



Northern NY Agricultural Development Program 2004 Project Report

Biological Control of Alfalfa Snout Beetle Using the Insecticidal Fungi *Metarhizium anisopliae* and *Beauveria bassiana*

Project Leader(s):

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Cooperator(s):

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Background:

The alfalfa snout beetle (ASB) has been deemed one of the more severe pests of alfalfa in North America, capable of destroying entire fields of alfalfa within a single growing season. The insect was first discovered in the late 1800's in Oswego County, NY. It is believed that the ASB was introduced from Europe by discharge of soil/gravel ballasts onto the shores of the St. Lawrence River and Lake Ontario from wooden sailing vessels. Since then, the flightless beetle has been expanding its range by walking, water movement, and transport on farm equipment.

Currently, ASB infestations occur in eight counties in New York, and the beetle has permanently infested up to 500,000 acres, or 13% of New York State's total cropland.

Because the beetles feed primarily on alfalfa, a major forage crop for dairy farms, the economic loss sustained by New York dairy farms has been substantial (20 –30% decrease in net profits).

With the discontinued use of DDT in 1972 and heptachlor in the early 1980's, ASB populations have been expanding rapidly without an effective and ecologically sound method of control. This has led to the exploration of controlling ASB outbreaks by use

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of biological control agents, such as the entomopathogenic (insecticidal) fungi *Metarhizium anisopliae* and *Beauveria bassiana*. These fungi have proven to be effective against locust outbreaks in Australia and South America and have been used commercially to make “green” pesticides in both areas.

The primary advantage of using biological control is that no obvious health risks to humans by entomopathogenic fungi or compounds secreted by the fungi are evident. Furthermore, with the increasing amount of legislation put forth to curtail the use of chemical pesticides (such as Diazinon, slated to be phased out in the next few years by the FQPA), research into the development of effective and relatively inexpensive bio-control methods will be crucial for sustainable agriculture in upstate New York.

Methods:

Currently, advanced soil ecology methods are being developed and used to examine the field efficacy of the insecticidal fungi and their potential non-target effects on native soil organisms. Both activities are key pieces of information that must be fully assessed before a large-scale control program can be developed.

In the spring of 2004, field experiments were initiated at both the New York Agricultural Research Station in Willsboro, NY, and in infested fields of a private operating farm site in Great Bend, NY (Peck Farm). The aims of these trials were to investigate two main factors associated with implementing an effective bio-control technique using insecticidal fungi:

- (1) increasing the overall effectiveness of the bio-control agent in the field and
- (2) generating soil conditions that promote continuous growth of the fungi for long periods of time after their initial application.

The field site in Great Bend provides us with an excellent opportunity to develop and test effective fungal-based bio-control methods for the control of ASB due to the high rate of infestation found in these fields.

Two different methods of applying the treatment fungi were developed. A soil inoculum which was tilled into the soil prior to planting was prepared in the laboratory and applied to the test plots in the spring of 2003 and in test plots in a different field in the spring of 2004 at two different rates (0.2 kg m⁻² and 0.1 kg m⁻²).

The second application method will be a fungal spore suspension that will be sprayed onto test plots in the spring of 2005 during the adult feeding/egg-laying stage of the beetle's life cycle.

In order to determine viability of fungi after soil inoculation, soil samples were taken at different points during the growing season and subjected to selective media plate counts

to ascertain total colony forming units (CFU's) of *M. anisopliae* in the field. Viability of the inoculant strain will be confirmed by using DNA fingerprinting methods on colonies isolated from the field. The effectiveness of the fungi against ASB will be measured by counting the number of ASB larvae found in treatments plots in the coming season and by overall stand quality for each treatment in the fall of 2005. These results will be compared to a chemical pesticide treatment applied at the same time as the spore suspension.

Soil samples from the 2003 test plots were subjected to microbial community analysis to look for possible changes in the soil microbial community structure which, if found, may indicate adverse effects on non-target organisms. These analyses were conducted for both soil bacteria and soil fungi. For soil fungi, the current methods available need modification in order to be of use for this project. Thus, we have been modifying current protocols to aid us in both assessing non-target impact of the inoculum on fungal communities as well as identifying the inoculum strain within a soil fungal community profile.

At the Willsboro site, a field experiment was established to examine how different crops and crop rotation strategies effect the population of insecticidal fungi in soil. Currently, preliminary greenhouse/growth chamber experiments of a similar nature have been conducted in which neutral potting mixtures planted with different families of crops (i.e. alfalfa, oats, and canola) were investigated for their ability to sustain and even promote establishment of a population of insecticidal fungi based on potential differences in the composition of root exudates from the different plant families.

