iPLANTA COST Action CA15223

Workshop on

"Biosafety issues associated with RNAi"

6-7 SEPTEMBER 2017

GHENT UNIVERSITY, FACULTY OF BIOSCIENCE ENGINEERING

GHENT (BELGIUM)

BOOK OF ABSTRACTS

*Grosshans H, **Filipowicz W**.Nature. **2008***

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) 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 (significally 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 cystine 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, 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.

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**

DAY 1 - WEDNESDAY 6TH (Joint Session with IOBC), Ghent University, Faculty of Bioscience Engineering

Session 1 – SPECIFIC BIOSAFETY ISSUES ASSOCIATED WITH RNAi (WG3)

AUTHORS	INVITED LECTURE	
JOE ZHOU (UNIVERSITY OF KENTUCKY)		

Session 1 – SPECIFIC BIOSAFETY ISSUES ASSOCIATED WITH RNAi (WG3)

INTRODUCTION TO RNAi RESEARCH AND DEVELOPMENT IN RELATION TO INVERTEBRATES AND CROPS.

JEREMY SWEET

J T Environmental Consultants, 6 Green St, Cambridge CB245JA, UK.

KEYWORDS : GM plants, pests, diseases, dsRNA, biosafety.

In the short 20 year period since the first reports of RNA interference there has been a tremendous growth in research and associated publications. Research has initially focused on using RNA to silence genes in order to study gene functions in the model species *C. elegans* (nematode) and *Drosophila* (Diptera) and in model plants such as Arabodopsis and tobacco . This research has contributed to revealing potential conserved target genes in other invertebrates, vertebrates, humans that can be used for remediating disorders and controlling pest species. RNAi systems have also been studied for inhibiting virus replication and virus control systems have been developed in plants and animals. More recently RNAi has been studied for controlling plant metabolism, plant pests and fungal and bacterial pathogens. The development of transgenic plants expressing dsRNA has resulted, and it is now recognised that plants can be modified to express dsRNA which target genes in pests and pathogens parasitizing plants as well as endogenous plant genes. This paper will review some of these developments and the biosafety issues they raise.

SUSCEPTIBILITY OF TWO LADYBIRD SPECIES TO dsRNA TARGETING THE *vATPase A* OF THE WESTERN CORN ROOTWORM

S.HALLER[1], F.WIDMER[2], B.D.SIEGFRIED[3], X.ZHOU[4], J.ROMEIS[1]

[1]Agroscope, Research Division Agroecology and Environment, Zurich, Switzerland

[2]Agroscope, Competence Division Method Development and Analytics, Zurich, Switzerland
[3]Department of Entomology and Nematology, University of Florida, Gainesville, FL, USA
[4]Department of Entomology, University of Kentucky, Lexington, KY, USA.

Environmental risk assessment, non-target effects

RNAi-based genetically engineered (GE) crops are a potentially powerful and highly specific new tool for insect pest control. One concern with the adoption of this technology is the potential to harm valued non-target organisms which might be affected by the double-stranded RNA (dsRNA) produced in GE plants that are designed to kill the target pest. Species of Coccinellidae (Coleoptera) are important natural enemies in many agricultural landscapes and might be exposed to the insecticidal dsRNA via pollen or herbivorous prey insects. In order to estimate their susceptibly to dietary RNAi, we fed *Adalia bipunctata* and *Coccinella septempunctata*, two common European ladybird species, with dsRNA designed to target the *vATPase A* of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), a major insect pest of maize belonging to the same insect order. Specific dsRNA designed to target the *vATPase A* of the two ladybird species serves as a positive control treatment. Our results revealed that both ladybirds were sensitive to dietary RNAi when ingesting their specific dsRNA, with *C. septempunctata* being much more sensitive than *A. bipunctata*. We could also observe adverse impacts caused by the dsRNA targeting *D. v. virgifera vATPase A* in the two ladybird species manifesting in significantly prolonged developmental time for *A. bipunctata* and significantly reduced survival rate for *C. septempunctata*, confirming the higher sensitivity in the latter species. The results are supported by bioinformatical analyses (aligning the target *D. v. virgifera vATPase A* sequence with the *A. bipunctata* / *C. septempunctata vATPase A* sequence) that revealed a higher number of possible 21 nucleotide matches, a requirement for RNAi, of the targeted dsRNA sequence with the *vATPase A* of *C. septempunctata* (34 matches) compared to that of *A. bipunctata* (6 matches). Gene expression analyses confirmed the bioactivity of the dsRNA treatments and the results from the feeding bioassays.

