OP-139 [Bee Health]
Use of RNA interference to silence target genes of “Aethina tumida””, an invasive pest species of apiaries

Salvatore Arpaia[1], Linda Bianco[1], Valeria Bonina[2], Giovanna Cangiulli[3], Paolo Facella[1], Carlo Fasano[1]
Giovanni Federico[2], Paolo Iovieno[4]
[1]ENEA - Italian national agency for new technologies, energy and sustainable economic development, TERIN-BBC, Research Centre Trisaia, Rotondella (MT), Italy\
[2]Istituto Zooprofilattico Sperimentale per il Mezzogiorno, sezione di Reggio Calabria e Vibo Valentia, Catona (RC), Italy;
[3]Università degli Studi di Bari ‘Aldo Moro', Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Bari, Italy
[4]Institute of Biosciences and Bioresources, National Research Council of Italy, Sesto Fiorentino (FI), Italy

Background/purpose
RNA interference (RNAi) is a naturally occurring mechanism enabling eukaryotic cells to regulate gene expression; the mechanism can be reproduced by introducing in vitro synthesized double strand RNA (dsRNA) molecules into an organism to selectively block a specific messenger RNA with consequent transient stop of protein production. The goal of our study was to investigate the biological effects of dsRNA on the small hive beetle “Aethina tumida””.

Problem description
“A. tumida”” is a species native to South Africa, endemic to sub-Saharan Africa, where its populations attack African subspecies of “Apis mellifera”” without severe consequences. In newly introduced countries where the hosts are the European subspecies of honeybees, the beetle is responsible for serious damage to beekeeping by feeding on bees' eggs and larvae, wax and causing honey fermentation. Since it was detected in Italy in 2014, “A. tumida”” is considered an invasive alien species of concern in the European Union under Regulation 2016/429.

Methodology
We synthesized dsRNAs against two fundamental genes (RPS13 and V-ATPase) of “A. tumida”” and administered them to first instars via artificial diet or topical application. The measurement endpoints in the bioassays were mortality, larval growth and development, adult eclosion and fecundity. To relate the observed phenotypic effects to the treatment, Real-Time PCR were performed.

Results and conclusion
Both dsRNAs have induced negative effects on beetle development, survival rates and fecundity of emerging adults. While mortality rates were below 40%, with most fatalities occurring during adult eclosion from pupae, dsRNA caused a marked decline in all measured life parameters, sufficient to slow down the population's growth rate in natural conditions. Molecular analyses confirmed the successful silencing of both genes after 24 hours following dsRNA administration.

Practical implications for beekeepers
Pest control in beekeeping is a delicate management activity, as it needs to efficiently control pests and diseases without impairing bee health or product quality. RNAi may represent a major advance in this area, since its very specific mode of action may guarantee effective pest control in beehives, without harming honeybees, which proved to be refractory to treatments with dsRNA.

