ERA CoBioTech

BIOTECH RESEARCH AND INNOVATION HACK 2021

Final seminar of the cofunded projects of ERA CoBioTech

Sustainable Bioproduction of Pheromones for Insect Pest Control in Agriculture

SUSPHIRE

SUSPHIRE

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

29.09.2021

SUSPHIRE CONSORTUM

PROJECT DURATION
36 Months 30/09/2021

TOTAL REQUESTED FUNDING
1.643.000 €

TOTAL COSTS
1.861.000 €

CONSORTIUM

P 1	Dr Diego Orzaez Agencia Estatal Consejo Superior de Investigaciones Científicas IBMCP	Fausto Elio SN, 46022, 46022 VALENCIA Spain	dorzaez@ibmcp.upv.es Tel.: 0034 963879933 Mobile: 0034 963879933 Fax: 0034 +34963879933
P 2	Dr Nicola Patron Earlham Institute Engineering Biology	Norwich Science Park, NR4 7UZ Norwich United Kingdom	Nicola.Patron@earlham.ac.uk Tel.: 0044 450845 http://www.earlham.ac.uk
P 3	Prof Heribert Warzecha Technische Universität Darmstadt Biologie	Schnittspahnstrasse 4, 64287 Darmstadt Germany	warzecha@bio.tu-darmstadt.de Tel.: 0049 61511620900 Fax: 0049 61511620902 https://www.tu-darmstadt.de/
P 4	Dr Spela Baebler National Institute of Biology Department of Biotechnology and Systems Biology	Vecna pot 111, 1000 Ljubljana Slovenia	Spela.Baebler@nib.si Tel.: 00386 59252825 http://www.nib.si/eng/
P 5	Mrs Teresa Gadea ECOLOGIA Y PROTECCION AGRICOLA SL NA	GREGAL 11, APDO. CORREOS 50, 46240 CARLET Spain	tgadea@epa-ecologia.com Tel.: 0034 962558098 Mobile: 0034 655853042 http://www.epa-ecologia.com

Introduction

Sustainable bioproduction of insect pheromones for pest control in agriculture

Chemically-synthetized pheromones are very efficient and highly selective pest control agents in agriculture

Limited use due to difficult and/or unsustainable chemical synthesis approaches

More economic strategies for both the synthesis of pheromones and formulation of active ingredients into dispensers can be developed using biotechnology

This could lead to increased viability of insect pheromones for pest control in agriculture

More environmentally friendly biosynthetic production methods for pheromone-based aligns with Europe's Bioeconomy objectives.

Sustainable bioproduction of pheromones

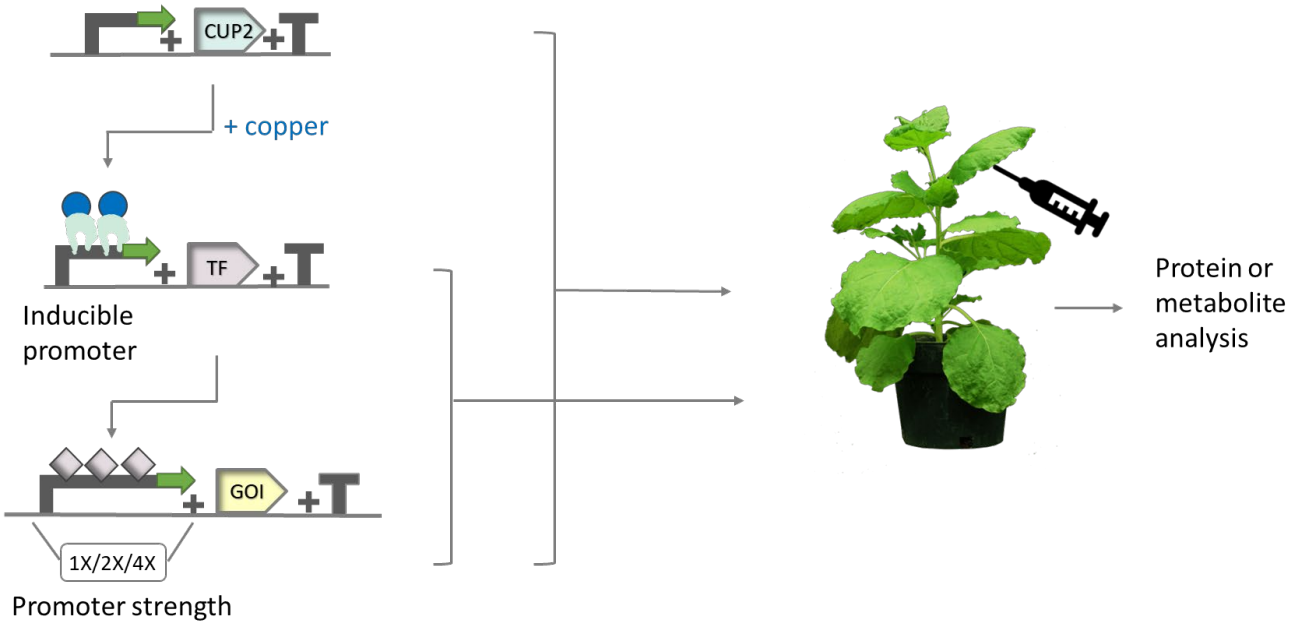
Previous studies demonstrated that it is possible to engineer *N. benthamiana* plants to produce moth sex pheromones via heterologous expression of the insect enzymes.

- Develop **synbio tools** for **conditional expression** of metabolic pathways in plants
- Create improved versions of lepidoptera (**moth) pheromone biofactories (fatty-acid derivatives).**
- Identify and validate key biosynthetic enzymes for the **bioproduction of Coccoidea (mealybugs) pheromones (irregular monoterpenoids).**
- Create plants producing mealybug pheromones

Technical overview

Molecular tools. Develop optimal **genetic constructs** for **control of** biosynthesis of moth and Coccoidea pheromones in **plants and fungi**.

