Brock University

Cool Climate Oenology & Viticulture Institute

Laboratory trials of entomopathogens (EPFs) as a biocontrol agent against Grape Mealybug (GMB)

CCOVI Lecture Series-March 27th, 2024

Malkie Spodek, PhD

Photo credit: M. Spodek

Partners:

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Ms. O. Devries (3rd year Biotechnology undergraduate student, Brock University)

Funders:

Ontario GRAPE & WINE RESEARCH Incorporated

Greenhouse Technology Network

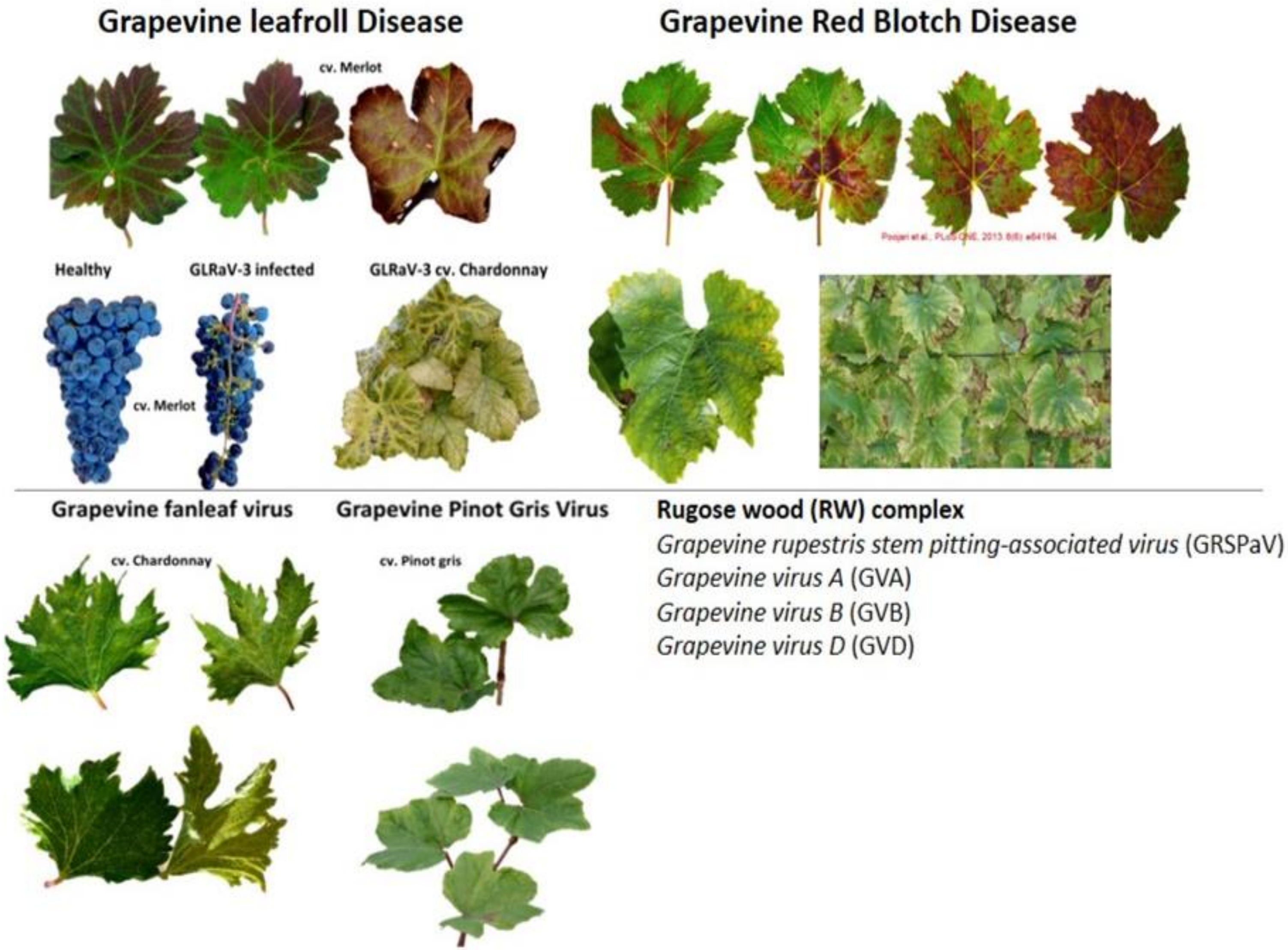
Brock University

Explore Grant

Outline of Talk

- Introduction to mealybugs and their importance in the wine industry
- Identity of Grape Mealybug in Ontario (GMB)
- Current management and treatment of Grape Mealybug in Ontario
- Establishing a laboratory rearing of GMB
- Experiments using entomopathogenic fungi (EPF) to treat GMB
- Future research directions

Major vineyard viruses in Canada



- Clean plant program
- Imported material
- Insect vectors

Photo credits: S. Poojari

Leafroll virus and insect vectors in North American vineyards

Photo credit: S. Poojari

Virus	Mealybugs	Soft scale insects
GLRaV-1	Bohemian mealybug (*Heliococcus bohemicus*)	*Pulvinaria vitis*
Grapevine	Apple mealybug (*Phenacoccus aceris*)	*Parthenolecanium corni*
leafroll virus-1	Obscure mealybug (*Pseudococcus viburni*)	*Neopulvinaria innumerabilis*
	Citrophilous mealybug (*Pseudococcus calceolariae*)	
	Grape mealybug (*Pseudococcus maritimus*)	
	Comstock mealybug (*Pseudococcus comstocki)*	
GLRaV-3	Bohemian mealybug (*Heliococcus bohemicus*)	*Pulvinaria vitis*
Grapevine	Vine mealybug (*Planococcus ficus*)	*Neopulvinaria innumerabilis*
leafroll virus-3	Citrus mealybug (*Planococcus citri*)	*Parthenolecanium corni*
	Longtailed mealybug (*Pseudococcus longispinus*)	*Coccus hesperidium*
	Citrophilous mealybug (*Pseudococcus calceolariae*)	*Coccus longulus*
		Saissetia sp.
	Grape mealybug (*Pseudococcus maritimus*)	*Parasaissetia nigra*
	Obscure mealybug (*Pseudococcus viburni*)	*Ceroplastes rusci*
	Comstock mealybug (*Pseudococcus comstocki*)	
GLRaV-4 and its strains -5, -6, and -9		
	Vine mealybug (*Planococcus ficus*)	*Ceroplastes rusci*
	Longtailed mealybug (*Pseudococcus longispinus*)	
	Apple mealybug (*Phenacoccus aceris*)	

Photo credit: M. Spodek

Table: Updated from Rayapati et al., 2014. Plant Disease / Vol. 98 No. 9

Grapevine leafroll on cv. Chardonnay

Grapevine leafroll on cv. Merlot

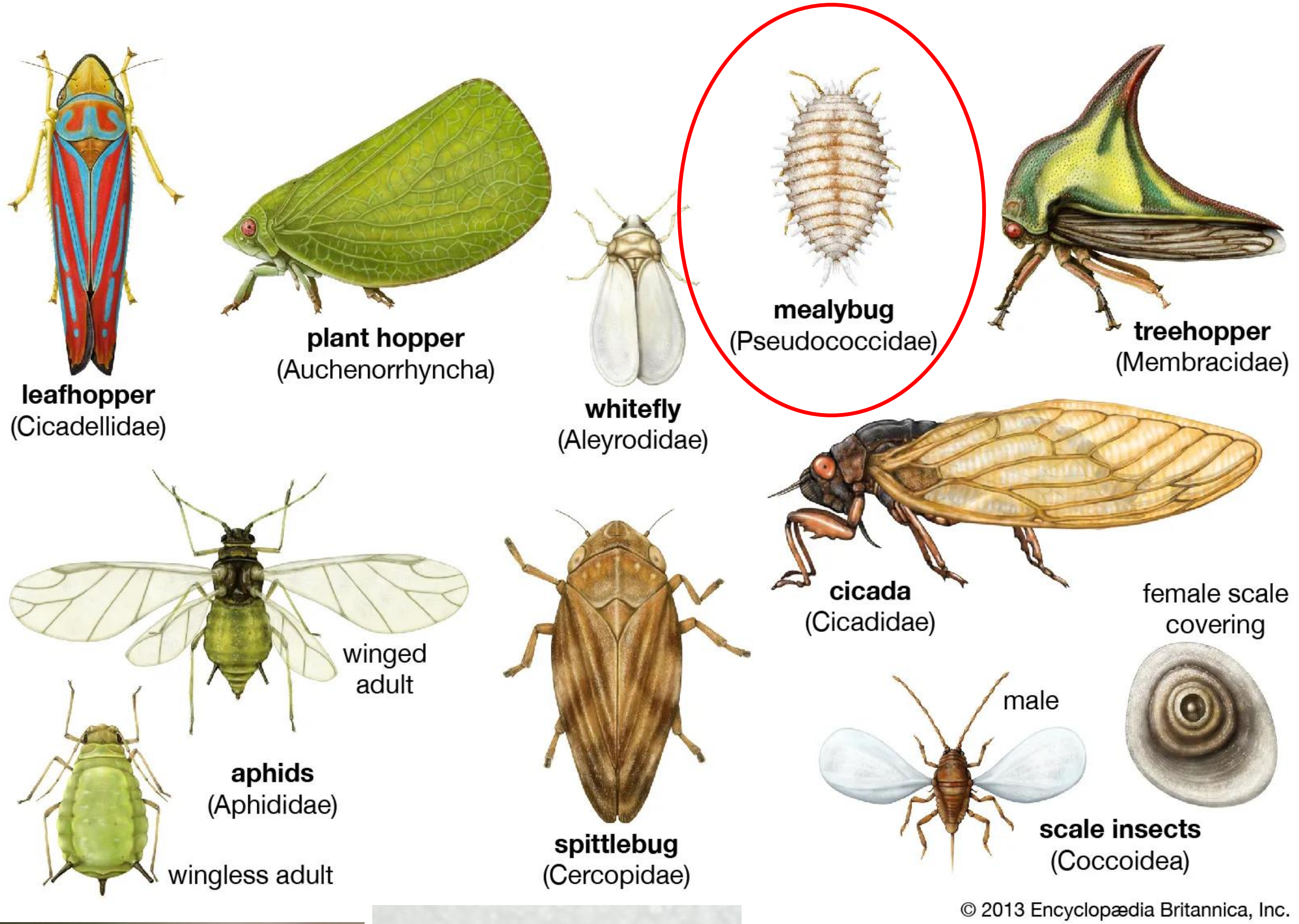
Virus symptoms:

- Red or purple coloration on mature leaves with green veins in red-fruited cultivars
- Yellowing on the mature leaves in white-fruited cultivars
- Backward rolling of mature leaf margins commonly found in both white and red fruited cultivars
- Uneven ripening of berries and thin clusters
- Reduced yield and poor quality of grapes would result in significant economic losses

Long-term productivity losses of up to CAD$298,784 per hectare (ha)