ITALIAN NATIONAL AGENCY FOR NEW TECHNOLOGIES, ENERGY AND SUSTAINABLE ECONOMIC DEVELOPMENT

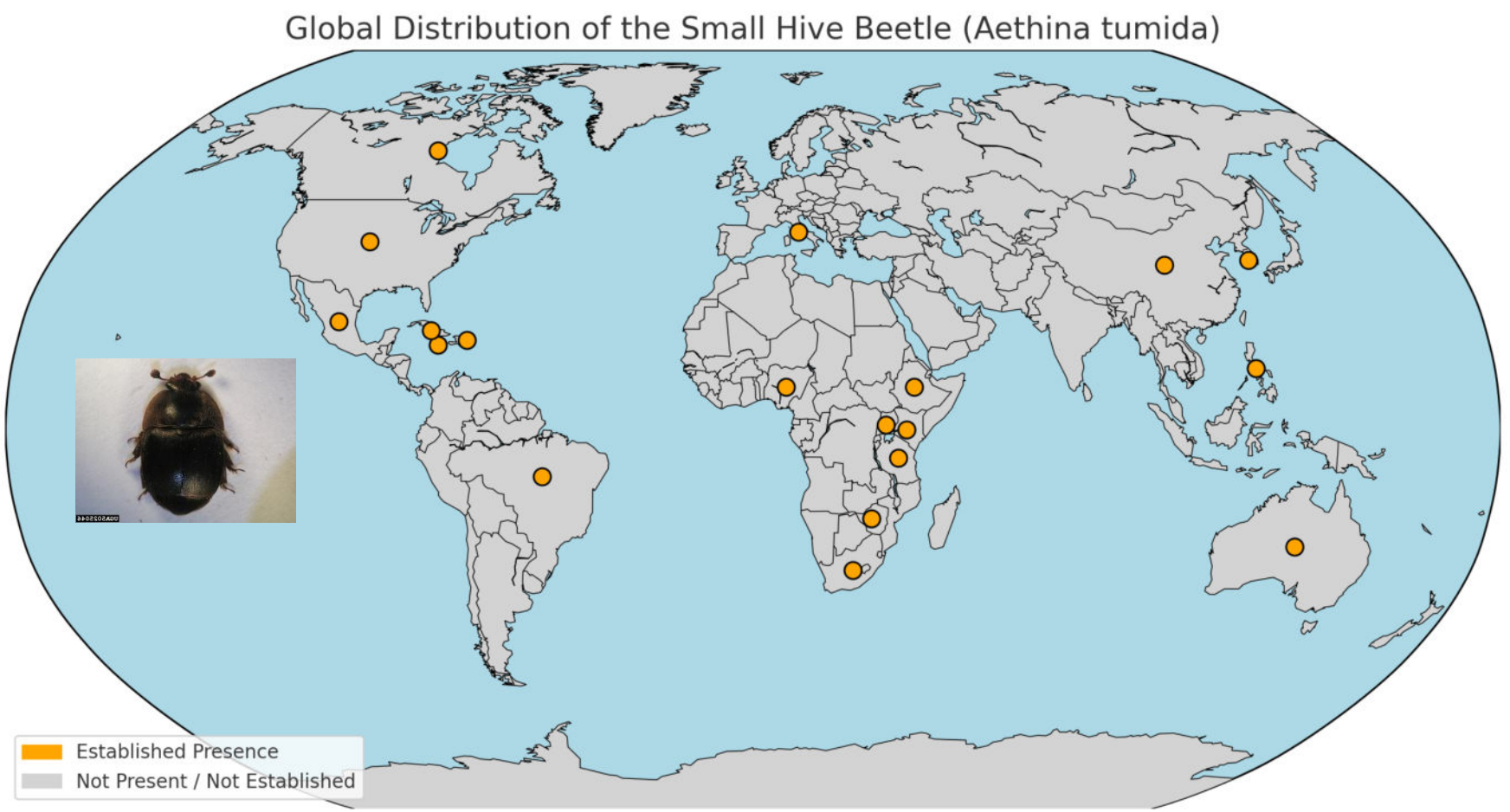
Istituto Zooprofilattico Sperimentale del Mezzogiorno

Use of RNA interference to silence target genes of *Aethina tumida*, an invasive pest species of apiaries

Salvatore Arpaia, Linda Bianco, Valeria Bonina, Giovanna Cangiulli, Paolo Facella, Carlo Fasano, Giovanni Federico, Paolo Iovieno

APIMONDIA SCANDINAVIA 2025
SWEDEN | DENMARK | NORWAY
23-27 SEPTEMBER
COPENHAGEN

The small hive beetle in the EU



Buffer zones, prohibited movement of colonies, and all beekeeping products

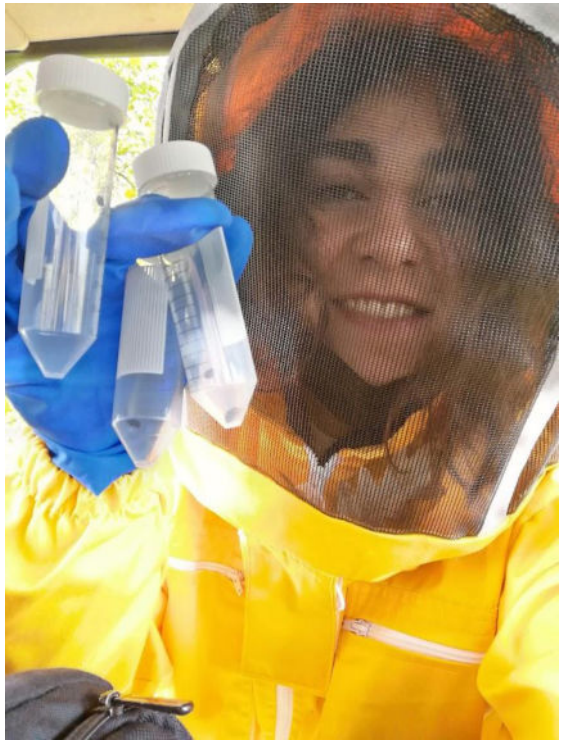
However, eradication failed. Needs for control measures