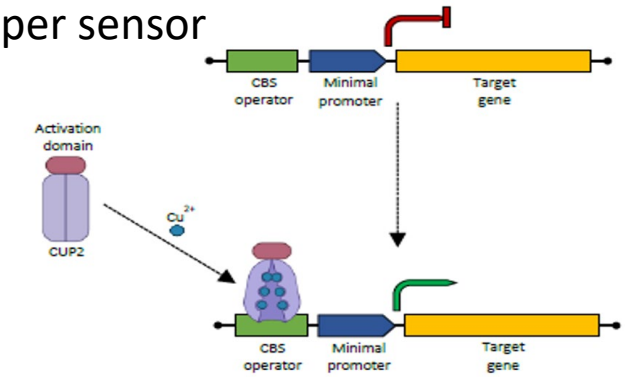
Development and implementation of a **copper switch** linked to a **synthetic regulatory cascade** made of **a TF and a collection of synthetic promoters**



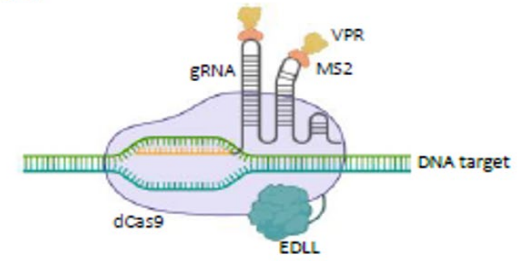
Technical overview

<u>Molecular tools</u>. Development and implementation of a **copper switch** linked to a **synthetic regulatory cascade** made of a **collection of synthetic promoters**

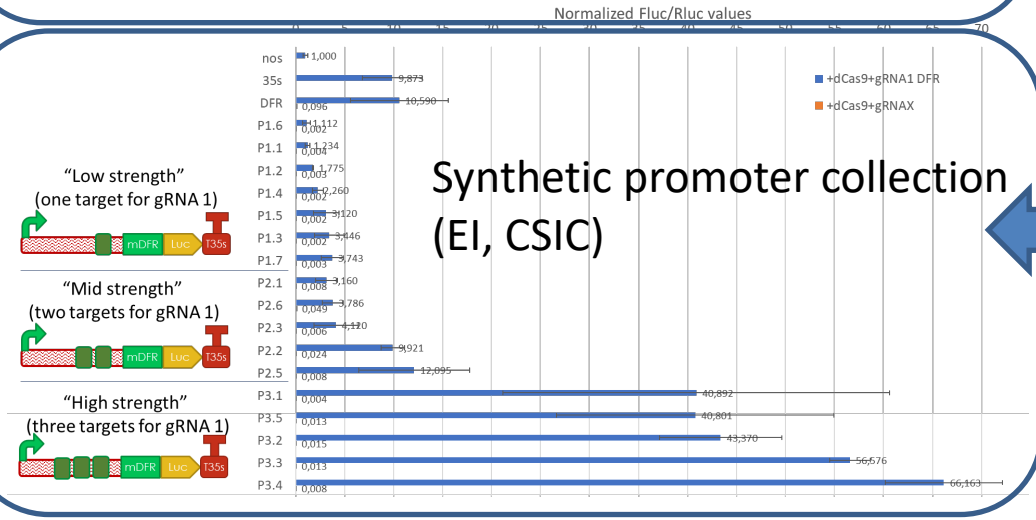
Copper sensor

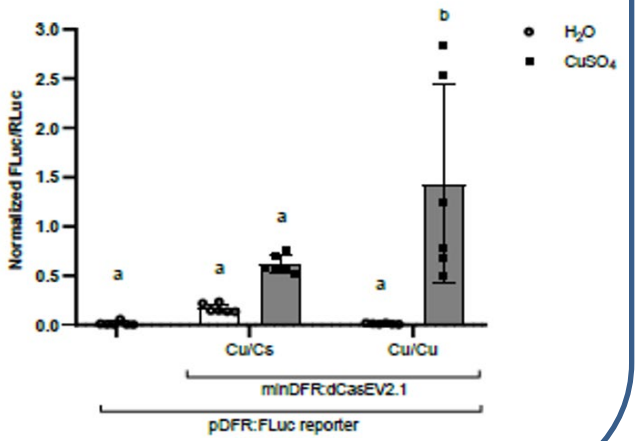


TFs: dCas9 (CSIC), PhiC31(EI)



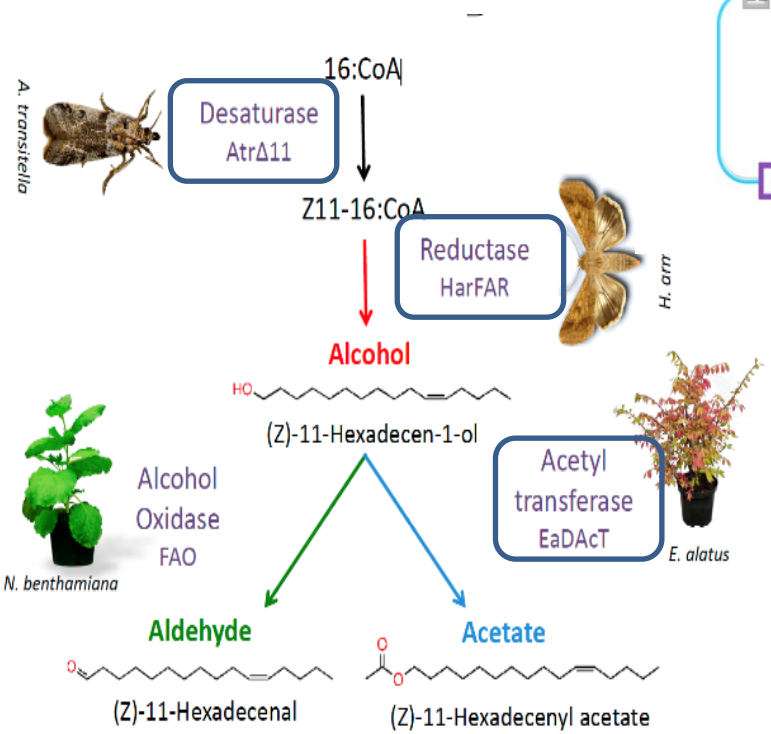
Synthetic promoter collection (EI, CSIC)

