**Modern Biotechnology in Integrated Crop Management**”, organized by the West Palearctic Section of the International Organization for Biological Control (see [www.eigmo.info](http://www.eigmo.info)) and 2) a practical demonstration of work with RNAi and insects at Ghent University (laboratory of Prof. Guy Smagghe).

The joint session was attended by ca 50 people and was opened with an invited lecture (via web) presented by Prof. Xuguo Zhou (University of Kentucky) on early-tiers risk assessment of transgenic RNAi plants on non-target arthropods. The main focus was on selectivity of dsRNA. In fact, while for several non-target organisms studied no adverse effects by specifically designed dsRNA are reported, more recent experiments indicate opposite results. While the taxonomical proximity between non-target and target organisms is a first indicator of possible similar RNAi effects, preliminary research about non-target effects even between different insect orders was highlighted in the presentation.

Further presentations were given by:

- J.B. Sweet who discussed European research initiatives on RNAi aimed at developing better understanding of interference mechanisms and activity, potential developments and the biosafety raised by this technology;
- J. Romeis presented data on feeding studies with two ladybird species fed with dsRNA designed to target the *vATPase A* of the coleopteran pest *Diabrotica virgifera virgifera*. Both ladybird species were sensitive to dietary RNAi upon ingestion, with *C. septempunctata* being much more sensitive (lethal effects detected) than *A. bipunctata* (significantly prolonged developmental time).
- S. Arpaia illustrated preliminary results of laboratory experiments conducted using larvae of the lacewing *Chrysoperla carnea*, for which no genomic sequences are publically available. Larvae were fed with dsRNA targeting the clathrin gene of the coleopteran pest *Leptinotarsa decemlineata*. No acute toxic effects were detected, further experiments will have to address the likelihood of sublethal effects.
- M. Edwards illustrated how information gained from the mode of action of traditional synthetic pesticides and arthropod inhibitory cysteine knot venoms (ICK) can be used to design RNAi-based insect control strategies.
- J. Polak reported about the results of 15 years of field trials with the genetically modified PPV-resistant plum cultivar HoneySweet.

- W. Jarausch illustrated the potential value of designing RNAi-mediated control of spotted wing *Drosophila suzukii* and highlighted differences in mechanisms between this pest species and *Drosophila melanogaster*
- O. Christiaens also centered on applications, challenges and biosafety considerations of RNAi as an insect pest control strategy.

During the laboratory visit in the afternoon (see picture), practical insight into different methods of dsRNA application in insects (e.g. feeding, microinjection) was given. About 20 people visited laboratories and it was possible to practically try the microinjection system to deliver dsRNA extracts into larval bodies. Further, a visit was paid to the mass rearing of different insect species used for laboratory bioassays.



The social dinner concluded Day 1 of the Meeting

During the second day the scientific session was exclusively attended by the participants to the IPlanta COST action and included four presentations on general biosafety issues associated with RNAi.

- Z. Martinez illustrated a technical microscope application for studies on mechanisms of action of dsRNA. In the presentation confocal microscopic analysis of cellular dsRNA uptake in cells of Lepidoptera were showed and discussed.
- A. Gallé reported about the differential expression of plant miRNAs involved in drought tolerance.

Two more presentations concerned PPV resistant HoneySweet plum cultivar obtained with RNAi mechanisms. Both presentations focused on possible benefits of this RNAi plant cultivar:

- I. Zagrai showed data on the preliminary assessment of the potential reduction of insecticide treatment for aphid vector control. The preliminary results revealed that a slight decrease in the number of insecticide treatments is possible (since insect vectors of the plum pox virus are not a threat when using this GM cultivar). On the other hand, this advantage was overshadowed by the need of insecticide treatments to control the pest *Laspeyresia funebrana*, whose flight curve partly overlaps with that of aphids;

- M. Ravelonandro discussed the possibly greater food safety in fruits of Honeysweet plums, due to the absence of viral replication in fruits.

During the following round table (chair: Salvatore Arpaia) future perspectives of RNAi GM plants in Europe were discussed.

It was communicated that EFSA is about to release their opinion on the request for import and processing of maize MON 87411 which will become the first case of a RNAi-based insect resistant crop under discussion for possible approval in the European Union.

The current major knowledge gaps about RNAi technology were discussed. They may represent relevant challenges for the biosafety assessment of RNAi applications, e.g. lack of genome sequences of many potential non-target organisms, doubts about the amount of dsRNA necessary to trigger silencing in a cell, lack of information on possible RNAi amplification mechanism in insects.

An upcoming call for preparing a cooperative Ph.D. student program (expected to start in 2019) was presented, where iPLANTA COST Action consortium could consider to submit an application. There was consensus that this topic should be brought up during the next general iPLANTA conference in Poznan in February.

In the afternoon, an internal WG3 meeting took place for planning future activities. The discussion was introduced by a short presentation from A. Dietz-Pfeilstetter who summarized the expected deliverables of WG3, and the different biosafety aspects addressed in presentations at the first and the second WG3 meeting. The subsequent discussion resulted in a number of decisions and plans for future activities:

- Solicit information from WG1 and WG2 for preparing case studies for risk assessments of RNAi plants and the consequent development of specific biosafety protocols. Case studies should not be limited to insect resistant RNAi plants, but also addressing, e.g., virus resistance and fungi resistance. Case studies should be preferably based on data produced by iPLANTA participants;
- Increase number of people actively involved in iPLANTA activities. In particular, only a limited contribution on food safety issues was received for this meeting. It was proposed to contact Gijs Kleter (Wageningen University) who might involve colleagues in this area of expertise. S. Arpaia will contact him.
- Start making a plan for publication of reviews. In this respect, it was decided to wait for the availability of the upcoming EFSA report on "Literature review of scientific information on RNAi that could support the environmental risk assessment of RNAi-based GM plants". A small group constituted by Jeremy Sweet, Guy Smagghe and Salvatore Arpaia (who are also involved in writing the EFSA report) will take the lead and organize the possible structure of the review. Within this scope a small meeting will be organized after the completion of the EFSA report (tentative date and location: January 2018, Rome).
- Apply for possible joint projects by members of the iPlanta Consortium, e.g. the above mentioned Ph.D. program.
- Solicit further applications for STSM with the deadline of 30 September 2017.
- Plan the next WG3 meeting. Given the good additional attendance generated by the joint meeting with IOBC this year, it was proposed to organize next working group meeting back-to-back with the European Congress of Entomology (Naples, Italy, 2-6 July 2018) where a symposium on the

applications of insecticidal RNAi is already planned. In order to proceed, S. Arpaia will contact the organizers to evaluate this possibility;

- A proposal was made to start considering one of the deliverables foreseen for the COST action, to compile a review on targets and off-target of known dsRNA and miRNA sequences.

Antje Dietz-Pfeilstetter

Salvatore Arpaia

26 September 2017