THE POSSIBLE ENVIRONMENTAL EXPOSURE OF NON-TARGET ORGANISMS TO dsRNA

I. URRU[1], K. KOSTOV[2], T. DJAMBAZOVA[2], J.B. SWEET[3], G. SMAGGHE[4], O. CHRISTIAENS[4], S. ARPAIA[1]

[1] ENEA- Italian National Agency for New Technologies, Energy and Sustainable Economic Development

[2] AgroBioInstitute, Bulgaria

[3] JT Environmental Consultants, UK

[4] Ghent University, Belgium

Environmental risk assessment, environmental fate, plant expression

Genetically modified plants (GMPs) can be designed to induce silencing of target genes in plants or in arthropod pests through RNA interference. In order to conduct an environmental risk assessment, two main components will need to be specifically evaluated: the intrinsic hazard of the GMPs for non-target organisms and the likelihood for these organisms to be exposed to the GMP and its products.

Exposure of organisms to dsRNA and/or its metabolites will depend on the expression levels of RNAi in the GMP, the potential routes of distribution in the environment, and the environmental fate of dsRNA.

The description of the novel attribute of the GM plant in terms of route, frequency, duration, and intensity of exposure is considered relevant information for characterizing exposure during environmental risk assessment.

We reviewed the existing literature on RNAi applications to characterize the main factors that could lead to exposure of non-target organisms to dsRNA derived from genetically modified plants, if these are introduced in commercial cultivations.

In general, the available information on dsRNA expression in plants or its environmental persistence is insufficient to run a robust exposure analysis. The only exception is constituted by studies conducted for the characterization of the maize transgenic event MON87411, recently authorized for cultivation in the US, for which several studies are available.

The value of available data and the knowledge gaps identified in our literature survey will be discussed.

Session 2 – SPECIFIC BIOSAFETY ISSUES ASSOCIATED WITH RNAi (WG3)

Session 2 – SPECIFIC BIOSAFETY ISSUES ASSOCIATED WITH RNAi (WG3)

AUTHORS	ORAL PRESENTATION	
MARTIN EDWARDS et al**.** **(NEWCASTLE UNIVERSITY)**		20 min

RNAI-MEDIATED CONTROL OF SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*): EFFICACY CHALLENGES AND BIOSAFETY CONSIDERATIONS

W.JARAUSCH, M.RUNNE, M.WASSENEGGER, G.KRZCAL

AlPlanta – Institute for Plant Research, RLP AgroScience, Neustadt/Weinstrasse, D

Drosophila suzukii, *SWD, pest control, RNAi delivery*

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is a rapidly emerging invasive pest in America and Europe. First described in Germany in 2011, high yield losses were already recorded in 2014 in cherries, small fruits and grapevine. Pesticide and mechanical control measures have been applied which are neither environmentally nor economically sustainable. As D. suzukii is well established in Germany, long-term solutions have to be developed. In this regard, RNAi-based pest control strategies would have some potential. However, a major bottleneck is RNAi delivery and efficacy of control. D. suzukii attacks intact maturing and ripening fruits and damage is produced by the developing larvae inside the fruits. D. suzukii can rapidly produce high population densities. Fruit damages caused by D. suzukii can be the entrance for further pests as well as microbes, which is of major concern in grapevine. Therefore, RNAi-mediated control has to attack adult flies before egg deposition in fruits and has to be efficient against high population densities. Furthermore, RNAi - mediated shut down of D. suzukii genes has to occur rapidly. Challenges are therefore finding the right target gene and developing an efficient delivery method. As RNAi in D. suzukii is not systemic, a constant dsRNA uptake either through transgenic plants or through oral feeding seems to be necessary. We tried to induce gene silencing through oral application of dsRNAs for essential genes like tubulin (γTub23C) or vacuolar ATPase (Vha26) with unsatisfactory success. Another challenge is biosafety considerations: many different Drosophila species share the same habitats as D. suzukii and we captured several of these species in monitoring traps. Some species are not well studied and molecular data are missing. In order to be sure that no off-target effects of D. suzukii RNAi control occur, these Drosophilids need to be considered in further studies as well.