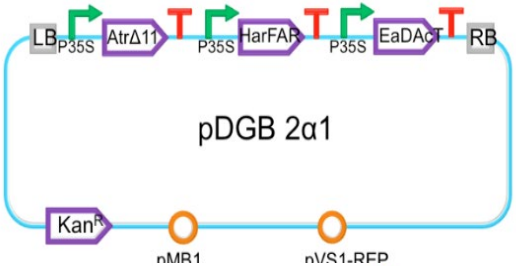




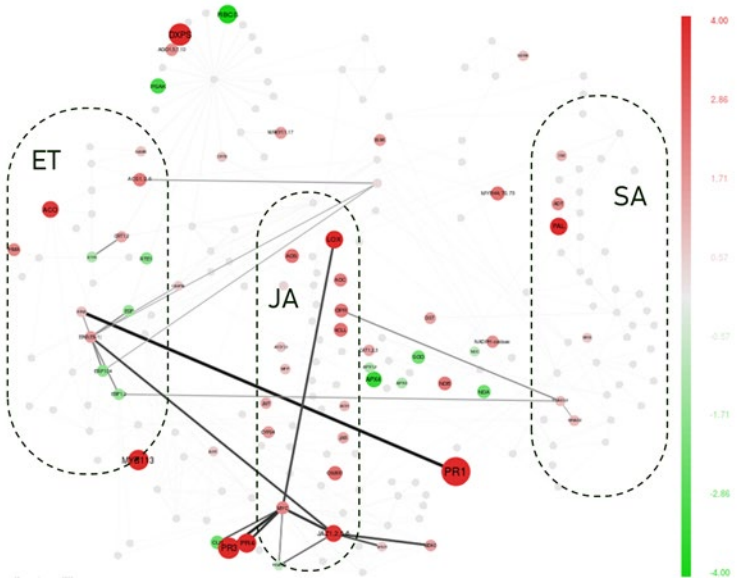
Technical overview

Lepidoptera Pheromone. New prototypes for **constitutive** bioproducers. System biology approaches to understand effects on fitness





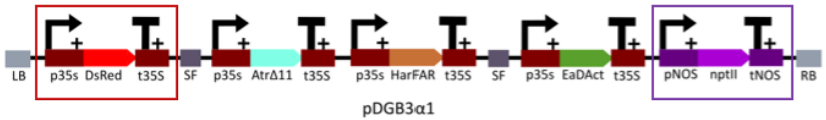
SxP1.0
Transient OK
Stable: Low Acetate
Strong growth penalty



NIB: Systems biology analysis, activated stress responses, truncated enzyme

Lepidoptera Pheromone. New prototypes for constitutive bioproducers. System biology approaches to understand effects on fitness

(a)

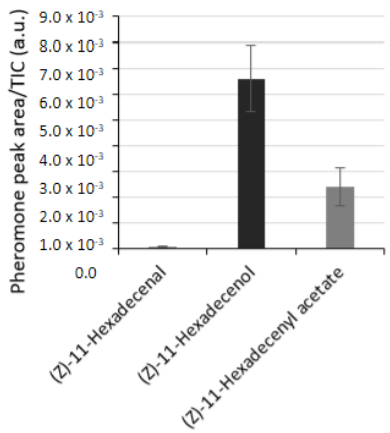


SxP1.1

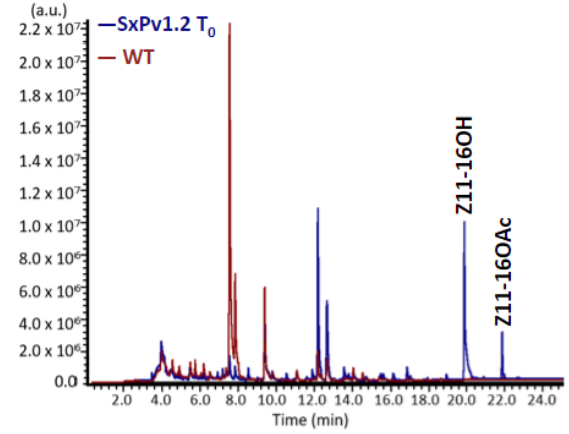
(b)

SxP1.2

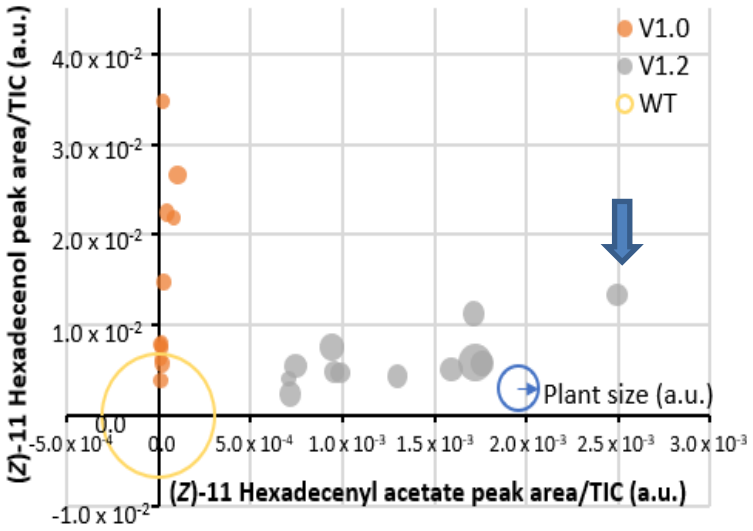
(c)



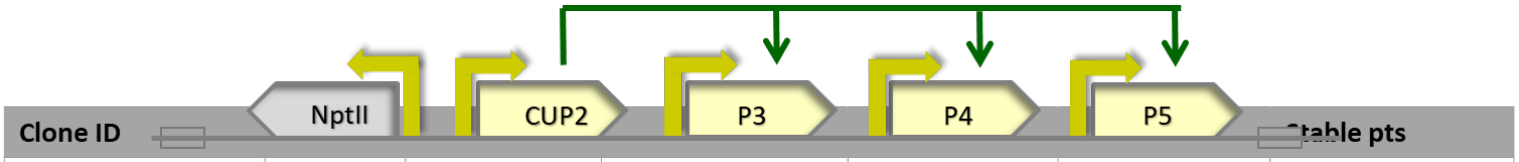
(d)



