Seed Eating Ground Beetles: Assessing Plant Primers in PCR to Determine Trophic Interactions in Carabids

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Study Introduction

Figure 1.1: *Harpalus sp.* in a hemp field.

- Beetles (Coleoptera) are the most dominant insect species on earth. Carabidae, commonly known as ground beetles, are a ubiquitous family of beetles with more than 40,000 species identified worldwide and more than 900 known carabid species in Canada[1].
- Ground beetles are known as to be generalist predators. They have a continuum of diets which range from specialist to generalist, and carnivore to omnivore to herbivore, with the omnivorous and granivorous species consuming weed seeds and having the potential to control weeds in agroecosystems[2].
- Carabids can eat 200[3] to 1,000 weed seeds daily[4] and can consume up to 74% of certain species of weed seeds in agroecosystems[5].

The main objective of this study is to research how effectively carabids act as a biological control agent for pest plants. Previous methods of unravelling their cryptic feeding habits date back nearly seventy years and have included sentinel prey cards and laboratory seed choice tests[6]. Currently, molecular gut content analysis (MGCA) is used to identify trophic interactions for insects. The application of MGCA requires sequenced DNA of the target species of prey to create species-specific primers, which will bind to the target DNA during PCR to make over a billion copies of the target region. The use of MGCA to identify plant species consumed has been used to determine if a pest or beneficial insect was collected on casual, feeding, or shelter plants in the field[7].

Molecular Collection

There are various different methods of collection in our fields. The first is pitfall traps (Figure 1.2), small cups are placed into the ground for live samples to climb into, depending on the type of collection, the cups are either left empty or filled with antifreeze to act as a preservative. The second method is sticky traps (Figure 1.3), where insects simply become immobilized on the card. The third method of collection is sweeptnetting in the field, taking a net and brushing the surrounding area.

Figure 1.2: Live carabid samples (*Pterostichus. melanarius*).

Figure 1.3: Sticky trap placed in a wheat field.

Primers and PCR Testing

This research aims to test species-specific primers designed for crops and weeds in Alberta agroecosystems in PCR to be later used in a multiplex PCR for molecular gut contents. In our PCR testing, we are using primers to look for a specific amplicon length, which is the desired number of base pairs of DNA that the primers were designed for, and these primers display whether the weed seed DNA actually resides in our specimen's gut content. One of the primers we use gives us a known amplicon length of approximately 450 base pairs. We first conduct a single plex test, where only one primer set is used. Once these primers have been optimized they will be used to investigate the carabids omnivorous diet by being used in multiplex PCR.

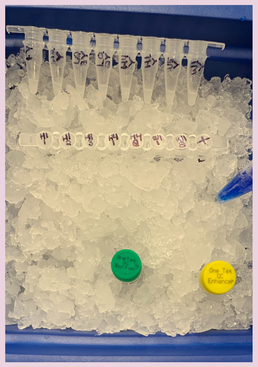

Figure 1.4: Buffers, subject DNA, Taq polymerase, electrophoresis ladder in an ice box.

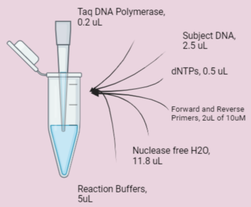

Figure 1.5: Procedure for preparing DNA and primer mix

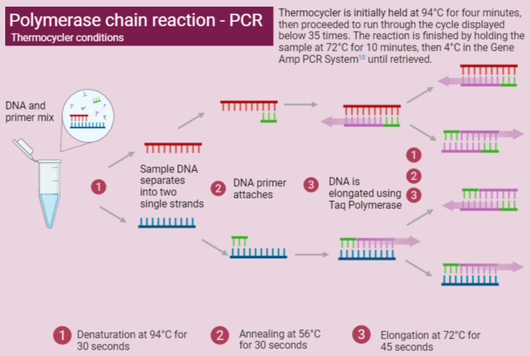

Figure 1.6: PCR test procedure and thermocycler conditions

DNA Confirmation

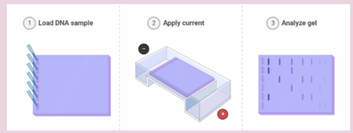

Figure 1.7: Agarose Gel Electrophoresis procedural diagram.