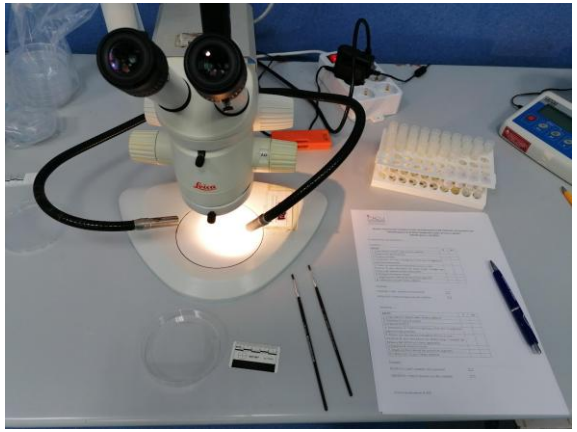
Damage

Fermentation of honey

Destruction of combs

Aethina tumida mass rearing facilities in Reggio Calabria



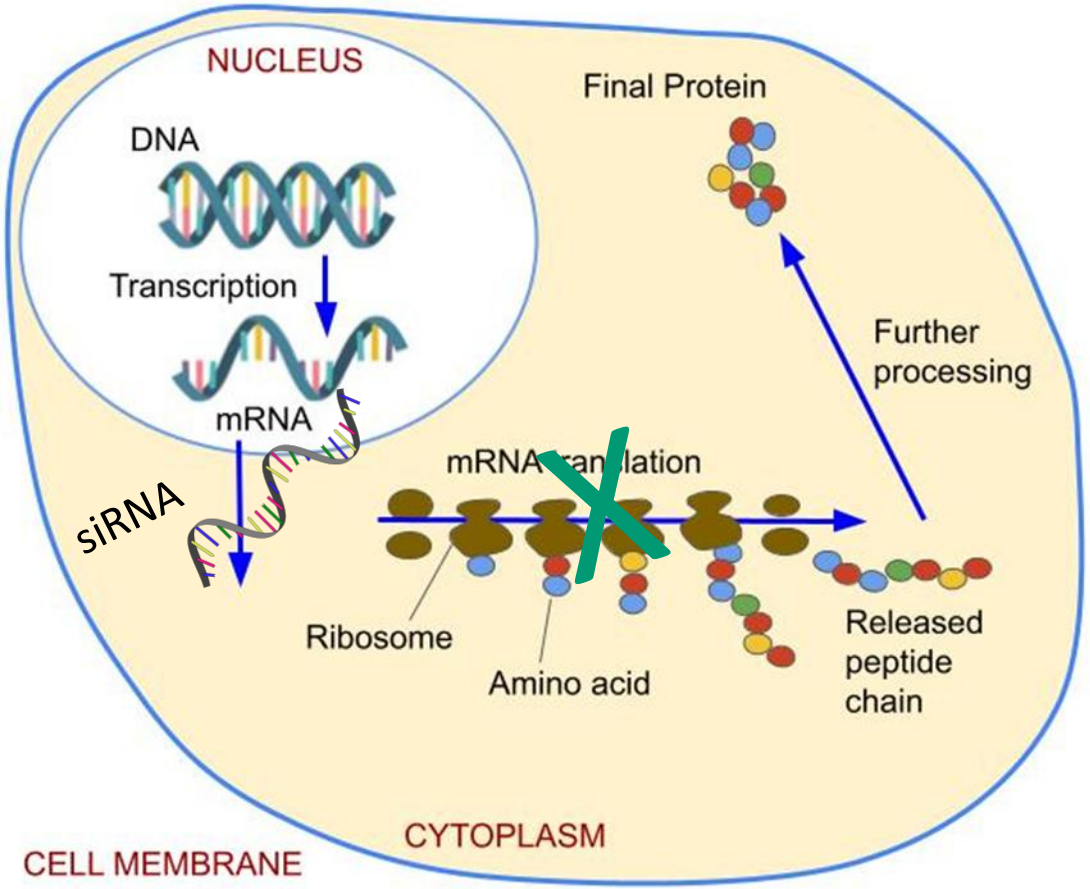


Discovery of RNA interference

RNA interference is a natural mechanism used as a defence by most eukariotic organisms against invading nucleic acids, e.g. viruses, or for internal gene expression regulation, leading to specific post-transcriptional silencing of a mRNA with appropriate base complementarity

