Alliances between beneficial insects, plants & fungi pollinate, protect, & promote crop production

Peter G. Kevan
School of Environmental Sciences, University of Guelph, ON
1 October, 2010
Pollinator Biocontrol Vector Technology (PBVT): Background

• Bees have shown the capacity to carry microscopic particles, other than pollen, such as fungal spores and bacterial cells.

• Can this capacity be used to deliver biological control agents?
Milkweeds, *Metschnikowia* & Pollinators

*Metschnikowia* infection prevents pollen germination:

Biocontrol potential?
Pollinator Biocontrol Vector Technology (PBVT): Since 1990

- The first trials of bee vectoring of biological control agents used
  - *honeybees* to vector
  - *fungal antagonist* to
  - *gray mold* on
  - *strawberry* & *raspberry*

Gray mold on strawberry

Pollinator Biocontrol Vector Technology (PBVT): Background

- The *antagonistic fungus* delivered by *honeybees* to *strawberries* resulted in *grey mold control* equivalent to the application of *commercial fungicides*.

Application of fungal biocontrol agent to strawberry by honeybees.
We called this the B52 bomber approach.
<table>
<thead>
<tr>
<th>Crop</th>
<th>Pathogen</th>
<th>Agent</th>
<th>Vector</th>
<th>Res</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pome</td>
<td>Fire blight</td>
<td><em>Pseudomonas</em></td>
<td>Hbee</td>
<td>+</td>
<td>Thomson et al. 1992; Johnson et al. 1993</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Grey mold</td>
<td><em>Clonostachys</em></td>
<td>Hbee</td>
<td>+</td>
<td>Peng et al. 1992</td>
</tr>
<tr>
<td>Raspberry</td>
<td>Grey mold</td>
<td><em>Clonostachys</em></td>
<td>Hbee &amp; Bbee</td>
<td>+</td>
<td>Yu &amp; Sutton 1997</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Grey mold</td>
<td><em>Trichoderma</em></td>
<td>Hbee</td>
<td>+</td>
<td>Maccagnani et al. 1999</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Grey mold</td>
<td><em>Trichoderma</em></td>
<td>Hbee &amp; Bbee</td>
<td>+</td>
<td>Kovach et al. 2000</td>
</tr>
<tr>
<td>Blueberry</td>
<td>Mummy berry</td>
<td><em>Bacillus subtilis</em></td>
<td>Hbee</td>
<td>+</td>
<td>Dedej et al. 2004</td>
</tr>
<tr>
<td>Canola</td>
<td><em>Sclerotinia</em></td>
<td>various</td>
<td>Hbee</td>
<td>n/a</td>
<td>Israel &amp; Boland 1992</td>
</tr>
<tr>
<td>Crop</td>
<td>Pest</td>
<td>Agent</td>
<td>Vector</td>
<td>Result</td>
<td>Reference</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------</td>
<td>-------------</td>
<td>--------</td>
<td>--------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Clover</td>
<td><em>Helicoverpa</em></td>
<td>NPHV</td>
<td>Hbee</td>
<td>+</td>
<td>Gross et al. 1994</td>
</tr>
<tr>
<td>Canola</td>
<td>Pollen beetle</td>
<td><em>Metarhizium</em></td>
<td>Hbee</td>
<td>+</td>
<td>Butt et al. 1998</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Moth</td>
<td><em>Bt</em> kurstaki</td>
<td>Hbee</td>
<td>+</td>
<td>Jyoti &amp; Brewer 1999</td>
</tr>
<tr>
<td>Canola</td>
<td><em>Lygus</em> (TPB)</td>
<td><em>Beauveria</em></td>
<td>Hbee</td>
<td>+</td>
<td>Al-Mazra’awi et al. 2006 a,b</td>
</tr>
<tr>
<td>Sweet pepper</td>
<td><em>Lygus</em> (TPB)</td>
<td><em>Beauveria</em></td>
<td>Bbee</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sweet pepper</td>
<td>WF Thrips</td>
<td><em>Beauveria</em></td>
<td>Bbee</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sweet pepper</td>
<td>GP Aphid</td>
<td><em>Beauveria</em></td>
<td>Bbee</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Crop</td>
<td>Pest</td>
<td>Agent</td>
<td>Vector</td>
<td>Result</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
<td>------------</td>
<td>--------</td>
<td>--------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Sweet pepper</td>
<td>White Fly</td>
<td><em>Beauveria</em></td>
<td>Bbee</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sweet pepper</td>
<td>GP Aphid</td>
<td><em>Beauveria</em></td>
<td>Bbee</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td><em>Lygus</em> (TPB)</td>
<td><em>Beauveria</em></td>
<td>Bbee</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>White Fly</td>
<td><em>Beauveria</em></td>
<td>Bbee</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>GP Aphid</td>
<td><em>Beauveria</em></td>
<td>Bbee</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
## B52 & Biocontrol of Insect Pests & Plant Pathogens Together

<table>
<thead>
<tr>
<th>Crop</th>
<th>Pest + Pathogen</th>
<th>Agent</th>
<th>Vector</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepper</td>
<td>Tarnished Plant Bug + Grey Mold</td>
<td><em>Beauvaria</em> + <em>Clonostachys</em></td>
<td>Bbee</td>
<td>+, +</td>
<td></td>
</tr>
</tbody>
</table>
Why the pollinator-vector approach?

