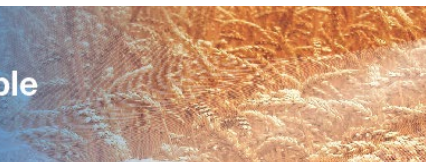




FELLOWSHIP SUMMARY REPORTS

- **Vojislava Grbic**
- **Functional genomics of *Tetranychus urticae* host adaptation**
 - Co-operative Research Programme: Biological Resource management for Sustainable Agricultural Systems: TAD/CRP JA00096812
- Host institution: **Centro Nacional de Análisis Genómico (CNAG-CRG)**
- Host collaborator: **Ivo Gut**
- Dates of the fellowship: 1 June 2018 to 12 October 2018
- I consent to posting this report on the Co-operative Research Programme's website





1. What were the objectives of the research project? Why is the research project important?

The overall objectives of the research project were:

I. To identify loci associated with TSSM adaptation to grapevine.

II. To examine biological role of selected loci.

III. To develop a bioinformatics and experimental pipeline for the identification of genetic loci of interest in TSSM.

IV. Dissemination and knowledge transfer of project results to scientific community, chemical industry and end users.

The importance of the research project:

Understanding molecular mechanisms that govern host adaptability of the important global agricultural pest is relevant for future policy decisions related to: development of new environmentally-friendly strategies for pest management, risk monitoring of pest infestations, precision agriculture (through the effective management of well diagnosed pest infestations), biodiversity and integration of agricultural production systems (through specificity of pest control strategies within the agro-eco system) and food safety. In addition, this research project contributes to development of genetic/genomic tools. TSSM is particularly suited for the proposed research as it is a global pest with staggering number of host plants, small genome and the genome sequence of an extremely high quality. These features secure the relevance of the proposed research to global agricultural production, relatively small cost of genomic analysis and feasibility of extracting relevant biological information from the bioinformatic data analysis. Our results will be directly relevant to the management of the two spotted spider mite, but this knowledge is also translatable to other agricultural herbivores, increasing the significance of this proposal to the sustainability of agricultural production.

2. Were the objectives of the fellowship achieved?

The objectives of the fellowship were *partially* achieved, but they are on the way to being achieved through the ongoing research. There are two main reasons for delay: a) the awarded fellowship was shorter than originally anticipated and thus, some objectives could not have been achieved; and b) the genetic analysis of host preference requires continuity in the research activities, however, there was an unanticipated engagement in dissemination and knowledge transfer during the summer of 2018, as I was invited speaker at two international conferences (The III Latin American Congress of Acarology (III CLAC) and the VI Brazilian Symposium of Acarology (VI SIBAC), Pirenopolis, Brazil (30 July 2018 to 3 August 2018); and XV International Congress of Acarology, Antalya, Turkey (2-8 September 2018). However, we are continuing with the research project, so that proposed objectives will be achieved.

3. What were the major achievements of the fellowship? (up to three)

- a) Toward the identification of loci associated with TSSM adaptation to grapevine we **sequenced the genome of the Murcia inbred line** that was derived from the mite population collected in vineyard around Murcia, Spain, referred to as Murcia strain. Sequencing was performed at the host institution (CNAG) using their Illumina platform. We obtained ~325 million read-pairs that corresponded to ~98.2 Gigabases of data. The obtained reads were mapped to the reference London genome sequence released in 2009 (turticae.200909). Importantly, 99.9% of reads were mappable to the reference genome with at least 30x of coverage (c30). Thus, the obtained sequence is of high quality and will allow detection of unique genomic differences between London mite strain (susceptible to grapevine defences) and Murcia mite strain (resistant to grapevine defences). Importantly, the analysis of synteny between genomes of these two different mite strains will identify potential inversions/deletions/translocations that would affect recombination of these genomes in genetic crosses.





- b) The second objective of our proposal was to examine biological role of selected loci using an *RNAi*. Even though we developed several protocols for inducing RNAi in spider mites, the RNAi had reduced penetrance (~50% of treated mites). We have further optimized the method that now results in ~100% efficiency. We optimized the length of dsRNA and the delivery of dsRNA itself, so that now we are ready to test genes for their biological function using RNAi as a reverse genetics tool.
- c) Further, with our host institution, we forwarded the *bioinformatics and experimental pipeline* for the identification of spider mite loci associated with the adaptation status. We are ready to use these protocols on mite isogenic lines that contain or not loci required for mite adaptation to grapevine. In addition, I actively *disseminated* our findings of mite adaptation to different hosts as an invited speaker to several conferences and meetings: The III Latin American Congress of Acarology (III CLAC) and the VI Brazilian Symposium of Acarology (VI SIBAC), Pirenopolis, Brazil (30 July 2018 to 3 August 2018); XV International Congress of Acarology, Antalya, Turkey (2-8 September 2018).

