Proceedings of the LXV SIGA Annual Congress
Piacenza, 6/9 September, 2022
ISBN: **978-88-944843-3-5**

Poster Communication Abstract – 5.48

HETEROLOGOUS PRODUCTION OF IRREGULAR MONOTERPENES IN TOBACCO AND BENTHAMIANA AS PRECURSORS FOR THE SYNTHESIS OF MEALYBUG PHEROMONES.

GIANOGLIO S.*, MATEOS-FERNÁNDEZ R.*, VACAS GONZÁLEZ S.**, NAVARRO FUERTES I.***, ORZÁEZ D.*

*) Instituto de Biología Molecular y Celular de Plantas (IBMCP-CSIC)
**) Instituto Agroforestal Mediterráneo, Centro de Ecología Química Agrícola
***) Universitat de València (UV), Departamento de Química Orgánica

sex pheromones, pest management, sustainable agriculture, plant biofactories

The use of insect pheromones for pest management, either to construct traps or for sexual confusion strategies, is an appealing alternative to broad-spectrum pesticides whose use is being reduced because of their toxicity. However, the chemical synthesis of insect pheromones is often costly, which makes their use affordable only for very high-value end products. Also, their chemical synthesis can cause the release of polluting by-products, hindering their sustainability. Hence, the growing interest for their biosynthesis in heterologous hosts which support more sustainable production practices, reduce costs and ensure stereoselectivity, which is crucial to their activity.

Mealybugs (Pseudococcidae) are a family of insects which rely heavily on sex pheromones for mating and constitute a relevant threat to crops in Mediterranean climates. Their sex pheromones typically contain various monoterpene-derived esters, among these irregular monoterpenes, which are unusual in nature. 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 genes capable of producing irregular monoterpenes: *LPPS* from *Lavandula* x *intermedia*, which synthesizes lavandulyl pyrophosphate, a linear branched irregular monoterpene; and *CPPS* from *Tanacetum cinerariifolium*, which produces the cyclic irregular monoterpene chrysanthemyl pyrophosphate. Both molecules are valuable as the monoterpene moieties of the sex pheromones of

various mealybug species, which can then be esterified to give the active product.

We transformed *Nicotiana tabacum* and *N. benthamiana* with *Li*LPPS and *Tc*CPPS to evaluate their ability to accumulate irregular monoterpenes. We measured their production levels through the GC-MS analysis of ground plant tissues and of the emission of intact flowers and leaves, and we evaluated the impact of the production of high levels of these compounds on plant development and flowering. In addition, we also over-transformed *LPPS*-expressing tobacco plants with the *AAT4* acetyltransferase from *L. intermedia*, successfully esterifying lavandulol to lavandulyl acetate, itself an active pheromone component of the mealybug D. grassii and of the Western flower thrips *Frankliniella occidentalis*. This work represents a first effort to fill the gap for the sustainable production of mealybug sex pheromones using plants as biofactories. We chose tobacco for its amenability to transformation and for its high biomass production. These plants will then be employed as chassis as more enzymatic activities are characterized for the synthesis of a wide range of monoterpene esters. The use of plants for the bioproduction of insect pheromones for sustainable pest control can be envisioned either to produce molecules to be extracted for the formulation of traps or, ideally, as live emitters to be used in the field for sexual confusion strategies.