SUPERPESTS NEWSLETTER

Volume 1 / June 2020

TOP 3 SUPERPESTS DISCOVERIES SO FAR

Identified and validated new resistance markers in different pest species.

Tested the anti-resistance potential of novel candidate insecticidal compounds

Identified and validated environmentally-friendly biopesticides

INNOVATIVE TOOLS FOR RATIONAL CONTROL OF THE MOST DIFFICULT-TO-MANAGE PESTS AND THE DISEASES THEY TRANSMIT

A new project funded within the H2020 topic "Innovations in plant protection" was recently launched (www.superpests.eu). It aims to develop and evaluate a suite of innovative products, tools and concepts, and integrate these with existing approaches using data driven mathematical models, to achieve effective and sustainable **Integrated Pest Management (IPM)** of the "super pests" (aphids, whiteflies, thrips and mites).

"SuperPests is an EU funded project that aims to develop and evaluate suite of innovative products and concepts towards effective and sustainable Integrated Pest Management"

To achieve this SuperPests will develop multiplex and automated sample-to-answer diagnostics, evaluate biopesticides (green chemistry – plant extracts & metabolites, synergists, RNAi and bio-stimulants), study host plant resistance to pests and compatibility with biological control, select natural enemies better suited to certain crops and IPM, and develop predictive mathematical models, iteratively validated against experimental data, to determine optional combinations for IPM.

THE CONSORTIUM

The project is coordinated by the **Agricultural University of Athens** (PI John Vontas, vontas@imbb.forth.gr). It has a number of participants (University of Ghent, University of Exeter, University of Amsterdam, INRA, DIMITER, CSIC, Univesidad Politècnica de Cartagena, ENDURA, Albert-Ludwigs-Universitaet Freiburg, ENDURA, BIPA NV, BioBest, University of Western Ontario) and will be developed in a 4 years period (2018-2022).

Superpests partners at the **Kick-off Meeting** that took place in the Agricultural University of Athens, Greece, from the 16th to 17th October 2018. The main aim of the two-day meeting was to plan how this project will move forward and develop the structure in which all partners will work.

FIRST ANNUAL MEETING

On 27 January 2020 the **SuperPests Annual Consortium Meeting** took place in **Montpellier, France,** where partners had the chance to review tasks and activities in preparation of the EU Report, clarify technical and/or managerial issues and define the action plan for Year 2 of the project.

PROGRESS SO FAR

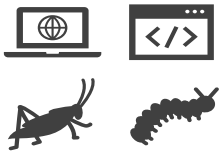
The following work has been carried out by the consortium towards the completion of SuperPests' during the project's first 18 months:

Evidence-based IPM

We have identified and validated **seven new resistance markers** in **four different pests**. Knowledge of the associated resistance genotype will **help decide which pesticides should, or should not, be applied** in order to successfully restrain these pests, and avoid a further spreading of resistance.

Environmentally-friendly biopesticides

We have performed tests of **non-conventional chemistry formulations**, which revealed very good potential for the **control of resistant pests**. Appropriate targets for RNAi-based spider mite control have been identified.



Optional combinations for effective and sustainable IPM

We developed a **web-based app** for exploring the dynamics of **tri-trophic predator-prey model**. The model has already produced some suggestions, e.g. addition of carnivore has significant and positive effects

Tools for screening anti-resistance potential of novel compounds

A **virtual insectary** of most highly resistant SuperPests has been created. ***In vitro* assays** based on **SuperPest cytochrome P450s** has been developed, as well as recombinant fruit-flies bearing defined resistant traits have been developed and used.

Biological control agents (BCA)

Tomato defense mutants and are ready to be used. Testing on the first **predator-prey community** combination has been completed. Work is ongoing to **evaluate the genetic variability and biodiversity of BCAs**.

Knowledge integration

Review documents on biopesticide pest control options and regulatory pathways have been drafted. A **workshop** on **translation research and regulatory pathways** for biopesticides has been planned. **E-learning units** are outlined. A series of quizzes by using a web platform for quiz hosting has been designed. Implementation of **practice abstracts** is ongoing.

NEWS - Top Picks

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SYNERGY FOR SURVIVAL

Synergies between target-site mutations and enhanced detoxification in pyrethroid resistance has been long hypothesized as a major evolutionary mechanism.

Combinations of target site and P450 overexpression lead to dramatic changes in insecticide resistance.