Photo credits: Poojari, 2020

Hemipteran insects

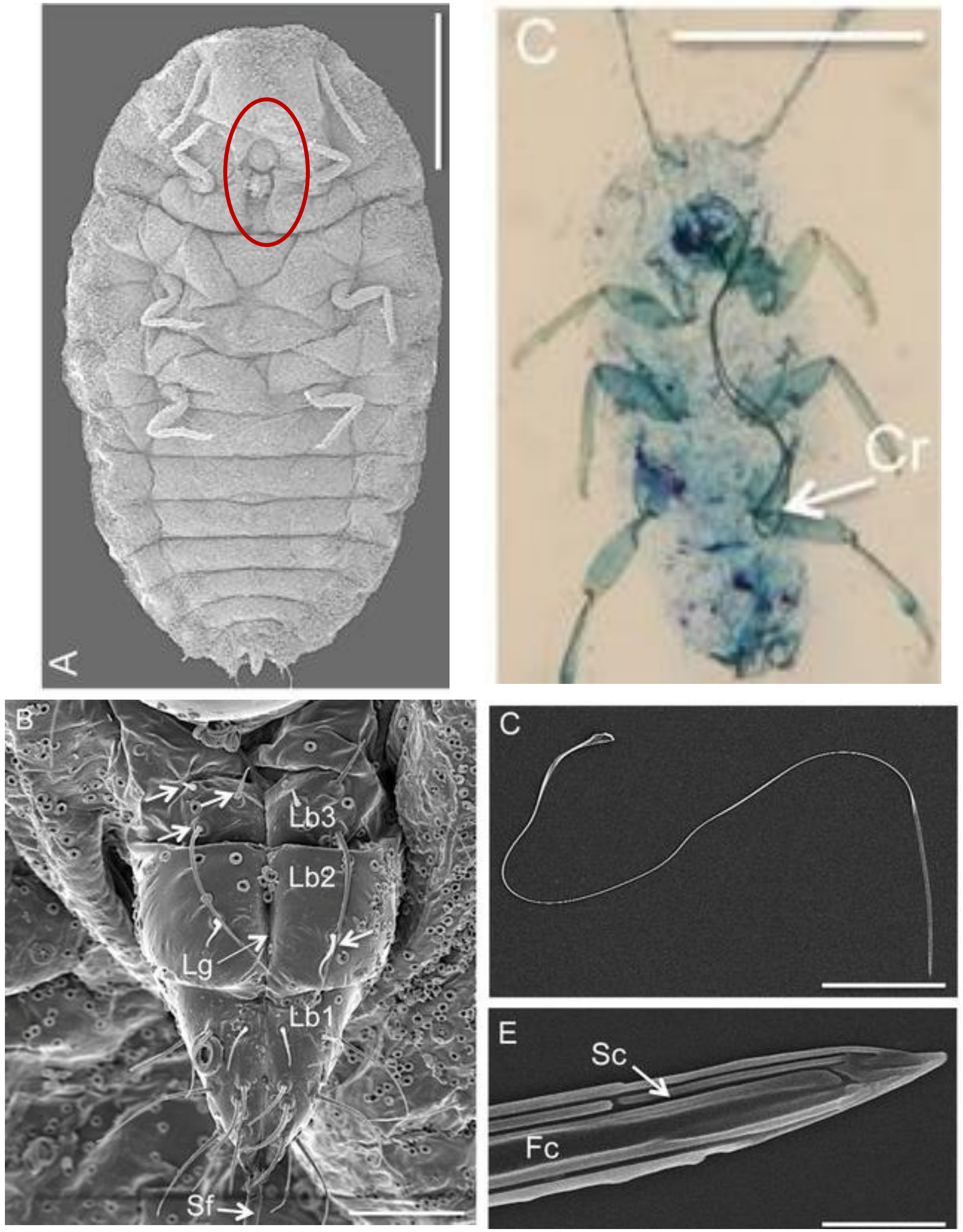


- Major agricultural and forestry pests
- Piercing and sucking mouthparts
- Direct feeding on plant sap (phloem) damages plant vitality and quality of fruit/vegetables
- Vectors of plant viruses

plant hopper

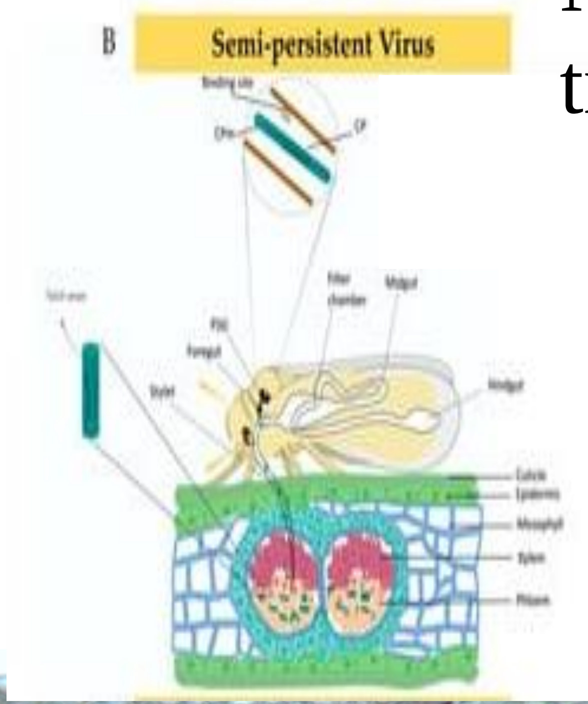
Source: Lawrence Barringer, Pennsylvania Department of Agriculture, Bugwood.org

Mealybug mouthparts

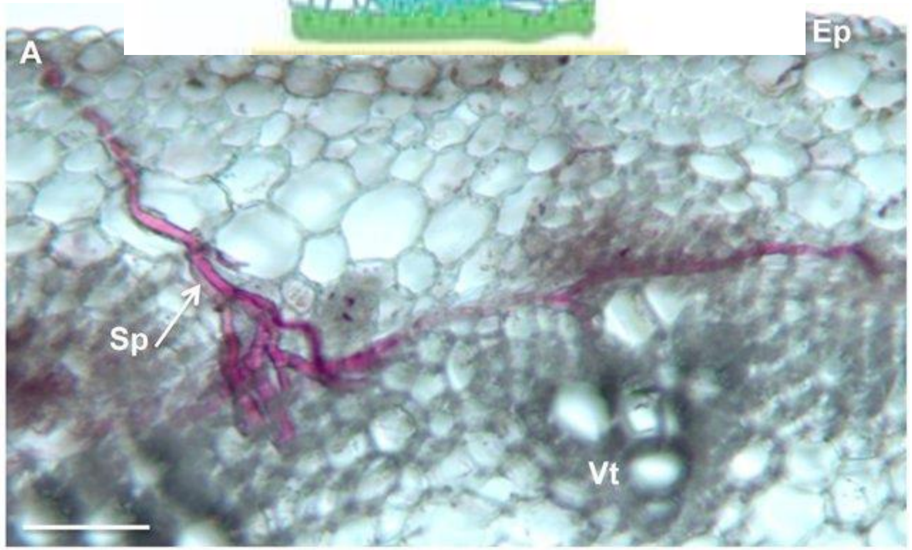


Source: Alliaume, A. et al. (2018). Bulletin of Insectology. 71.

Mode of virus transmission



Source: Catto, M.A. et al (2022). Cells



Light micrograph of a grapevine petiole cross section showing the stylet pathway (Sp) from epidermis (Ep) to vascular tissues (Vt);

Grape Mealybug in Ontario (GMB)

- Soft bodied insects

- Sexual dimorphism
(different appearance of males and females)

- Males don’t have mouthparts and live a few days

- Feed on vine sap (phloem) not on grapes

- Live most of life under the bark of vines

- Not known to feed on other plants in Canada

- Instars are vectors of vine viruses; Leafroll 1 and 3

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Photo credits: M. Spodek

GMB Lifecycle

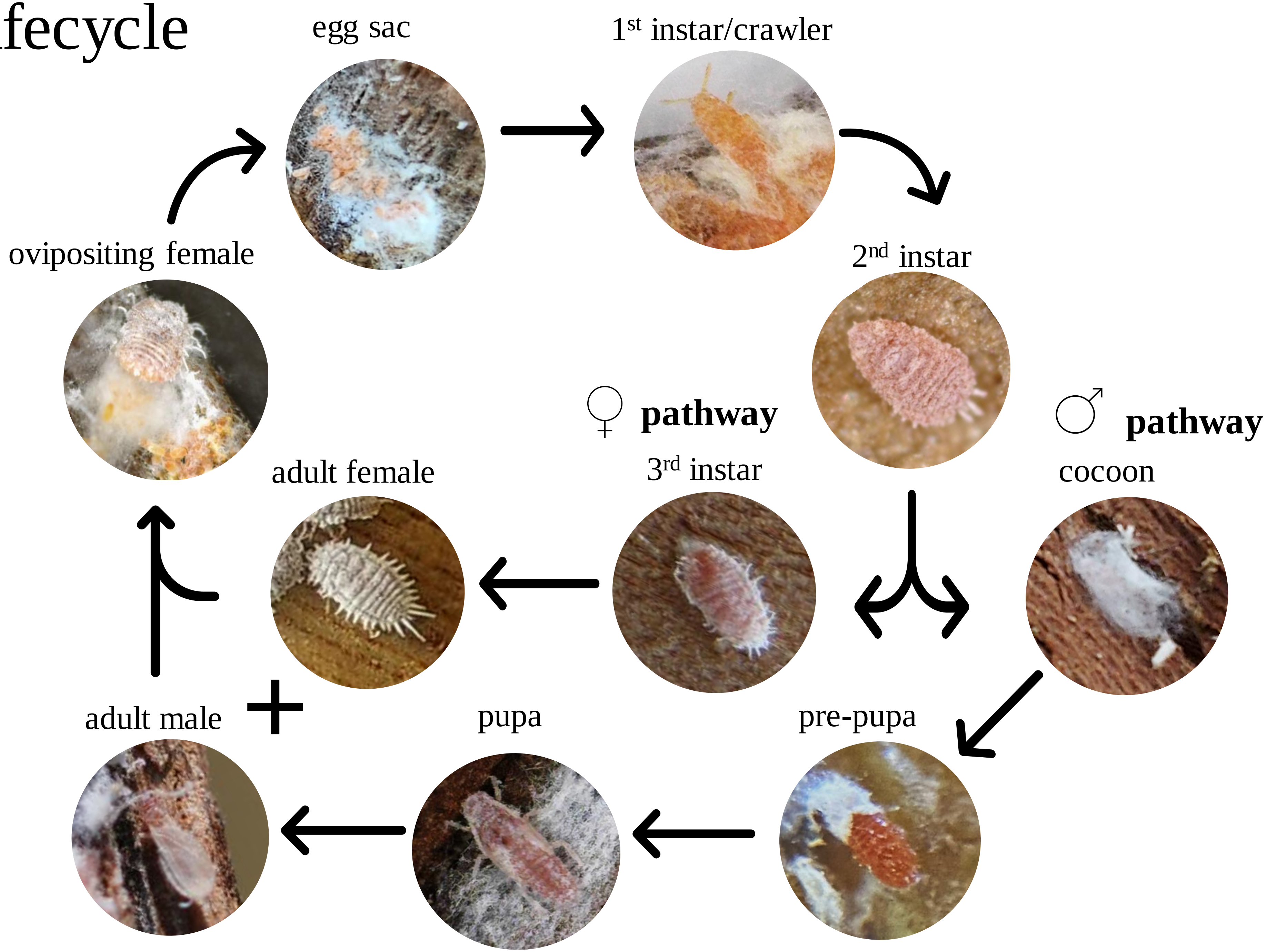
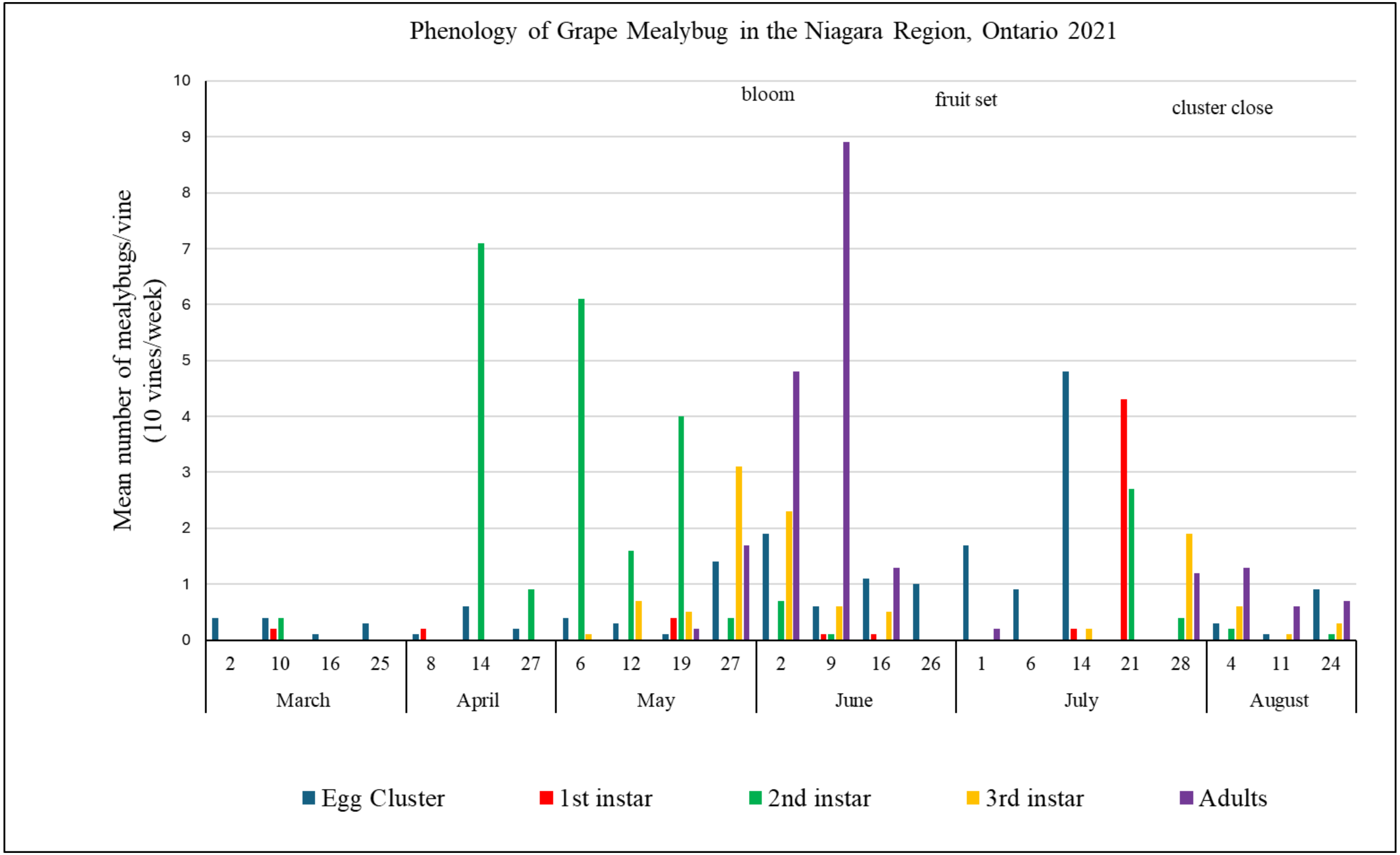


