Evaluation of Endophytic *Beauvaria bassiana* as a Targeted Insecticide in Tomato

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Background

Beauvaria bassiana is a fungus known to endophytically colonize a broad range of host plants in tissues both above and below ground, with no documented negative effects to the host (Xu, 2008, Behei, 2014). *B. bassiana* is also a known entomopathogen of many insect species (Gurulingappa et al., 2010). Gurulingappa et al. (2010) documented decreased survival, reproduction, and fecundity of *Aphis gossypii* (greenfly) when exposed to *B. bassiana* on cotton plants. The combination of *B. bassiana*'s ability to colonize a broad host range of plants endophytically as well as to function as an entomopathogen of a wide spectrum of insects offers great potential for use of this fungus as a bio-insecticide.

Domesticated tomato (*Solanum lycoperscicum*) is the most widely cultivated horticultural crop in the world (Schwartz, 2014). It is also an excellent crop to research as it grows quickly and has many genomic resources. For this study, tomato was chosen as the host for the fungal endophyte *Beauvaria bassiana*, which has previously been documented as able to endophytically colonize tomato plants (Ownley et al., 2009). However, the research methods can be applied to any plant host species which can be colonized by *B. bassiana*.

Methods

Leaf tissue of four three week old tomato seedlings (Heinz 1706-BG and Early Girl varieties) were inoculated with a spore suspension 1x 10^7 spores/mL of *B. bassiana* using vacuum infiltration. Four seedlings were inoculated with water as a control. On each plant, six nodes were chosen and the three terminal leaves in each node were inoculated at each site. Tissue samples were taken after 1, 4, and 8 days, ground in liquid nitrogen, and frozen in RNA extraction buffer. An RNA analysis is planned for these samples.

Four weeks after inoculation, an insect herbivore of tomato, the beet armyworm larvae (*Spodoptera exigua),* was applied to the leaves in a no-choice experiment. On each plant, two sites inoculated with *B. bassiana* and two control sites, each containing three terminal leaves, were selected and tagged. Three larvae were enclosed in a bag of mesh that was affixed securely to the stem above the leaves with thread and tape in order to prevent insects from escaping their intended feeding site. Plants were also placed inside insect cages to prevent larval escape (Fig. 1). Four days after the insect inoculation, plant tissue and insect samples were taken for culturing and RNA analyses. The leaves at each site were rated for herbivore damage on a scale of 0 to 5, where 0 was no damage, 1 was 1-20%, 2 was 21-40%, 3 was 41-60%, 4 was 61-80% and 5 was 81-100% of leaf consumed.

A subset of the plant tissue sample was prepared for RNA analysis, while the remainder was plated on a selective media for *B. bassiana* (Strausser media). Insects were cut in half lengthwise and also plated on selective media. Plant growth measurements (shoot height, No. flowers, No. nodes, and root and shoot biomass) were taken and used to analyze the effect of *B. bassiana* on tomato plant growth.

Fig. 1. Experimental Setup

Abstract

A targeted insecticide treatment has great value in agriculture because it would mitigate insect damage while causing no harm to non-harmful insects, especially pollinators. *Beauvaria bassiana* is an endophyte which has been shown to improve plant growth and is a known insect pathogen. It was hypothesized that the combination of these two traits would make *B. bassiana* a potential targeted insecticide. Two tomato (*Solanum lycoperscicum*) varieties, Early Girl and Heinz 1706-BG, were inoculated with *Beauvaria bassiana*. The Early Girl variety was tracked to assess plant-fungal interactions, while the Heinz variety was inoculated with beet armyworm larvae (*Spodoptera exigua*) to examine effects of *Beauveria* on herbivory. Our results show that *B. bassiana* was transferred from the leaf tissue to the beet armyworm larvae, infecting and killing some insects, and slowing insect damage. Our results also show some indication that *B. bassiana* of pathogenic, rather than mutualistic, interaction with the Early Girl variety of tomato.

Results

Effects of *B. bassiana* on Herbivory by *S. exigua*

Inoculation of the Heinz 1706-BG variety of tomato with *B. bassiana* showed decreased levels of herbivory on leaves (Fig. 2, Table 1). The total area of leaf consumed was lower in *B. bassiana* infected leaves.

