



**HAL**  
open science

## **A metabarcoding exploration of ecological interactions among hoverflies, spontaneous vegetation and aphids in apple orchards.**

Pierre P. Franck, Ludivine Laffon, Jérôme Olivares, Françoise Lescourret, Armin Bischoff

### **► To cite this version:**

Pierre P. Franck, Ludivine Laffon, Jérôme Olivares, Françoise Lescourret, Armin Bischoff. A metabarcoding exploration of ecological interactions among hoverflies, spontaneous vegetation and aphids in apple orchards.. IOBC/WPRS Working Groups "Landscape Management for Functional Biodiversity", 170, IOBC-WPRS, pp.71-74, 2024, Landscape Management for Functional Biodiversity. <hal-04668804>

**HAL Id: hal-04668804**

**<https://hal.science/hal-04668804v1>**

Submitted on 7 Aug 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC0 1.0 - Universal - International License

# A metabarcoding exploration of ecological interactions among hoverflies, spontaneous vegetation and aphids in apple orchards

Pierre Franck,<sup>1</sup> Ludivine Laffon<sup>1</sup>, Jérôme Olivares<sup>1</sup>, Françoise Lescourret<sup>1</sup>, Armin Bischoff<sup>2</sup>

<sup>1</sup>PSH, National Research Institute for Agriculture, Food and Environment, INRAE, 84000 Avignon, France; <sup>2</sup>Mediterranean Institute of Biodiversity and Ecology, IMBE, Avignon University, CNRS, IRD, Aix-Marseille University, IUT, Agroparc, 84000 Avignon, France.

**Abstract:** Interaction of hoverfly with plant and aphid taxa were explored in two apple orchards to identify hoverfly trophic preferences based on a metabarcoding analysis. Only four hoverfly taxa (among the thirteen taxa observed in the apple orchards) were directly involved in *Dysaphis plantaginae* biological control. In average 16 plant taxa were detected per hoverfly taxa. Hoverfly taxa hardly differed in their plant distribution.

**Key words** pollen resources, hoverflies, *Dysaphis plantaginae*, conservation biological control, apple orchard, metabarcoding

## Introduction

Hoverfly larvae are major aphid predators that contribute limiting Rosy Apple Aphid (RAA) outbreaks because of their early appearance in apple orchards (Dib *et al.*, 2010). The amount of flower resources from the spontaneous vegetation or from perennial strips can significantly increase the abundance of hoverfly adults in apple orchards (Rodríguez-Gasol *et al.*, 2019; Laffon *et al.* 2024). Few studies have demonstrated effects of flower resources in apple orchards on aphid biological control (Albert *et al.*, 2017; Laffon *et al.* 2024) and on reducing fruit damage (Cahenzli *et al.*, 2019). In this work we carried out a metabarcoding analysis to explore the trophic relationships of hoverflies with both plant and aphid taxa and to specify pollen resources likely to improve RAA biological control in apple orchards. We aimed to answer two questions:

- (1) Are hoverflies visiting the spontaneous flowers in orchards involved in RAA control?
- (2) Do the consumed plants differ among the hoverfly taxa?

## Material and methods

### *Hoverfly sampling*

Hoverfly adults and larvae were collected in spring 2022 (three sessions: late April, mid-May and mid-June) in two apple orchards from the Lower Durance Valley (<https://site-atelier-basse-vallee-durance.fr/>). These two commercial orchards were selected based on the level of RAA damages and hoverfly abundances previously observed in 2021 (Laffon *et al.*, 2024). Adults observed on the spontaneous flowering vegetation inside the orchards and their borders were sampled during two hours using nets (2 persons/orchard). Larvae were collected in RAA colonies (30 min search/orchard). The hoverflies were caught between 9 a.m. and 12 p.m., on sunny days with little wind. Each hoverfly was collected individually, stored in 70° alcohol and

kept at -20°C. A total of 178 hoverflies were collected: 118 adults (29 in April, 59 in May and 30 in June) and 60 larvae (22 in April and 38 in May). Adult hoverflies were identified and photographed prior to DNA analysis

### ***Metabarcoding analysis***

Individual hoverfly DNA extractions were performed on dissected thorax and abdomen or on whole larvae using Qiagen DNeasy 96 kit for plant. Four independent PCRs were run in 30 µL for each DNA extract, two PCRs with different sets of specific *COI* primers to amplify hoverfly and aphid DNA respectively, and two PCRs with *rbcL* and *ITS2* primer pairs to amplify plant DNA. The four PCR products were pooled to index each hoverfly sample independently (Nextera index combination). Finally, paired-end sequencing (2x150 pb) using the Illumina NGS technology (NextSeq 2000 P1 Reagent kit) was performed on PCR products from the 178 hoverfly samples and from eight negative control samples (DNA extractions without biological material or PCR consisting of water or from instead of DNA templates) and two plant DNA positive control). Indexing PCRs and NGS sequencing were performed on the PGTB platform (INRAE, Bordeaux).