Photo credits: M. Spodek
O. Devries
S. Poojari
W. McFadden-Smith



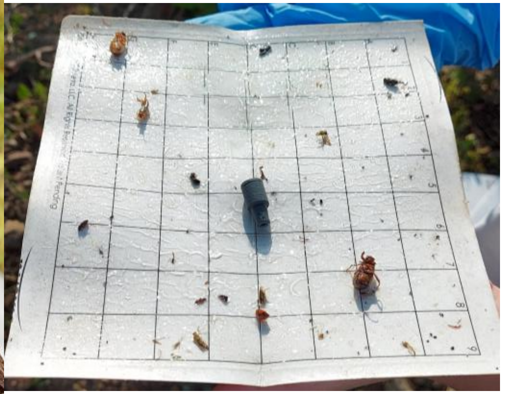
Source: Dr. W. McFadden-Smith, OMAFRA

Monitoring using pheromone traps for male mealybugs

pheromone baited sticky trap in vineyard

Photo credits: M. Spodek

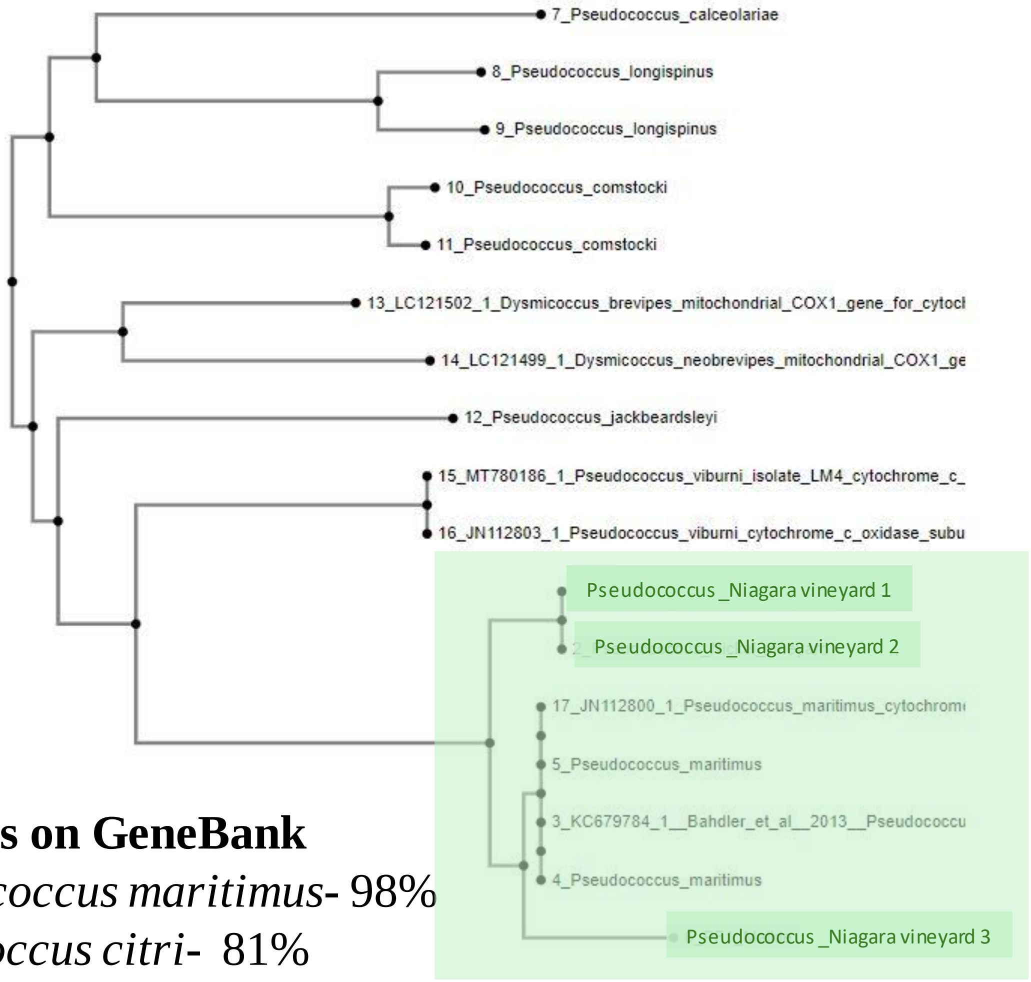
Photo credit: W. McFadden-Smith



insects on sticky trap with pheromone bait in the center

male mealybug on sticky trap

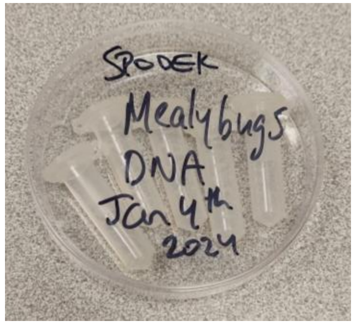
Identity of Ontario's grape mealybug



Matches on GeneBank
Pseudococcus maritimus- 98%
Planococcus citri- 81%

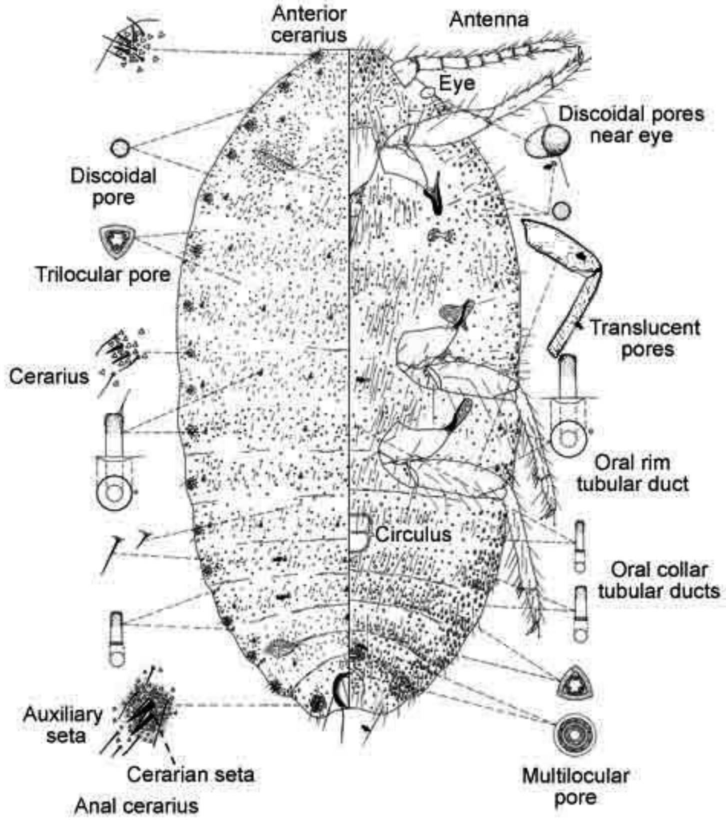
Tree credit: Dr. V. Cezar Pacheco da Silva, Universidad de la República, Uruguay

unpublished data



Partial CO1 Sequencing
CAATGCATATTATTCTGCCATA
TTAATAATTTATTTTAAAAT-
TTTTATTTTATAAT
AATTAAATTTTCATTAAATGTA
TGATTTAAATTTGGATTATTAT
TTAATCATTCAGGATT

Pseudococcus maritimus (Ehrhorn)



Adult female

Miller, D., A. Rung, G. Parikh, G. Venable, A.J. Redford, G.A. Evans, and R.J. Gill. 2014. *Scale Insects, Edition 2*. USDA APHIS Identification Technology Program (ITP). Fort Collins, CO. <https://idtools.org/scales/>

Treatment options for GMB in Ontario

Very toxic for Honeybees	Very toxic for Honeybees	Unknown toxicity for Honeybees	Unknown toxicity for Honeybees	Non toxic for Honeybees	Non toxic for Honeybees
IRAC 1B	IRAC 23	NC	NC	NC	NC
Insecticide & Miticide*	Insecticide*	Insecticide, Miticide & Fungicide*	Insecticide, Miticide & Fungicide*	Insecticide & Miticide*	Insecticide*
Malathion 85 E	**Movento 240 SC**	**Vegol Crop Oil**	**Purespray Green Spray Oil 13E**	**Kopa Insecticidal Soap**	**Opal Insecticidal Soap**
a.i.(s): malathion	*a.i.(s): spirotetramat*	*a.i.(s): canola oil*	*a.i.(s): mineral oil*	*a.i.(s): potassium salts of fatty acids*	*a.i.(s): potassium salts of fatty acids*
1/1 matched pests: Mealybug	1/1 matched pests: Mealybug	1/1 matched pests: Mealybug	1/1 matched pests: Mealybug	1/1 matched pests: Mealybug	1/1 matched pests: Mealybug

