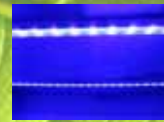


New developments in biological control of greenhouse vegetable pests



Les Shipp

Greenhouse and Processing Crops Research Centre, Harrow, ON



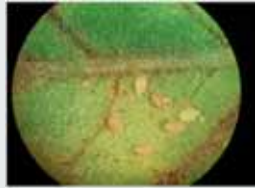
Agriculture and
Agri-Food Canada

Agriculture et
Agroalimentaire Canada

Talk Outline

- ❖ Quality of fresh vs stored biological control agents
- ❖ New predatory mite on the market
- ❖ Pest and biological control agent development under supplemental lighting
- ❖ Bee vectoring of microbial control agents

Effects of cold storage on the quality of whitefly parasitoids



Eretmocerus eremicus



Encarsia formosa



Fresh vs Cold stored parasitoids

Will the quality of whitefly parasitoids be affected if parasitoid pupae are cold stored compared to that of fresh ?



Experiments (60% RH, 16:8 L:D)

Supplier Control: Tested immediately on receipt at 24°C.



Acclimatized: Stored at 12°C for one week and cold stored at 7°C for one more week.



Cold stored 1 week: Cold stored at 7°C, for 1 week.

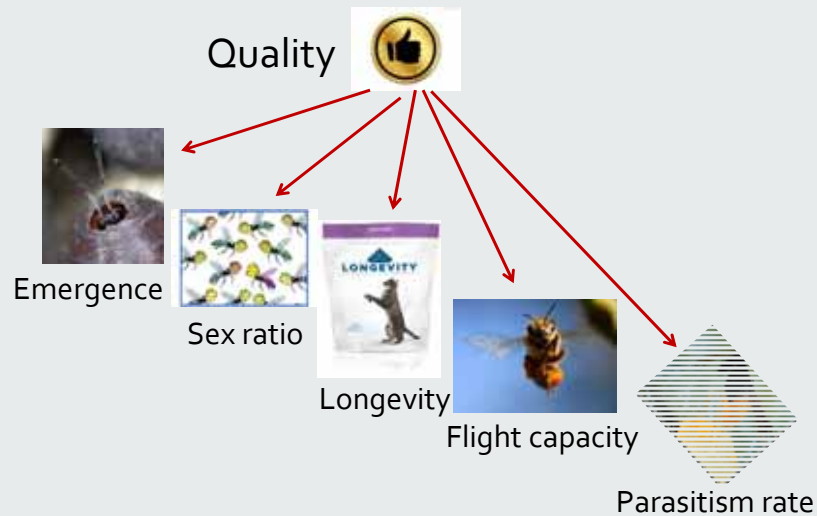


Cold stored 2 weeks: Cold stored at 7°C, for 2 weeks.

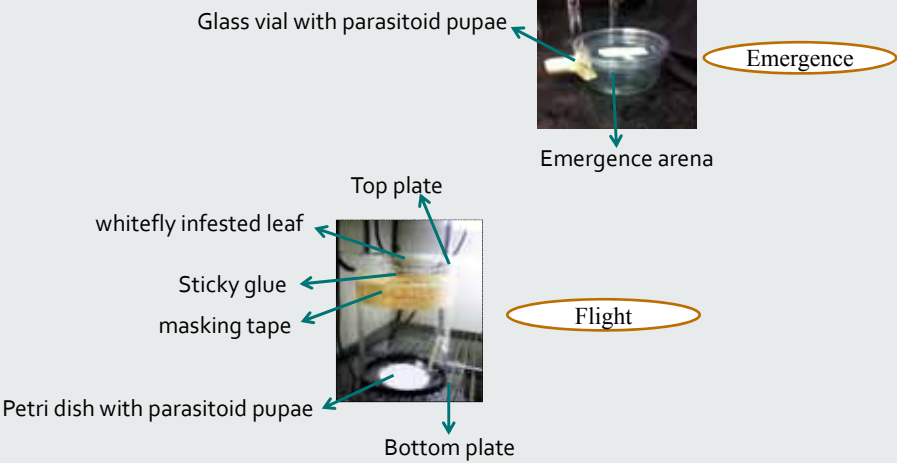
AAFC Control: Parasitoids were reared in the AAFC laboratory and used as fresh at 24°C.



