Molecular method for monitoring greenhouse insect populations

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Introduction

- Greenhouses are controlled environments containing communities of plant-associated insects and their natural enemies.
- CO1 is a mitochondrial gene that codes for the electron transport protein cytochrome oxidase 1 and is highly conserved across insect taxa.
- The primer set HCO/LCO is widely used to amplify CO1.
- HCO/LCO is not a viable primer set for a small amount of insect taxa, including the family *Pseudococcidae* (mealy bugs) and superfamily *Coccoidea* (scale insects).
- The preliminary objective of this study is to develop a primer set that is viable for a wide variety of insects including *Pseudococcidae* and *Coccoidea*.
- The end objectives of this study are to develop standardized PCR and qPCR methods for the rapid identification of greenhouse insect pests and the insects that control those populations.

Methods

- Insect specimens were collected from the WISER Center greenhouses and vouchered in 96% ethanol at -20° C.
- DNA was extracted via the Qiagen DNeasy Blood and Tissue extraction kit.
- DNA extractions from five species, *P. longispinus, P. citri, S. coffeae, C. hesperidum* and *A. nerii,* were diluted 1:10, and then amplified via PCR using primer set HCO 2198/LCO 1490.
- PCR product underwent thermocycling, with an annealing temperature of 50° C.
- The amplicon was visualized using UV gel electrophoresis in a 1.2% agarose gel, ran for 45 minutes at ~100 v and stained for two hours in a 0.05 M Ethidium Bromide bath.
- The process was repeated with four additional taxa, *Parthenothrips dracaenae , C. montrouzieri, Formicidae* and *A. solani*, modifying the annealing temperature to 52° C.
- The process was repeated for all nine taxa, decreasing the dilution of *P. longispinus, P. citri, S. coffeae, C. hesperidum* and *A. nerii,* to 1:5, then amplifying with the primer set Calvin/LCO 1490, and annealing at 50° C.

P. citri "Citrus Mealy Bug", photo by Chase Bond

Results

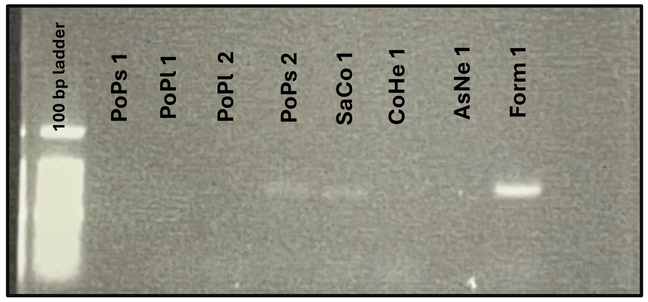

Fig. 1, 1.2% agarose gel containing PCR of samples PoPs1, PoPl1, PoPl2, PoPs2, SaCo1, CoHe1, AsNe1 and Form1.

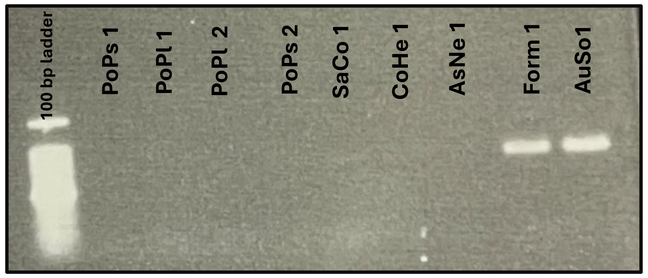

Fig. 2, 1.2% agarose gel containing PCR of samples PoPs1, PoPl1, PoPl2, PoPs2, SaCo1, CoHe1, AsNe1, Form1 and AuSo1.

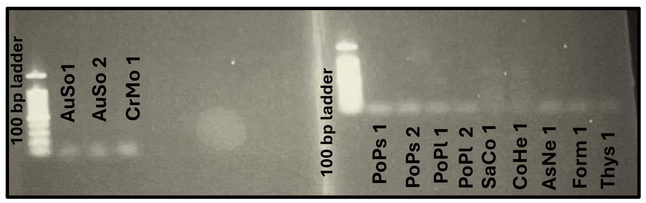

Fig. 4, 1.2% agarose gel containing PCR of samples PoPs1, PoPs2, PoPl1, PoPl2, SaCo1, CoHe1, AsNe1, Form1, Thys1, AuSo1, AuSo2 and CrMo1.

Table 1. details each taxa and their corresponding codes.

