Managed bumble bees – A new delivery system for microbial agents for pest and disease management in protected and outdoor crops

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Why the pollinator-vector approach?

- Bees have shown the capacity to carry microscopic particles other than pollen such as fungal spores and bacterial cells.
- So why not beneficial microbes?

Honey bees carrying pollen from a pollen dispenser.
Using bumble bees as a novel application strategy for microbial agents for pest control and disease suppression

- 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
Summary of bee vectoring technology development

• Early 1990’s, Sutton and Kevan showed that bee vectored *Clonostachys rosea* suppressed grey mould on strawberries.

• 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 *C. rosea* simultaneously as a single inoculum for insect pest and plant disease control.

• 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.
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
Pest Management Regulatory Agency (PMRA) has approved the use of pollinator biocontrol vector application for the delivery of BotaniGard® 22WP in 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.
Bee vectoring of AcMNPV baculovirus and *Bacillus thuringiensis* subsp. *kurstaki* for cabbage looper control.
Mortality (± SE) of *Trichoplusia ni* to bee vectored and
spray application of *Bacillus thuringiensis* subsp. *kurstaki*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>4 days post treatment</th>
<th>7 days post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mortality (%)</td>
<td>Corrected Mortality (%)</td>
</tr>
<tr>
<td>Dipel 2X DF</td>
<td>3</td>
<td>100.0 ± 0.00</td>
<td>100.0 ± 0.00a</td>
</tr>
<tr>
<td>Dipel (undiluted)</td>
<td>3</td>
<td>100.0 ± 0.00</td>
<td>100.0 ± 0.00a</td>
</tr>
<tr>
<td>Dipel (½ rate)</td>
<td>3</td>
<td>100.0 ± 0.00</td>
<td>96.6 ± 1.70a</td>
</tr>
<tr>
<td>Dipel (¼ rate)</td>
<td>3</td>
<td>93.0 ± 6.70</td>
<td>89.8 ± 7.76a</td>
</tr>
<tr>
<td>Dipel (⅛ rate)</td>
<td>3</td>
<td>87.0 ± 8.80</td>
<td>84.7 ± 7.76a</td>
</tr>
<tr>
<td>Dipel (inactivated)</td>
<td>3</td>
<td>13.0 ± 8.80</td>
<td>10.7 ± 9.09b</td>
</tr>
<tr>
<td>Control (bees and no</td>
<td>3</td>
<td>2.0 ± 1.70</td>
<td>-</td>
</tr>
<tr>
<td>Dipel)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>7 days post treatment</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mortality (%)</td>
<td>Corrected Mortality (%)</td>
<td></td>
</tr>
<tr>
<td>Sprayed AcMNPV (full rate)</td>
<td>3</td>
<td>98.3 ± 1.67A</td>
<td>98.3 ± 1.73A</td>
<td>100.0 ± 0.00A</td>
</tr>
<tr>
<td>Sprayed AcMNPV (½ rate)</td>
<td>3</td>
<td>98.3 ± 1.67A</td>
<td>98.3 ± 1.73A</td>
<td>100.0 ± 0.00A</td>
</tr>
<tr>
<td>Bee vectored AcMNPV (full rate)</td>
<td>3</td>
<td>55.0 ± 13.23B</td>
<td>53.5 ± 13.67B</td>
<td>96.7 ± 1.67A</td>
</tr>
<tr>
<td>Bee vectored AcMNPV (½ rate)</td>
<td>3</td>
<td>40.0 ± 12.58B</td>
<td>37.9 ± 13.00B</td>
<td>81.7 ± 8.82A</td>
</tr>
<tr>
<td>Bee vectored AcMNPV (inactivated)</td>
<td>3</td>
<td>25.0 ± 5.00BC</td>
<td>22.4 ± 5.20B</td>
<td>38.3 ± 16.41AB</td>
</tr>
<tr>
<td>Control (bees and no AcMNPV)</td>
<td>3</td>
<td>3.3 ± 1.67C</td>
<td>-</td>
<td>28.3 ± 6.01B</td>
</tr>
</tbody>
</table>

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.
Bumble bee vectoring of *Metarhizium anisopliae* (Met52)

- Bees carried $6.12 \times 10^5$ to $1.02 \times 10^6$ CFU
- Tomato flowers had $1.97 \times 10^4$ CFU
- Leaves had 164.9 CFU per cm$^2$
- Adult greenhouse whitefly (*Trialeurodes vaporariorum*) infection level – 58% (43% mycosis)
- Bee mortality

Inoculum concentration was $5.0 \times 10^9$ spores/g
New dispenser designs

Korean

Belgium/Biobest

Koppert

Enviroquest

Sutton
Measuring bee activity in and out of the different dispenser types
New bee vectoring dispenser
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.
Strawberry Disease management

<table>
<thead>
<tr>
<th></th>
<th>Grey mould (Botrytis) incidence</th>
<th>Leaf blight (Phomopsis) incidence</th>
<th>Whiskery rot (or “leak”) (Rhizopus) incidence/berry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treated field</strong></td>
<td>0 – 10%: average 6%</td>
<td>0%</td>
<td>3%</td>
</tr>
<tr>
<td><strong>Untreated field</strong></td>
<td>60%</td>
<td>12%</td>
<td>41%</td>
</tr>
</tbody>
</table>

Bee vectored *Clonostachys rosea*
Using pollinators to delivery *Clonostachys* for mummyberry control on blueberry

PEI Organic/Pesticide-free Blueberry Farm, 2009 – 2011 trials
Bee vectoring of *Clonostachys* and *Bacillus thuringiensis* subsp. *kurstaki* for disease and pest control
Future directions

- Expand the bee vectoring technology to canola, apples and coffee
- Evaluate the possibility of combining three microbial agents into a single inoculum
- Investigate the idea of using other insects as vectors of microbial agents
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