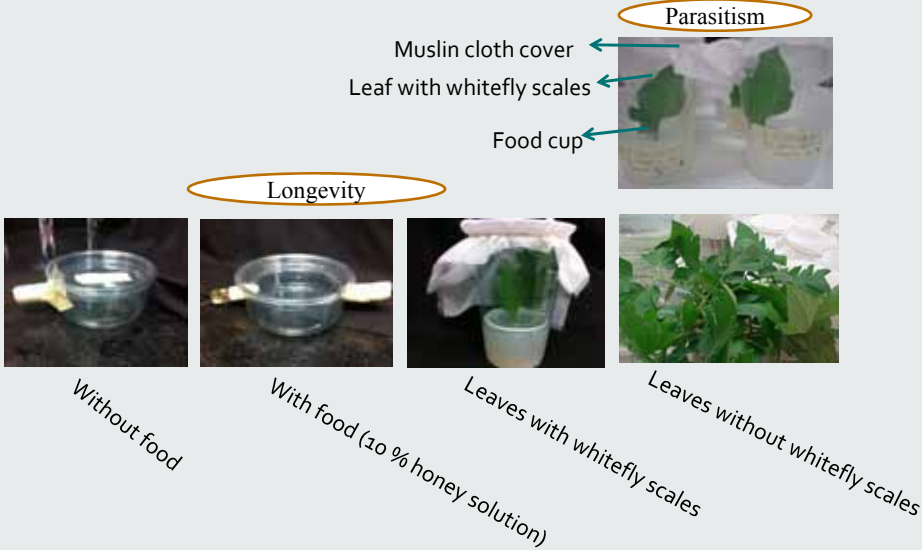
Objectives



Materials and methods (Laboratory experiments)



Materials and methods (Laboratory experiments)



Data Collection



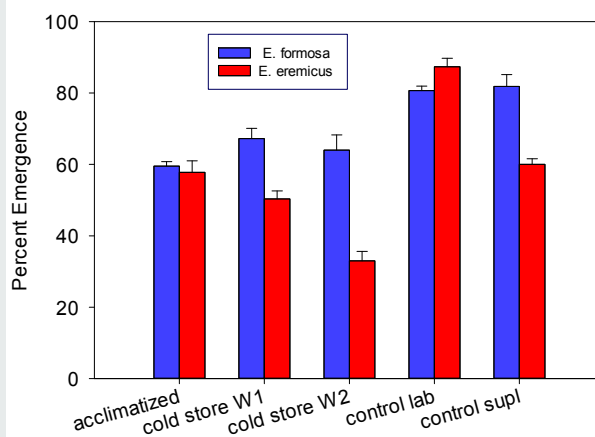
- Percentage emergence?
- Percentage of adults capable of flying?
- Sex ratio?
- Parasitism rate?
- Longevity?

Results



I can not emerge properly when stored at cold temperature

The average percentage (\pm SE) of adult parasitoids emerged under the different treatments.

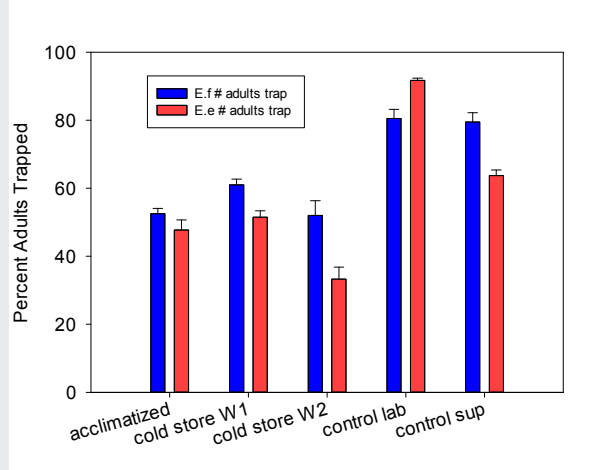


Results



I can not fly properly when stored at cold temperature

The average percentage (\pm SE) of adult trapped in flight chamber under different treatments.

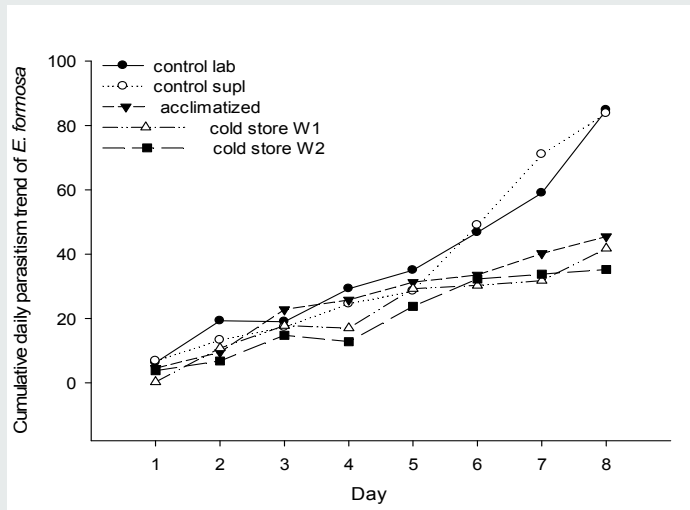


Results



I can not parasitize properly when stored at cold temperature

Mean numbers of scales parasitized by *E. formosa* over 8 days under different treatments.

