Validated SOPs for MALDI mass fingerprints

Summary of confidential Deliverables D9.2, D9.3 and D9.4

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PoshBee

Pan-european assessment, monitoring, and mitigation of stressors on the health of bees

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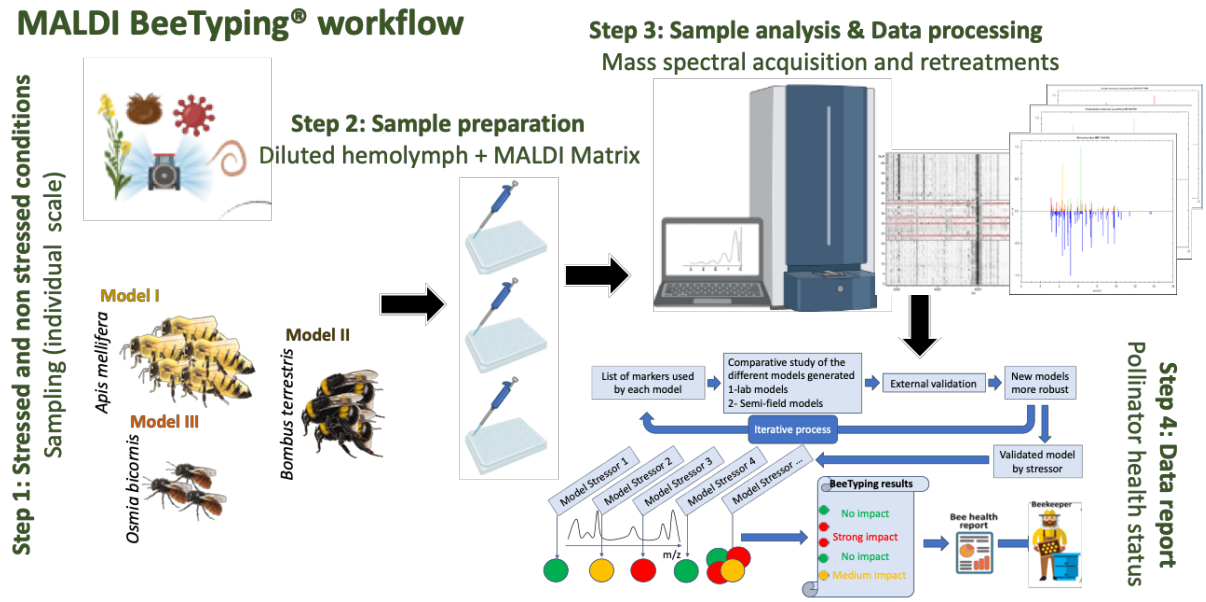
Workflows for MALDI BeeTyping® for three pollinator models

PREFACE

MALDI BeeTyping® is an innovative analytical "blood test" inspired from MALDI BioTyping (used daily in clinical microbiology for bacterial speciation) for bee health monitoring. This technique provides molecular mass fingerprints/spectral molecular fingerprints (MFPs) of peptides and small proteins (<18 kDa) circulating in bee haemolymph. MALDI BeeTyping® is used for bee health monitoring in the same way a medical practitioner proposes a blood test to evaluate the health status of a patient. The present standard operating procedure (SOP) summary merges three deliverables "Validated SOPs for MALDI mass fingerprints I" on *Apis mellifera* (model I, D9.2) adjusted for *Bombus terrestris* (model II, D9.3) and *Osmia bicornis* (model III, D9.3). The models (I-III) were generated form laboratory experiments, applied to individual haemolymph samples raised in lab and semi-field experiments and validated on field samples. The conditions proposed in this SOP were optimised to obtain the most exhaustive and reproducible list of molecular masses for an optimal MFP of the sample. This includes sample preparation homogeneity, mass spectral resolution and ion intensity. The general workflow of the MALDI BeeTyping® analytical method is illustrated in the figure below.

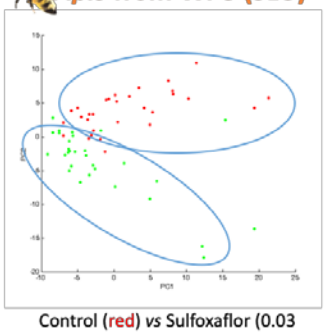
MALDI BEETYPING® WORKFLOW



1. **Sampling**: control *versus* stressed (individual and in combination) conditions (abiotics or/and biotics).
2. **Haemolymph sample** dilution and **matrix preparations** (HCCA*, dry droplet). * alpha-Cyano-4-hydroxycinnamic acid
3. **Spectral molecular recording** (1 to 18 kDa), Data processing (stack view gel-view) and Statistical analysis.
4. **Data reporting** of the impact level in a user friendly three color code to view the impact level, **green no impact**, orange moderate and red high impact.

EXAMPLES OF APPLICATIONS OF MALDI BEETYPING® IN THE POSHBEE PROJECT

Apis from WP3 (SLU)



Control (red) *vs* Sulfoxaflor (0.03 µg/bee) (green)

Sulfoxaflor: Impact on *Apis* haemolymph

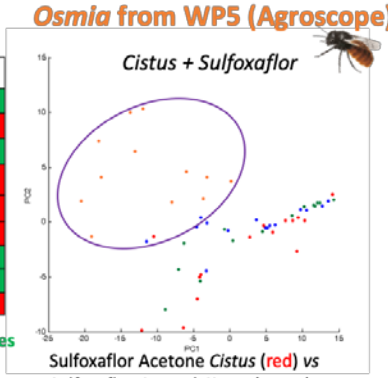
Bombus from WP3 (EMU)

Type of Agrochemical	Agrochemical Classes	Gender	Dose / Comment	Impact
Sulfoxaflor	Insecticide	Workers	Exposure time: 24h and 48h	
		Queens	Only from 110 µg/bee	
		Males	Exposure time: 24h and 48h	
Azoxystrobin	Fungicide	Workers	Exposure time: 24h and 48h	
		Queens	Only from 200 µg/bee	
		Males	Only from 200 µg/bee	
Glyphosate	Herbicide	Workers	Exposure time: 24h and 48h	
		Queens	Exposure time: 24h and 48h	
		Males	Only exposure time 48h	

Green: No discrimination compared to control bees

- **Sulfoxaflor: Impact variation on *Bombus* haemolymph depends on the caste**
- **Azoxystrobin: Impact on different castes (workers, queens and males)**
- **Glyphosate: impact 48h on males only**

Osmia from WP5 (Agroscope)



Sulfoxaflor Acetone *Cistus* (red) *vs* Sulfoxaflor Control Cistus (green) *vs* Sulfoxaflor low concentration *Cistus* (blue) *vs* Sulfoxaflor high concentration Cistus (orange)

The insecticide at high dose may have an effect on bees if the nutrition is poor

LESSONS TO LEARN

- MALDI BeeTyping® is based on a "blood test" to monitor the impact of stressors on bees at an individual scale;
- Haemolymph is the equivalent to the human blood and is used as a readout of the immune status in the same way a medical practitioner proposes a blood test to evaluate the health status of a patient;
- This technique allows to define each species' specificity: i.e. molecular composition of the haemolymph, response to stressors, immune status;
- Databases of spectral molecular models of fingerprints featuring the impact of stressors on bees, under laboratory and semi-field experiments were built;
- The statistical models can be applied to any field samples;
- MALDI BeeTyping is cost- and time-effective compared to molecular biology techniques and represents a user-friendly approach than can be applied to any bee model and stressor condition.