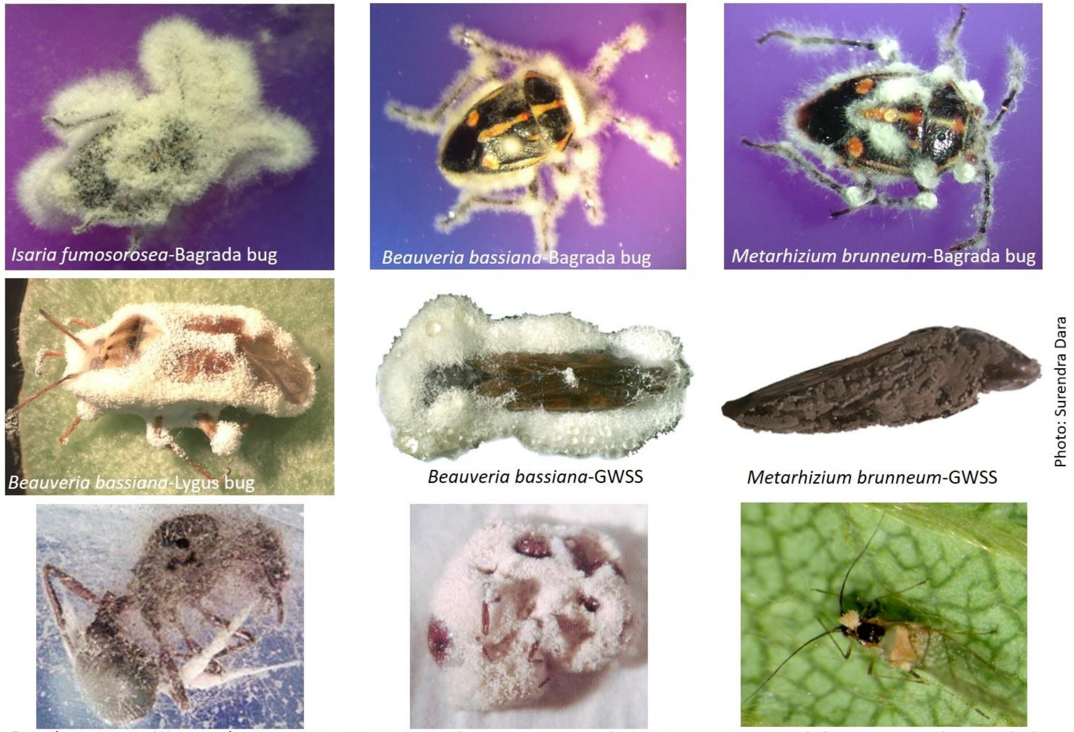
= Potentially organic

= View details to see warning.

Ontario | Crop Protection Hub

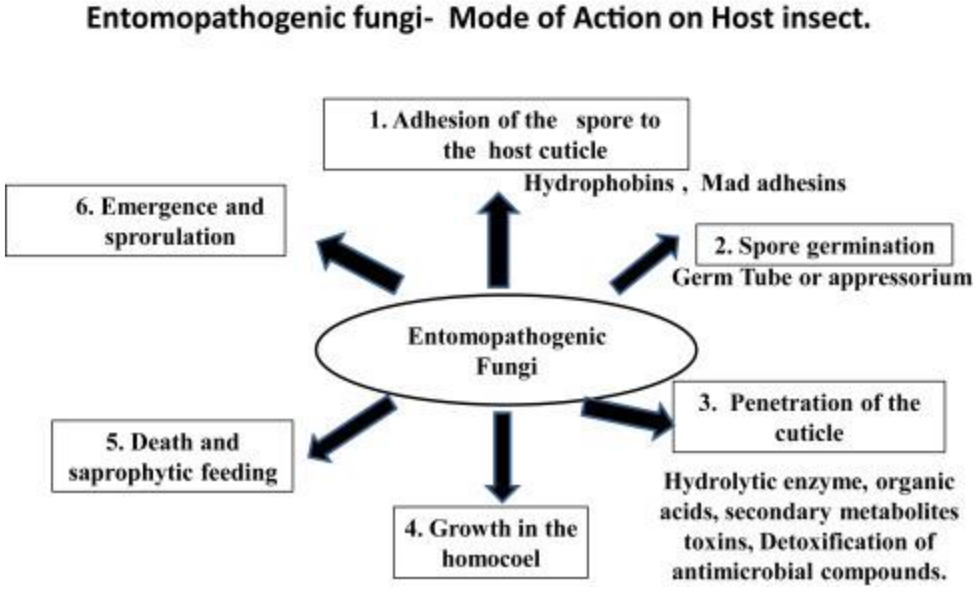
Alternative treatment for mealybugs- Entomopathogenic fungus (EPF)

- Entomo-insects
- pathogenic-disease causing
- fungal spores directly penetrates through the exoskeleton of the insect and kill it



Isaria fumosorosea-Bagrada bug
Beauveria bassiana-Bagrada bug
Metarhizium brunneum-Bagrada bug
Beauveria bassiana-Lygus bug
Beauveria bassiana-GWSS
Metarhizium brunneum-GWSS
Paecilomyces sp.-Western harvester ant
Beauveria bassiana-Western harvester ant
Entomophthora sp.-Strawberry aphid

Photo: Surendra Dara



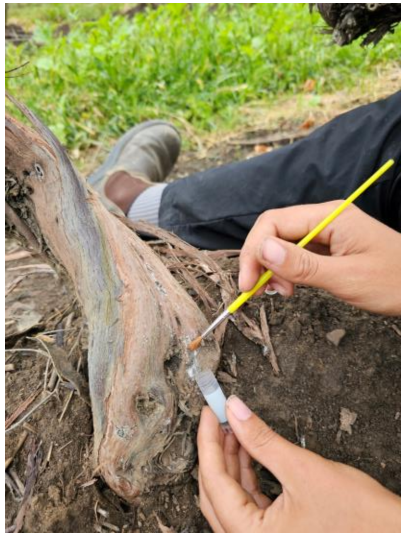
Sinha et al. (2016) Chapter 15 - Entomopathogenic Fungi, Ecofriendly Pest Management for Food Security, Academic Press, 475-505

Objectives

- To establish a mealybug rearing for lab trials
- To test 2 commercial EPF products against Ontario’s Grape Mealybug (GMB)
- To determine which product and what concentration is most effective again GMB