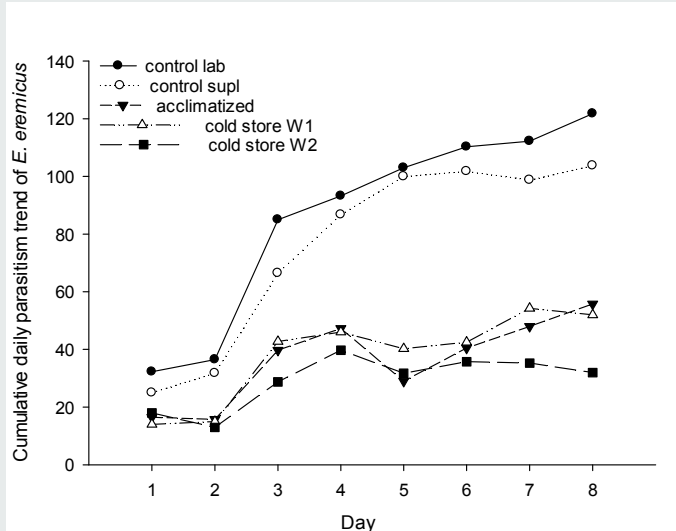


Results



I can not parasitize properly when stored at cold temperature

Mean numbers of scales parasitized by *E. eremicus* over 8 days under different treatments.

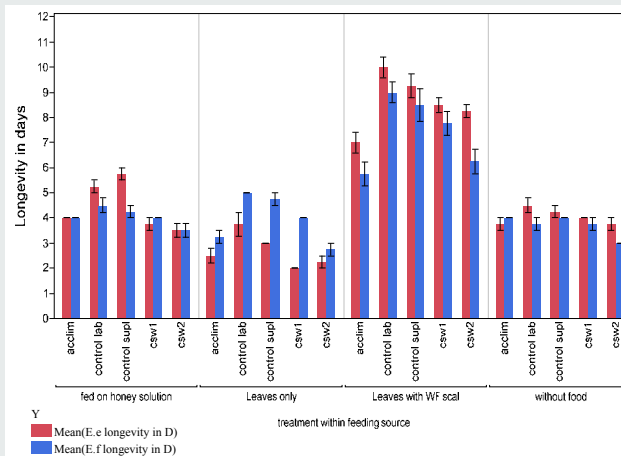


Results



I can live longer when have good food

The average (\pm SE) longevity (in days) of *E. formosa* and *E. eremicus* under different sets of food resources and storage regimes.



Conclusions



- ✓ More than 80% of the parasitoid adults should emerge from fresh pupae.
- ✓ Emergence and parasitism capacities of parasitoids decreased with increase in storage time.
- ✓ Storage has no effects on sex ratio.
- ✓ Reduction in parasitism rate is higher than decline in emergence rate following cold storage.
- ✓ *E. eremicus* lays maximum number of eggs during first five days of its life; *E. formosa* lays uniform number of eggs throughout its life.

New predatory mite



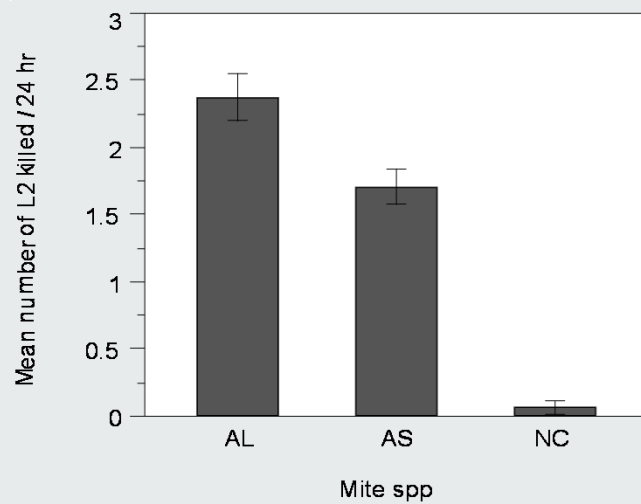
Amblydromalus limonicus

- Higher predation and egg laying rates than *Neoseiulus cucumeris* and *Amblyseius swirskii*
- Feeds on both larval instars of western flower thrips
- Active at lower temperatures (13 – 15°C)
- Feeds on immature stages of thrips, whiteflies and other mites