Craig Mello & Andrew Fire 1998

Caenorhabditis elegans



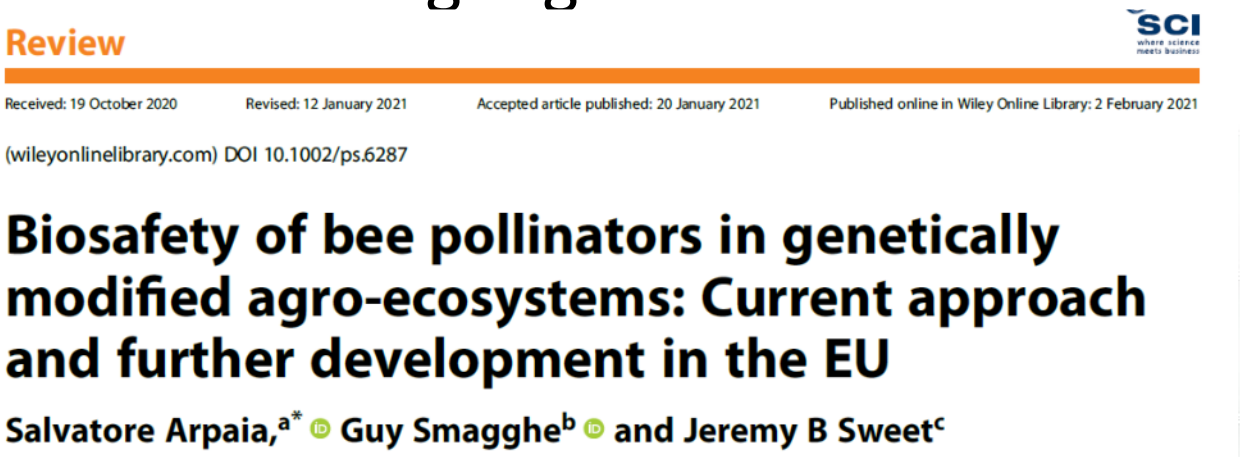
Victor Ambros e Gary Ruvkun 2024

General aspects of RNAi approaches

- Based on a mechanism naturally present in plants and animals
- dsRNAs are ubiquitous in nature
- No new proteins expressed
- Potentially highly selective
- The change in the genome is only functional (the gene is still there)
- Can be used as any common pesticide

Harmless to bees?

- Honeybees have the RNA machinery;
- no negative effects recorded in absence of sequence similarity with the target genes



Review

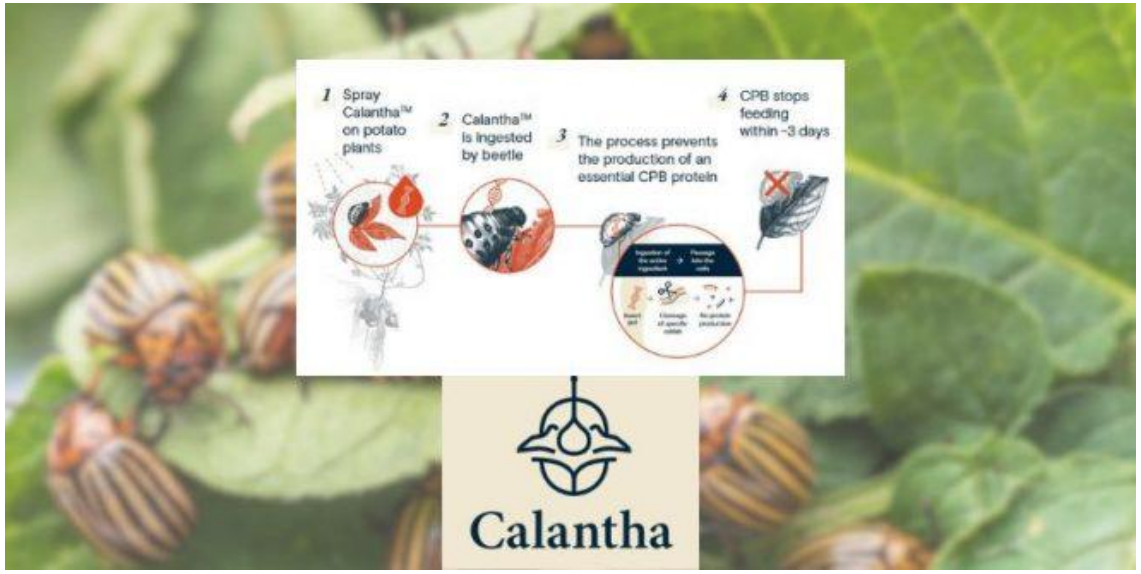
Received: 19 October 2020 Revised: 12 January 2021 Accepted article published: 20 January 2021 Published online in Wiley Online Library: 2 February 2021

(wileyonlinelibrary.com) DOI 10.1002/ps.6287

Biosafety of bee pollinators in genetically modified agro-ecosystems: Current approach and further development in the EU

Salvatore Arpaia,[a*] Guy Smagghe[b] and Jeremy B Sweet[c]

ds-RNA based pesticides



Authorized for commercialization in the USA, December 2023

Vadescana proposed for registration by EPA (May, 2025)

Goal of the study

- Evaluating the efficiency of *RNA interference* applications against *A. tumida*
- Evaluating *lethal and sublethal* effects of *different dsRNA molecules* and *different delivery systems*

Research Article

SCI

Systemic RNAi in the small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae), a serious pest of the European honey bee *Apis mellifera*