***MONILIA SP*. INFECTION WEAKENS RESISTANCE OF TRANSGENIC *PLUM PRUNUS DOMESTICA* L., CV. HONEYSWEET TO THE PLUM POX VIRUS**

J. POLÁK, J. K. KUNDU, P. KOMÍNEK, T. NEUBAUEROVÁ, R. SCORZA

1Division of crop protection and plant health, Crop Research Institute, Drnovská 507, 16106 Prague, Czech Republic (polak@vurv.cz)

2Usda-ars appalachian fruit research station, Kearneysville, wv 25430, USA

keywords *plum pox virus, resistance, transgenic plum, cv. honeysweet, monilia sp., infection, resistance weakening*

Sharka caused by plum pox virus (PPV) is the most harmful disease of stone fruits in Europe and elsewhere in the world. Biotech approach has led to the development of resistance through genetic engineering. In this study, we evaluated a transgenic plum prunus domestica l., clone c5 (cv. Honeysweet), where the ppv resistance is based on RNA interference (RNAi). Resistance in c5 plums has been evaluated for ppv, and combinations of PPV with prune dwarf virus (PDV), and apple chlorotic leaf spot virus (ACLSV) in a regulated field trial in the Czech Republic for fifteen years (2002-2016). Even under high and permanent infection pressure introduced through graft inoculation of the viruses, PPV has been detected in c5 trees only in several leaves situated close to the point of inoculum grafting in the first nine years. Mild symptoms of PPV disappeared year by year. No PPV symptoms were observed in the years 2011-2013 and results of elisa detection tests were negative. Similar results were obtained, when rt-pcr was used for PPV detection.

There was a severe attack of transgenic plum trees by monilia sp. In the twelfth year. Mild PPVsymptoms have appeared again in several leaves in the next two years (2014-2015) after the monilia sp. Infection and disappeared again in the fifteenth year. The presence of PPV was confirmed by elisa and rt-pcr not only in symptomatic leaves, but also in several fruits showing no symptoms. The low presence of PPV was confirmed in several asymptomatic leaves and fruits in the fifteenth year of evaluation, too. Engineering nutrient content in plants through RNAi

RNAi AS AN INSECT PEST CONTROL STRATEGY: BIOSAFETY CONSIDERATIONS

O. CHRISTIAENS1, G. SMAGGHE1

1Laboratory of Agrozoology, Department of Crop protection, Gent University

RNAi, insects, biosafety, specificity, risk assessment

RNA interference (RNAi), a post-transcriptional gene silencing mechanism is a promising next-generation biopesticide. The technology has, in theory, great advantages in terms of species specificity, biosafety and environmental safety in general. However, there are still considerable gaps in our knowledge at present, which might urge a cautious approach. Here, we give an overview on what is known in the literature in terms of species-specificity of dsRNA, the potential implications for non-target species, the environmental fate of dsRNA, the food/feed safety and potential requirements for risk assessment. We also explore whether bioinformatics could be of use in risk assessment.

DAY 2- THURSDAY 7TH, Ghent University, Faculty of Bioscience Engineering

09:00

Session 1 – SPECIFIC BIOSAFETY ISSUES ASSOCIATED WITH RNAi (WG3)

AUTHORS	**ORAL PRESENTATION**	
GALLE et al. **(GODOLLO UNIVERSITY)**	WOUNDING AND WATER STRESS ALTERS LATERAL ROOT FORATION VIA MIRNA REGULATED NETWORK: SOME INTERESTING MIRNAS IN *Brachypodium distachyon*: CONTRIBUTION TO THE ENVIRONMENTAL RISK ASSESSMENT OF RNAi BASED PLANTS	20 min

Session 1 – SPECIFIC BIOSAFETY ISSUES ASSOCIATED WITH RNAi (WG3)

CONFOCAL MICROSCOPY ANALYSIS OF POLYMER-COATED DSRNA UPTAKE IN LEPIDOPTERA MIDGUT CELLS.