These are major considerations from the insecticide resistance management viewpoint in both public health and agriculture.

'What I cannot create, I do not understand': functionally validated synergism of metabolic and target site insecticide resistance

PROCEEDINGS B
royalsocietypublishing.org/journal/rspb
Research

George-Rafael Samantsidis[1,2,†], Rafaela Panteleri[1,2,†], Shane Denecke[1], Stella Kounadi[1,2,‡], Iason Christou[1,2], Ralf Nauen[3], Vassilis Douris[1,4] and John Vontas[1,5]

[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, 100 N. Plastira Street, 70013 Heraklion, Crete, Greece
[2]Department of Biology, University of Crete, Vassilika Vouton, 71409 Heraklion, Crete, Greece
[3]Bayer AG, CropScience Division, R&D Pest Control, 40789 Monheim, Germany
[4]Department of Biological Applications and Technology, University of Ioannina, 45110 Ioannina, Greece
[5]Laboratory of Pesticide Science, Department of Crop Science, Agricultural University of Athens, 118 55 Athens, Greece

G-RS, 0000-0002-8279-2114; SD, 0000-0002-7291-1394; VD, 0000-0003-4608-7482; JV, 0000-0002-8704-2574

The putative synergistic action of target-site mutations and enhanced detoxification in pyrethroid resistance in insects has been hypothesized as a major evolutionary mechanism responsible for dramatic consequences in malaria incidence and crop production. Combining genetic transformation and CRISPR/Cas9 genome modification, we generated transgenic *Drosophila* lines expressing pyrethroid metabolizing P450 enzymes in a genetic background along with engineered mutations in the voltage-gated sodium channel (*para*) known to confer target-site resistance. Genotypes expressing the yellow fever mosquito *Aedes aegypti Cyp9J28* while also bearing the $para^{V1016G}$ mutation displayed substantially greater resistance ratio (RR) against deltamethrin than the product of each individual mechanism ($RR_{combined}$: 19.85 > $RR_{Cyp9J28}$: 1.77 × RR_{V1016G}: 3.00). Genotypes expressing *Brassicogethes aeneus* pollen beetle *Cyp6BQ23* and also bearing the $para^{L1014F}$ (*kdr*) mutation, displayed an almost multiplicative RR ($RR_{combined}$: 75.19 ≥ $RR_{Cyp6BQ23}$: 5.74 × RR_{L1014F}: 12.74). Reduced pyrethroid affinity at the target site, delaying saturation while simultaneously extending the duration of P450-driven detoxification, is proposed as a possible underlying mechanism. Combinations of target site and P450 resistance loci might be unfavourable in field populations in the absence of insecticide selection, as they exert some fitness disadvantage in development time and fecundity. These are major considerations from the insecticide resistance management viewpoint in both public health and agriculture.

NEWS - Top Picks

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HIGHLIGHTS

Resistance monitoring is not common practice in agriculture.

Molecular markers can be a crucial tool in resistance management of agricultural pests.

Strength and predictive value of a diagnostic marker depend on many factors.

New technologies (MinION, ddPCR) will allow determining mutation frequency at low levels.

Molecular markers 🧬🧪can be a crucial tool in resistance management of agricultural pests. 🍅🐛But when it comes to operational decision making which is their true value ❓🤔Find out more in SuperPests' recent review article:

Current Opinion in Insect Science

Volume 39, June 2020, Pages 69-76

Significance and interpretation of molecular diagnostics for insecticide resistance management of agricultural pests

Thomas Van Leeuwen [1] ✉, Wannes Dermauw [1], Konstantinos Mavridis [2], John Vontas [2, 3]

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NEWS - Top Picks

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HIGHLIGHTS

Virgin T. urticae females were injected in the ovary with a mix of Cas9 and sgRNAs.

SgRNAs were designed to target phytoene desaturase, a pigmentation gene.

Albino males were detected in the progeny of Cas9-sgRNA injected T. urticae females.

Lines derived from the albino males show typical CRISRP-Cas9 events.

CRISPRed mites? 🕷 🧬 🎯 👍
Now it is possible!!! Check out SuperPests' latest research to find out more.