FROGS workflows were used to filter paired-end reads of accurate sizes, to discard chimeric sequences, and to realize multiple taxonomic affiliations (Escudie *et al.*, 2018). The *BOLD\_1percentN\_2019* data base implemented in FROGS was used to assign *COI* sequences to hoverfly and to aphid taxa. A list of 112 plant taxa previously observed in apple orchards from the Basse Durance Valley (Laffon *et al.*, 2024) was used to construct our own *ITS2* and *rbcL* reference databases to assign sequences to local plant taxa. Taxonomic affiliations were based on at least 97% identity between sequences. A conservative threshold was used to remove any taxonomic assignments whose number of reads fell below the average plus 1.645 times the standard deviation of the number of reads detected in negative controls (Arstingstall *et al.*, 2023). Above this threshold, taxonomic assignments were considered positive for the sample.

### ***Analysis of the trophic interactions***

First, the hoverfly taxa identified in the RAA colonies were compared to those collected on the spontaneous vegetation. Second, bipartite networks were computed (to represent relationships between hoverfly and aphid taxa, one the hand, and between hoverfly and plant taxa on the other hand (*bipartite* package; Dormann, 2011). Assignment of the plant taxa with *ITS2* and *rbcL* makers were combined for each hoverfly sample.

## **Results and discussion**

In total, 93,542,427 out of 99,704 026 reads were conserved after the FROGS treatments (93%): 62,516,478 *COI* hoverfly reads (67%), 10,033,761 *COI* aphid reads (11%), 4,703,530 *rbcL* reads (5%) and 16,288,658 *ITS2* reads (17%). The number of reads in a negative control sample did not exceed 0.02% of the total number of reads per marker.

### ***Are hoverflies visiting orchard flowers involved in RAA control?***

Among the 13 hoverfly genera identified, 12 were collected on flowers and 4 in RAA colonies (Fig. 1). *Sphaerophoria scripta*, *Episyrphus balteatus* and *Syrirta pipiens* were the most frequent taxa observed among the hoverfly adults collected on flowers. *Epistrophe nitidicollis*, *E. balteatus* and *Syrphus vitripennis*, *Pipiza noctiluca* were the four taxa identified among the larvae collected in RAA colonies. No *Pipiza noctiluca* adults was collected. These

results suggest that most of the hoverfly taxa foraging on orchard flowers were not directly involved in RAA biological control.

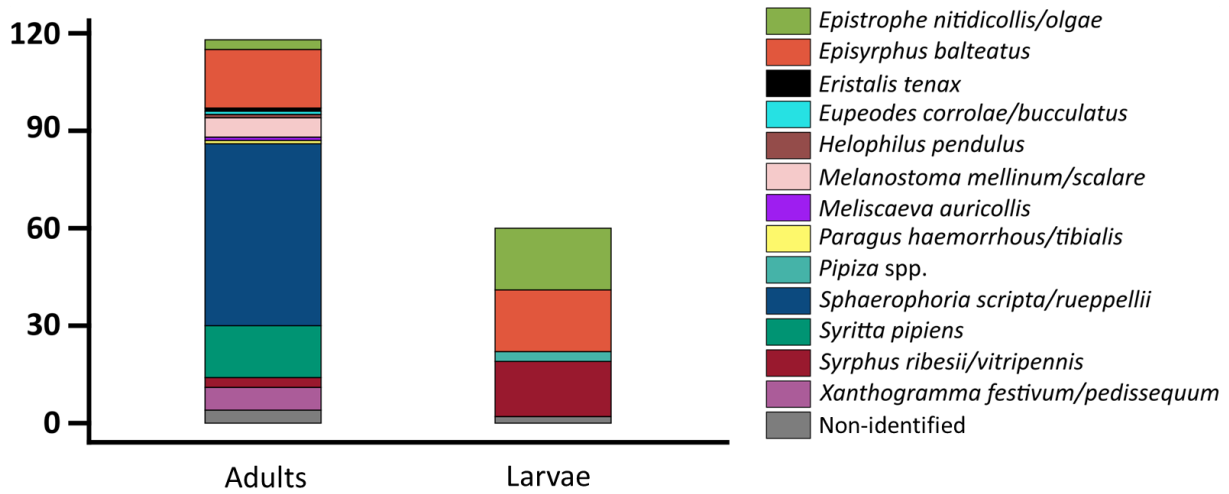


Figure 1. Distribution of the hoverfly taxa collected on flowers (adults) and in RAA colonies (larvae).

All the 60 hoverfly larvae were detected positive to *D. plantaginae* and 5 were also positive to *Aphis pomi* or *A. spiraeicola*. Furthermore, 30% of the hoverfly adults (8 taxa) were also positive to at least one aphid taxa, notably *D. plantaginae* (7 taxa), and/or *A. spiraeicola/pomi* (4 taxa). The consumption of honeydew may explain the large number of positive aphid signal on hoverfly adults (Pinheiro *et al.*, 2015).