(b)

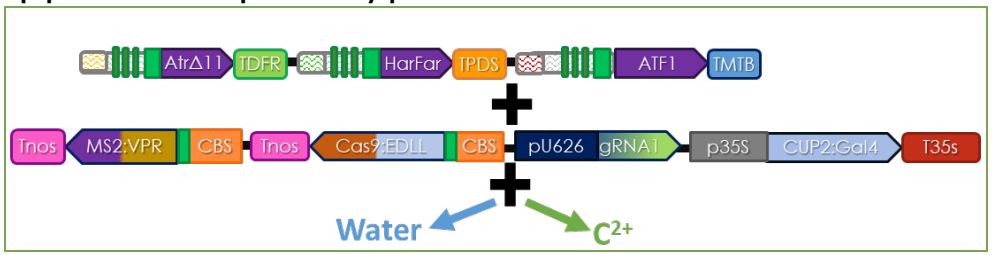


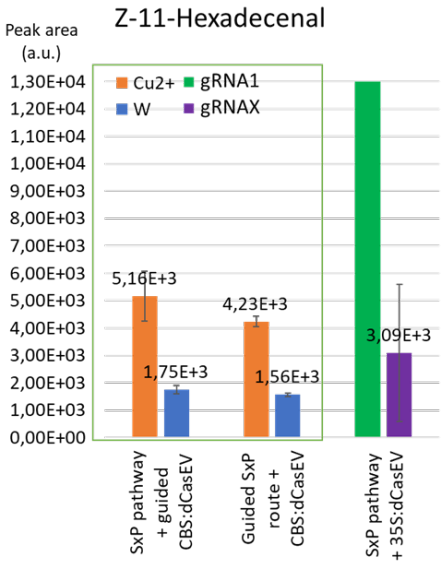
<u>Lepidoptera (moth) pheromone</u>. Creation of conditional versions (SxPv2) using tools in WP1

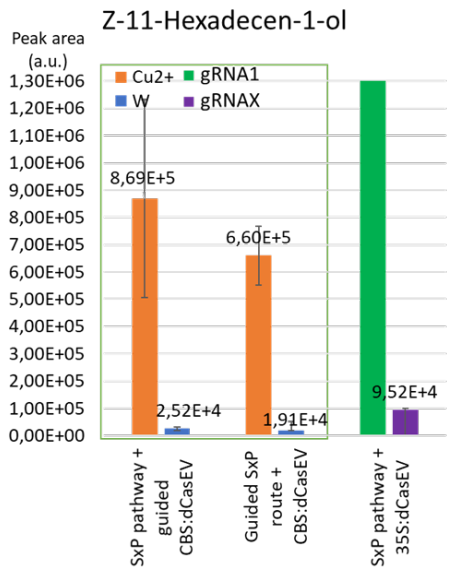


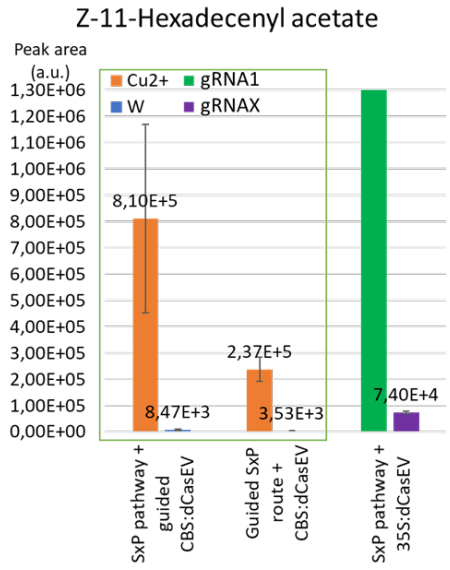
Clone ID						Stable pts
pEPKKΩ1SP0492	NptII	35Sp-CUP2	cbs-AtrΔ11	cbs-int_ScATF1	cbs-HarFar	3
pEPKKΩ1SP0493	NptII	35Sp-CUP2	cbs-HarFar	cbs-AtrΔ11	cbs-int_ScATF1	3
pEPKKΩ1SP0494	NptII	35Sp-CUP2	cbs-int_ScATF1	cbs-HarFar	cbs-AtrΔ11	0
pEPKKΩ1SP0495	NptII	35Sp-CUP2	cbs-AtrΔ11	cbs-int_EfDAct	cbs-HarFar	2
pEPKKΩ1KN0496	NptII	35Sp-CUP2	cbs-HarFar	cbs-AtrΔ11	cbs-int_EfDAct	3+2 in lab
pEPKKΩ1KN0497	NptII	35Sp-CUP2	cbs-int_EfDAct	cbs-HarFar	cbs-AtrΔ11	2
pEPKKΩ1KN0519	NptII	35Sp-CUP2	cbs-AtrΔ11	cbs-ScATF1	cbs-HarFar	0 (TC)
pEPKKΩ1KN0520	NptII	35Sp-CUP2	cbs-HarFar	cbs-AtrΔ11	cbs-ScATF1	0 (TC)
pEPKKΩ1KN0522	NptII	35Sp-CUP2	cbs-AtrΔ11	cbs-EfDAct	cbs-HarFar	0 (TC)
pEPKKΩ1KN0524	NptII	35Sp-CUP2	cbs-EfDAct	cbs-HarFar	cbs-AtrΔ11	0 poorly calli
pEPKKΩ1KN0678	**NptII**	**35Sp-CUP2**	**cbs-AtrΔ11**	**cbs-SpATF1-2**	**cbs-HarFar**	**Not started**
pEPKKΩ1KN0679	**NptII**	**35Sp-CUP2**	**cbs-HarFar**	**cbs-AtrΔ11**	**cbs-SpATF1-2**	**Not started**
pEPKKΩ1KN0680	**NptII**	**35Sp-CUP2**	**cbs-SpATF1-2**	**cbs-HarFar**	**cbs-AtrΔ11**	**Not started**