ELSEVIER

Insect Biochemistry and Molecular Biology
Volume 120, May 2020, 103347

Targeted mutagenesis using CRISPR-Cas9 in the chelicerate herbivore *Tetranychus urticae*

Wannes Dermauw [a], Wim Jonckheere [a], Maria Riga [b], Ioannis Livadaras [b], John Vontas [b, c], Thomas Van Leeuwen [a]

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Visibility of SUPERPESTS

List of scientific publications in peer reviewed journals

Samantsidis et al (2020). 'What I cannot create, I do not understand': Functionally validated synergism of metabolic and target site insecticide resistance. *Proc Biol Sci.* 287 (1927):20200838.

Van Leeuwen T et al (2020). Significance and interpretation of molecular diagnostics for insecticide resistance management of agricultural pests. *Curr Opin Insect Sci.*39:69-76.

Alavijeh ES et al (2020). Molecular and genetic analysis of resistance to METI-I acaricides in Iranian populations of the citrus red mite Panonychus citri. *Pest Biochem Physiol.* 164:73-84.

Dermauw W et al (2020). Targeted mutagenesis using CRISPR-Cas9 in the chelicerate herbivore Tetranychus urticae. *Insect Biochem Mol Biol.* 120:103347

Wei P et al (2020). Overexpression of an alternative allele of carboxyl/choline esterase 4 (CCE04) of Tetranychus urticae is associated with high levels of resistance to the keto-enol acaricide pirodiclofen. *Pest Manag Sci.* 76(3):1142-1153.

Fotoukkiaii SM et al (2020). Identification and characterization of new mutations in mitochondrial cytochrome b that confer resistance to bifenazate and acequinocyl in the spider mite Tetranychus urticae. *Pest Manag Sci.* 76(3):1154-1163.

Katsavou E, et al (2020). Identification and geographical distribution of pyrethroid resistance mutations in the poultry red mite Dermanyssus gallinae. *Pest Manag Sci.* 76(1):125-133.

Snoeck S et al (2019) Substrate specificity and promiscuity of horizontally transferred UDP-glucosyltransferases in the generalist herbivore Tetranychus urticae. Insect Biochem Mol Biol. 109:116-127.

İnak E et al (2019). Resistance incidence and presence of resistance mutations in 2 populations of Tetranychus urticae from vegetable crops in Turkey. *Exp Appl Acarol.* 78(3):343-360.

Wybouw N et al (2019). Convergent evolution of cytochrome P450s underlies independent origins of keto-carotenoid pigmentation in animals. *Proc Biol Sci.* 286(1907):20191039.

Snoeck S et al (2019). High-resolution QTL mapping in Tetranychus urticae reveals acaricide-specific responses and common target-site resistance after selection by different METI-I acaricides. *Insect Biochem Mol Biol.* 110:19-33.

Rameshgar F et al (2019). Characterization of abamectin resistance in Iranian populations of European red mite, Panonychus ulmi Koch (Acari: Tetranychidae). *Crop Prot.* 125: 104903.

Kurlovs et al (2019). Trait mapping in diverse arthropods by bulked segregant analysis. *Curr Opin Insect Sci.* 36:57-65.

List of publications in conferences, meetings, public events, patents and other activities

Tixier MS. (INRAE) - Biodiversity assessment within the species Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae), Entomological Society of America (ESA) Annual Meeting, 17-20 November, St Louis, Missouri, USA (poster presentation).

Tsagkarakou A. (DIMITRA) - Monitoring for ketoenol resistance in Mediterranean populations of the whitefly Bemisia tabaci (Hemiptera: Aleyrodidae), 'Resistance 2019', 16-18 September 2019, Rothamsted Research, Harpenden, UK (poster presentation).

Riga M, (AUA) - Genetics, molecular and functional characterization of insecticide/acaricide resistance in Tetranychus urticae, 14th International Congress of Crop Protection Chemistry (IUPAC 2019), 19-24 May 2019, Ghent, Belgium (poster presentation).

Kant M. (UvA) - Spitting whiteflies: effector proteins of Bemisia tabaci, Annual Meeting Experimental Plant Sciences (EPS), 8-9 April 2019, Lunteren, Wageningen (oral presentation).

Pekas A. (Biobest) Innovations and challenges for arthropod biological control, Agrotica, 1-4 February 2018, Thessaloniki (Invited talk).

CONTACT US

Postal Address

Pesticide Science lab,

Agricultural University of Athens,

Iera Odos 75, 11855,

Athens, Greece

Project Coordinator: John Vontas

https://www.aua.gr/vontas

E-mail: vontas@aua.gr

Phone: +30 2105294545

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https://superpests.eu

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