Michelle E Powell, Hannah M Bradish, John A Gatehouse and Elaine C Fitches

Proof of concept

Assessing suitability for pest control

Experimental setup

METHODS

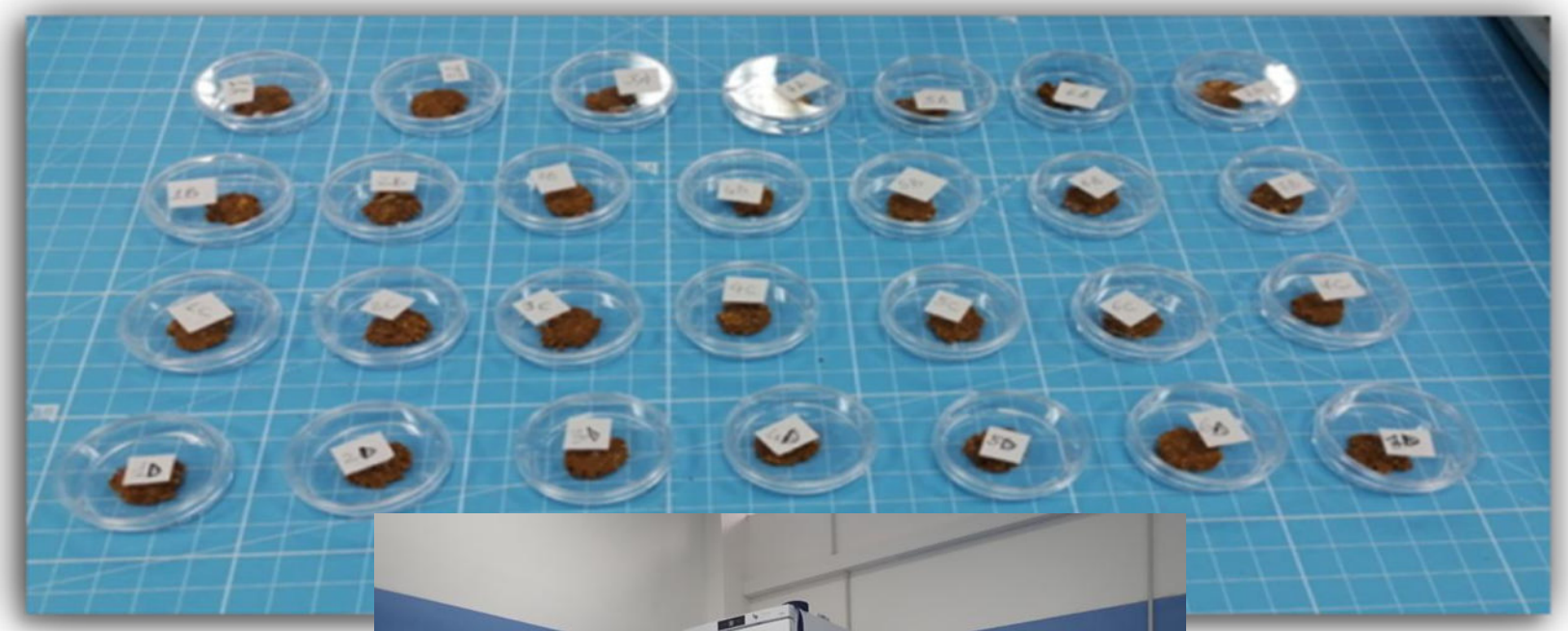
- Ingestion
- contact

