

## **Objectives and Rationale**

The objectives of this project were to i) identify (morphologically and molecularly) the diversity of parasitic wasp species in South Africa and their relative importance in controlling fruit flies; ii) search for biocontrol agents in the area of origin of fruit flies (rest of Afrotropical region) in view of future introductions; iii) highlight the trophic associations between fruit flies and their parasitoids (utilizing a DNA metabarcoding approach), and iv) perform initial testing of augmentoria, which could greatly assist with orchard sanitation.

## **Methods**

Surveys were conducted in several regions of South Africa and in the rest of the afrotropical region through collaborators. Specimens were collected by direct sampling in the orchards and by rearing fruits infested by fruit flies. Samples were identified based on morphology and were subject to DNA extraction, PCR amplification and sequencing the mitochondrial Cytochrome Oxidase 1 to construct a DNA barcode database using conventional molecular methods. A 2-step PCR and an illumina sequencing technology was applied to freshly emerged samples collected in the field in order to assign each parasitoid wasp to its specific fruit fly host. Lastly, an augmentorium (orchard sanitation method) was tested to optimize and adapt the technique according to optimal size of netting to match size of local parasitoids, and optimal time for fruit collecting.

## **Key Results**

- Two main Opiinae wasps (biological control agents) of fruit flies have been identified in South Africa: *Fopius ceratitivorus* and *Psytalia humilis*.
- A substantial contrast was observed in the distribution of fruit fly parasitoid species between the regions studied and between fruit types.
- In appropriate locations and for specific fruits, augmentoria allow a mass-release of Opiinae biological control agents of fruit flies.
- Up to 50% of fruit flies can be controlled by Opiinae in appropriate conditions.
- Native trees in the vicinity of orchards could be used as an important reservoir of Opiinae wasps when combined with augmentoria.
- In total, 70 morphospecies of Opiinae wasps from the Afrotropical region have been identified and sequenced for the standard COI gene. They constitute a list of candidates for potential future introductions into South Africa.
- Despite the application of the appropriate calibration step, the current protocols (DNA metabarcoding) failed to provide accurate data on trophic relationships between Opiinae wasps and their fruit fly hosts.

## **Conclusion and Discussion**

Except for the analysis of the host-parasitoid interaction analysis, significant progress has been made for all objectives of the project. South Africa shows two main species of Opiinae

that are efficient biocontrol agents and that can be used via augmentative techniques. However, additional research is needed to understand the variation of the abundance of these parasitoids among the localities and fruit types considered.