Taxa	Code
Cryptolaemus montrouzieri	CrMo 1
Aulacorthum solani	AuSo 1
Aulacorthum solani	AuSo 2
Aulacorthum solani	AuSo 3
Formicidae	Form 1
Parthenothrips dracaenae	Thys 1
Pseudococcus longispinus	PoPs 1
Pseudococcus longispinus	PoPs 2
Planococcus citri	PoPl 1
Planococcus citri	PoPl 2
Saissetia coffeae	SaCo 1
Coccus hesperidum	CoHe 1
Aspidiotus nerii	AsNe 1

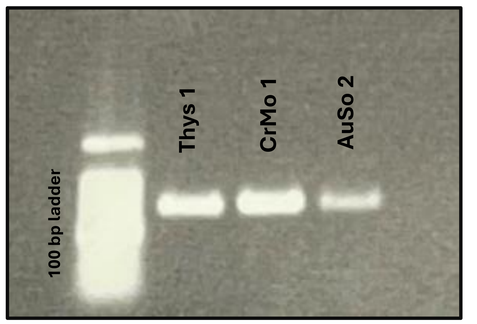

Fig. 3, 1.2% agarose gel containing PCR of samples Thys 1, CrMo 1 and AuSo 2.

We found that there is significant variability in the CO1 barcoding region across insect taxa. Strong single banding, and thus successful amplification, was achieved using primer set HCO 2198/LCO 1490 for *C. montrouzieri, A. solani*, *Formicidae* and *P. dracaenae* when annealing at both 50° C and 52° C (see figs. 1-3). However, either extremely faint banding or nothing at all, and thus unsuccessful amplification, was achieved using primer set HCO 2198/LCO 1490 for *P. longispinus, P. citri, S. coffeae, C. hesperidum* and *A. nerii* annealing at both 50° C and 52° C (see figs. 1-3). None of the taxa sampled were successfully amplified using the primer set Calvin/LCO 1490 and annealing at 50° C (see fig. 4).

Discussion

Our research goal is to develop a qPCR analysis method to identify all taxa, and their respective populations, represented in the greenhouses. This process requires PCR products which yield strong amplification of the target sequence, and thus far we have not been able amplify the CO1 barcoding region in any mealy bug or scale insect samples. Therefore, analysis of these samples has halted for the time being, and qPCR analysis of the working PCR products is imminent. If we discover a universal primer set, analysis of the mealy bug and scale samples may proceed. Future investigation includes testing the viability of the primer set PcoF1/LepR1 as proposed by Park et al [2] in their development of a universal primer set which was successful at amplifying a diverse range of taxa, including several species of mealy bug and scale insects. Another possible primer set proposed by Lin et al [3] is the combination of the primers PcoF1 [2] / HCO 2198, which successfully amplified several species of mealy bug and scale insect. Identifying an ideal primer set for the range of taxa present in the greenhouse is the next step towards developing a standardized PCR and qPCR method for our research.

Acknowledgements

This research was made possible by the Kilmer Fund, the LOUGHEED Center For Applied Learning and the SUNY Potsdam Biology Department. We would like to specially thank Rachel Wallace for her expertise, Raymond Bowdish for his care of the WISER Center and Dr. Robert Snyder for his patience and for lending his photography equipment.

Resources

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2. Park DS, Suh SJ, Oh HW, Hebert PD. Recovery of the mitochondrial COI barcode region in diverse Hexapoda through tRNA-based primers. BMC Genomics. 2010 Jul 9;11(1).
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C. hesperidum "Brown Soft Scale", photo by Chase Bond

P. longispinus "Longtail Mealy Bug", photo by Chase Bond

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WISER Center

Assessing the insect taxa present in the WISER Center greenhouses

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Introduction

- Extreme outdoor conditions, like cold and hot temperatures, heavy rain, or predation, can periodically reduce insect populations.
- Greenhouses are controlled environments where parameters such as temperature, humidity and amount of light can be set to a narrow range.
- Thus, greenhouses create optimal conditions for pest population growth, requiring releases of beneficial insects or other pest control strategies.
- Within the WISER Center greenhouses (Potsdam, NY), there are a variety of insect taxa including occasional large populations of aphids, mealy bugs and scale insects.
- Integrated Pest Management (IPM) uses cultural, chemical and biological controls to manage pests and diseases on crops.
- IPM methods utilized in the WISER Center include the use of biological controls and the application of the organic pesticide neem oil.

Table 1, sampled insect taxa and their corresponding common name, order and order common name. * indicates biological control.

Order	Order Common Name	Taxa	Common Name
Coleoptera	Beetles and weevils	*Cryptolaemus montrouzieri*	Mealy Bug ladybird*
Diptera	Flies	*Aphidoletes aphidomyza*	Aphid midge*
Hemiptera	True bugs	*Pseudococcus longispinus*	Longtail Mealy Bug
Hemiptera	True bugs	*Planococcus citri*	Citrus Mealy Bug
Hemiptera	True bugs	*Saissetia coffeae*	Hemispherical scale
Hemiptera	True bugs	*Coccus hesperidum*	Brown soft scale
Hemiptera	True bugs	*Aspidiotus nerii*	Armored scale
Hemiptera	True bugs	*Aulacorthum solani*	Glasshouse potato aphid
Hemiptera	True bugs	*Macrosiphum euphorbiae*	Potato Aphid
Hymenoptera	Bees, wasps and ants	*Formicidae*	Ants
Hymenoptera	Bees, wasps and ants	*Aphidius ervi*	Ervi wasp*
Hymenoptera	Bees, wasps and ants	*Aphidius colemani*	Colemani wasp*
Thysanoptera	Thrips	*Parthenothrips dracaenae*	Palm thrips