dsRNA targets

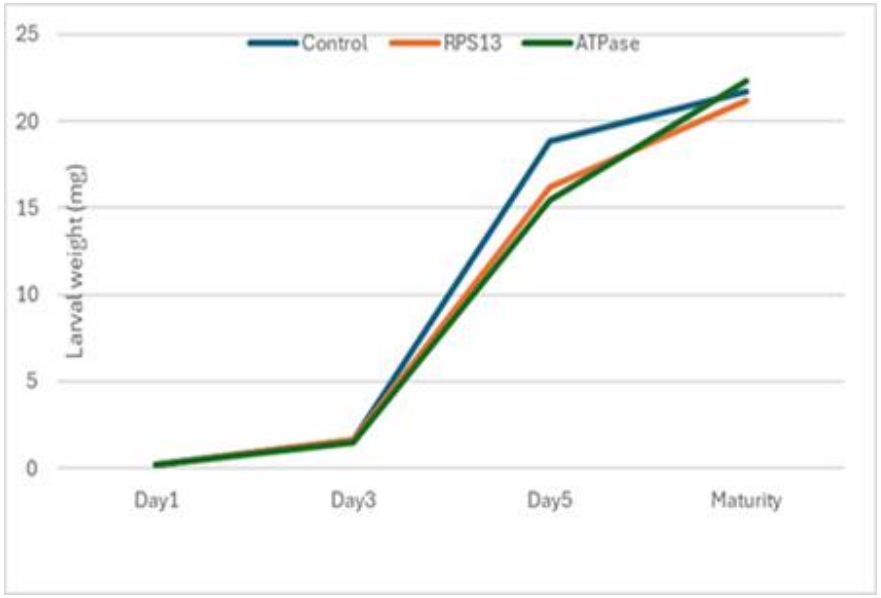
V-ATPase and *RPS13*

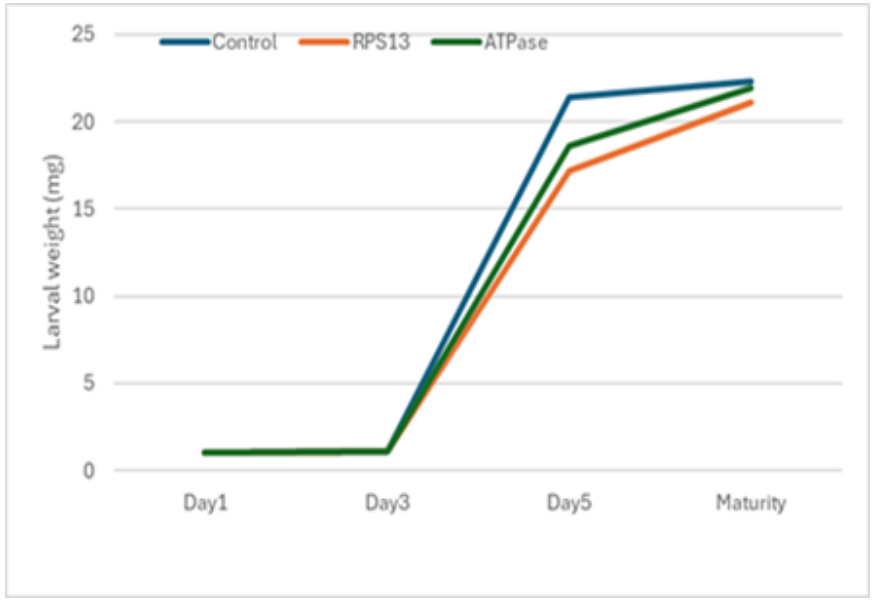
ENDPOINTS

Growth, development, mortality, fecundity



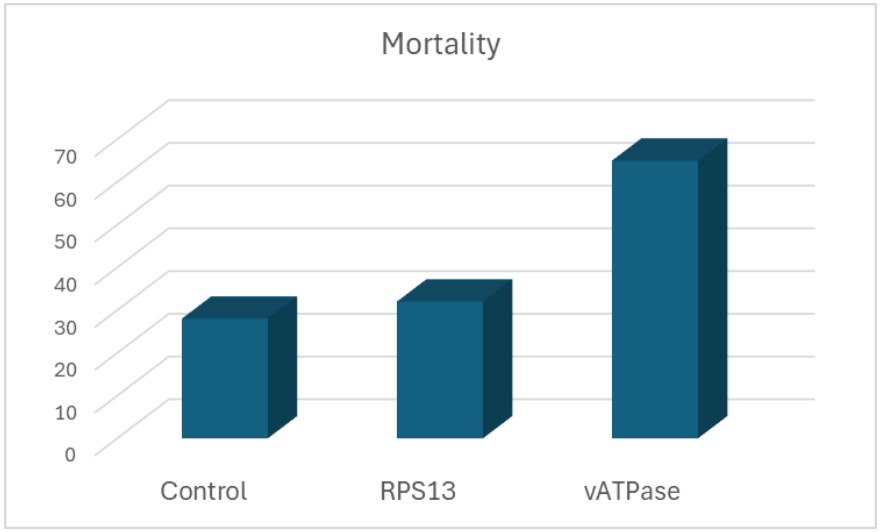
Ingestion

