Bioproduction of insect sex pheromones and other volatile compounds in plants

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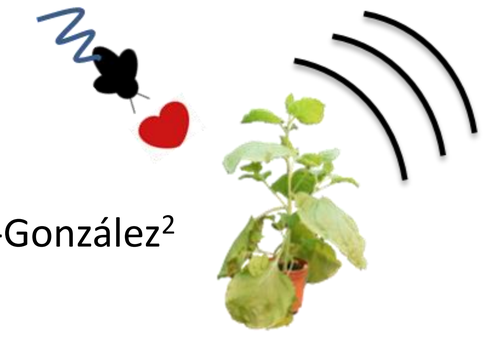

Ph.D. Program in Biotechnology

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1 INTRODUCTION

- **Insect pheromones** are a sustainable alternative to broad-spectrum pesticides in pest control.
- Global insect pheromone market was worth 1,9 billion USD in 2017.
- Their **chemical synthesis** implies high costs, complexity, and generation of polluting by-products.

Biological synthesis as a solution!

SUSPHIRE
Sustainable Bioproduction of Pheromones for Insect Pest Control in Agriculture

Plants can be an ideal chassis for metabolic engineering, showing high scalability and counting on photosynthetic precursors.

SUSPHIRE project, in the framework of this thesis, aims to use genus ***Nicotiana*** plants to establish an efficient system to synthesize insect pheromones.

OBJECTIVES 2

Generate constitutive and inducible metabolic pathways in plants for the biosynthesis of insect pheromones and potential precursors of pheromones of Lepidoptera and Coccoidea, by the use of Synthetic Biology, and optimize their production in a biofactory way.

Optimize the bioproduction of **moth** sex pheromones (**fatty alcohols metabolic pathway**)

Generate transgenic plants producing sex pheromones of mealybugs or at least **monoterpene** moieties of the sex pheromones of various mealybug species

3 OPTIMIZATION OF THE MOTH SEX PHEROMONE METABOLIC PATHWAY IN PLANTS

First attempts of recreating the moth sex pheromone metabolic pathway in plants reached the creation of the called "**Sexy Plant (SxP)**": a *Nicotiana benthamiana* transgenic plant expressing constitutively a desaturase (AtrΔ11) and a reductase (HarFar), both from moth genes, and a plant acetyltransferase (EaDact) (Fig 1).

The result: a plant producing and emitting two main volatile components in many Lepidoptera sex pheromone blends: (*Z*)-11-hexadecenol (Z11-16OH) and (*Z*)-11-hexadecenyl acetate (Z11-16OAc).

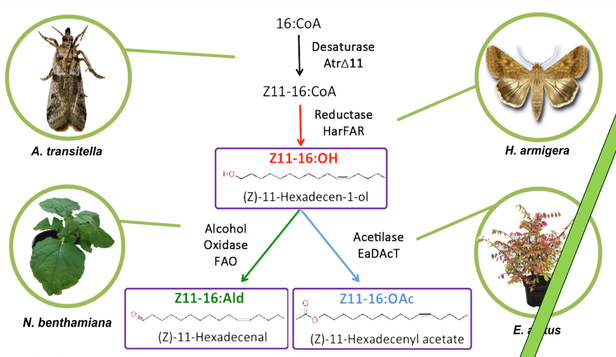


Figure 1: Schema of moth sex pheromone synthetic pathway

By regulating the expression of the metabolic pathway by the control of **synthetic promoters**.
Only in the presence of all elements, when the system is complete, the metabolic pathway is activated and the pheromone production is switched on.

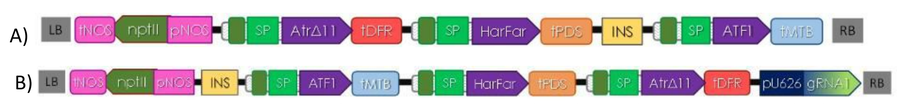


Figure 3: Schema of DNA constructs: A) NonGuidedPathway (NGP) and B) GuidedPathway (GP). Synthetic promotors (SP) regulate the activation of genes responsible of the synthesis of pheromones.

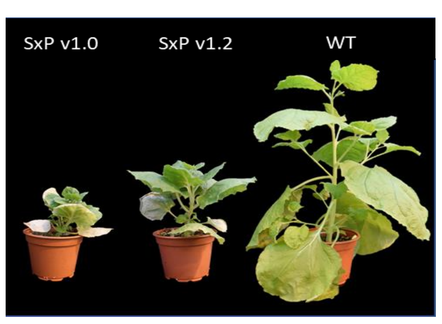
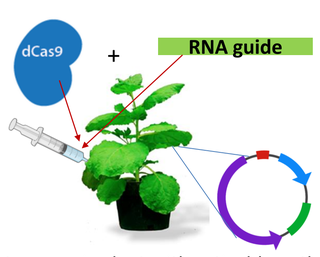


Figure 2: Comparition of size between SxP (diff versions) and WT *N.benth* plants

In a recent work (Mateos-Fernández et al., 2021), we have characterized this plant (v1.0) and even created another version (v1.2) with a different transgene configuration, producing more acetate form. This allowed us to exploit the system, but a **growth penalty**, more minor in v1.2 but still remarkable, was always noticed (Fig 2).

