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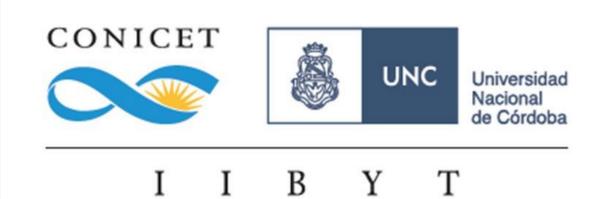
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ELECTROANTENNOGRAPHY



OF TERPENE-VOLATILE COMPOUNDS WITH INSECTICIDAL ACTIVITY

Natalia A. Corvalán^{1,2}, Christelle Monsempès², Mariela E. Sánchez-Borzone, Daniel A. García¹, Philippe Lucas²

¹Instituto de Investigaciones Biológicas y Tecnológicas (IIBYT), CONICET-Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611, Córdoba 5016, Argentina.

²INRAE, UMR 1392 iEES-Paris, Route de Saint Cyr, F-78026 Versailles, France.

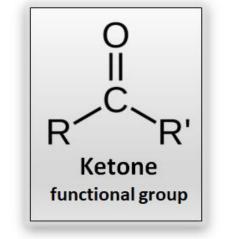
ABSTRACT

Synthetic insecticides have adverse effects on non-target organisms, ecosystems and human health due to their high toxicity and residual accumulation in different environmental components and organism tissues. Moreover, their long-term applications generate the development of pest resistance. This has led to a growing interest in novel, more environmentally friendly insecticides. Essential oils and their active constituents, especially monoterpenoids, which play an important role in plant defense system against microorganisms, insects, herbivores and allelopathic interactions, are considered as an alternative to synthetic insecticides.

Electroantennography (EAG), a technique widely used in insect olfaction studies, is performed in the present work on males of the Noctuid *Agrotis ipsilon* to characterize the electrical response evoked by well-known natural insecticides from the peripheral nervous system of insects.

MATERIALS and **METHODS**

VOLATILE PLANT COMPOUNDS (VPCs)



Ketones:

Menthone, Carvone, Thujone, Pulegone and Dihydrocarvone (all cyclic monoterpenes with *insecticidal properties*)

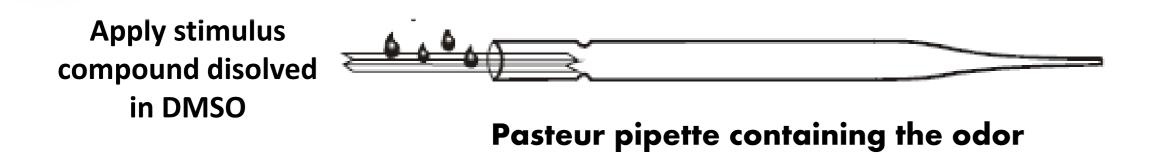
Control (-):

Eucalyptol: (cyclic monoterpene) does not evoke a significant neural activity by Single Sensillum Recordings (SSR) technique (Conchou, et al. 2021)

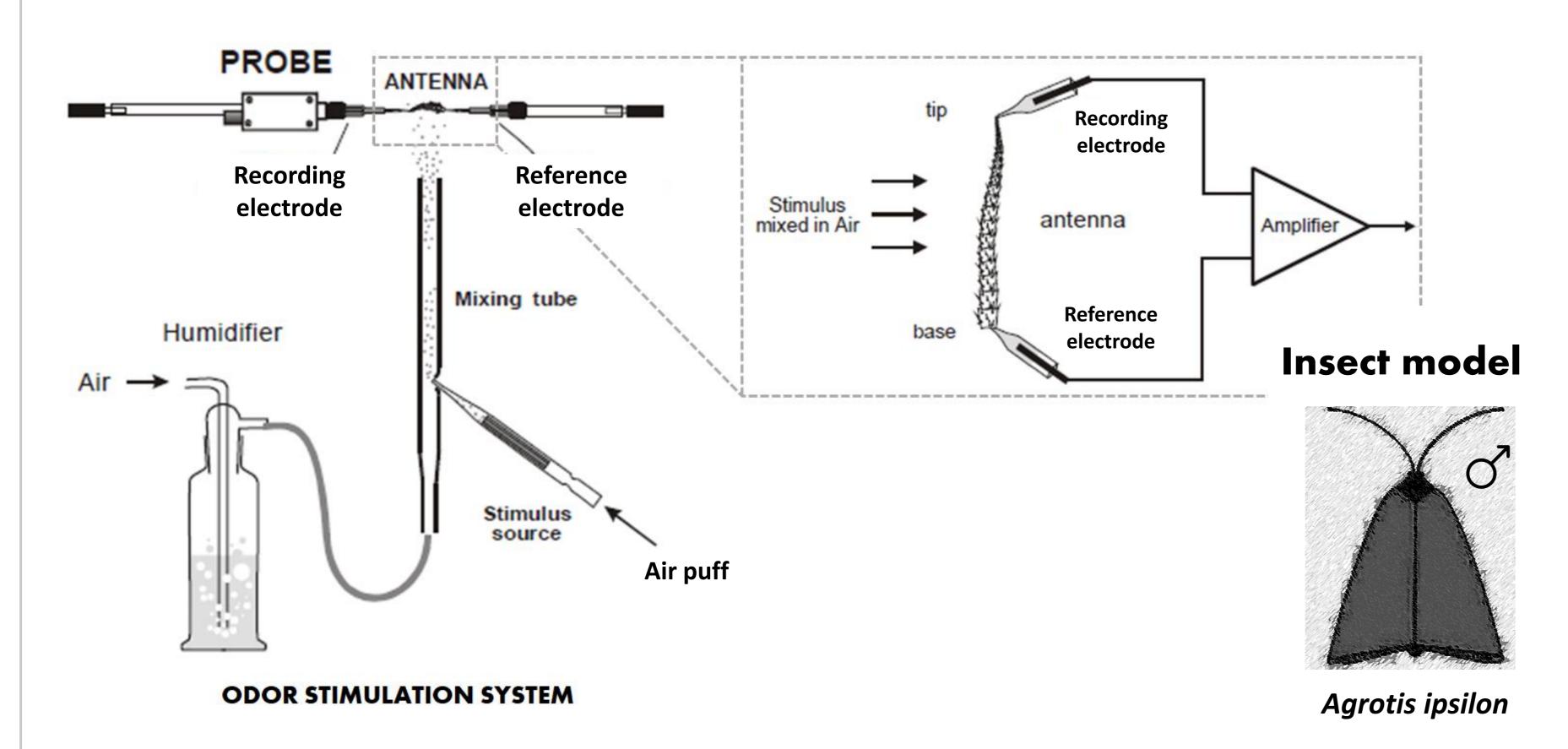
Control (+):