However, greenhouse trials are only indicative, rather than conclusive, of what may happen in the field, and it was therefore necessary to conduct field trials. Unfortunately, the field trial in Willsboro had to be abandoned because of a severe weed infestation in the plots. Because we are looking at how the roots of each specific plant type may effect the microbial populations in the soil, the presence of a large number of different weeds from all different plant families made it impossible to continue this field experiment in 2004.

It is our intention to attempt this experiment once more, with a better handle on how to prevent another weed infestation. For this study numerous techniques ranging from more traditional methods, such as selective plating, to more advanced and accurate techniques, such as molecular microbial community analysis, have been developed in the past year in order to assess the effects of crop rotation on the population of insecticidal fungi in soil.

Results

Principal components scatter plots provide a visual representation of the similarity between samples – the closer the points, the greater the similarity in the communities analyzed between the samples. The inoculated and non-inoculated samples are fairly close together, while distinct groups are resolved based on sampling time (as expected). See Figure 1.

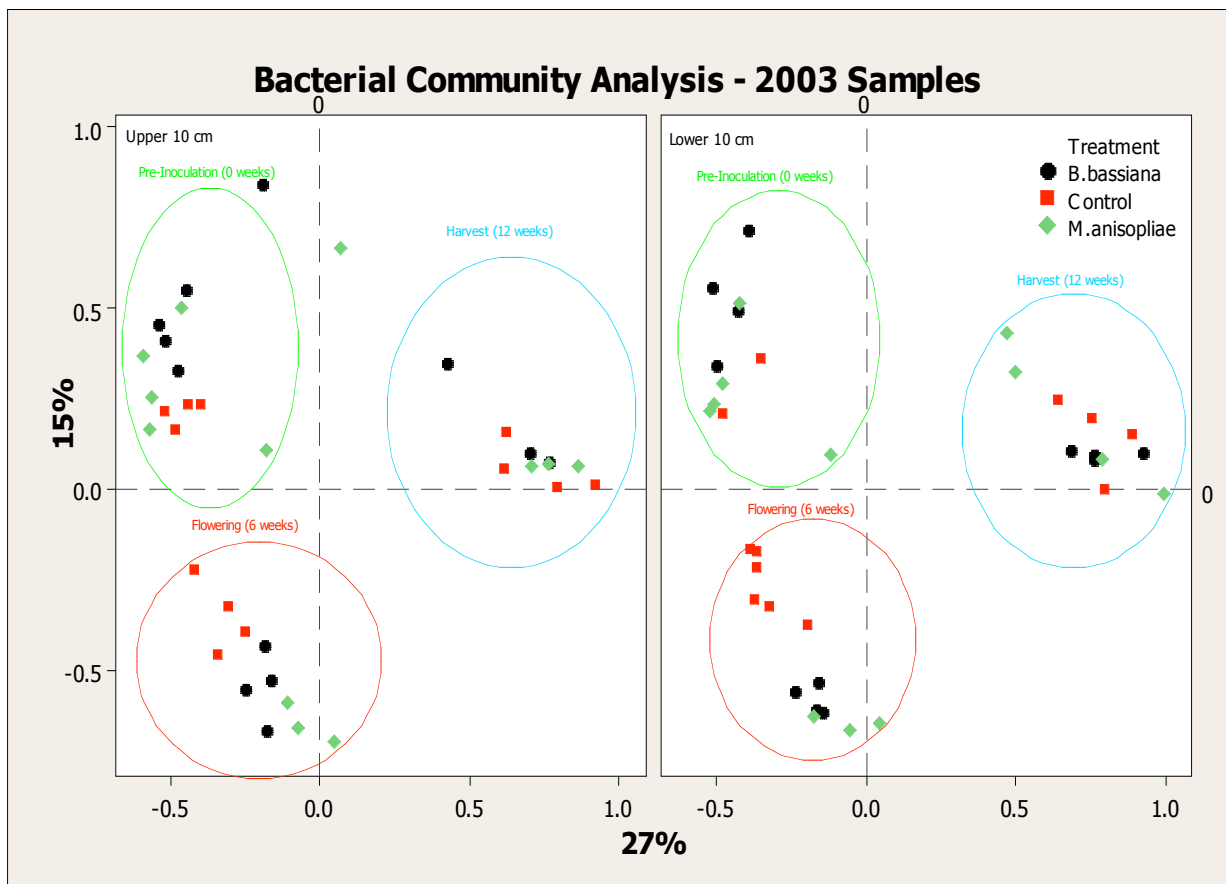


Figure 1. Bacterial community analysis in soils inoculated with *M. anisopliae*, *B. bassiana*, and non-inoculated soils, divided into upper and lower 10 cm of soil samples. Principal components scatter plots provide a visual representation of the similarity between samples – the closer the points, the greater the similarity in the communities analyzed between the samples. The inoculated and non-inoculated samples are fairly close together, while distinct groups are resolved based on sampling time (as expected).