Z.MARTINEZ[1], O. CHRISTIAENS[1], M. GOMEZ TARDAJOS[2], P.DUBRUEL[2], G. SMAGGHE[1]

[1]Laboratory of Agrozoology, Department of Crop protection, Gent University

[2] Polymer Chemistry & Biomaterials group, Department of Organic and Macromolecular Chemistry, Gent University

Confocal microscopy, CF203, uptake, dsGFP. fluorescence intensity

Cellular uptake of double-stranded RNA (dsRNA) is an important factor impacting RNAi efficiency. One of the most powerful tools to investigate cellular uptake of these nucleic acids is confocal fluorescence microscopy. It is capable of creating sharp images of a specimen providing a window into the physiology of living cells at sub-cellular levels. This is attained by the confocal pinhole, which allows the exclusion of most of the light from the specimen that is not from the microscope's focal plane.

Using this technology we aim to analyze the uptake of naked dsRNA and also polymer-coated dsRNA (polyplex) in Choristoneura fumiferana midgut cells (CF203). To investigate whether the polyplex is taken up as a complex by cells and if cellular uptake is maximized when using the polyplex compared to the naked dsRNA, simultaneous experiments were setup. Labeled dsRNA was obtained using the Label IT® siRNA Tracker Intracellular Localization Kit, Cy®3 (Mirus) and the polymer was labeled with FITC. The polyplex and dsRNA were incubated for 4 hour at 27o C in CF203 cell culture. After incubation the nucleus and plasma membrane of the cells where stained using Hoechst 33342 and CellMask™ Plasma Membrane probes. Images were taking using 40-60x microscope lens.

Our initial results suggest that the polyplex is mostly taken up by the cell as a complex, however free dsRNA can also be seen inside the cells. Using Lysotracker and CellLight™ Late Endosomes-GFP we ambition to confirm the location of de polyplex inside the cell.

PRELIMINARY ASSESSMENT ON THE POTENTIAL REDUCTION OF TREATMENTS WITH INSECTICIDES FOR APHIDS BY USING RNAi TECHNOLOGY IN PLUM AGAINST PLUM POX VIRUS

I. ZAGRAI[1], L. ZAGRAI[1], M. RAVELONANDRO[2], R. SCORZA[3], Z. JAKAB[1], G. GUZU[1]

[1]Fruit Research & Development Station Bistrita, Romania

[2]Institut National de la Recherches Agronomique Bordeaux, France

[3] Ralph Scorza LLC , Shepherdstown, WV USA (formerly USDA-ARS)

Plum pox virus, genetic engineering, resistance, insecticide treatments

Pathogen derived resistance through RNAi technology has been obtained in 'HoneySweet' transgenic plum and it was demonstrated as an efficient strategy to obtain high level resistance to Plum pox virus (PPV), the most detrimental viral pathogen of stone fruits. Regulatory authorities in the U.S. have found no safety concerns and approved 'HoneySweet' for cultivation. Fruit growers are facing safety issues related to the use of pesticides and will need to comply with EU requirements to reduce the use of pesticides for crop protection. We have raised the question as to whether some treatments against aphid vectors might be avoided in plantings of 'HoneySweet'. To investigate this a field trial including 'HoneySweet' and two conventional plums cultivars ('Stanley' and 'Reine Claude d'Althan') is currently underway with the aim of evaluating the requirements for pesticide use. Theoretically, since 'HoneySweet' cannot be naturally infected with PPV by aphids there is a possibility on decreasing the number of treatments with insecticides without promoting PPV spread. The situation is complex because avoiding some treatments, raises questions of direct damages done by aphid feeding and, more importantly, of controlling other insect pests. *Eurytoma schreineri* Schr. and *Laspeyresia funebrana* Tr. have economic importance in plum production and are present in our experimental plot. The preliminary results revealed that a slight decrease in the number of insecticide treatments did not led to significant aphid damage due to their feeding on young shoots and leaves. On the other hand, the flight curve of *Laspeyresia funebrana* Tr. partly overlaps those of aphids and, consequently, treatments with insecticides are necessary to control this pest. The identification of short periods when the flight curve of aphids and other pests do not overlap might allow avoiding some treatments with insecticides. We will continue these investigations to provide additional data based on flight curves along the multiple vegetative periods.