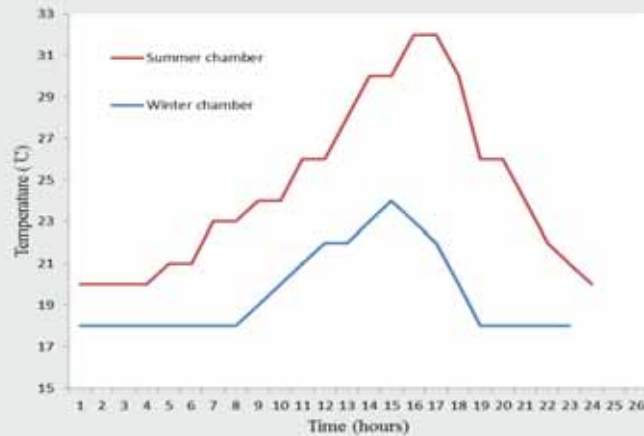
Life stages of western flower thrips



Predation rate of *A. limonicus*, *A. swirskii* and *N. cucumeris* on 2nd instar western flower thrips

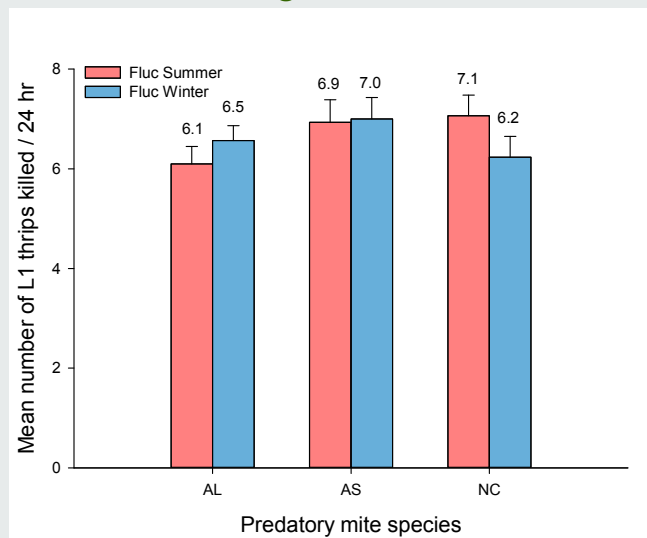


Predation and oviposition rates of *A. limonicus*, *A. swirskii* and *N. cucumeris* under fluctuating simulated summer and winter greenhouse conditions

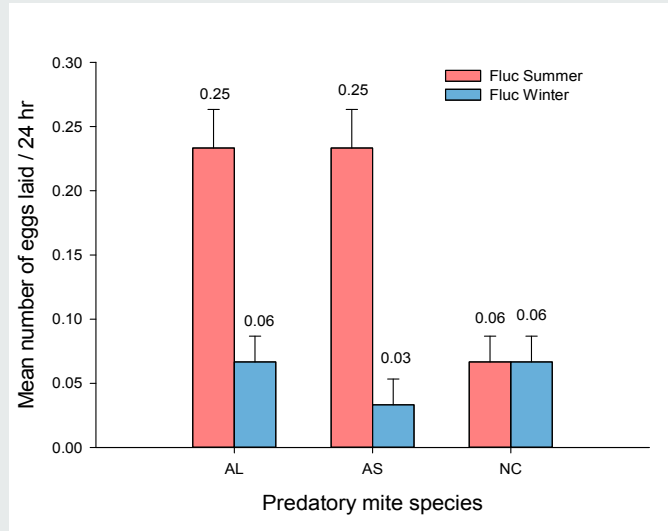


Summer: 24.5°C, 16L:8D and 83 W/m²
 Winter: 19.6°C, 8L:16D and 11W/m²

Predation rates of *A. limonicus*, *A. swirskii* and *N. cucumeris* under fluctuating simulated summer and winter greenhouse conditions



Oviposition rates of *A. limonicus*, *A. swirskii* and *N. cucumeris* under fluctuating simulated summer and winter greenhouse conditions