- The bees usually place the inoculum precisely on the flowers of crops.
- Bees forage the crop almost daily, thus continually carry inoculum to newly opened flowers.
- Cost effective.
- Bees improve crop pollination resulting in better yield and quality of crops.
Formulating the biocontrol agent

Biocontrol agent from commercial suppliers is highly concentrated
e.g. $2 \times 10^{11}$ conidia of *Beauveria bassiana*/gram of product (Botanigard®)

Must be diluted with suitable carrier
Trials with talc, flours, clay, beads, etc ...

Best results with finely ground corn (maize) flour
Talc is the most irritating to the bees and they groom it off their bodies
Honeybees, *Beauveria bassiana*, *Lygus* bugs and canola: a field crop

- We used pollinator-vector technology using *B. bassiana*, a fungus, against the tarnished plant bug (TPB) on canola.
Canola experimental set up

Screened cages were placed over blooming canola.

A honeybee nucleus was placed inside each cage.
Canola experimental set up

Each hive was equipped with an inoculum dispenser.
Canola experimental set up

- Honey bees leaving the dispenser carry inoculum to flowers of canola where they forage for pollen and nectar.
Canola experimental set up

- Honeybees, canola flowers, canola leaves and *Lygus* (TPB) were sampled on two dates to evaluate the biological control agent dissemination and pest mortality.
Results from the canola field

Percentage of honey bees, flowers, leaves and TPB with detected densities of *B. bassiana* collected from canola plants caged with honey bees and *B. bassiana*.
Field results of *Lygus* bug mortality on canola.
Pollinator biocontrol vector technology: Protecting sunflowers

*Bacillus thuringiensis kurstaki* vectored by honeybees to sunflower to control the banded sunflower moth (Jyoti & Brewer 1999 in North Dakota)
Pollinator biocontrol vector technology: Protecting sunflowers

Sunflower Heads of hybrid 894 treated with *Bacillus thuringiensis*, means of two years

<table>
<thead>
<tr>
<th>BT Delivery Method</th>
<th>Damaged Seed</th>
<th>Seed set (%)</th>
<th>Oil (%)</th>
<th>Yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee carried</td>
<td>12.2c</td>
<td>78.4a</td>
<td>40.8a</td>
<td>60.7a</td>
</tr>
<tr>
<td><em>Spray</em></td>
<td>12.9b</td>
<td>74.5b</td>
<td>40.2ab</td>
<td>57.9ab</td>
</tr>
<tr>
<td>Control</td>
<td>21.7a</td>
<td>74.6b</td>
<td>38.9b</td>
<td>51.5c</td>
</tr>
</tbody>
</table>

Joyoti & Brewer, 1999
Bumble bees, *B. bassiana*, *Lygus* bugs, thrips and sweet pepper.

- The same approach was used against:
  - *Lygus* and western flower thrips on greenhouse
  - sweet peppers
  - using bumble bees as the vectors.

*Lygus* damage on sweet pepper
Greenhouse pepper experimental set up

Screened cages placed inside a greenhouse.

Potted pepper plants inside each cage.
Greenhouse pepper experimental set up

Bumble bee hives inside the cage
Greenhouse pepper experimental set up

Each bumble bee hive was equipped with an inoculum dispenser.

Inoculum was placed inside a removable tray.
Greenhouse pepper experimental set up

Bumble bee passing through the inoculum.

Bumble bee exiting the dispenser and carrying inoculum.
Greenhouse pepper: sampling

- *Lygus*, thrips, bumble bees, flowers and leaves were collected to assess inoculum acquisition and infection rates.

- Individual *Lygus* were sampled, placed in containers and monitored for mortality.
Greenhouse pepper: sample processing

Surface sterilization of *Lygus* to determine levels of internal infection

Individual *Lygus* were kept in Petri dishes and fed organic lettuce to determine mortality
Greenhouse pepper: sample processing

Each dead *Lygus* was kept on moist filter paper in a Petri dish and stored in the dark for 7 days to monitor disease (mycosis).

When dead *Lygus* showed white mycelia they were scored as killed by *B. bassiana*. 
Results from the greenhouse:

*Lygus* mortality

Percentage of bumble bees, flowers, leaves and TPB with detected densities of *B. bassiana* collected from pepper plants caged with bumble bees and *B. bassiana*.