ENGINEERING NUTRIENT CONTENT IN PLANTS THROUGH RNAi

J. PEJIN, V. TUMBAS ŠAPONJAC, A. FIŠTEŠ, J. VULIĆ, M. PRIBIĆ

Faculty of Technology, University of Novi Sad, 21000 Novi Sad, Serbia

Nutrients, RNAi technology, gene silencing

Over the years the RNAi proved to be a promising tool in agriculture and gene silencing has been used to improve many plant characteristics. RNAi has been used to: improve natural defense mechanism to increase disease and pathogen resistance, enhance plant stress adaptation, improve insect pest control, increase crop yield, and generate male sterility in hybrid seed production. In addition, the RNAi also provided a way to modify plant metabolic pathways to enhance nutrient content and reduce toxin production. The two most common approaches utilizing stable transformation technology for the analysis of gene function are either to over-express the gene of interest or to silence it using RNA interference. In biotechnology, the use of plants as producers of compounds of interest for humans, invites studies on the regulation of gene expression, for the better use of its qualities. For example, resistant starch, a source of short chain fatty acids (SCFA) which are linked to several health benefits, is preferentially derived from amylose which can be increased by suppressing amylopectin synthesis by silencing of starch branching enzymes (SBEs). Further, bioactive compounds have raised consumers and researchers attention, because their consumption plays an essential role in the prevention of several diseases. Among the most studied bioactive compounds are flavonoids, phenolic acids, carotenoids and tocols, which cooperate in reducing aging-related, chronic and intestinal diseases. In spite of their importance, the mechanism of their regulation in plants is still partly unknown. Given the possibilities of using the RNAi in functional genomics, some studies have used this technology for the overproduction of these bioactive compounds. This paper gives an overview of current results on the use of RNAi to increase nutritional value in crops and plants. Work is focused on two things: host - the type of plant that has been RNAi modified and application - the compound which content has been increased/decreased following the RNAi modification. In conclusion, RNAi-silencing approach has the potential to reengineer metabolic pathways to increase production of commercially important metabolites or reduce the risk by decreasing the production of harmful components.

ARE THERE ANY ISSUES IN EATING FRUITS BEARING EITHER RNAI OR VIRUS?

M. RAVELONANDRO[1,], I. ZAGRAI[2], L. ZAGRAI[2], R. SCORZA[3], A. CALLAHAN [3], C. DARDI CK[3] , P. BRIARD[1,]

[1]UMR-1332, Biologie du Fruit et Pathologie, INRA-Bordeaux, France

[2]Fruit Research & Development Station Bistrita, Romania

[3] Appalachian Fruit Research Station USDA-ARS, Kearneysville, WV, USA

Genetically modified fruit, RNAi, resistance, *plum pox virus*

Basic research has reached the potential to successfully achieve the targeted goal of protecting an economically fruit-tree like Prunus domestica from a devastating virus disease like sharka. Genetically engineered plum-trees are resistant to the severe quarantine plum pox virus (PPV). Since, as like for conventional cultivars, these transgenic fruit-trees produce fruits. Initial evaluations of fruit composition between transgenic and conventional fruits did not reveal any significant differences. Nevertheless, evaluations were expanded. These studies showed that fruits from transgenic resistant trees contained RNAi and they did not accumulate PPV as did the conventional PPV infected fruits. Efforts to better understand the role of fruits were performed through the comparison with shoot apical meristem, that is located in the vicinity of fruits. Experimental studies through the injection of PPV via syringe, showed that PPV RNA can replicate in both apical meristem and fruit tissue in conventional cultivars however PPV cannot be detected in fruits of transgenic resistant trees. Advances in our understanding of fruits, as a sink/source of PPV, were gained through these studies because PPV RNA can replicate in conventional fruits. These controlled studies were corroborated with the detection of PPV RNA from a commercial fruit picking. While biorisks associated with genetically modified (GM) food were raised from non-advertised consumers, we drew an interesting conclusion that the RNAi mechanisms in resistant GM fruit-trees are apparently robust. The production of RNAi in GM fruits appears to be essential for PPV resistance. It eliminates the presence of PPV and also suggests the quality-labelled fruit to be a safer virus-free stone-fruit.