Possible solution? **Regulate the activation** of the pathway. HOW??

Elements required in the activation system:



Stable transgenic plant with activable pathway

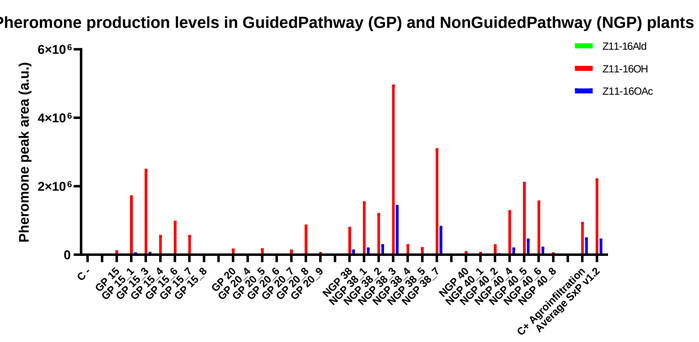
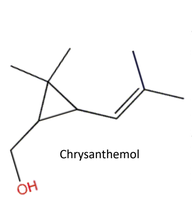
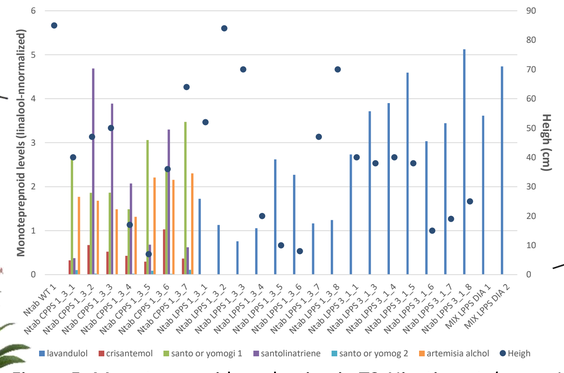


Figure 4: Pheromone production in different GP and NGP *Nicotiana benthamiana* T1 plants after activation by agroinfiltration:

4 GENERATION OF TRANSGENIC PLANTS PRODUCING MONOTERPENE MOIETIES OF SEX PHEROMONES OF VARIOUS MEALYBUG SPECIES

Mealybug sex pheromones typically contain various monoterpene-derived esters. Unfortunately, their biosynthesis remains unclear and insect candidate genes for their production are yet to be identified.
An **alternative approach** to the bioproduction of mealybug sex pheromones is to exploit two **plant-derived genes** capable of producing irregular monoterpenes: lavandulyl-PP synthase (**LPPS**) from *Lavandula* x *intermedia*, which synthesizes lavandulyl pyrophosphate; and chrysanthemyl-PP synthase (**CPPS**) from *Tanacetum cinerariifolium*, which produces the cyclic monoterpene chrysanthemyl pyrophosphate.





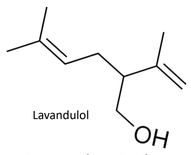


Figure 5: Monoterpenoid production in T2 *Nicotiana tabacum* LPPS and CPPS lines, measured by HSPME GCMS.

Lavandulol and **chrysanthemol** are valuable as the monoterpene moieties of the sex pheromones of various mealybug species, which can then be esterified to give the active product.

First successful esterification in stable plants by LiAAT4 of lavandulol to lavandulyl acetate, itself an active pheromone component of the mealybug *D. grassii* and of the Western flower thrips *Frankliniella occidentalis*.

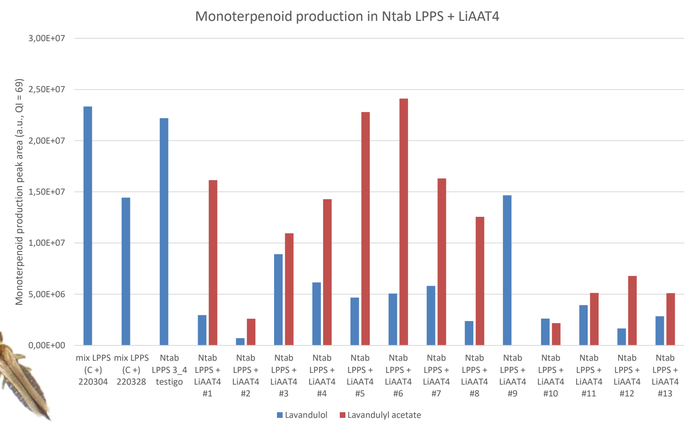


Figure 6: Monoterpenoid production in T0 *Nicotiana tabacum* LPPS + LiAAT4 lines.

5 NEXT STEPS

- Different activation systems of SxP plants (eg: viral vectors…) for stronger activations
- Field assays with T1 LPPS + LiAAT4 Ntabacum plants.

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