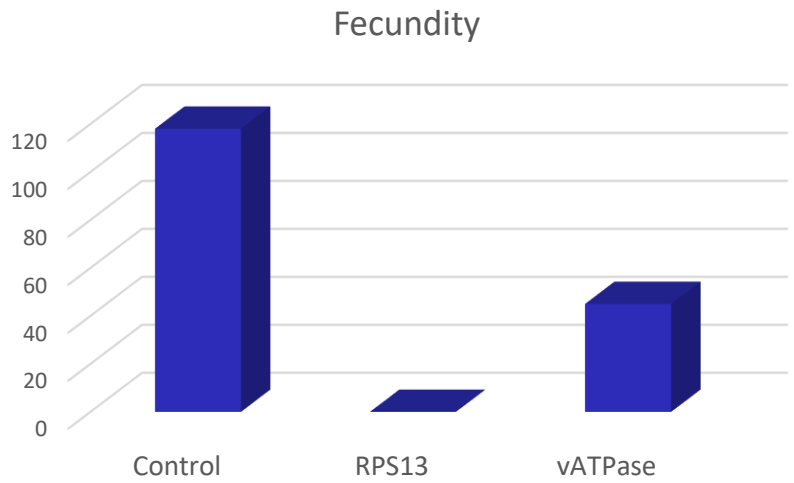


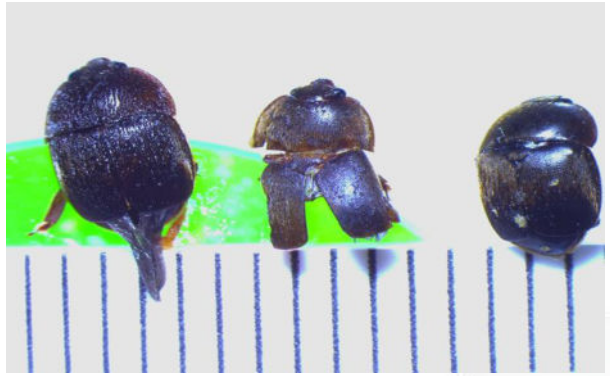


Both dsRNAs impair early larval growth upon feeding

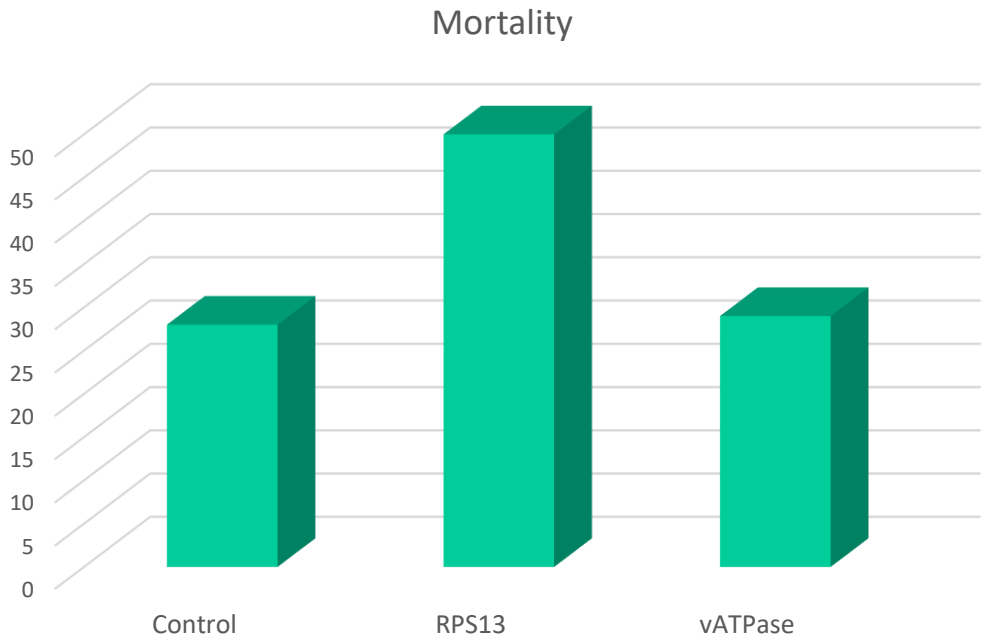
Doubling time: RPS 4,7 ATPase 3,9 compared to control populations