Collecting mealybug egg sacs and crawlers September/October 2023

Vineyard in Beamsville, ON

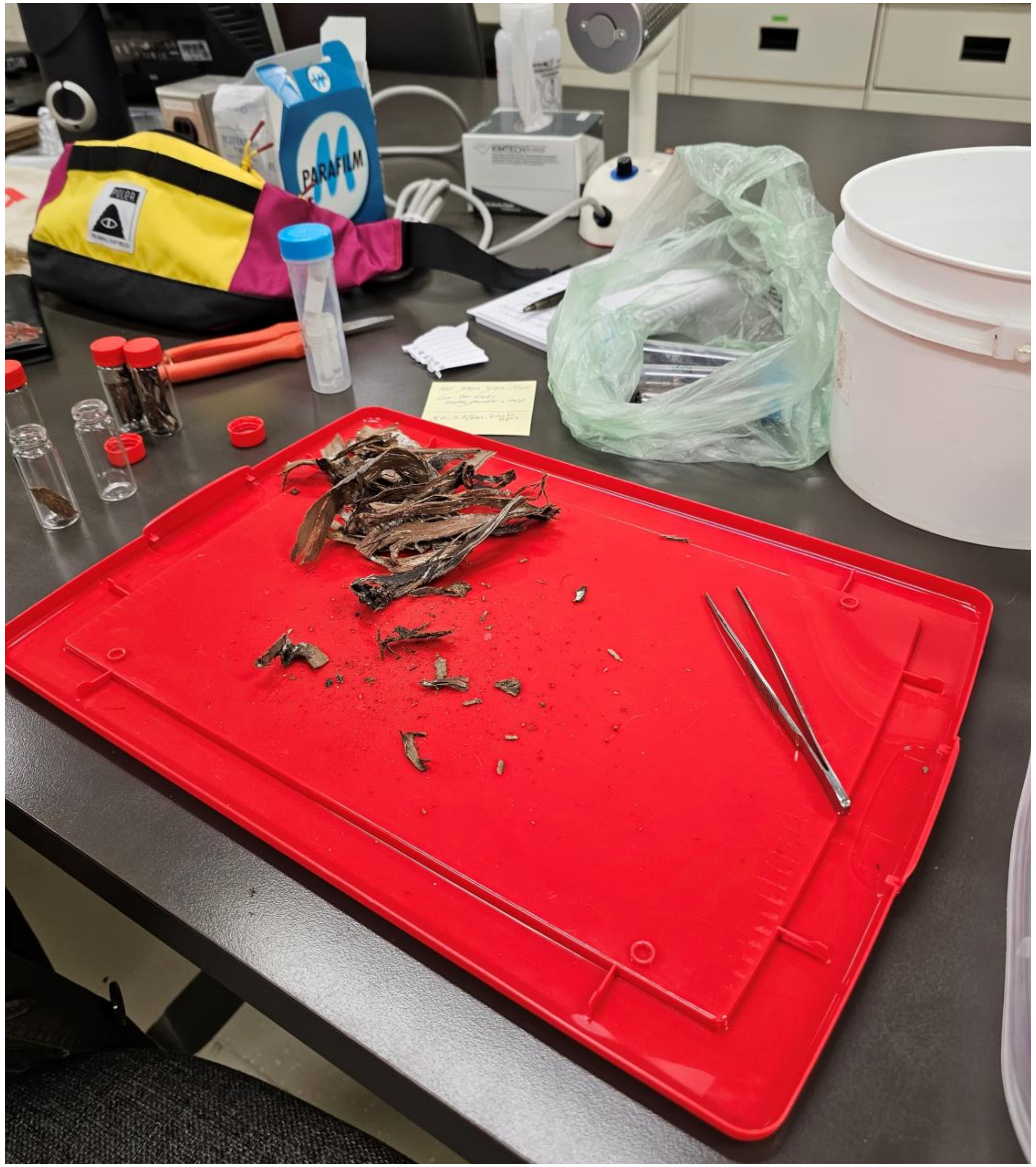


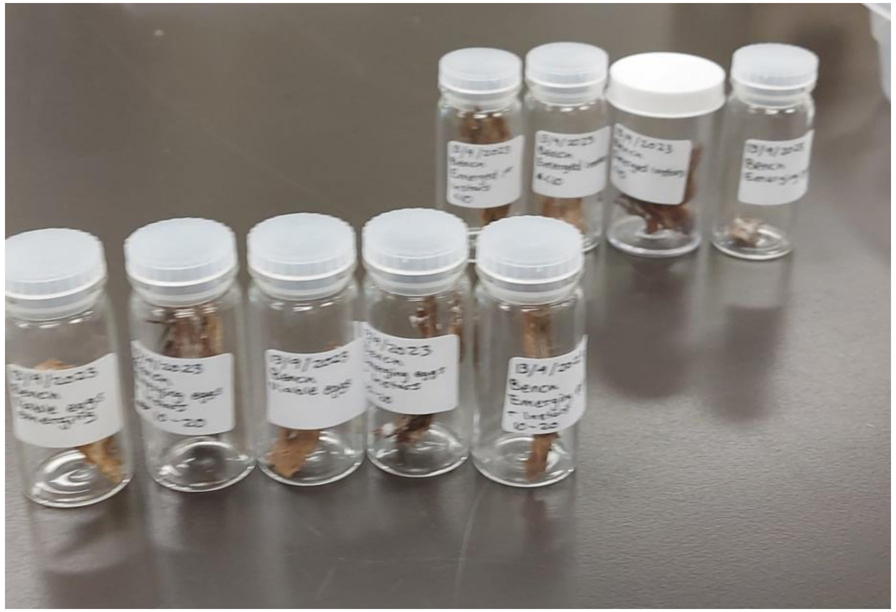
egg sac

1st instar/crawler

Photo credits: M. Spodek

Sorting through collected material in lab

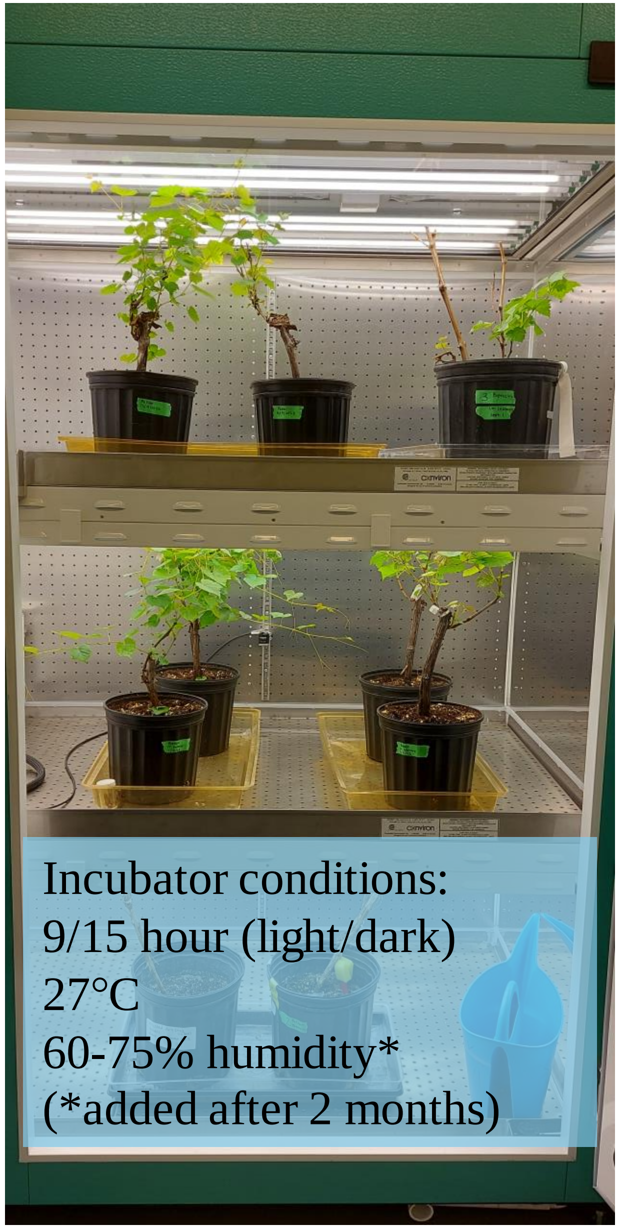




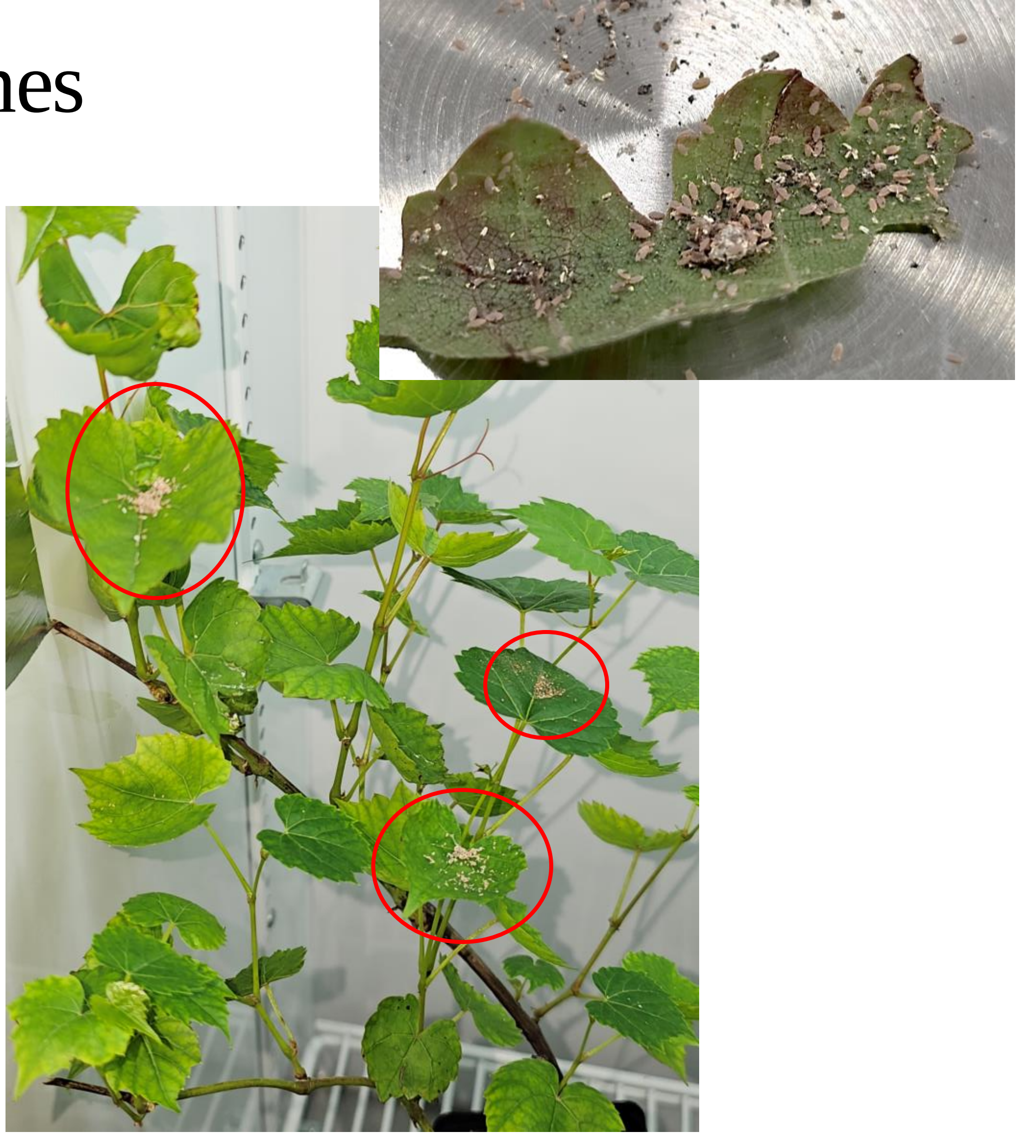
Egg sacs and 1st instars on bark preserved in refrigerator at 4°C

Photo credits: M. Spodek

Infesting potted organic and "treated" vines



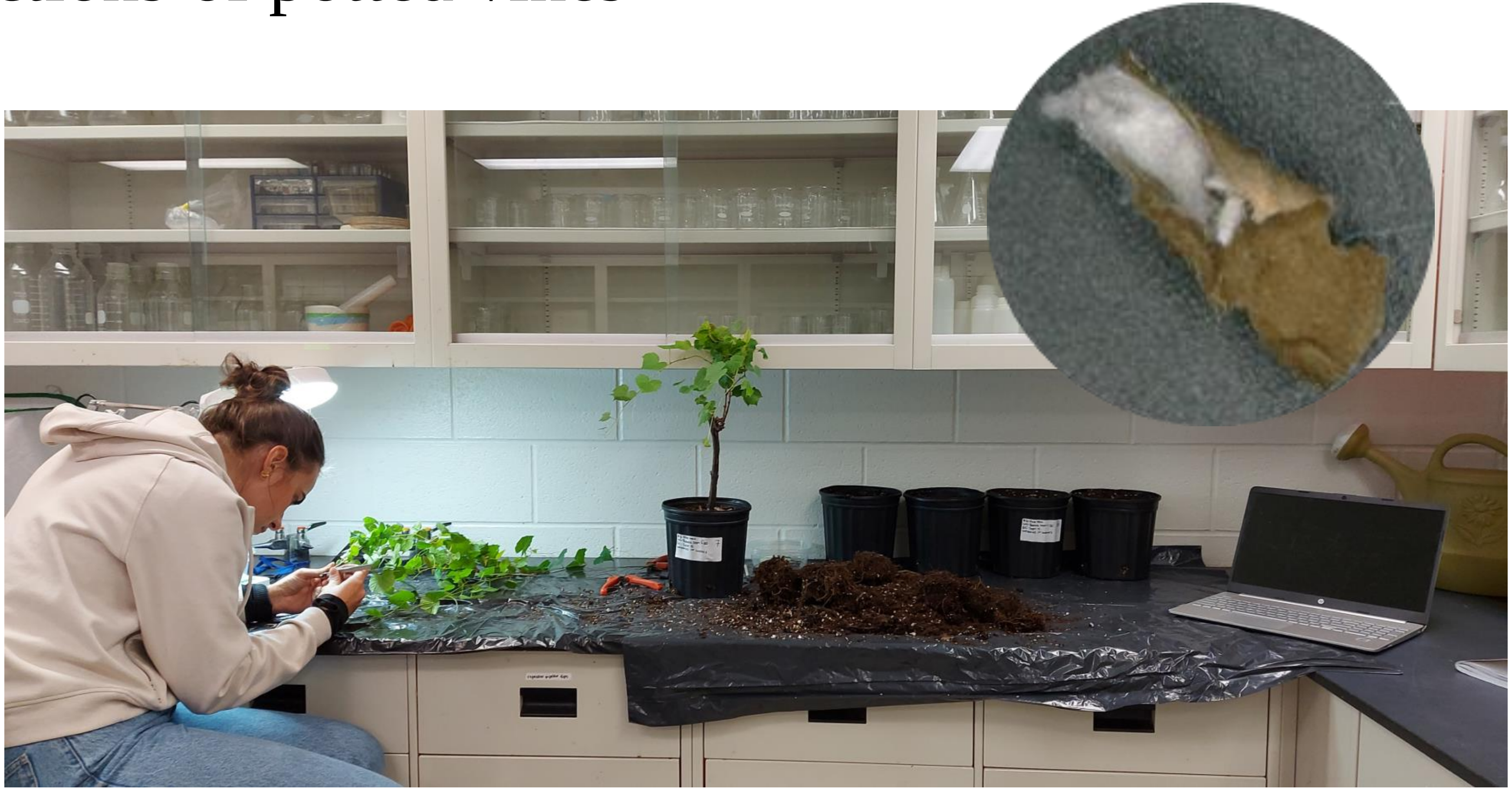
Infesting potted vines with infested bark collected from vineyards



Infesting potted vines with crawlers collected from vineyards

Photo credits: M. Spodek

Weekly inspections of potted vines



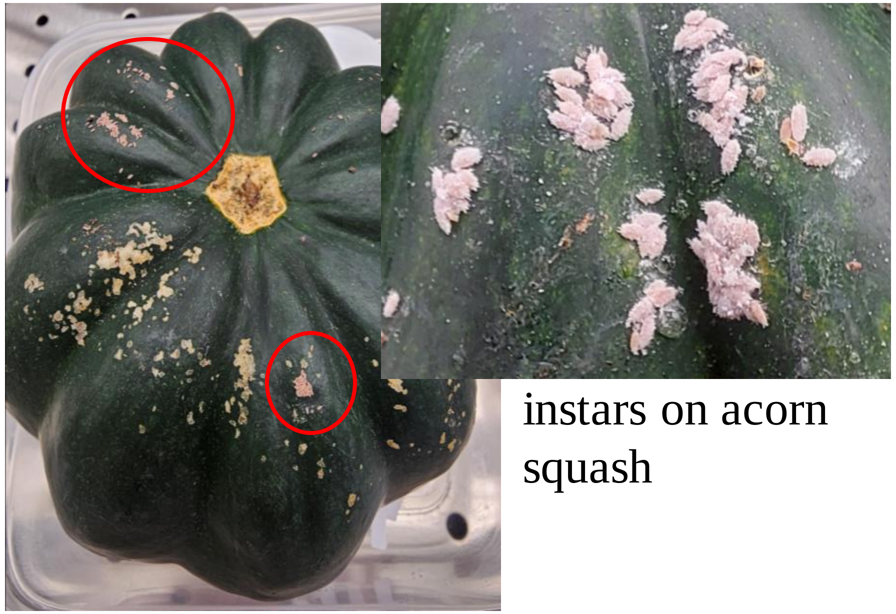
male cocoon in leaf

- All insects that were infested 2 months earlier were found dead, except for evidence of male cocoons
- Eliminated 10 potted vines from rearing after inspecting all parts of vine, including roots
- Cause of mealybugs death is potted vines were treated with a broad spectrum neonicotinoid insecticide, just prior to infestation