Development of fungal community analysis techniques

Currently, fungal community analysis methods are still in development. This work is anticipated to be completed in the spring of 2005 and implemented in the analysis of the 2004 field site samples.

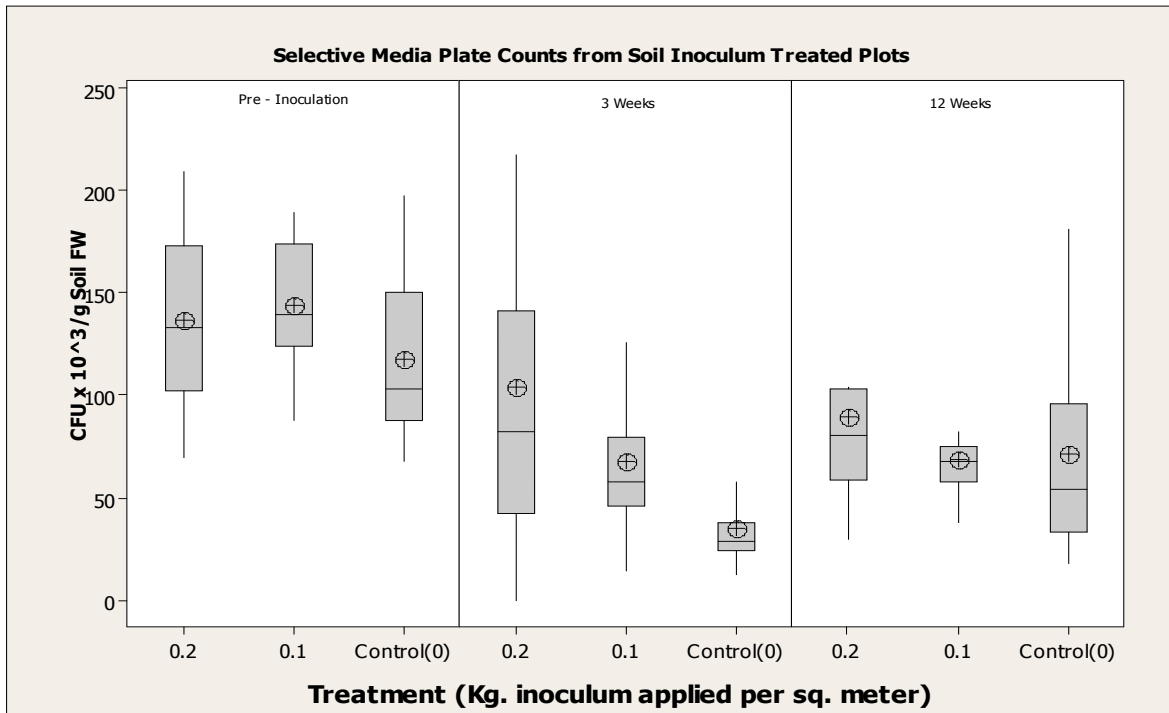


Figure 2. Viability of *M. anisopliae* based soil inoculum in 2004 field plots.