Pest and biological control agent development under supplemental lighting

- Greenhouse whitefly development and egg laying



- Parasitism levels of *Encarsia formosa* and *Eretmocerus eremicus*



Controlled environmental chamber trials: Greenhouse whitefly development



Treatments:

Summer = 24°C, 16L:8D, 83W/m²

Winter = 20°C, 8L:16D, 11W/m²

High pressure sodium lights (HPS) = 20°C, 8L:16D, 11W/m²

High intensity blue LED (HB) = 20°C, 20L:4D, 10.83W/m²

Low intensity blue LED (LB) = 20°C, 20L:4D, 2.7 W/m²

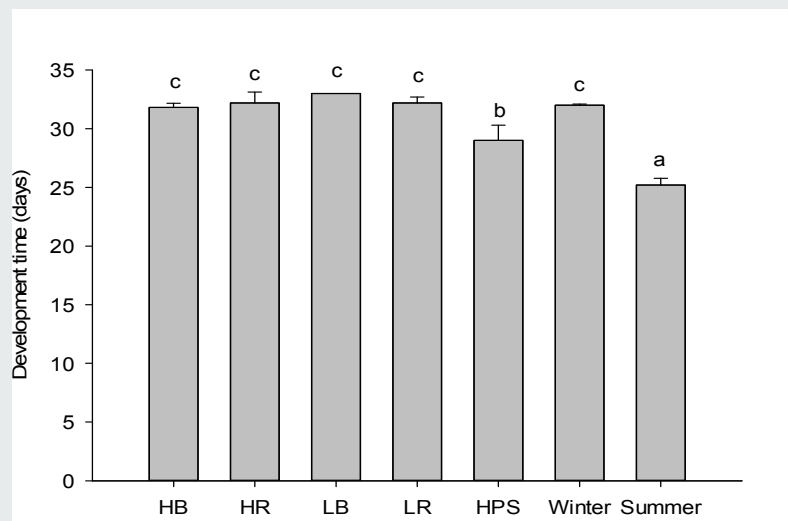
High intensity red LED (HR) = 20°C, 20L:4D, 10.83 W/m²

Low intensity red LED (LR) = 20°C, 20L:4D, 2.7 W/m²

4 replications

- 15 – 20 eggs per leaf

Controlled environmental chamber trials: Greenhouse whitefly development



Controlled environmental chamber trials: Greenhouse whitefly oviposition

Treatments: Summer, Winter and HPS

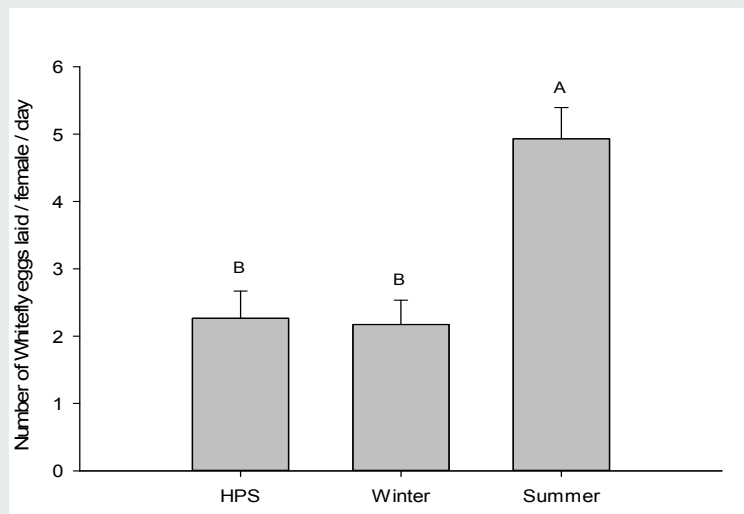
A single pair of adult (male and female) whiteflies (< 48 h old) was released into the oviposition arena.



After 7 days, the leaves were removed and the number of eggs counted. A new leaf was placed in the arena and the process repeated after another seven days.