Aphids

M. euphorbiae "Potato Aphid", photo by Chase Bond

A. solani "Glasshouse Potato Aphid", winged female, photo by Chase Bond

Aphids are "true bugs" in the order Hemiptera, suborder Sternorrhyncha and the family Aphididae. Aphids mostly reproduce parthenogenetically[1], which means that their offspring are clones of the females and require no fertilization for reproduction. This method of reproduction allows aphids to reproduce very rapidly, which is a major problem in a greenhouse setting. Most aphids are polyphagous, which means that they feed on many different plants. The species pictured above generally feed on plants in the family Solanaceae, which includes tomatoes, potatoes and peppers, but in our specific greenhouse setting, the main host plants for aphids are leafy greens like lettuce and arugula that we grow in our tower gardens (pictured below).

Mealy Bugs

P. citri "Citrus Mealy Bug", photo by Chase Bond

P. longispinus "Longtail Mealy Bug", photo by Chase Bond

Mealy bugs are "true bugs" in the order Hemiptera, suborder Sternorrhyncha and family Pseudoccoccidae. Females are covered in a waxy flocculence of powder or spikes. Most species lay egg sacs, also covered in the waxy material, which protects their offspring from predation. Mealy bugs are stationary feeders that feed on the sap of plants, which is mostly water and nutrient poor. Thus, once they find a location to begin feeding, they will remain there indefinitely, unless disturbed, to intake large volumes of sap. Mealy bugs produce a waste product called honeydew on the leaves of their hostplant, which promotes the growth of mold and bacteria, and reduces photosynthetic abilities for the plant and exposes them to disease[1]. Mealy bugs mostly affect ornamental plants, but certain species are also known to feed on citrus plants, and tropical crops like pineapple and sugar cane[1].

Scale Insects

C. hesperidum "Brown Soft Scale", photo by Chase Bond

C. hesperidum "Brown Soft Scale", photo by Chase Bond

S. coffeae "Hemispherical Scale", photo by Chase Bond

Scale insects are "true bugs" in the order Hemiptera, the suborder Sternorrhyncha and two families, Coccidae (soft scales) and Diaspididae (armored scale). They often reproduce parthenogenetically, so their offspring are female clones. Their reproduction is limited by seasonality and temperature[1]. Greenhouses have regulated temperature, sunlight and humidity within a narrow range, so their population growth goes nearly unchecked, sometimes producing between three and six generations per year[1]. Like mealy bugs, scale insects are also stationary feeders that attach to the leaves of plants to feed, often near veins or stems, inhibiting sunlight and causing defoliation[1]. In particular, Soft Scale causes damage similar to mealy bugs because they produce honeydew, which grows mold[1]. Armored scale insects cause damage by injecting toxins into the plant's cells during the feeding process, causing brown or yellow patches to appear on the leaves, eventually killing them[1].

IPM (Integrated Pest Management)

Integrated Pest Management (IPM) is a means for controlling pests in a way that reduces chemical pest control methods. This involves various methods, for example; scouting for pests, identifying them and tracking their life cycles, and determining the pest's host preference and dispersal patterns. With this information, biological controls are strategically released to control pests. If pest populations continue to grow, minimally damaging insecticides are sprayed[2]. The objective of IPM is to effectively control pest populations below an economic threshold. In the WISER Center, we generally adopt the mantra "scout, identify, monitor, and spray (as needed)". This program minimizes pest populations without overprescribing chemical application. Chemical control with Neem oil (extracted from the Neem Tree, *Azadirachta indica),* is sprayed as a safe and organic insecticide[3].

Biological Controls

A. aphidomyza "Aphid Midge", photo by Chase Bond

A. ervi "Parasitic Aphid Wasp", photo by Chase Bond

Biological controls are organisms which are introduced to help regulate the populations of certain species of concern. Some bio-controls regulate populations through predation, like *Cryptolaemus montrouzieri,* of the order Coleoptera and the family Coccinellidae, a ladybird beetle that eats mealy bugs. However, many bio-controls regulate populations through parasitism. The parasitoid wasps, of the order Hymenoptera and the family Braconidae, *Aphidius ervi* and *Aphidius colemani*, lay their eggs inside aphids, which then hatch and parasitize the aphid from the inside out[1]. *Aphidoletes aphidomyza* is a type of fly known as a midge, which lays its eggs on leaves near aphid colonies[1]. When the larvae hatch, they inject a digestive toxin into the aphids and then sucks the digested fluid out[1].

Resources

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