4. Will there be any follow-up work?

The proposed program is ongoing and will ultimately result in publication. Upon genetic and introgression analysis of Murcia x London cross we will resequence the introgressed line at CNAG (as a part of our extended collaboration) and will ultimately test candidate genes with RNAi. These results would ultimately inform both scientific and industrial community on grapevine defense compounds and mite adaptation to it. These findings may have practical application in development of plant-based biopesticides to control mite populations in agricultural production systems.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Spider mites (TSSM) are controlled by the application of acaricides whose global market is ~\$1.6B. The biopesticides (that can stem from this project) is expected to be specific for TSSM, with zero residue and with low impact on the environment. It is anticipated it will penetrate rapidly and remain stable over time on the ~\$1.6B acaricide market. In addition to the direct economic benefit of the project deliverables to agricultural sector, the project also directly links to social benefits that include: a) reduced impact of pesticides on human and environmental health; b) reduced environmental footprint of agricultural production; and c) increased sustainability of agriculture and food security.

6. How was this research relevant to the CRP research theme:

This proposal is submitted to Theme II. MANAGING RISKS IN A CONNECTED WORLD that focuses on “investing in research into anticipating, pre-empting, coping with and managing risks that treat agricultural production”. The project contributes to:

1) *Risk assessment*: This project represents the first step in our understanding of molecular mechanisms governing TSSM host-adaptability and its high-risk pest status. This knowledge will lead to development of diagnostic tools that will be able to anticipate the virulence potential of local TSSM populations, allowing the assessment of the risk of TSSM infestation. Such tools will “help the management of agricultural systems through the ability to anticipate, avoid and react to TSSM challenge and so minimize impacts at local or more global scale”.

2) *Climate risks to production*: A recent modeling study sponsored by NASA indicated that “many regions around the world will experience declines in crop and livestock production from increased stress due to weeds, diseases, insect pests, as well as climate-change induced stresses” (Melillo et al., 2014). This panel recommended urgent measures to reduce risk of crop yield decline. High temperatures favour mite population expansion as spider mites’ rate of development increases (with





up to 37 generations in a year, as observed on grapevines in Brazil). Further, recent results show that the number of eggs laid by TSSM females increases 2-fold on drought-stressed plants (Ximenez-Embun et al., 2017). Thus, under hot and dry conditions, spider mite populations are expected to flourish resulting in more frequent TSSM outbreaks, and broader host range and the geographic area of its economic damage. This project focuses on genetic analysis of TSSM host-adaptability and development of new crop protection strategies against mite pests and directly contributes to mitigation of climate-change imposed risks on global agricultural production.

3) *Emerging Diseases/Pests, new threats to major commodity crops, and Biosecurity*: The importance of spider mites as pests is increasing significantly in Canada and worldwide. For example, numerous media headlines have recently reported mite outbreaks on corn, soybean and cotton in North America (thecropsite.com/news/11500/spider-mites-attack-soybeans-already-hit-by-drought; dairybusiness.com/se/article.php?title=west-conversations-spider-mites-in-corn-silag&date=2013-05-15;

pioneer.com/home/site/us/agronomy/library/template.CONTENT/guid.822E5C6F-885F-161B-5FC3-C8DAE12ABC22; southeastfarmpress.com/cotton/spider-mites-increasing-tennessee-cotton.; today.agrilife.org/2012/12/26/2012-produced-extreme-spider-mite-infestations-in-corn/).

This project will uncover a mechanism that underlies the global TSSM spread, contributing to our understanding of the emergence of TSSM as a high-risk pest for global crop production under climate change.

4) *Food safety*: Pest control is essential for food production and crop security. Recent estimates indicate that without crop protection by pesticides, pests and diseases would destroy over 40% of global food production. Pesticides, with costs predicted to be >\$65 billion, represent the main tool for pest control, but they have serious limitations that include development of pesticide resistance in pests, environmental pollution, negative effects on beneficial insects and health repercussions in humans. The impact of pesticides on human health is widely underestimated, but consumer awareness for this problem is rapidly increasing. A review performed by the US Environmental Protection Agency found that "most studies on non-Hodgkin lymphoma and leukemia showed positive associations with pesticide exposure" (*Pesticides: Health and Safety, National Assessment of the Worker Protection Workshop*. 2007. US Environmental Protection Agency). In addition, pesticide use increases the risk of fetal defects and neurological and neurodevelopmental disorders (Jurewicz et al., 2008). The Stockholm Convention on Persistent Organic Pollutants, effective from May 2004, identified nine pesticides amongst the 12 most dangerous and persistent chemicals in the environment. Awareness of the effects of pesticides on human health has led many countries, including Canada and the EU, to approve amendments restricting their use. Such situation makes development of highly specific and environmentally friendly pesticides the highest priority, however, the identification of the species-specific pest targets with an independent mode of action is a major bottleneck. This project aims to identify TSSM gene(s) that could be potential targets for development of the next generation acaricides that will support sustainability of agricultural production and human and environmental health.

7. Satisfaction

The OECD fellowship conformed to my expectations and enabled the establishment of collaboration with a world-class research institution such as CNAG and Ivo Gut's research group.

8. Advertising the Co-operative Research Programme

I am on the OECD's mailing list. I think that this is good venue to advertise this program.