15 replications per treatment

Controlled environmental chamber trials: Greenhouse whitefly oviposition

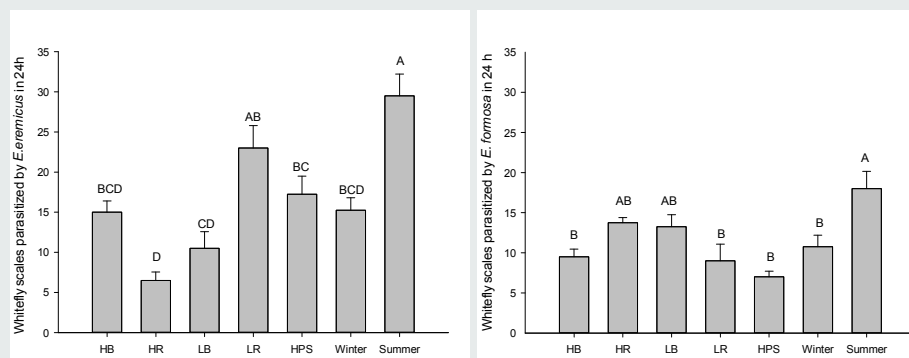


Controlled environmental chamber trials: Parasitism rates for *Eretmocerus eremicus* and *Encarsia formosa*

- 7 light treatments
- Tomato leaves containing 100 – 200 whitefly eggs were placed in plastic arenas with 1 48-h old mated *E. eremicus* or *E. formosa*.
- Parasitoids were removed after 24 h and after 15 days, the number of parasitized whitefly pupae were recorded.
- 4 replications per treatment



Mean (\pm SE) number of whitefly scales parasitized by *E. eremicus* and *E. formosa* within 24 hours under different supplemental light treatments



BUMBLE BEES – A NEW DELIVERY SYSTEM FOR MICROBIAL CONTROL AGENTS FOR GREENHOUSE CROPS

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¹Agriculture & Agri-Food Canada, ²University of Guelph, ³Al-balqa' Applied University, ⁴National Academy of Agricultural Science and ⁵Ontario Ministry of Agriculture, Food and Rural Affairs



Agriculture and
Agri-Food Canada

Agriculture et
Agroalimentaire Canada

Canada

Why the pollinator-vector approach?

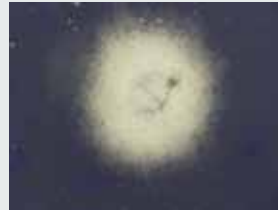


- Simultaneous combination of pollination and distribution of a biocontrol agent
- Continuous dissemination of the inoculum to the target plants
- Bees deliver the biocontrol agent (inoculum) to the right place
- Environmentally and economically friendly
- Potential compatibility with other bio-based IPM programs

***Beauveria bassiana* is an entomopathogenic fungus that attacks a wide host range of arthropods**

In the greenhouse, it attacks:

- greenhouse whitefly
- western flower thrips
- tarnished plant bug
- green peach aphid



Background of bee vectoring technology on greenhouse crops

- In 2003, showed that bee pollinators can also be used to deliver the entomopathogen, *B. bassiana*, for insect pest control (*Lygus* and thrips) on greenhouse pepper.
- By 2005, determined the optimal concentration of bee vectored *Beauveria* for pepper and tomato for whiteflies, thrips, aphids and *Lygus*.
- By 2006, proved that bumble bees can vector *B. bassiana* and *Clonostachys rosea* simultaneously as a single inoculum for insect pest control and plant disease suppression.
- 2006 and 2008, conducted commercial greenhouse trials to determine the effect of inoculum dispensers on bee foraging activity (pollination and fruit yield) and to determine where the inoculum was deposited on the crop (ie., flowers and leaves).
- 2008-09, determined that bee vectored *B. bassiana* had minimal impact on greenhouse biocontrol agents (predatory mites and parasitoids).
- 2010-11, demonstrated that bumble bees can vector AcMNPV baculovirus and *Bacillus thuringiensis* subsp. *kurstaki* (Bt) for cabbage looper control.
- 2011-13, demonstrated that bumble bees can vector *C. rosea*, *B. bassiana* and Bt in outdoor crops (strawberries, blueberries, sunflowers) for disease and pest management.

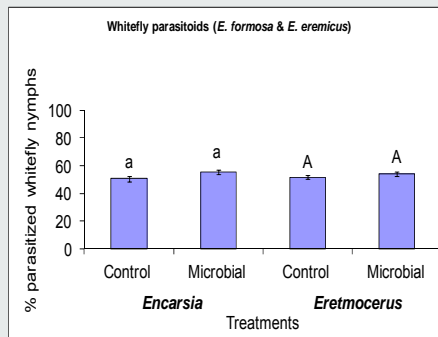
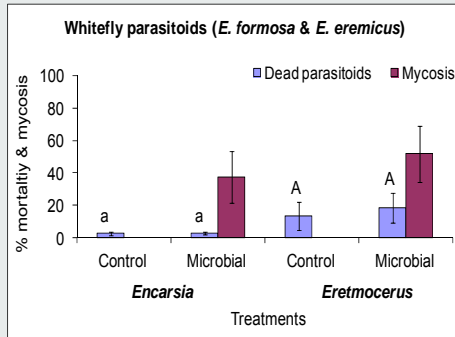
Impact of bee vectored *Beauveria* on greenhouse biocontrol agents