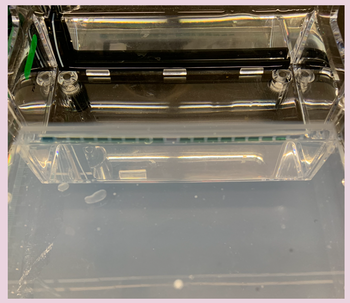

Figure 1.8: Gel loaded with DNA samples and ladder.

Figure 1.9: Electrophoresis test testing some general plant primers and species-specific plant primers (where C is canola, WO is wild oat, A-B are extracted DNA samples, 1-2 are general plant primers, and 3-6 are species-specific plant primers).

Electrophoresis tests are used for many different things, in our study it is used to confirm the presence of DNA. A 1% agarose gel was made with SYBR Safe[10]. and left to set with a 20-well gel comb where the DNA is loaded into. Since DNA is negatively charged, it is loaded on the negative end. Once there is a current the DNA moves towards the positive end and because DNA has the same amount of charge per mass, the small fragments move through the gel further than the larger ones. The Tritrack 6X[10] dye and 1k kB+ ladder[10] were loaded with the DNA and the gel ran for 1 hour. Photographs of gels were taken using the ChemiDoc Imaging System[11], since DNA bands are not typically visible to the naked eye.

Technology manufacturers: 9. (Thermo Scientific, Wilmington, DE) 10. (Thermo Scientific, CA, USA) 11. (Biorad, Hercule, California, USA)

Conclusion

These general plant primers effectively determine what carabid species are regularly ingesting. The testing methods and processes we use will help determine what weed seeds carabids are eating, and if they are consuming the same weed seeds in the field as they are in controlled lab settings. This research can be applied to other opportunistic omnivorous insects to determine if they are consuming weed seeds and could be considered beneficial insects. Many carabid species prove to be beneficial in laboratory testing, we hope that this research involving molecular gut content analysis leads to more conclusive results in terms of biological control to enhance integrated pest management of weeds in our cropping systems.

Table 1.1: The DNA concentration, 260/280 ratio, and 260/230 ratio measured using Nanodrop 2000E[9].

Sample ID	Concentration (ng/ul)	260/280 ratio	260/230 ratio
Canola #2	25.4	1.94	1.13
Canola #3	16.3	2.05	1.13
Wild Oat #2	27.5	1.97	1.08
Wild Oat #3	27.2	1.93	1.12

References & Acknowledgements

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1. (Lindroth C.H., 1961-1969. The Faunal Connections between Europe and North America. New York: Wiley 344) 2. (Westerman P.R., Hofman A., Vet L.E.M. & Van der Werf W. 2003. Relative importance of vertebrates and invertebrates in epigeic weed seed predation in organic cereal fields. Agric. Ecosys. Environ. 95: 417– 425) 3. (Gaines H.R., & Gratton C. 2010. Seed predation increases with ground beetle diversity in Wisconsin (USA) potato agroecosystems. Agric. Ecosys. Environ. 13: 329-336) 4. (Honek A., Martinkova Z., Saska, P.,& Pekar, S. 2007. Size and taxonomic constraints determine the seed preferences of Carabidae (Coleoptera). Basic Appl. Ecol, 8:343– 353) 5. (Jonason D., Smith H. G., Bengtsson J. & Birkhofer, K. 2013. Landscape simplification promotes weed seed predation by carabid beetles (Coleoptera: Carabidae). Landsc Ecol. 28(3): 487-494). 6. (Davies M.J. 1953. The contents of the crops of some British carabid beetles. Entomol. Mon. Mag. 89:18-23.) 7. (Garcia-Robledo C., Erickson D.L., Staines C.L., Erwin T.L., & Kress W.J. 2013. Tropical plant-herbivore networks: Reconstructing species interactions using DNA barcodes. PLoS ONE, 8, e52967)