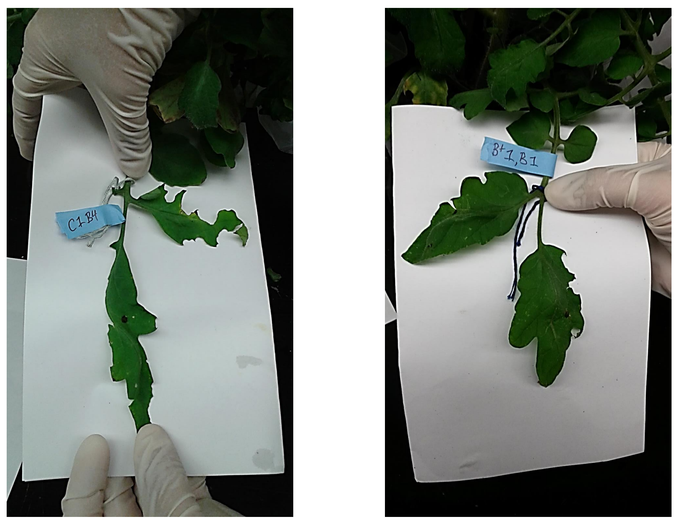

Fig. 2. Control (non-inoculated) leaves exposed to *S. exigua (Left)*, and leaves inoculated with *B. bassiana* exposed to *S. exigua* (Right).

Table 1. Herbivory damage ratings on Heinz 1706-BG tomato leaves 4 days after *S. exigua* inoculation

Treatment	Repetition	Damage Classification	No. of Insects
Control	Average	4.125	2
B. bassiana	Average	3.75	1.75

Transfer of *B. bassiana* from Leaf Tissue to Larvae

In order to test the hypothesis that *B. bassiana* may directly infect larvae feeding on infected leaves, surface sterilized leaf tissue and insects recovered from inoculated leaves were plated on a selective media. Two main morphotypes were isolated from armyworm larvae. One was white, and identified morphologically as *B. bassiana*. The other was green, and is currently unidentified. Molecular methods will be used to determine the identity of these fungal strains.

Table 2. Fungal morphotypes recovered from 16 larvae

Treatment	*B. bassiana*	Green Fungus	Other
Control	0	15	1
Inoculated	8	9	0

Fig. 3. Two main fungal morphotypes (white and green) isolated from beet armyworm larvae in herbivory experiment.

Plant Responses to *B. bassiana*

Inoculation with *B. bassiana* slightly decreased plant growth and reproduction (Table 3).

Table 3. Plant Growth Response in Heinz 1706-BG

	Control	*B. bassiana*
Height	21.71±2.36	19.36±0.92
Flower Count	27.00±8.84	29.50±1.00
Fruit Count	5.00±1	1.75±0.5
Node Count	15.33±3.16	13.00±3.30
Wet Weight (Shoot)	164.66±59.83	108.26±10.10
Wet Weight (Root)	90.46±70.08	72.09±1.46
Dry Weight (Shoot)	20.23±5.19	16.52±17.72
Dry Weight (Root)	13.73±3.45	10.21±0.89

There was some indication that *B. bassiana* may also illicit a pathogenic response. In some cases, the inoculated leaves fell off of the plant. Although this did happen to both the control treated and *B. bassiana* treated leaves, the fall rate was much greater in treated leaves. The response to *Beauveria* may also depend on the tomato variety. A pathogenic response was observed with greater frequency in the Early Girl variety inoculated with *B. bassiana* spores rather than the control (Table 4).

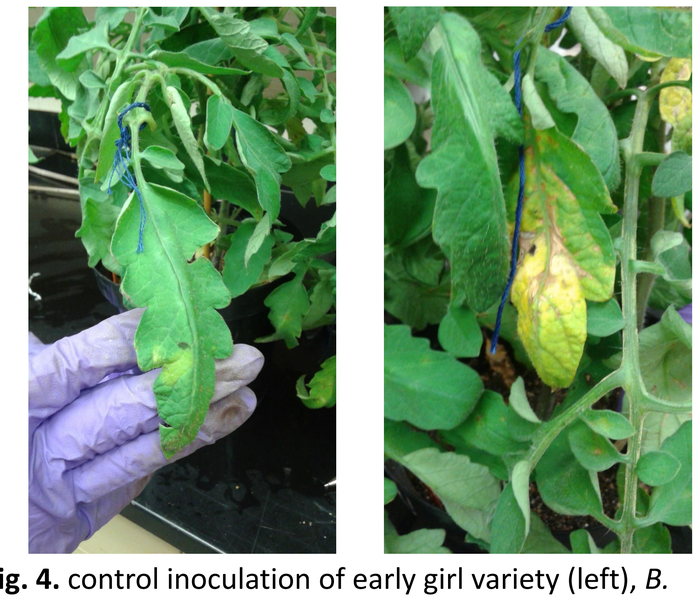

Fig. 4. control inoculation of early girl variety (left), *B. bassiana* inoculation (right).

Table 4. Indication of Pathogenic Response to *B. bassiana*

Treatment	Lost Nodes	Diseased Nodes	Healthy Nodes
Control	9	3	0
B. bassiana	*4*	*0*	*8*

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