Photo credits: M. Spodek

Alternative food trials for mealybugs

acorn squash | potato (Russet) | pumpkin | butternut squash



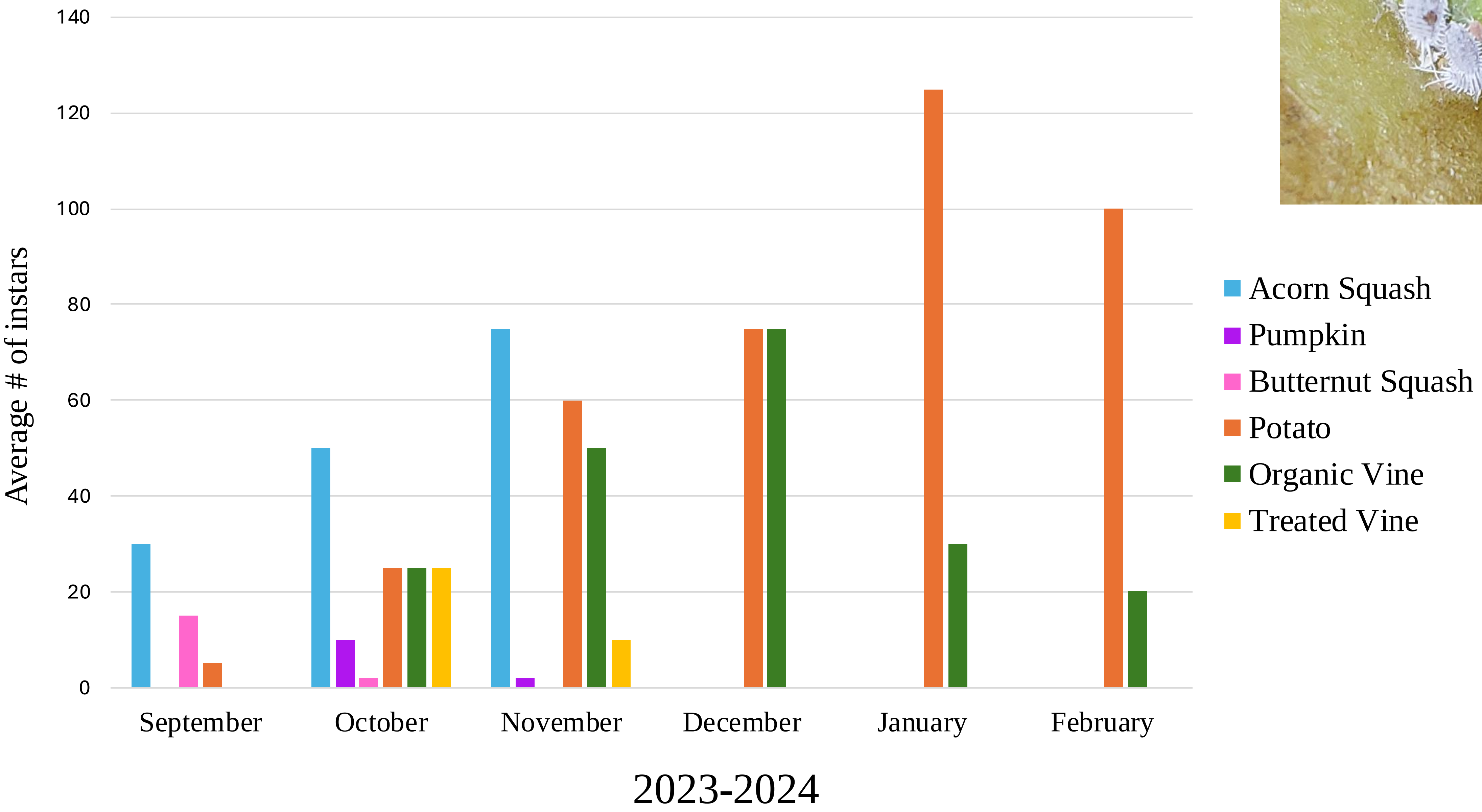
instars on acorn squash

adult females and ovisac on potatoes

instars on pumpkin

Photo credits: M. Spodek

Mealybug Host Trials



Rearing surprises and challenges

- Sprouting potatoes
- Rotting potatoes after one month
- Migrating females for ovipositing (egg laying)
- Slow development, dry eggs and low eclosion (hatching) rates
- Humidity levels

Photo credit: M. Spodek

Sprouting potatoes and variety trials

wrapped in moist paper towel and cloth

sprouted under plastic

sprouted in soil

sprouted in greenhouse

sprouted in dark and dry conditions

Potato Varieties (organic)

Sweet Potato

Yellow

Red

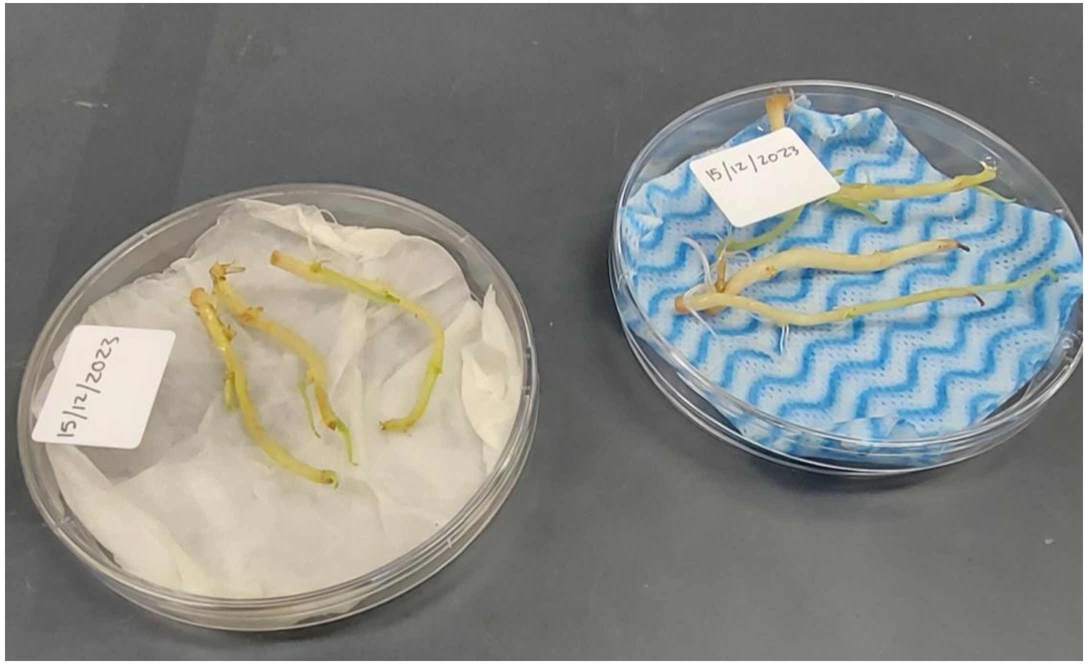
Russet

Kennebec

Photo credits: M. Spodek

Rearing surprises and challenges

Work around for rotten potatoes



sprouts removed from potato

half potato on moist cloth

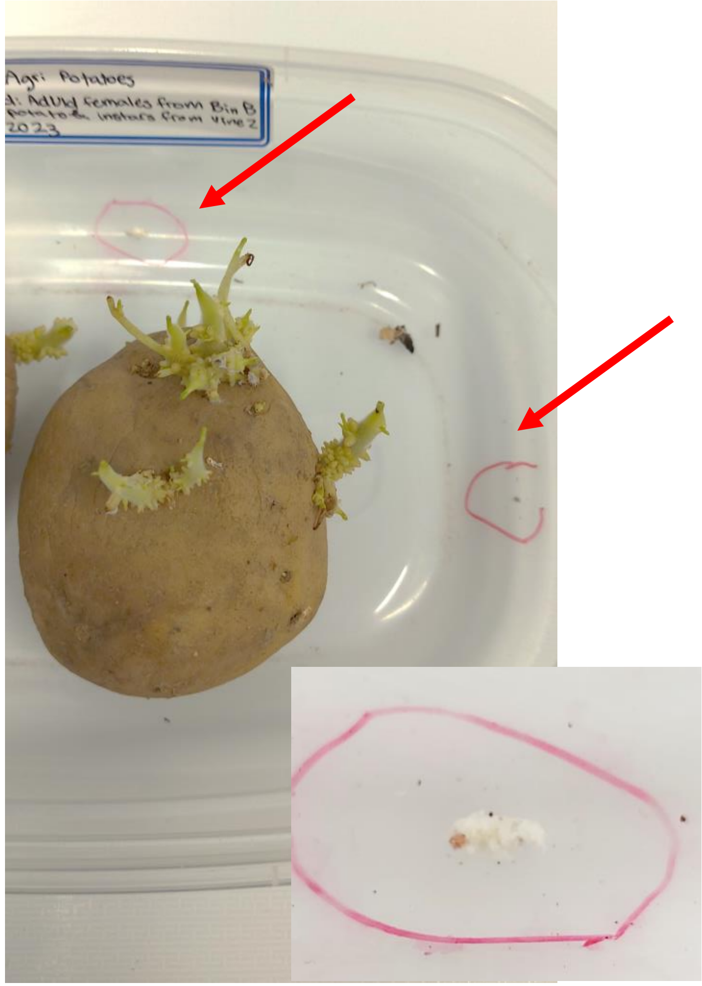
male cocoon on rotten potato

leaf bioassay

Photo credits: M. Spodek

Migrating females for ovipositing (egg laying)