![Bar chart showing the percentage of bumble bees, flowers, leaves, and TPB with detected densities of B. bassiana collected from pepper plants caged with bumble bees and B. bassiana.](chart)

- **Insect or plant**: Bumble bees, Flowers, Leaves, TPB
- **Percentage**: 0, 20, 40, 60, 80, 100, 120
- **Sampling dates**: 1st, 2nd
Greenhouse results: *Lygus* mortality

### TPB mortality

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>B. Bassiana + bumble bees</th>
<th>Bumble bees only</th>
<th>No treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>% Mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>% Mortality</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Greenhouse results: Thrips mortality

WFT infection rate

% Infection

B. bassiana + bumble bees  Bumble bees only  No treatment

1st 2nd

Sampling date

0 10 20 30 40 50
Co-vectoring *Beauveria* + *Clonostachys* on Bumblebee Pollinators for Greenhouse Tomatoes and Bell Peppers

**Effect of co-vectoring Beauvaria & Clonostachys on suppression of grey mold and death of insect pests**

- **WF** = Whitefly
- **GM** = Grey mold
- **TPB** = *Lygus*

![Graph showing the effect of co-vectoring Beauvaria & Clonostachys on suppression of grey mold and death of insect pests](image_url)
Vector/Pollinator safety is important

Honeybees dead from overdose of Beauveria bassiana: a situation to be avoided.
Optimizing dose for maximum pest control and minimum vector risk

<table>
<thead>
<tr>
<th>Treatments (Beauveria g of inoculum)</th>
<th>Dead Lygus (%)</th>
<th>Dead bees (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9x10^9 conidia of Beauveria/g</td>
<td>46.67±7.1^b</td>
<td>10.93±0.6^e</td>
</tr>
<tr>
<td>6.24x10^{10} conidia of Beauveria/g</td>
<td>77.92±4.0^a</td>
<td>10.93±0.9^e</td>
</tr>
<tr>
<td>2x10^{11} conidia of Beauveria/g</td>
<td>77.50±7.3^a</td>
<td>45.38±2.4^d</td>
</tr>
<tr>
<td>Heat inactivated Beauveria</td>
<td>11.67±0.9^c</td>
<td>6.10±0.4^ef</td>
</tr>
<tr>
<td>No Beauveria, no bees (Control)</td>
<td>11.00±1.5^c</td>
<td>5.48±0.8^f</td>
</tr>
</tbody>
</table>

Mortalities marked with the same letter are not significantly different at α = .05 (ANOVA and Tukey grouping).
Optimizing dose for maximum pest control and minimum vector risk

✔ 6.24x10^{10} conidia of *Beauveria*/gram of inoculum resulted in:

✔ 78% kill of *Lygus*,

✔ and 11% of bumblebees (statistically the same as in the controls at 6%)
Refining R & D for PBVT

1. Other control agents
2. a. Vector Safety
   b. Vector & Agent Compatibility
   c. Dispenser Design
3. Human/Consumer Safety Registration & labeling
New: *Clonostachys* on Blueberry Pollinators for Mummyberry control

PEI Organic Blueberry Farm
Pioneering Canadian Research Commercialized!

- BioBest & Koppert dispensers are in use on Bumble Bee hives.
  - No longer “science-fiction”!
Conclusions

• Both bumble bees and honeybees successfully disseminated Beauvaria bassiana to greenhouse sweet peppers and field canola.

• Bee-delivered B. bassiana reduced the numbers of Lygus and Thrips on greenhouse sweet pepper as well as the former on field canola.

• Bee-delivered B. bassiana reduced the numbers of Lygus, Thrips, Whitefly, and Green Peach Aphid on greenhouse tomato and sweet pepper.

• Bee-delivered Clonostachys rosea reduced the incidence of Grey Mold on field grown strawberries, raspberries, and greenhouse grown tomato and sweet pepper.

• Both B. bassiana and C. rosea can be bee-delivered simultaneously and effectively suppress several insect pests and plant pathogens (grey mold) on several crops.

• Pollinator biocontrol vector technology (PBVT) is a win-win situation; it brings benefits of pest control with better pollination and crop yield.
Combining Pollination with Biological Crop Protection Results in Improved Yields. Larger crops. Fewer chemicals.
Acknowledgements

Funding for the research has come from the Biocontrol Network of Canada (NSERC), Improved Farming Systems and Practices Initiative (AAFC), NSERC-CANPOLIN (The Canadian Pollination Initiative) & NSERC-Engage

We acknowledge the assistance we have enjoyed from various companies in Ontario, BC, NS, PEI involved in biological control and the greenhouse industry.

Special thanks go out to all the assistants who have laboured with us over the years.