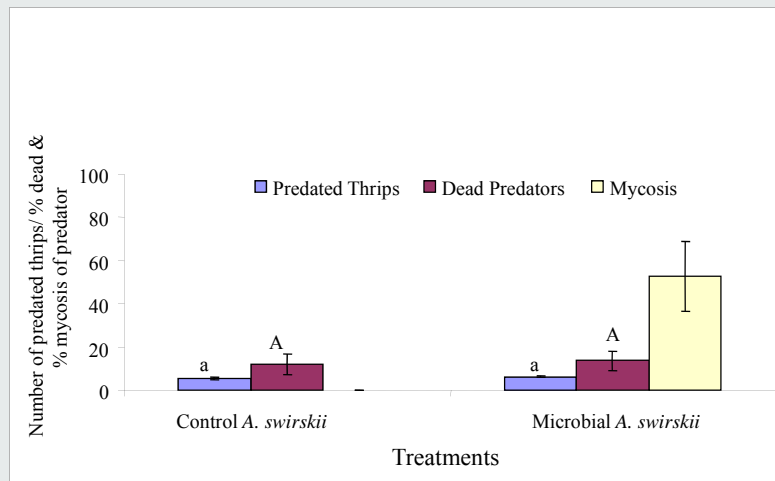
Encarsia formosa



Eretmocerus eremicus



Impact of bee vectored *Beauveria* on the predatory mite, *Amblyseius swirskii*



Impact of bee vectored *Beauveria* on the predatory bug, *Orius insidiosus*



Control mortality = 16.9%

Beauveria mortality = 41.2%

Control predation rate = 19.5

Beauveria predation rate = 21.1

Summary

- Bee-vectored *B. bassiana* at a concentration of 1.37×10^{10} conidia of *Beauveria* per gram of inoculum did not have a negative impact on *E. formosa*, *E. eremicus*, *A. colemani* or *A. swirskii* as indicated by percentage mortality and mycosis compared to non-*Beauveria* control treatment.
- Parasitism or predation levels were also not negatively impacted.
- Bee mortality was not significantly different between the microbial and control treatments.

Bee vectoring of AcMNPV baculovirus and *Bacillus thuringiensis* subsp. *kurstaki* for cabbage looper control



Mean mortality (\pm SE) of *Trichoplusia ni* exposed to bee vectored and spray application of AcMNPV

| Treatment | N | 4 days post treatment | | 7 days post treatment | |
|-----------------------------------|---|-----------------------|-------------------------|-----------------------|-------------------------|
| | | Mortality (%) | Corrected Mortality (%) | Mortality (%) | Corrected Mortality (%) |
| Sprayed AcMNPV (full rate) | 3 | 98.3 \pm 1.67A | 98.3 \pm 1.73A | 100.0 \pm 0.00A | 100.0 \pm 0.00A |
| Sprayed AcMNPV (½ rate) | 3 | 98.3 \pm 1.67A | 98.3 \pm 1.73A | 100.0 \pm 0.00A | 100.0 \pm 0.00A |
| Bee vectored AcMNPV(full rate) | 3 | 55.0 \pm 13.23B | 53.5 \pm 13.67B | 96.7 \pm 1.67A | 96.5 \pm 1.73A |
| Bee vectored AcMNPV (½ rate) | 3 | 40.0 \pm 12.58B | 37.9 \pm 13.00B | 81.7 \pm 8.82A | 81.0 \pm 9.12A |
| Bee vectored AcMNPV (inactivated) | 3 | 25.0 \pm 5.00BC | 22.4 \pm 5.20B | 38.3 \pm 16.41AB | 36.2 \pm 16.98B |
| Control (bees and no AcMNPV) | 3 | 3.3 \pm 1.67C | - | 28.3 \pm 6.01B | - |

N = numbers of samples collected (a total of 20 individuals were collected per sample). Within a column, means followed by different letters are significantly different at $P < 0.05$ using Tukey HSD test.

