

Biological Control of Chinch Bug Research Project, 2005-2006

Final Report, Year 3

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Year 3. Biological Control of Chinch Bug Research Project. 2006-2007.

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Executive Summary For Year 3

During year 3 both field and lab bench testing was carried out as part of an ongoing effort to identify methods to control chinch bugs. The project commenced late in the season (August) and insect numbers were low, but certain conclusions could none-the-less be drawn. The field tests, made on the lawn at the firehall in Rothesay NB confirmed that Sevin (carbaryl) killed the insects within about two weeks. However, this chemical insecticide had low specificity, killing most other insects. Its effects did not persist; Treated plots became re-infested within a few weeks. Shading the plot surface reduced chinch numbers dramatically, and also improved the appearance of the turf. Application of soluble N:P:K also decreased chinch numbers and improved appearance of the turf. Organic fertilizer improved turf appearance but did not decrease chinch numbers. Some proprietary organic fertilizers *increased* chinch numbers, presumably by attracting them. Watering had little effect on either turf appearance or chinch numbers, but this may relate to the poor water holding capacity of the soil at the test site. Interestingly, the best chinch control in this field test was obtained by topdressing with compost.

Implanted sod (either Kentucky bluegrass or sod cut from the 'chinch resistant' turf at Angelview park) both became colonized by chinch, but neither sod type dramatically attracted the insects. The sod from Angelview became colonized with as many bugs as did the commercially-produced Kentucky bluegrass sod, some chinch bugs migrating into both types within a few weeks of them being implanted.

As a follow-up to the field work, the field plot soils and also soils that came from sites that differed in their susceptibility to chinch bugs were assessed for abiotic factors (e.g. chemical and physical characterization) and biotic factors. The latter included both general soil microbiological factors and factors included in the analysis done by Soil Foodweb Canada East.. For purposes of comparison, some manufactured topsoils were also analysed. Not enough soils were analysed to show clear trends, but the results were consistent with the idea that nutrient rich soils with active populations of beneficial microbes are more likely to support lawns without chinch problems.

The results from the microplots (the outdoor turf-containing buckets) that were started in year 2 were disappointing. The main focus this year was to use them to assess the effects of the *Beauveria bassiana* fungal strains that had been shown to kill chinch bugs in the lab-scale tests. When chinch bugs were added to the microplots, and these were then inoculated with the *B. bassiana*, there was no evidence for the fungus killing the chinch bugs on either of the two soils tested, regardless of whether a 'high' or 'low' watering regime was used. Dilution plate counts confirmed that the *B. bassiana* spores remained alive in the plots for the duration of the experiment, and lab tests, made in parallel confirmed the virulence of the isolate towards the insects. These results substantiate those that were obtained last year on these microplots.

When a similar test was subsequently made using pots of sod and chinch bugs growing in clear, vented plastic bags held in a plant growth room (ca 25°C, 16hr day length), the *Beauveria* did kill a high percentage of the chinch bugs within three weeks. Temperature may have been a key factor differentiating these encouraging results from those obtained in the outdoor microplots. Other explanations are also possible.

In subsequent lab (*in-vitro*) tests, the *Beauveria*'s ability to kill various age classes of chinch was assessed. The fungus was much more active against younger insects. Over 80 % of the 1st, 2nd and 3rd stage nymphal instars died within three days of *Beauveria* inoculation while relatively few (<20%) of the inoculated older insects, or insects in the non-inoculated control treatments died. These results suggest that for *Beauveria* to be effective, it should be applied earlier in the season, before the insects have matured. The literature suggests that entomopathogenic fungi (*Metarhizium* and also *Beauveria*) might also kill chinch eggs. This should be investigated.

