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Unveiling the diet of predatory mites through DNA metabarcoding—can abiotic factors affect prey detectability?*

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Despite the importance of predatory mites as biological control agents, the way that generalist species can maintain in agrosystems, the alternative prey they can feed on, the way they choose to eat one prey, or another are poorly known. For some phytoseiid predatory mite species, prey consumption has been characterized by lab tests (Cavalcante *et al*., 2015, 2017; Juan-Blasco *et al*., 2012; Oliveira *et al*., 2007). However, those approaches are sometimes difficult to perform, very time consuming and do not totally reflect interactions occurring in field conditions. New technologies that allow determining the diet of predatory mites in situ are highly desirable to supporting biological control programs. A promising avenue for deciphering the diet of predatory mites is offered by DNA metabarcoding. Although this approach has been used in the study of insects (Hosseini *et al*., 2008; Kaunisto *et al*., 2017; Paula *et al*., 2022), only starts to be applied to microarthropod biological control agents, as predatory mites (Navia *et al*., 2019). DNA metabarcoding was successfully applied to identify prey species of phytoseiid mites using group-specific primers. However, biotic and abiotic factors can affect the detectability of predatory mite preys through metabarcoding, as previously showed for studies using traditional molecular methods (=PCR Multiplex and Sanger sequencing) (Pérez-Sayas *et al*., 2015). This information is relevant to understanding the limits of using the methodology, to guide sample collection procedures, and to assure the correct interpretation of the results.

In this study we evaluated if the detectability of preys through DNA metabarcoding can be affected under different temperatures and post-feeding times for different phytoseiid species. Mites evaluated in this study, both prey species (*Tetranychus urticae* Koch and *Tetranychus ludeni* Zacher) and predators (*Phytoseiulus macropilis* (Banks), *Neoseiulus californicus* (McGregor) and *Amblyseius tamatavensis* (Blommers), were obtained from laboratory rearing under controlled conditions. The effect of post-feeding time (0, 2, 4, 6, 8, 12 h) and temperature (for *N. californicus* 15, 18, 25 and 28°C and for three post-feeding times: 4, 8 and 12 h) were evaluated. Mites were starved for 12 hours before starting essays and then put during 3 hours in presence of *Tetranychus* mites to feed on. After that, mites were transferred to an arena without food, and, at each temperature considered, time was counted. Specimens were then collected at the selected post-feeding times and stored in microtubes with alcohol 100% at -20 ºC. On the temperature tests, after feeding the mites were left in climatic chambers with each one of the studied temperatures for the reported times. For each essay, 10 replicates were performed, except for *P. macropilis* (n=5), totaling 270 specimens. Total DNA was extracted from individualized specimens. DNA was then amplified by PCR with group-specific primers (MiniCOI 4F&7R, a short COI fragment (280 bp)), designed by the team for amplifying the prey within the predators. High-throughput sequencing was performed on a NovaSeq lane (2x250nt). Sequence analyses were performed using the QIIME2 (Quantitative Insights Into Microbial Ecology) (Bolyen *et al*., 2019) platform. A final data cleaning treatment was performed using R software (R Core Team, 2021) package metabaR

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(Zinger *et al*., 2021), to evaluate and exclude false positive reads based on the controls from the sequencing plate, detect and exclude tagjumps and spurious reads. The filtered reads were converted into a binary presence/absence (of prey detection) type of data.

Among the total 150 predators sampled, 131 (87,33%) presented prey amplification. The methodology allowed to detect prey even after 12 hours post-feeding; detectability was not affected during the tested times. No difference in detectability was observed for the three phytoseiid species; 86.6%, 88.3% and 86.6% of positive reads were obtained for *P. macropilis*, *N. californicus* and *A. tamatavensis*, respectively. Similarly, prey was detected at all temperatures and times without significant differences; of the 120 predators evaluated, 95 (79.16%) led to positive detection results. Results are encouraging and showed that the tested abiotic factors did not significantly affect the metabarcoding detectability. So, it is now known that i) it is not necessary to store samples at low temperatures (not below 28°C) during the period between plant sample collection and storage of the predatory mites for the molecular analysis; ii) and that this period can be up to 12 hours (even if no prey is available for the mites), without impairing prey detectability. Obtaining this information makes easier the collection procedures, especially field collections. These results also highlight the higher sensitivity, efficiency, and stability of the metabarcoding methodology for detecting prey of predatory mites, when compared to traditional molecular methods. As a next step, it would be interesting to conduct experiments to evaluate the efficiency of the metabarcoding for detection of multiple prey species.

Keywords: Phytoseiidae, high throughput sequencing, biological control, trophic interactions

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