incubator on loan from Prof G. Tattersall, Biological Sciences, Brock University



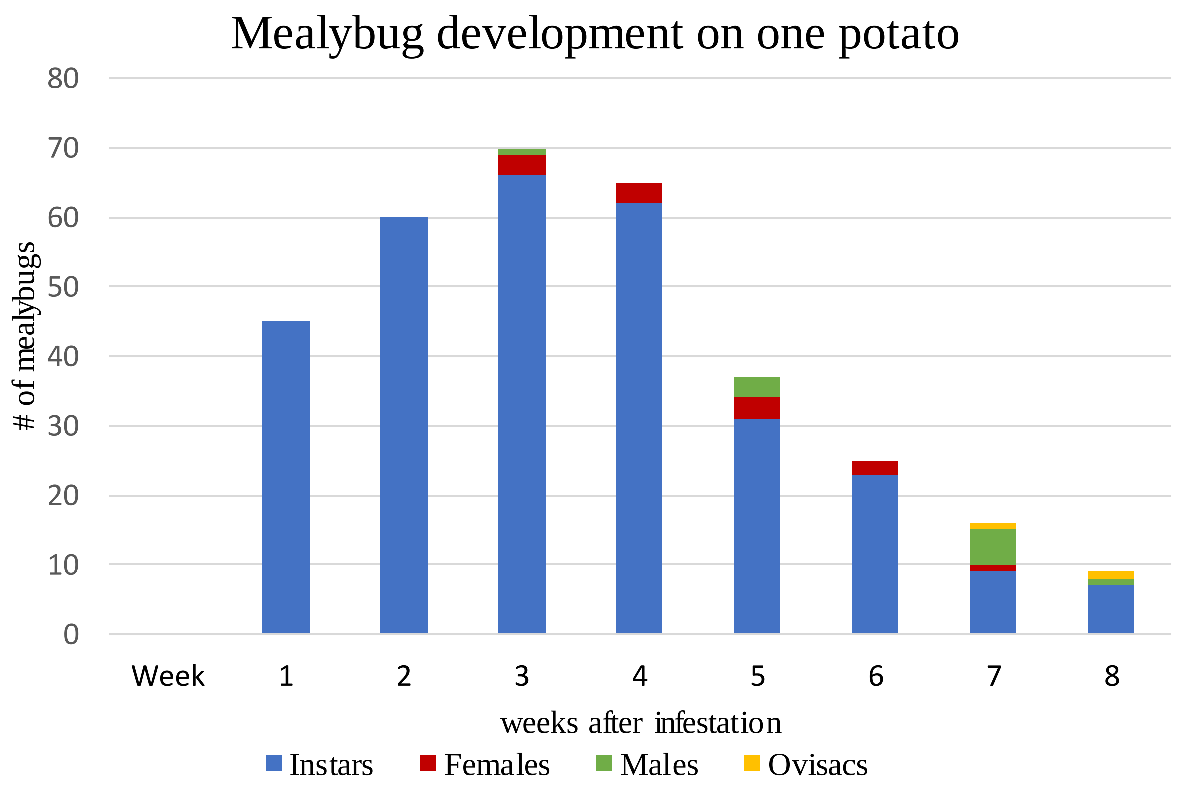
egg sacs on plastic container

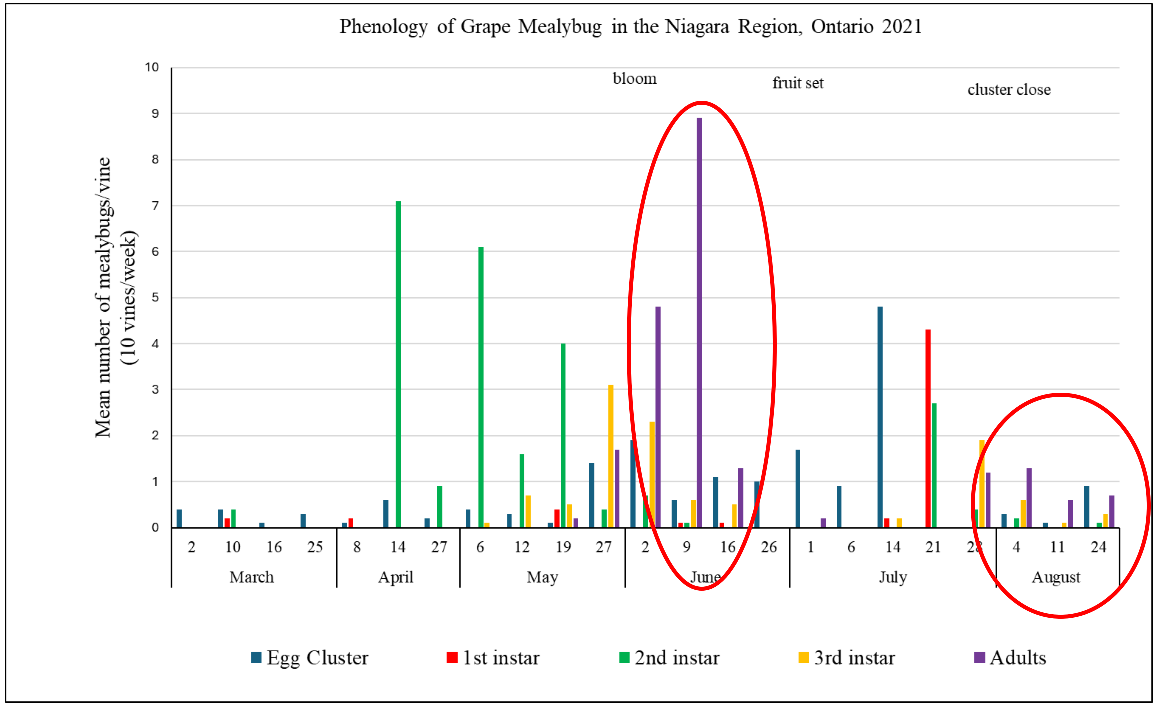
egg sac with emerging crawlers on vial cap

egg sac rescued from water bin

Photo credits: M. Spodek

Slow development, dry eggs and low eclosion (hatching) rates





Unpublished data

Source: Dr. W. McFadden-Smith, OMAFRA

Incubator and humidity trials

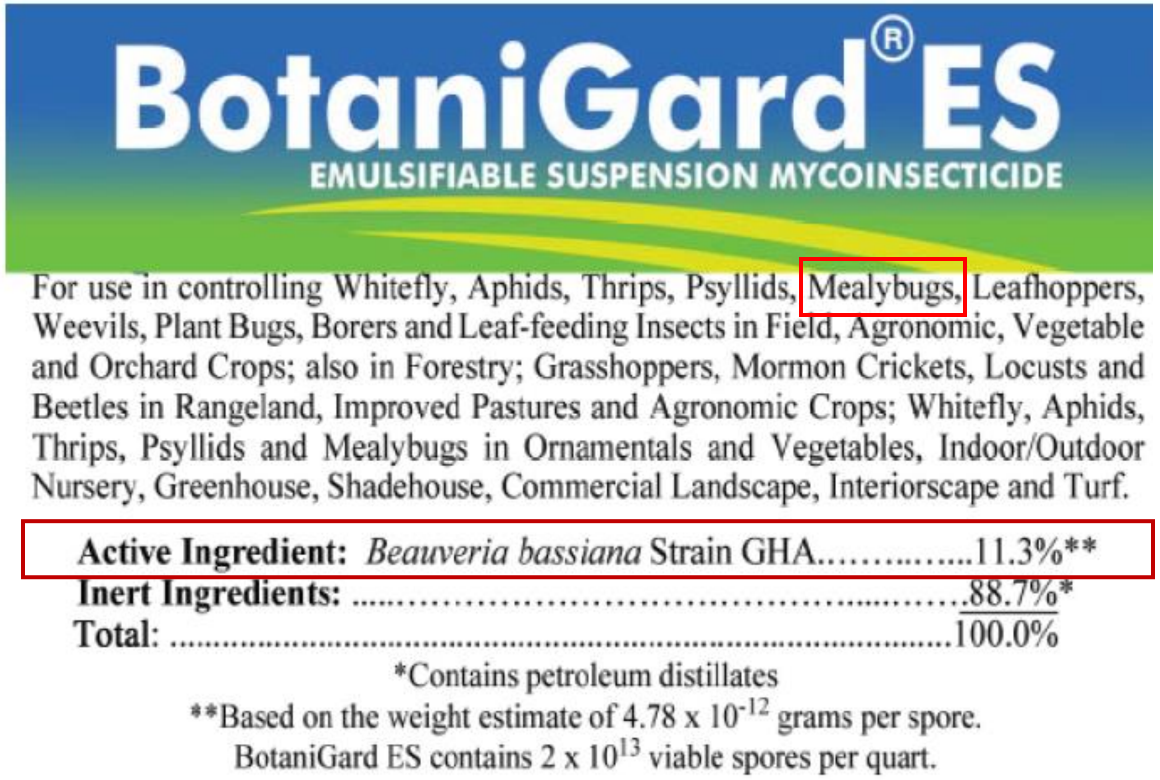
chicken egg incubator adapted for insect rearing

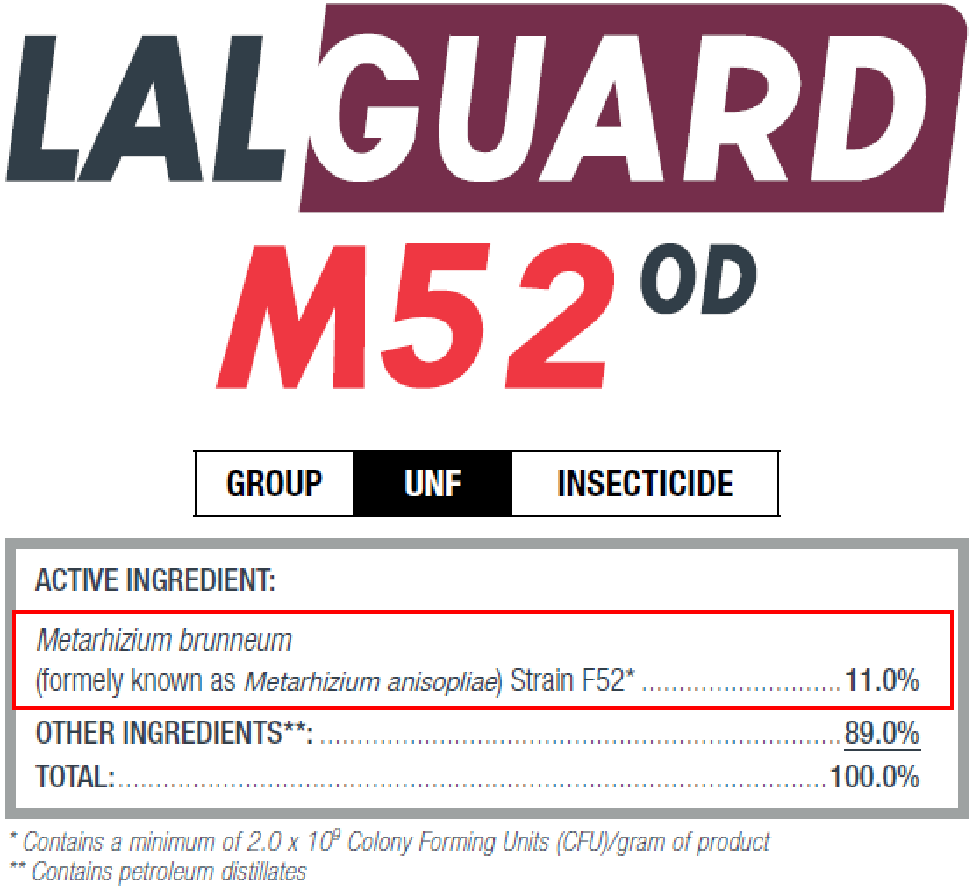
Conclusions from rearing trials

- Mealybugs require a minimum of 80% humidity to develop
- Organic sprouted potatoes work best as alternative food; Russet and Kennebec varieties
- Preserving 1st instars and eggs in the fridge for several months for successful reinfestation
- Potatoes sprouted in greenhouse killed mealybugs (Solanin toxin)
- Need to add vine bark for females to oviposit (lay eggs)

Photo credit: M. Spodek

EPFs used in experiments





Products donated by Plant Products

Methods

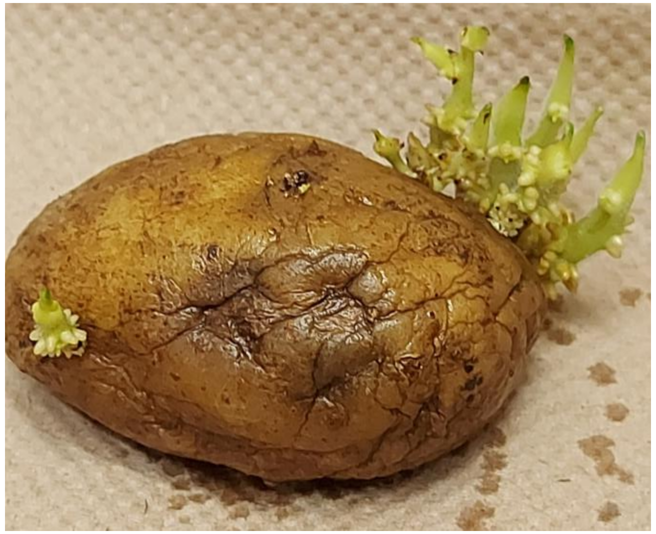
Each treatment with five 2nd instar mealybugs