### Do the consumed plants differ among the hoverfly taxa?

In total 39 plant taxa were identified on hoverfly samples with the combination the combination of *ITS2* and *rbcL* markers. The number of plant taxa detected differed between the two orchards (17 and 37 respectively). The distribution of the plant taxa changed among the three sessions according the plant phenology: *Veronica persica* and *Malus domestica* in April, *V. persica* and *Potentilla reptans* in May and *Torilis arvensis* and *Crepis* spp. in June. In average, 16 plant taxa were detected per hoverfly taxa (Fig. 2). Most of plant taxa were detected on several hoverfly taxa with little difference on the proportion of each plant detected. *Melanostoma mellinum* differ from other hoverfly taxa mainly due to higher consumption of pollen from grass taxa. These results support that hoverfly taxa are opportunistic about their use of pollen resources. These is mainly true for *E. balteatus*, which forage the most abundant forb taxa.

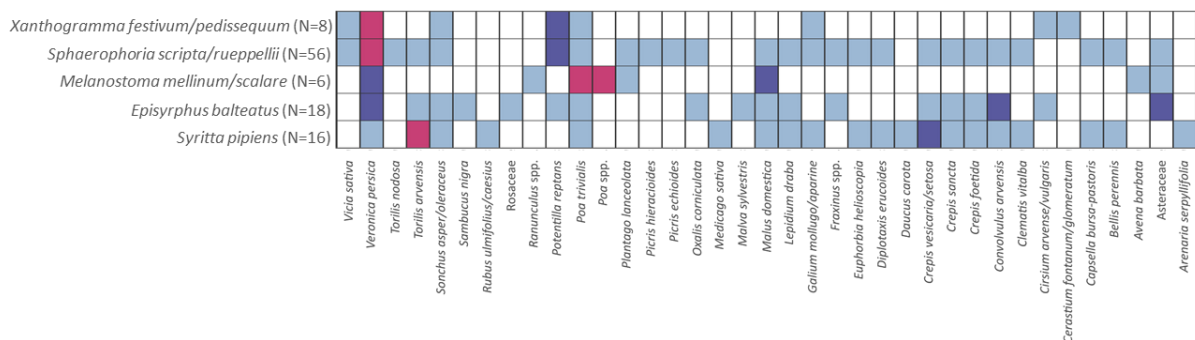


Figure 2. Proportion of hoverflies that were detected positive for a plant taxon among the five main hoverfly taxa (light blue: ]0;10]%; dark blue: ]10;20]%; red : ]20;30]%).

## Acknowledgements

This work was partially supported by the FRAMEwork (H2020 grant agreement number 862731) and 2P3P (SFR Tersys, Avignon University) projects. INRAE and Region Sud PACA funded LL's PhD grant.

## References

- Arstingstall, K. A., DeBano, S. J., Li, X., Wooster, D. E., Rowland, M. M., Burrows, S., & Frost, K. (2023). Investigating the use of pollen DNA metabarcoding to quantify bee foraging and effects of threshold selection. *PLOS ONE*, 18 (4), e0282715.
- Cahenzli, F., Sigsgaard, L., Daniel, C., Herz, A., Jamar, L., Kelderer, M., Jacobsen, S.K., Kruczyńska, D., Matray, S., Porcel, M., Sekrecka, M., Świergiel, W., Tasin, M., Telfser, J., Pfiffner, L., 2019. Perennial flower strips for pest control in organic apple orchards - A pan-European study. *Agriculture, Ecosystems & Environment* 278, 43–53.
- Dib, H., Simon, S., Sauphanor, B., Capowiez, Y., 2010. The role of natural enemies on the population dynamics of the rosy apple aphid, *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) in organic apple orchards in south-eastern France. *Biological Control* 55, 97–109.
- Dormann, C. F. (2011). How to be a specialist? Quantifying specialisation in pollination networks. *Network Biology*, 1 (1), 1-20.
- Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., Maman, S., Hernandez-Raquet, G., Combes, S., Pascal, G., 2018. FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics* 34, 1287–1294.
- Laffon, L., Bischoff, A., Blaya, R., Lescourret, F., Franck, P., 2024. Spontaneous flowering vegetation favours hoverflies and parasitoid wasps in apple orchards but has low cascading effects on biological pest control. *Agriculture, Ecosystems & Environment* 359, 108766.
- Pinheiro, L. A., Torres, L. M., Raimundo, J., & Santos, S. A. P. (2015). Effects of pollen, sugars and honeydew on lifespan and nutrient levels of *Episyrphus balteatus*. *BioControl*, 60 (1), 47-57. <https://doi.org/10.1007/s10526-014-9621-8>
- Rodríguez-Gasol, N., Avilla, J., Aparicio, Y., Arnó, J., Gabarra, R., Riudavets, J., Alegre, S., Lordan, J., Alins, G., 2019. The contribution of surrounding margins in the promotion of natural enemies in Mediterranean apple orchards. *Insects* 10.