<u>Lepidoptera Pheromone</u>. Creation of conditional versions **SxPv2** using new molecular tools (EI and CSIC approaches/prototypes)



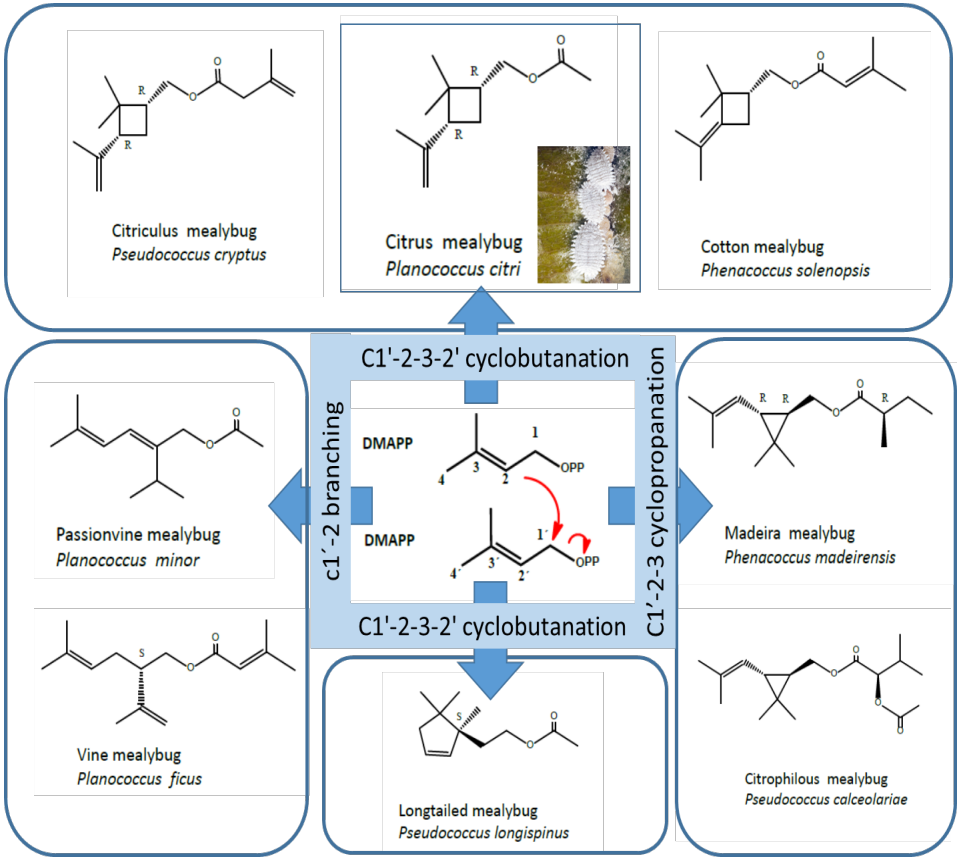






Technical overview

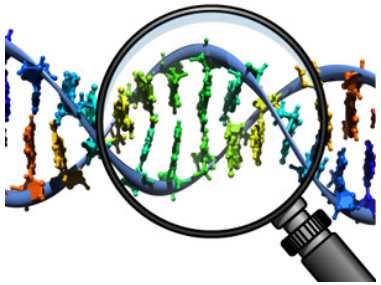
<u>Coccoidea pheromone</u>. Generate transcriptomic data and identify key enzymes. Create bioproduction prototypes.



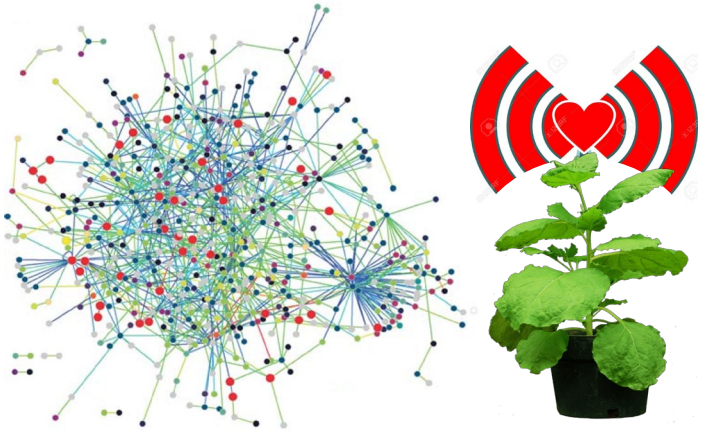
Technical overview

<u>Coccoidea pheromone</u>. Generate transcriptomic data and identify key enzymes. Create bioproduction prototypes.

NIB, **TUDA**, CSIC



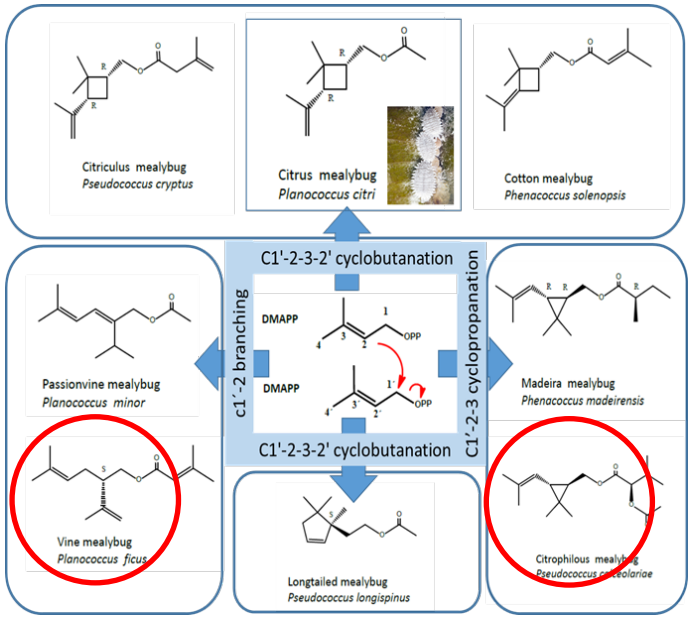
- PACBIO transciptome
- Differential expression (mated vs virgin females)
- Phylogenetic analysis
- Bioinformatics