Bb1= *Beauveria bassiana*, 62 µl/50 ml distilled water; low concentration
Bb2= *B. bassiana*, 125 µl/50 ml distilled water; high concentration
Ma1= *Metarhizium brunneum*, 25 µl/50 ml distilled water; low concentration
Ma2= *M. brunneum*, 250 µl/50 ml distilled water; high concentration
Control= 50 ml distilled water

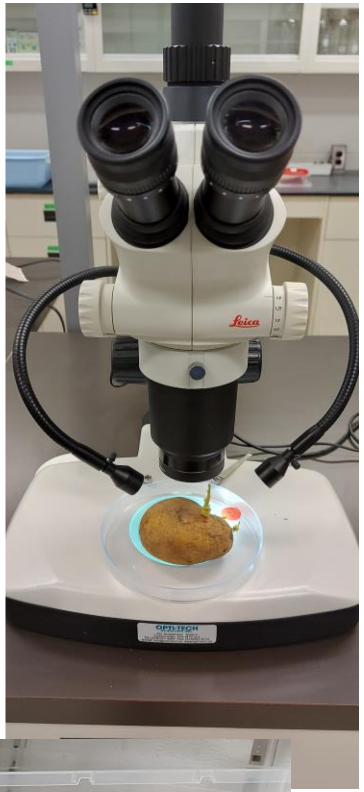
repeated 2x

Photo credits: M. Spodek

Methods

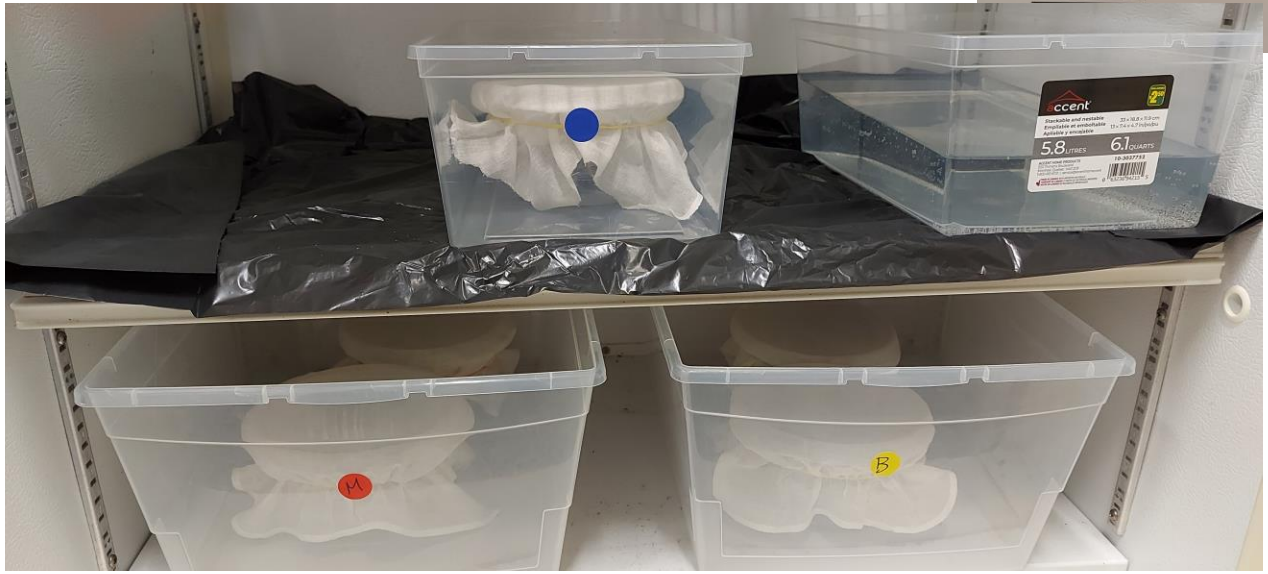


infested potato after treatment



daily observations and recording dead/alive status for 10 days after treatment spray

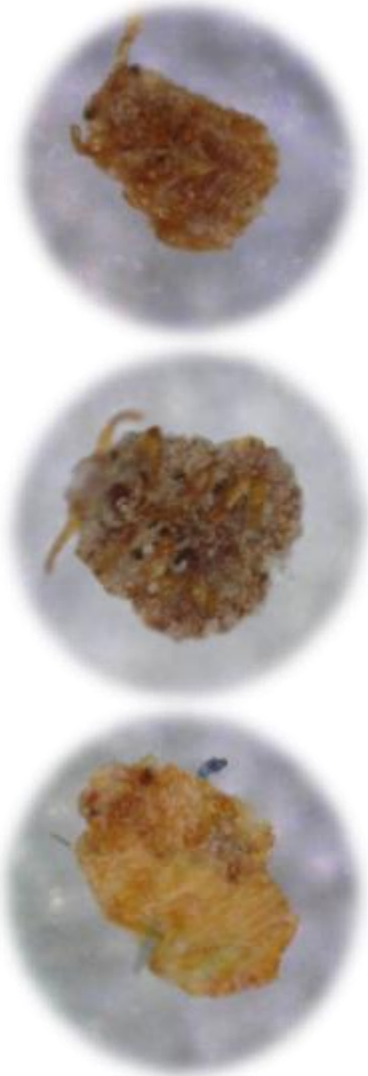
preparing treatments



storing treated potatoes in incubator, 27°C, 80% humidity, dark

Photo credits: M. Spodek

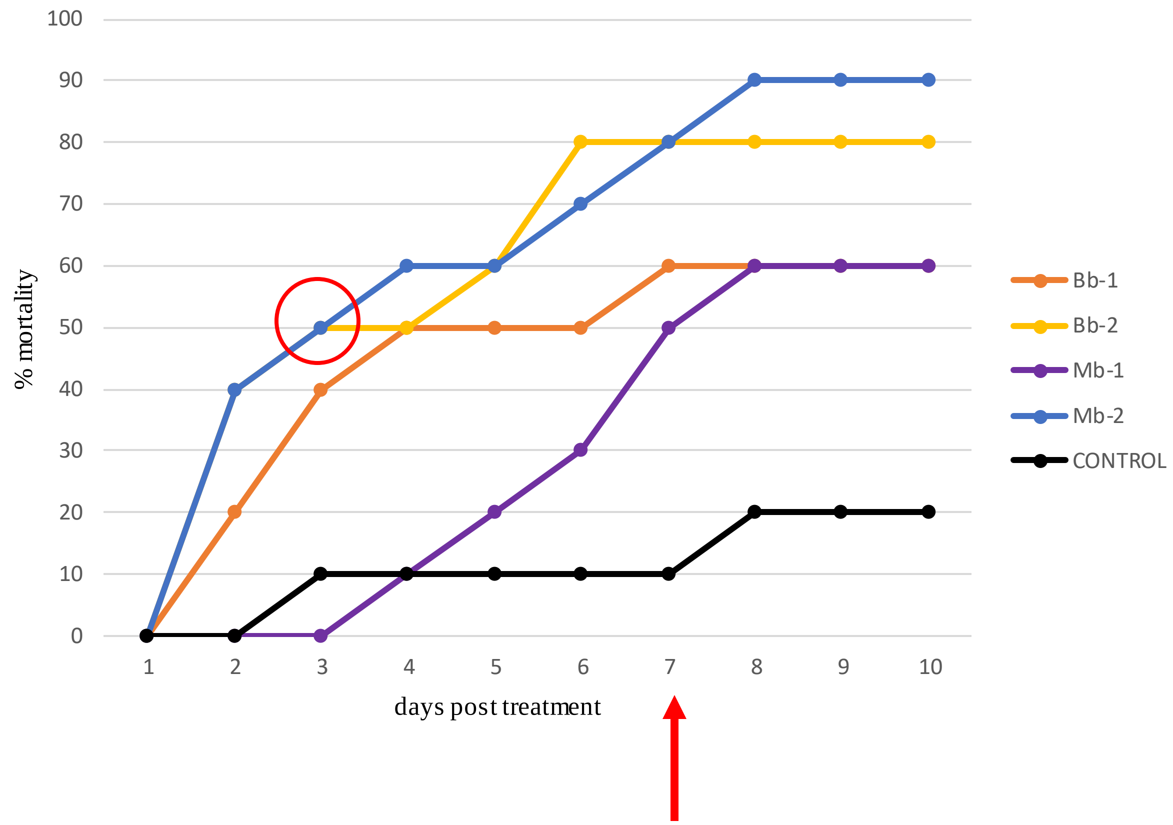
Results



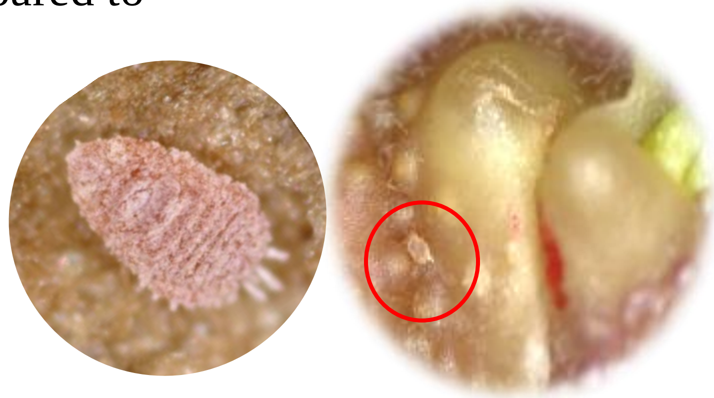
dead 2nd instar mealybugs after treatment

- Both the products showed mycoinsecticidal activity on the insects tested.
- Higher concentrations of both products (Bb2+Mb2) were most effective than the lower concentrations (Bb1+Mb1) from Day 2
- Both Bb2+Mb2 had 80% mortality on Day 7
- Mb2 had 90% mortality compared to Bb2 with 80%

Mycoinsecticidal activity of 2 EPF products against GMB



Bb1= *Beauveria bassiana*, low concentration
Bb2= *B. bassiana*, high concentration
Ma1= *Metarhizium brunneum*, low concentration
Ma2= *M. brunneum*, high concentration
Control= 50 ml distilled water



live 2nd instar mealybug

unpublished data

Conclusions and future research directions

- Improve rearing conditions to increase mealybug numbers
- Use higher number of mealybugs and adult female mealybugs and ovisacs
- Isolating fungi from dead instars with each treatment to confirm the presence of EPF in the cadavers
- Vineyard trials with both commercial EPFs
- National study of GMB genetics-potential cryptic species
- Other mealybug trials, greenhouse applications

Acknowledgements

- Grape growers for collecting mealies from their vineyards
- Casey Boroski- Plant Products-for donating products for trials
- Wes Wiens- Vine tech- for donating 20 potted vines
- Rachel Bird and Quinn Nixon, summer students “grape IPM crew”, Dr. W. McFadden-Smith, OMAFRA
- Prof. G. Tattersall- incubator loan
- Tony Wang, CCOVI Virus Testing Services for DNA extraction of mealybugs
- Dr. I. Scott, AAFC, London Research and Development Center for sprouted Kennebec potatoes
- Dr. H. Fisher, retired, University of Guelph for potted vines from Quebec
- Steven Renda and crew- Technical Services, Brock University, for creating my lab greenhouse