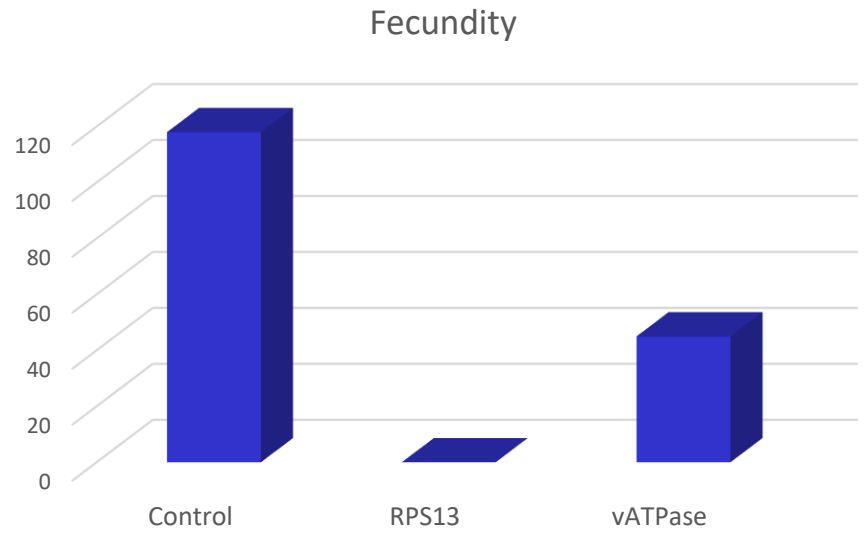




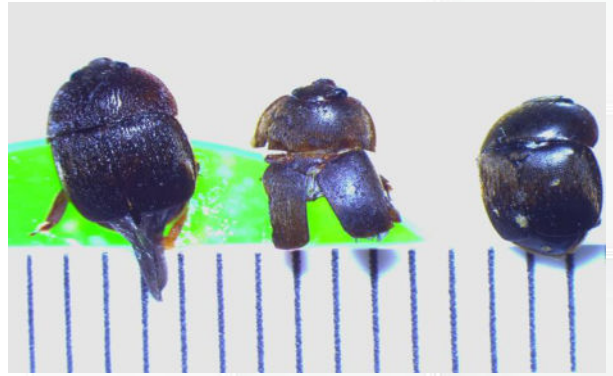


Topical application

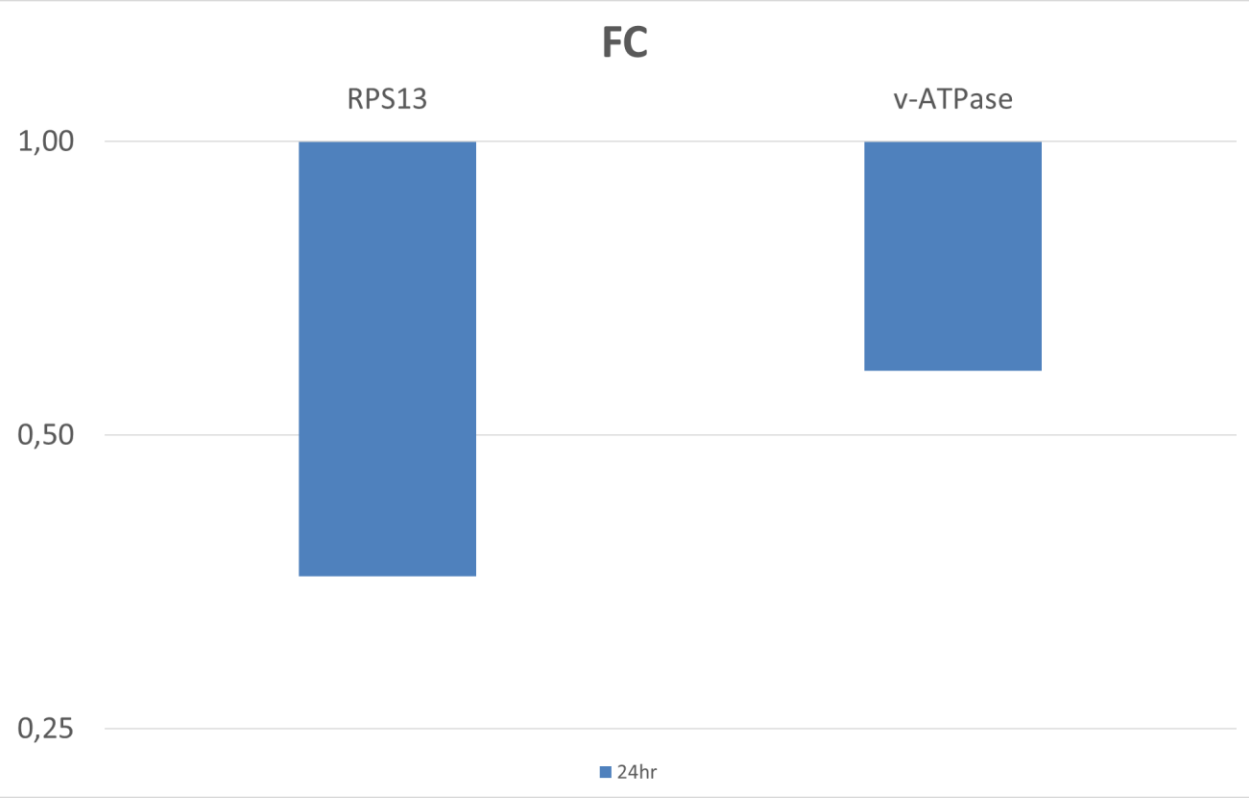




Doubling time: RPS 4,5 ATPase 2,6 compared to control populations



Gene silencing in *A. tumida*

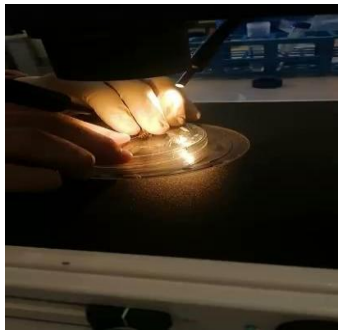


Gene silencing results consistent with vATPase, activity starts in 24 hours and lasts for up to 3rd day

Results were not reproducible with RPS13

Conclusions

- dsRNA is effective in silencing target genes in *Aethina tumida*
- Acute mortality is not very high
- Sublethal effects concur in severely affecting beetle population
- Molecular analyses confirm that phenotypical effects are due to silencing
- Administration by contact showed more consistently silencing (expected main delivery metod for possible insecticidal use)
- Target genes may explain differences in phenotypical results



The «Aethina team»

Paolo Facella

Linda Bianco

Carlo Fasano

Giovanna Cangiulli

Istituto Zooprofilattico Sperimentale del Mezzogiorno
Campania | Calabria

Giovanni Federico

Valeria Bonina

Paolo Iovieno – CNR IBBR

Much obliged for your time and attention, Salvatore