A DNA-based approach to study predator-prey trophic interactions within *Brassica* crops: a search for predators of diamondback moth (*Plutella xylostella*)

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Abstract

Brassica vegetables and oilseeds are economically important crops worldwide. These crops are associated with several destructive and widespread insect pests. In Australia these pests include six species, *Plutella xylostella* (Linnaeus), *Pieris rapae* (Linnaeus), *Hellula hydralis* Guenée, *Helicoverpa punctigera* (Wallengren), *Brevicoryne brassicae* (Linnaeus) and *Myzus persicae* (Sulzer), which are the focus of this research. Among them *P. xylostella* (diamondback moth or DBM) is the most serious and destructive insect pest.

Recently integrated pest management (IPM) strategies and the use of biological control methods have become the preferred approaches to controlling these pests over synthetic insecticides, due in part to the prevalence of insecticide resistance in *P. xylostella*. Pathogens and especially parasitoids play important roles in the control of diamondback moth. As a result, wide-ranging investigations of these natural enemies have been published. However little is known about the potential of predators, which may be able to contribute to control diamondback moth, although some field studies have shown the overall importance of predators in controlling this key pest. The aim of this study was to develop a method that allows study of predator-prey trophic interactions in the field.

While studies of trophic relationships are essential to understand the dynamics of predator-prey interactions, the actual experiments are difficult to perform because arthropod prey and predators are both small and often cryptic. Thus determination of the diets of predatory arthropods in the field can be complicated. Much of the published research on predators has involved manipulation of predators' habitats or use of

artificial arenas. As a result data gathered by these studies may not be realistic and therefore may not show how predators truly behave in the field.

Among techniques that do not interfere with the behaviour of predators, post mortem gut analyses, especially DNA-based methods, have recently received a great amount of attention. A variety of DNA-based approaches have been used to study trophic interactions including restriction fragment length polymorphisms, amplified fragment length polymorphisms, microsatellite variation and amplification of species-specific DNA sequences.

In this research the main objectives were to develop and evaluate a PCR-based approach that allows investigation of trophic relationships among important predators of diamondback moth and five other major pests under field conditions. The research was carried out in five main steps:

1) The abundance and activity of predators in the field were monitored with pitfall traps, sticky traps and a vacuum sampler. Identified predators included species of Coleoptera, Hymenoptera, Hemiptera, Diptera, Neuroptera, Dermaptera, Chilopoda, and Araneae.

2) A partial gene region of the Cytochrome Oxidase subunit I was sequenced from the six selected pest species. Species-specific primers were designed and developed for each of the six pests. Specificity tests confirmed each primer pair specifically amplifies prey DNA without cross-reactivity to predators or other non-target species that are commonly found in the same habitats. These molecular markers also allow amplification of a very small amount of target DNA in the presence of substantially

greater amounts of predator DNA. Although multiplexing of primers could potentially be used to detect the presence of multiple prey species in a single assay, the sensitivity of it compared with singleplex PCR was lower.

3) Some factors that could affect the detectability were evaluated. The detectability was negatively correlated with time. Prey detectability was different in three different predators with the longest retention time observed for *Venator spenceri,* an intermediate time for *Nabis kinbergii,* and the shortest for *Hippodamia variegata*. Subsequent food intake, sex and weight of predator did not influence detection of prey DNA. In *H. variegata* the rate of detection was decreased with increasing temperature. In addition, study uncovered potential sources of error caused by detection of prey DNA following secondary predation.

4) A multiplex PCR-primer system was developed for accurate identification of juveniles of seven species of wolf spiders that are difficult to identify based on morphology. These wolf spiders occur commonly in *Brassica* crops in South Australia. They are *Hogna crispipes, Hogna kuyani, Lycosa godeffroyi*, *Trochosa expolita*, *Venator spenceri, Venatrix pseudospeciosa*, and a new species in a new genus ('Species A'). Diagnostic DNA fragments for each of the target species allowed species identification.

5) Selected predators from the field were evaluated to determine trophic interactions among them and selected species of prey. Study of trophic relationships among all selected predators and their prey in *Brassica* fields by the DNA-based technique showed most selected prey are found in the diets of selected predators therefore

potentially they can be considered as a predators of them. These results will enable future researchers to confidently identify predators that attack six pests of *Brassica* crops.

Overall, this study demonstrated that a PCR-based technique is a valuable and promising tool to investigate trophic interactions among predators and their prey within *Brassica* crops. Many factors affect detection efficiency or rate of target prey DNA detection in the guts of predators, such as time since feeding and temperature. Therefore, future studies of trophic interaction among arthropods should focus on a particular prey and elucidate possible factors affecting detection before undertaking field investigations. If densities of prey and predator can be determined in the field, then determination of key predators of a particular pest will be possible. Moreover, future research may rely on multiplexing of primers for a quicker method to study large numbers of field-collected samples.