- Exhaustive analysis of >50 candidates for activity in E. coli and N. benthamiana
- No planoccocyl (cyclobutane) biosynthetic activity was isolated from *Planococcus citri* genome.
- A **new enzyme with irregular Isopenthenyl transferase** activity *in vitro* was characterized (TUDA), but regular activity was predominant.
- Several new regular IPT and terpene cyclases have been identified from insects
- Alternative strategies: irregular monoterpenes from plant origin LPPS and CPPS

Technical overview

Coccoidea pheromones: Transgenic plants producing alternative irregular monoterpenes (pheromone precursors) chrysantemol and lavandulol ant their acetylated forms



Tobacco

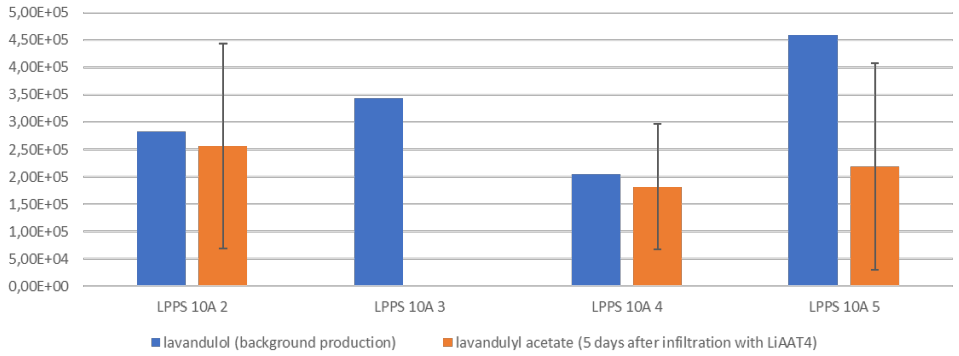
T0 plants	GC-MS
LPPS_1	+
LPPS_2	+
LPPS_3	+
LPPS_4	+
CPPS_1	+
CPPS_2	+
CPPS_3	+
CPPS_4	+
CPPS_5	+

N. benthamiana

T0 plants	GC-MS
LPPS_1	-
LPPS_2.1	-
LPPS_4	-
LPPS_5	+
LPPS_6	-
LPPS_7A	-
LPPS_7C	+
LPPS_10A	+
LPPS_10D	+
LPPS_11A	-
LPPS_11B	-

T0 plants	GC-MS
CPPS_1	-
CPPS_2	-
CPPS_3	-
CPPS_4A	+
CPPS_4B	+
CPPS_5	-
CPPS_6	+

Conversion of lavandulol to lavandulyl acetate by *Li*AAT4



RRI aspects

Ethics, dissemination and outreach. Responsible Research and Innovation. Communication and dissemination plan. Data stewardship according to the FAIR principles.

ETHICS

Provenance, pedigree, debates in RRI within the consortium (Prof.. Normand TUDA)

DISSEMINATION and OUTREACH

- **Producers**

  2018 Achema
  2018 Agritech East, UK
  2019 Meeting at Merck, Darmstadt

- ***Government and Regulators***

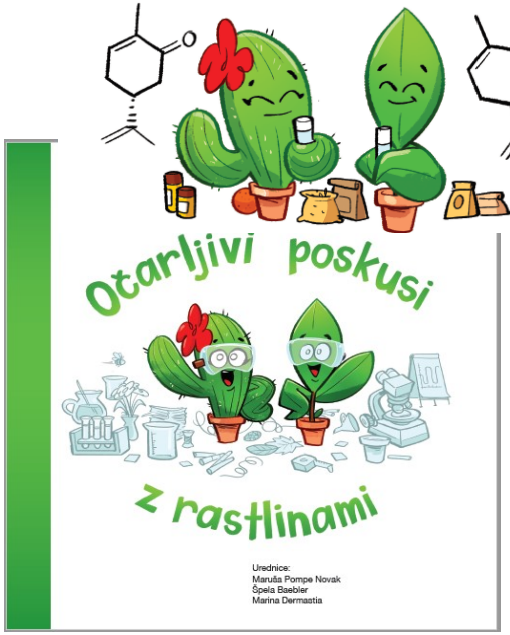
  2019 UK House of Commons (EI).