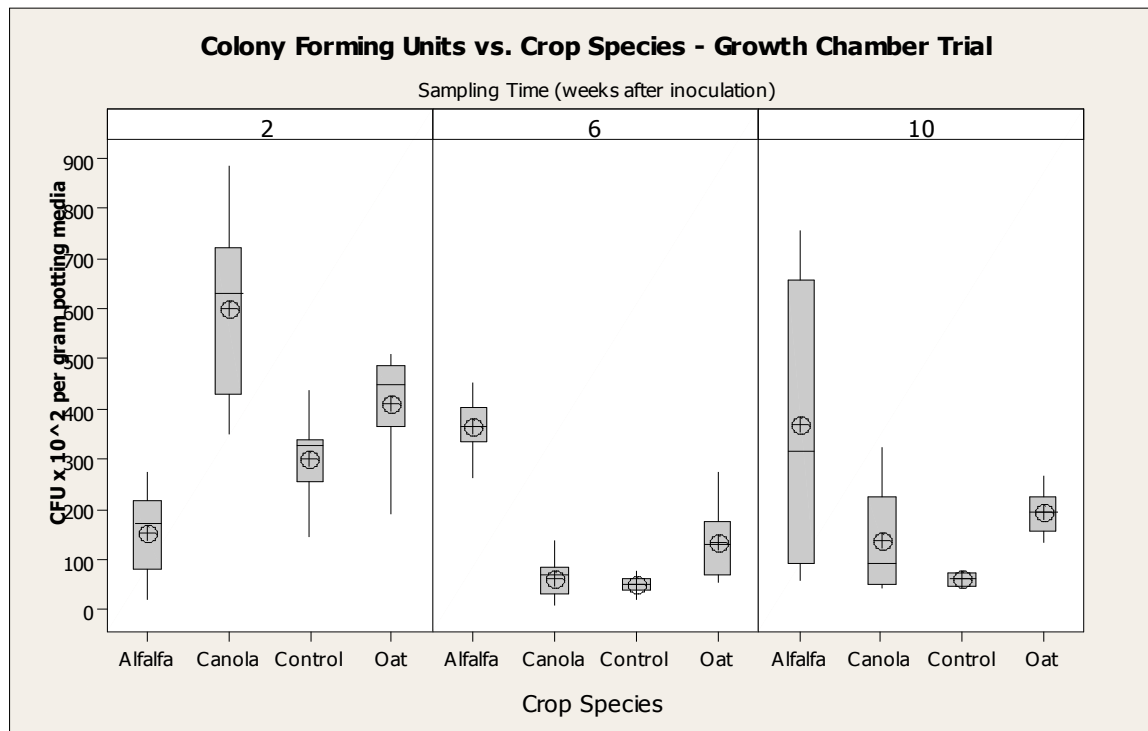


Figure 3. Growth Chamber experiments – Rhizosphere effect on *M. anisopliae* populations.

Conclusions/Impacts:

As can be seen in Figure 1, the bacterial community analysis so far suggests no non-target effects of the inoculum on bacteria, and work on the fungal community analysis is currently underway. The results from the selective media plate counts in Figure 2 suggest that plots inoculated with *M. anisopliae* maintain an increased level of CFU's throughout the growing season, which is of some importance in relation to the beetle's life cycle. By spring of 2005, the beetles will be laying eggs at the base of the alfalfa stands, and the larvae upon hatching will begin to burrow into the soil to depths of about 15 cm. It is anticipated that plots with increased number of *M. anisopliae* CFU's will exhibit lower numbers of ASB larvae and better overall stand quality.

The results of the growth chamber experiment (Figure 3) indicate that there may be some significance to the use of different crops to promote *M. anisopliae* populations. It appears that the highest levels of fungi are initially seen in the canola planted soil, yet after 6 and 12 weeks the highest level of fungal colonization is seen in the alfalfa planted soils. Because these data are somewhat conflicting with current knowledge about the ASB problem (if alfalfa supports high levels of fungi, why is there a beetle problem?), field trials will be of the utmost importance to confirm the accuracy and potential impact of this result.

Presentations, Abstracts and Fact Sheets:

Jones, CM and Thies, JE. 2004. Soil bacterial and fungal community response to application of entomopathogenic fungi in the field. *American Society of Agronomy, Agronomy Abstracts*, Seattle, WA

Jones, C and Thies, JE. 2004. Microbial community analysis in biocontrol research: Creation of a regional sequence database and refinement of T-RFLP for use with fungal populations. *10th International Symposium on Microbial Ecology (ISME)*, Cancun, Mexico.

Shields, EJ; Thies, JE and Viands, D. 2004. Developing biological controls for Alfalfa Snout Beetle, a Northern New York Agricultural Development Program Fact Sheet, #1 in a series on alfalfa snout beetle.

Future work:

The most important work to be conducted in the future will be the completion of the 2004 field trial to determine the efficacy of the fungal treatment on ASB populations in test plots. CFU counts and isolate DNA fingerprinting from soil samples and infected beetles found in the field should also be performed to confirm the presence of the treatment isolate.

Furthermore, work on potential non-target effects of the fungal treatment should be completed for both fungal communities and non-target insects in the 2005 growing season.

Finally, a field trial replicating the growth chamber experiment in the field should be conducted during the 2005 growing season. This may be of importance in that if alfalfa does indeed support a higher population of *M. anisopliae* in the field, it may be advisable to combine crop rotation with the use of a fungal-based biocontrol agent. This is due to the possibility that the beetles may be able to adapt to an environment with a large number of fungal propagules, thus rendering a fungal biocontrol agent less effective over time.

Acknowledgments:

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Northern New York Agricultural Development Program:

The Northern New York Agricultural Development Program provided funding for this alfalfa snout beetle research project. The Northern New York Agricultural Development Program is a farmer-driven research and education program specific to New York state's six northernmost counties: Jefferson, Lewis, St. Lawrence, Franklin, Clinton and Essex.

Thirty-three farmers serve on the Program board led by Co-Chairs Jon Greenwood of Canton (315-386-3231) and Joe Giroux of Plattsburgh (518) 563-7523. For more information, contact Jon, Joe or R. David Smith at 607-255-7286 or visit www.nnyagdev.org

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