cis-3-Hexenyl acetate: (carboxylic acid ester)/Linalool: (acyclic monoterpenoid alcohol). Both compounds exhibit neural activity by SSR technique (Conchou, et al. 2021)

ODOR STIMULI: Stimuli were delivered as air puff (10 L/h, 500 ms) through a Pasteur pipette containing a piece of filter paper loaded with 1 μ L of one VPC diluted at 10⁻², 10⁻¹ or 1 M in DMSO. The Pasteur pipette tip was inserted into a hole of a glass tube traversed by a permanent stream of humidified and charcoal-filtered air (70 L/h) focused on the moth antenna.



EAG RECORDINGS:

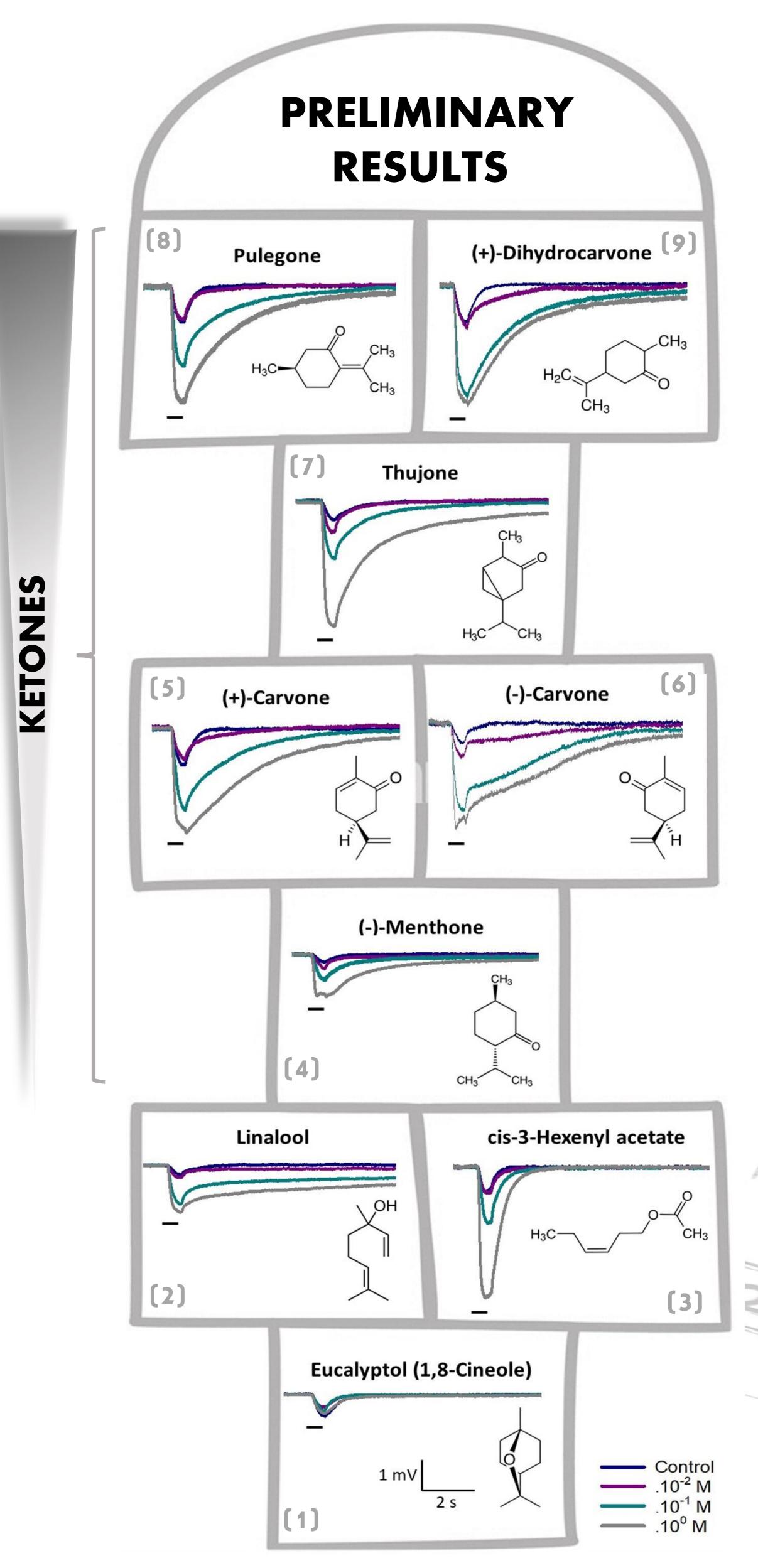


DISCUSSION

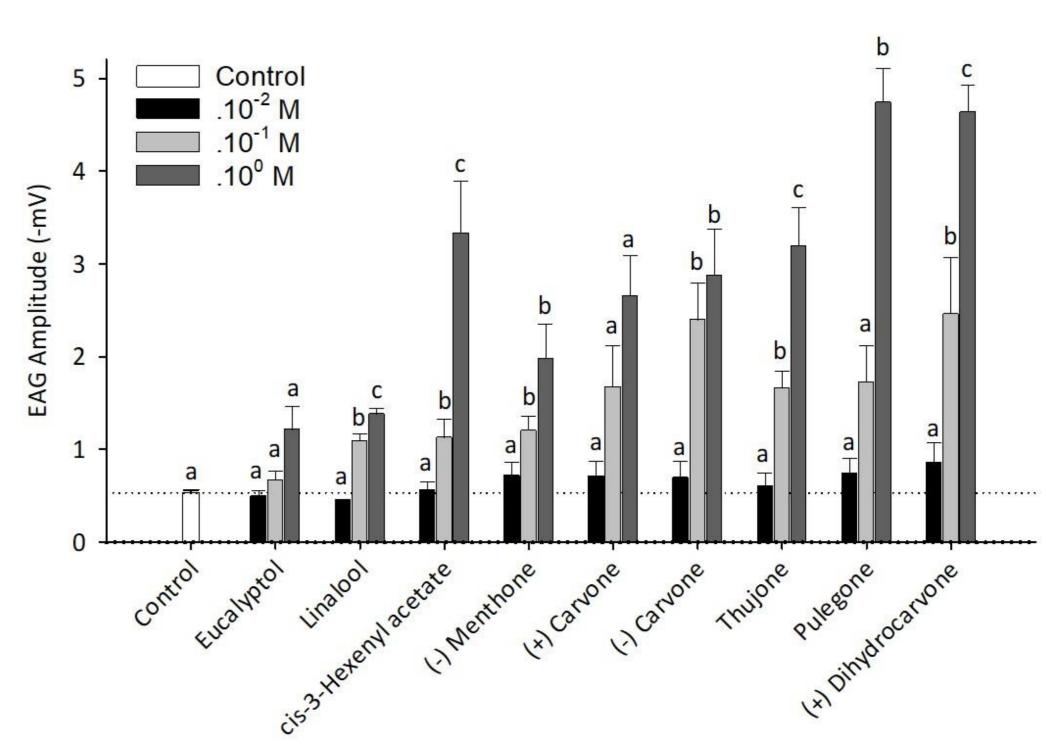
All ketones elicited dose-dependent EAG responses, with some compounds more active than others.

EAG is a simple, rapid method for measuring the activity of volatile compounds on insect sensory neurons.

We plan to use this technique for screening bioactive VPCs of interest previously identified by *in silico* techniques as putative GABAergic insecticide agents in the search of potential neurotoxic insecticides.



EAG responses to VPCs. Representative EAG traces elicited by increasing concentrations of the Ketones (4-9) and the reference compounds (1-3). Stimulus pulses lasted 500 ms. The EAG response evoked by each odorant was tested from lowest to highest concentration to avoid contamination. DMSO were tested as negative control (blue trace) immediately before and after measuring the triad of concentrations of each compound.



Comparison of VPC-evoked EAG response amplitudes at three different compound concentrations $(.10^{-2} \text{ to } .10^{0} \text{ M})$. Each bar represents the mean (±SEM) of five to eight tested antennae. Means sharing no letter on the top of bars are significantly different (p < 0.05), as determined by one-way analysis of variance with Tukey's test. Differing letters only have meaning within EAG responses evoked by different concentrations of a compound, not between compounds.