  2020 EU parliament policy debate (with BASF) Workshop not possible due to pandemic (EI)

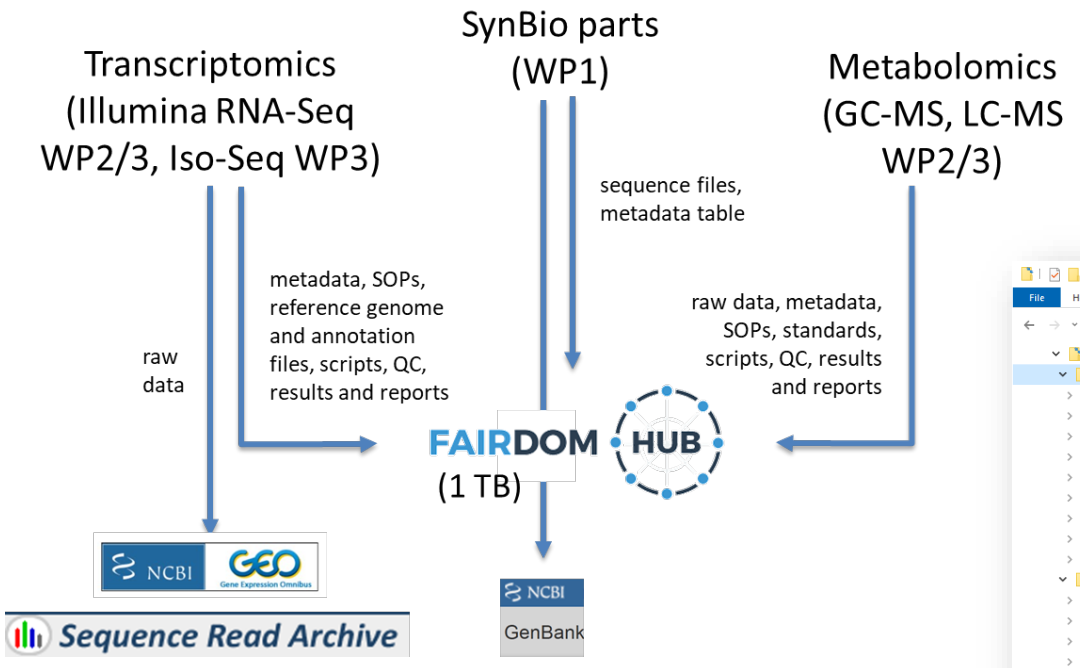
Consumers, including the general public

- *Media-releases highlighting project funding, results, publications provided to EU biotechnology networks and relevant industry magazines e.g. International Pest Control, EuroFruit, [All partners] Years 1, 2 & 3 (see http://susphire.info/susphireproject/media/ for full list) (BBC world, BBC radio, The Guardian, etc.)*
- *2019 EI Open Day, UK;*
- *2019 Fascination of Plants, Slovenia.*
- *2019/2020 collaboration with SAW trust*
- *2021 Children's book 'Fascinating Experiments with Plants'*

Note: less activities in year 2 and 3 due to the pandemic



Data management

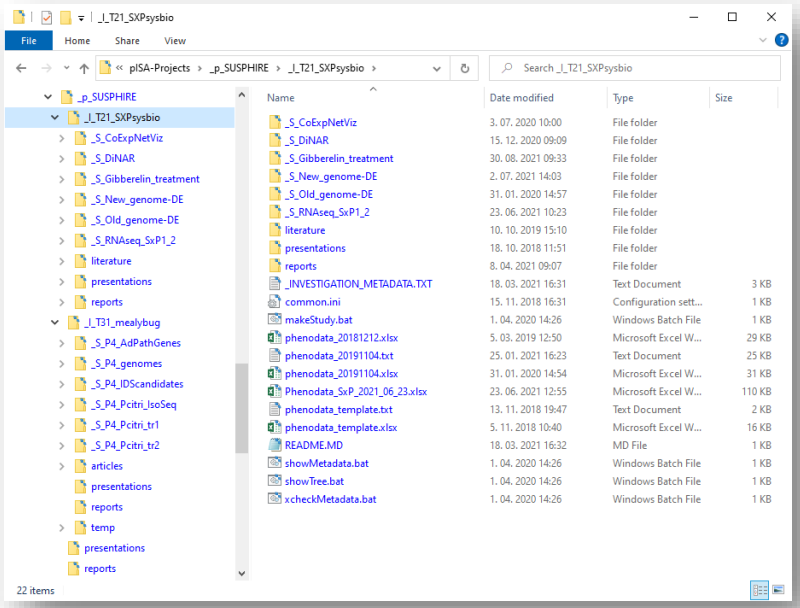


pISA-tree

Investigation

Study

Assay



Technology Transfer

- ***Moth pheromones***. ***TRL3*** *(SxPv1.0) PoC was* ***advanced to TRL4*** *(SxP1.2) live biodispenser, measurement production and emission rate. TRL5 not achieved. Release rates low. Not worthwhile*
- ***Mealibugs pheromones:*** *Principle TRL1-2 was not advanced further for citri (gene not found). As alternative, other irregular plant emitters were moved from TRL1-2 to* ***TRL3-4***
- *Moth pheromones: What do you plan after the project? Finalize search for elusive citri.*

Summary

- ***What was proposed**: Bioproduction of 2 types of pheromones (moth and mealybugs)*

- ***What was achieved:** Bioproduction of 2 types of pheromones (moth and mealybugs)*

***BUT**:*

Moth pheromone (SxP1.2) release rates need to be improved. Conditional expression phenotypes not finished yet

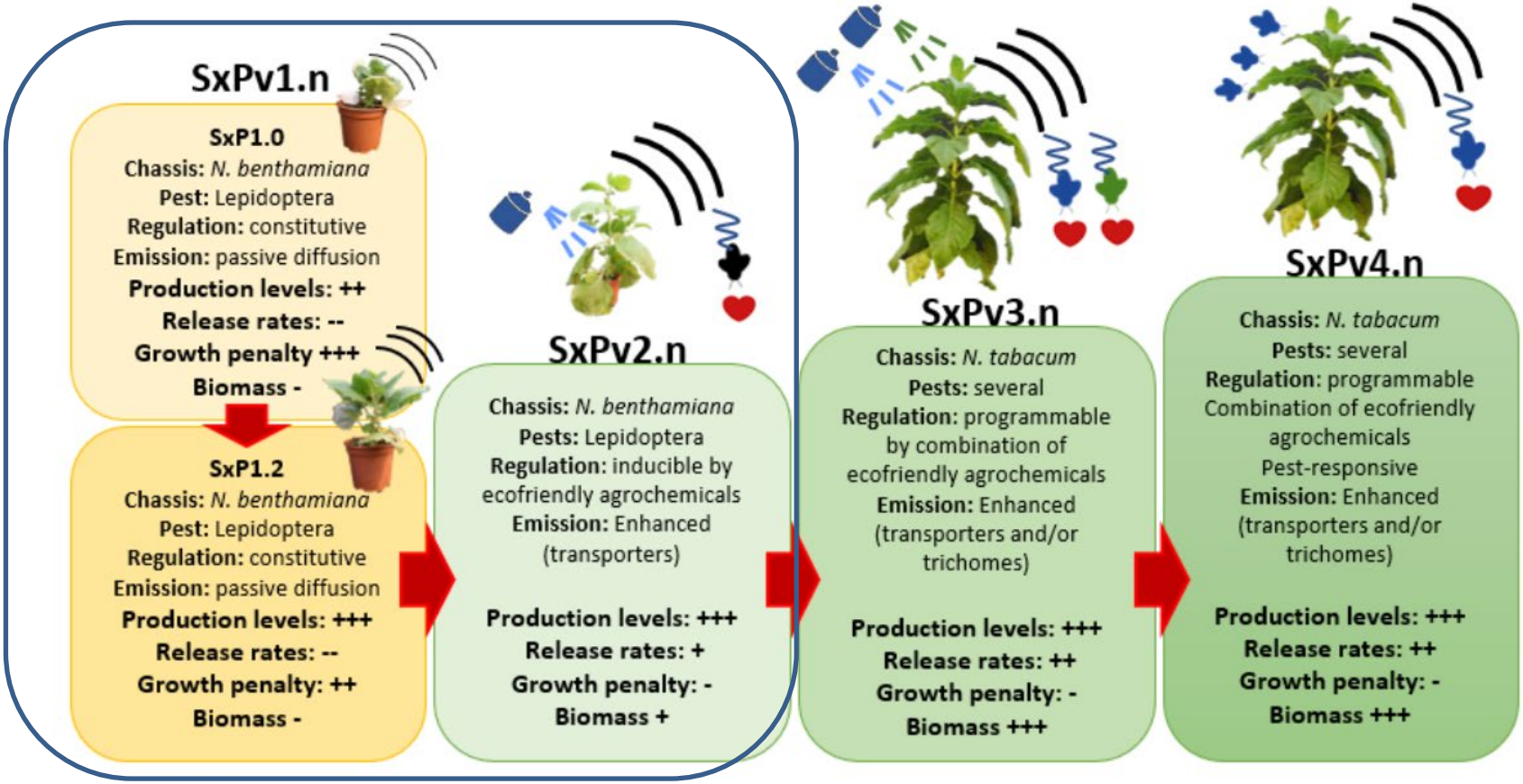
Discovery of key biosynthetic steps Planococus citri not achieved