Mortality (\pm SE) of *Trichoplusia ni* to bee vectored and spray application of *Bacillus thuringiensis* subsp. *kurstaki*

| Treatment | N | 4 days post treatment | | 7 days post treatment | |
|-----------------------------|---|-----------------------|-------------------------|-----------------------|-------------------------|
| | | Mortality (%) | Corrected Mortality (%) | Mortality (%) | Corrected Mortality (%) |
| Dipel 2X DF | 3 | 100.0 \pm 0.00 | 100.0 \pm 0.00a | 100.0 \pm 0.00 | 100.0 \pm 0.00a |
| Dipel (undiluted) | 3 | 100.0 \pm 0.00 | 100.0 \pm 0.00a | 100.0 \pm 0.00 | 100.0 \pm 0.00a |
| Dipel (1/2 conc.) | 3 | 100.0 \pm 0.00 | 96.6 \pm 1.70a | 100.0 \pm 0.00 | 100.0 \pm 0.00a |
| Dipel (1/4 conc.) | 3 | 93.0 \pm 6.70 | 89.8 \pm 7.76a | 100.0 \pm 0.00 | 100.0 \pm 0.00a |
| Dipel (1/8 conc.) | 3 | 87.0 \pm 8.80 | 84.7 \pm 7.76a | 88.0 \pm 6.00 | 88.1 \pm 6.11a |
| Dipel (inactivated) | 3 | 13.0 \pm 8.80 | 10.7 \pm 9.09b | 2.0 \pm 1.70 | 1.1 \pm 1.13b |
| Control (bees and no Dipel) | 3 | 2.0 \pm 1.70 | - | 2.0 \pm 1.70 | - |

N = numbers of samples collected (a total of 20 individuals were collected per sample). Within a column, means followed by different letters are significantly different at $P < 0.05$ using Tukey HSD test.

Sutton – University of Guelph



New bee vectoring dispenser

Bee Vectoring Technology Inc.



Biobest Belgium NV

FLYING DOCTORS®



Carrier issues



Caking and crusting over of the inoculum surface due to fecal and fluid deposition by the bees and high humidity

A new carrier was developed and patented which included corn flour, desiccant, sticker, etc.

Pest Management Regulatory Agency (PMRA) recently announced the approval of a novel pollinator biocontrol vector application method for the delivery of BotaniGard® 22WP to the target in pollinated greenhouse crops.

BOTANIGARD 22WP
Wettable Powder Mycoinsecticide
 For use in controlling whitefly, aphids, and thrips in Greenhouse Ornamentals and Vegetables
 COMMERCIAL
 WARNING – EYE IRRITANT
 CAUTION – SKIN IRRITANT
 POTENTIAL SENSITIZER
 READ THE LABEL BEFORE USING
 GUARANTEE: *Beauveria bassiana* strain GHA.....4.4X10¹³ conidia / kg
 KEEP OUT OF REACH OF CHILDREN
 CAUTION
 REGISTRATION NO. 29321 PEST CONTROL PRODUCTS ACT
 Warning: This product contains the allergen sulfite.
 NET CONTENTS: 500 grams
 Laverlam International Corporation (LVM)
 P.O. Box 4109,
 Butte, MT 59702
 USA
 Phone: BioWorks (North American Distributor)
 1-800-877-9443
 2011-2974
 2012-11-16



POLLINATOR BIOCONTROL VECTOR APPLICATION METHOD

For suppression of whiteflies, aphids and thrips in bumble bee pollinated greenhouse crops

Introduction

This application method uses a microbial inoculum dispenser that is attached to the front of a bumble bee hive. As bumble bees exit the hive through the dispenser, BotaniGard 22WP accumulates on their legs and body hairs. The bumble bees then transport and deposit the product on plant foliage, flowers and fruit during foraging and grooming. This continuous delivery of the bioinsecticide to the target sites on the plants enables management of whiteflies, aphids and thrips in bumble bee pollinated greenhouse crops.

The pollinator biocontrol vector application method facilitates the deposition of BotaniGard 22WP directly on the plant where the pests are found, reduces loss to ground deposition, continuously exposes the pest to the product, and limits aerosol concentration. Furthermore, this application method provides value added benefit to normal crop pollination, combining pollination with simultaneous pest control.

This application method can be used to deliver BotaniGard 22WP with bumble bees for the management of pests.

Future directions

- Conduct commercial greenhouse trials to determine the number of dispensers per hectare
- Investigate the possibility of combining three microbial agents into a single inoculum
- Investigate the idea of using other insects as vectors of microbial agents

Acknowledgements

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of GUELPH

biobeT
BIOLOGICAL SYSTEMS



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Gillian Ferguson
and numerous coop and
summer students

Bee Vectoring Technology Inc.

ADJUVANTS
plus



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농촌진흥청

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Improved Farming Systems and Practices Initiative