Evaluation of Beauvericin as a Marker for *Beauveria bassiana* Virulence and its Implication for Greenhouse Pest Management

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Introduction

Insect pests such as western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), are considered to be of great concern to the greenhouse ornamental and vegetable industry due to their potential for extensive damage and revenue losses (Lewis, 1997). At present, biocontrols such as entomopathogenic fungi are in the process of being evaluated as an alternative to synthetic chemicals for greenhouse pest control. Due to the broad host range of many entomopathogenic fungi and some other biocontrol agents, routine screening efforts are required to determine which isolates are better than others for targeting a specific pest insect (Figure 1).

To evaluate entomopathogenic fungi for increased efficacy or high levels of virulence, conventionally, *in vivo* studies using whole animals are conducted. Although whole insect bioassays provide useful information, such tests are laborious and time-consuming (Fornelli *et al*., 2004). Another approach is screening fungal isolates based on their metabolites (i.e. mycotoxins) (Fuguet and Vey, 2004).

To our knowledge there are no studies available which attempt to correlate whole animal virulence due to the exposure of *B. bassiana* isolates and their respective abilities to produce beauvericin for potentially seeking an indication of virulence (Figure 2). In this study we investigate *B. bassiana* isolates exhibiting either high or low levels of virulence for their ability to produce beauvericin for the purpose of determining if isolates can be rapidly screened for high levels of whole animal virulence based on beauvericin production. We included the commercially available isolate GHA (Emerald Bioagriculture, Lansing, Michigan, USA) to serve as an industry standard.

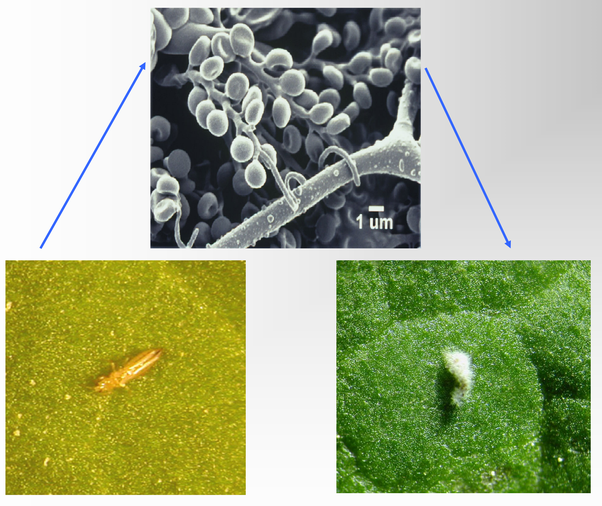

Figure 1. Pathogenesis of western flower thrips by *Beauveria bassiana*.

Primary objective

B. bassiana isolates that had been previously evaluated against western flower thrips were further evaluated for beauvericin production and to determine if beauvericin can be used as an indicator of whole animal virulence.

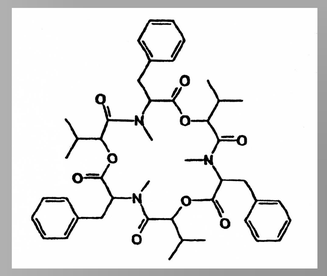

Figure 2. Chemical structure of the beauvericin mycotoxin (Thakur and Smith, 1997).

Materials and methods

- Five isolates were selected based on degree of virulence to WFT in comparison to GHA.
- Mature (14 day old) liquid cultures of *B. bassiana* were harvested and freeze-dried for 24 hours. To extract beauvericin, each sample was ground and homogenized using a tissue homogenizer and methanol (HPLC grade).
- Extracts were concentrated under reduced pressure at 40°C. Extracts were resuspended in 15 ml of methanol and filtered through a 0.22 µm syringe filter before HPLC analysis.
- HPLC analysis was performed using a Bio-Rad Hi-Pore® reversed phased C_{18} column (250 x 4.6 mm, 5 µm) and a photo diode array detector. All samples were filtered through a 0.22 µm syringe filter prior to injection (20 µl) onto the column. Beauvericin was detected at 205 nm.
- Beauvericin was identified by comparing retention times and UV spectra of samples with that of an authentic standard. Further confirmation was obtained by co-injecting pure standards with each sample. Beauvericin was quantified by comparing peak areas from samples to calibration curve of standards.
- Two out of the 5 isolates were replicated (i.e. replicate extractions) two more times to account for possible variability in toxin production and to ensure experimental consistency.

Results

- All five isolates produced beauvericin, ranging from 26.6 µg/g – 2377.5 µg/g (Figure 3).
- Using isolate GHA as an industry standard (Table 1):
  - Isolate SR-11 produced the least amount of beauvericin and performed worse than GHA in WFT whole animal bioassays.
  - Isolates SR-25 and SR-35 produced the most amount of beauvericin (each 10-fold more than GHA) and performed better than GHA in WFT whole animal bioassays.
  - Isolate SR-3 performed better than GHA, however it produced a lesser amount of beauvericin in comparison to GHA (10-fold less).

Figure 3. Chromatogram representing signal generated at 205 nm by injecting 20 µl of *B. bassiana* methanol extractions to determine if beauvericin is present and if so, quantity. Signal measured in milliabsorbance units (mAU). Isolate (a) GHA (b) SR-3 (c) SR-11 (d) SR-25 (e) SR-35.

Table 1. Beauvericin production of *Beauveria bassiana* isolates selected based on indirect exposure against adult female western flower thrips by spraying four conidial concentrations ranging from 10^6-10^8 spores/ml.

Isolate	LC_{50} (conidia/ml)	Beauvericin concentration (ug/g)
SR-11	1.88E+08 - 2.44E+10	26.6
GHA	**1.26E+08 - 8.38E+09**	**828.3**
SR-3	**2.63E+07 - 3.18E+07**	**91.9**
SR-35	1.86E+07 - 3.31E+07	1821.6
SR-25	1.49E+07 - 5.34E+07	2377.5

Conclusions

- Beauvericin was commonly produced in all isolates tested.
- Our results indicated a weak correlation between beauvericin production and whole animal virulence. A low concentration of beauvericin does not mean the isolate has a low degree of virulence. In this case, other screening tools such as *in vitro* morphological characteristics may be used.
- The uncoupling of whole animal virulence and beauvericin production may give us more flexibility in selecting isolates to control pests in different production systems.

Future research

- Examination of more isolates is required to further support the uncoupling conclusion of beauvericin production and whole animal virulence.
- Is there another *B. bassiana* toxin(s) which may relate to whole animal virulence? Or is whole animal virulence, in part, attributed to toxins acting in concert?
- Further downstream analysis of the effects of beauvericin on *in vitro* cell culture systems to determine potential impacts and to support the registration of mycoinsecticides for use in Canada is required.

Literature cited

Fornelli, F., Minervini, F. and Logrieco, A. 2004. Cytotoxicity of fungal metabolites to lepidopteran (*Spodoptera frugiperda*) cell line (SF-9). *Journal of Invertebrate Pathology*. **85**: 74-79.

Fuguet, R. and Vey, A. 2004. Comparative analysis of the production of insecticidal and melanizing macromolecules by strains of *Beauveria* spp.: *in vivo* studies. *Journal of Invertebrate Pathology*. **85**: 152-167.

Lewis, T. 1997. Pest thrips in perspective. In: Lewis, T. (editor). *Thrips as crop pests*. CAB International (Wallingford): 1-13.

Thakur, R.A. and Smith, J.S. 1997. Liquid chromatography/ thermospray/mass spectrometry analysis of beauvericin. *Journal of Agriculture and Food Chemistry*. **45**: 1234-1239.

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