Mealybugg pheromones bioproduced are not those initially planned.

- *What are the plans for future (any follow-up projects?)*

FOLLOW UP projects at national level in all three aspects. New discovery approaches, alternative chassis.

Project outcomes



Production of Volatile Moth Sex Pheromones in Transgenic Nicotiana benthamiana Plants BioDesign Research 2021. In press. https://www.biorxiv.org/content/10.1101/2021.03.31.437903v1

Project outcomes

Table 1. Quantity (µg) of (*Z*)-11-hexadecenol (OH) and (*Z*)-11-hexadecenyl acetate (OAc) extracted from SxPv1.2 individuals by solvent extraction and GC/MS/MS quantification.

plant	material	µg OH/g plant	µg OAc/g plant
SxPv1.2 T_1-3	fresh leaves	164,9	9,6
SxPv1.2 T_1-4	fresh leaves	129,9	8,6
SxPv1.2 T_1-5	frozen -20C	78,1	10,5
SxPv1.2 T_1-6	frozen -20C	115,1	17,3
SxPv1.2 T_1-7	frozen -80C	75,8	11,8
SxPv1.2 T_1-8	frozen -80C	104,8	12,9
	mean ± se	**111.4 ± 13.7**	**11.8 ± 1.3**

We would require between 1,000 Kg and 25,000 Kg of pheromone-producing intercropping biomass per Ha for effective mate disruption.
NEED to improve RELEASE
Alternative chassis

Table 2. Quantity (ng) of (*Z*)-11-hexadecenol (OH) and (*Z*)-11-hexadecenyl acetate (OAc) released by SxPv1.2 individuals obtained by volatile collection and GC/MS/MS quantification.

plant	ng collected OH	ng OH/day	ng collected OAc	ng OAc/day
SxPv1.2 T_1-1	209,3	69,8	193,3	64,4
SxPv1.2 T_1-2	225,6	75,2	359,4	119,8
SxPv1.2 T_1-3	222,6	74,2	250,7	83,6
SxPv1.2 T_1-4	293,9	98,0	256,8	85,6
mean ± se	**237.8 ± 19.0**	**79.3 ± 6.3**	**265.0 ± 34.6**	**88.3 ± 11.5**

Project outcomes

PUBLICATIONS

- *Production of Volatile Moth Sex Pheromones in Transgenic Nicotiana benthamiana Plants BioDesign Research 2021. In press. https://www.biorxiv.org/content/10.1101/2021.03.31.437903v1*
- *Insect pest management in the age of synthetic biology. Mateos-Fernández et al, Plant Biotechnology Journal 2021 DOI: 10.1111/pbi.13685*
- *Multigene Engineering by GoldenBraid Cloning: From Plants to Filamentous Fungi and Beyond. Vazquez-Vilar, et al. Current Protocols in Molecular Biology, 2021 https://doi.org/10.1002/cpmb.116*
- *Cai Y, Kallam K, Tidd H, Salzman A & Patron NJ (2020) Rational design of minimal synthetic promoters for plants. Nucleic Acids Research. https://doi.org/10.1093/nar/gkaa682*
- *Copper-regulated genetic switch and some successful synthetic regulatory elements (https://www.biorxiv.org/content/10.1101/2021.09.07.459151v1.full.pdf)*
- *Normand A. The ties that bind: collective experimentation and participatory design as paradigms for responsible innovation. Handbook of Responsible Innovation: A Global Resource, Edward Elgar, 2019. https://doi.org/10.4337/9781784718862*
- ***Several publications in prep***

PATENTS

- *Irregular enzyme activity discarded*
- *SxP2.1 discarded*
- *New SxP versions to be decided*
- *New conditional expression tools for plants to be decided*

General Evaluation

- *Benefits of international collaboration; publications; exchange of researchers etc.*

Ideal consortium size, fruitful interactions despite CoVid.

- *Comments, feedback to ERA CoBioTech*

Positive experience, collaborations will expand beyond the end of the project

Contact details

http://susphire.info/

DIEGO ORZAEZ

IBMCP-CSIC

» DIEGO ORZAEZ

(Project coordinator) Instituto de Biología Molecular y Celular de Plantas (IBMCP), Spain

» NICOLA PATRON

The Earlham Institute (EI), UK

» ŠPELA BAEBLER

National institute of Biology (NIB), Slovenia

» HERIBERT WARZECHA

Technische Universität Darmstadt (TUDA), Germany

» ISMAEL NAVARRO

Ecologia y Proteción Agrícola (EPA), Spain