Some preliminary tests were made to ascertain if there was potential to increase the efficacy of *Beauveria* by using it in conjunction with other potential "green" control agents. To this end survival of *Beauveria* conidia in insecticidal soap, various other surfactants, an essential oil based product named "MuscleTM" a few essential oils and compost tea was measured. Scientific or anecdotal evidence can be found on the internet or in the literature that each of these can control insects. Survival of the *Beauveria* conidia in Safer's soap was good, providing the concentration was not too high. Survival was also good in various surfactants, but interestingly was not good in Sunlight Lemon FreshTM dish detergent. This could result in problems for the unwary who chose to use "benign" dish detergent on their lawn. The MuscleTM killed the *Beauveria* conidia when used at the concentration recommended for chinch control, but they might be used together if it were diluted more. MuscleTM's constituent essential oils (eugenol, peppermint oil) also killed the spores, unless their concentrations was low. The compost tea stopped germination, but the spores were not killed. They did germinate when they were put onto nutrient medium. These results suggest that *Beauveria* conidia could be applied with insecticidal soap or compost tea, possibly as a spray. Direct application of *Beauveria* with concentrated MuscleTM or other essential oil-based treatments would not be advised, but the fungus could be used with diluted oils. Any of the materials might be synergistic with the *Beauveria*, possibly by stressing the insects. It seems likely that if the formulated *Beauveria* were added when younger insects were present, and if the temperature was high enough for the fungus to be active, chinch control could be accomplished. This could be the subject of further testing.

Introduction and Background. Year 3.

The overall goal of this project is to develop methods for controlling chinch bug (*Blissus leucopterus hirtus*) that do not depend upon the use of synthetic chemical insecticides. This is the final report for the third year of the project. More background information has been presented in the final reports for Year 1 and Year 2. Copies of these are attached and are also available on request (dboyle@nbnet.nb.ca).

Throughout this project we have been trying to identify soil factors that might make a site susceptible to chinch bugs. To gain insight into this, we compared soil from Angelview park in Fredericton to soil taken from the Rothesay firehall lawn. The former has been essentially chinch bug free for at least four years, while the latter has an on-going, serious chinch problem. We also looked at two manufactured topsoils, since these are being used with increasing frequency for lawn construction, and there seems to be a tendency for lawns made with manufactured topsoils to have chinch bug problems (Gregor MacAskill, Ecology Action Centre, Halifax, Pers. Comm.).

The soils were analysed for the normal spectrum of plant nutrients (N, P, K, etc.) by A&L labs, Ontario. In addition, they were characterized for their biological activity. Some of the latter assays were done by *Maritime MicroBiologicals* with others being done by the Soil Foodweb Canada East lab (www.sfce.ca) in Halifax. We decided to have the latter analysis done after hearing a talk by Dr. Elaine Ingham, President of Soil Foodweb International, who presented a talk at the opening of the Halifax lab. Her presentation convincingly underlined the importance micro-organisms play in plant-soil relationships, and in growing healthy lawns.

As indicated in the final report for Year 2, we established some micro plots outside of Maritime MicroBiologicals Inc's lab. The original intent was to use these to test the effects of a range of factors (soil type, watering regime, soil depth, soil microbes, entomopathogenic fungi) on chinch bugs. Unfortunately, due to poor over-winter survival of the plots and also to the late start of this year's project, we had to abandon some aspects of this testing. We did however succeed in carrying out the tests with the entomopathogen *B. bassiana*. Unfortunately, the results confirmed last year's microplot results, showing that the fungus did not kill the chinch bugs. To reconcile these results with the ones obtained in the lab, we also set up some better controlled, "micro plot" tests under lab conditions. These showed that the *B. bassiana* did kill the chinch bugs. The conflicting results are discussed.

Some replicated field plot testing was done in which the effects of various edaphic and cultural factors on chinch bugs on the lawn of the Rothesay Fire hall was assessed. Unfortunately, the late start made it impossible to adequately assess effects the treatments might have had on the earlier stages (nymphal instars) of the chinch, but the effects on the adults were of interest. These results suggest some potential non-chemical chinch control strategies.

More in-vitro tests were made looking at the effects of *Beauveria* on chinch bugs. Immature insects were collected from the field and the effects of *Beauveria* on these was assessed in in-vitro. The results showed that *Beauveria* is more active against young insects. Future microplot or field testing should take this into account.

The literature (e.g. <http://versicolor.ca/lawns/docs/SoapFeb05/soapNEW.html#Overview>) and some of our preliminary findings suggested some other treatments that might be effective against chinch bugs. These included Safer's insecticidal soap, a new GRAS (Generally recognized as safe) product called Muscle™ (Soil Technologies Corp., 2103 185th St., Fairfield, IA 52556) that is being used in the United States, and compost and/or compost tea. It also seemed possible that these might be synergistic with *Beauveria*, by for example weakening the insect making it more susceptible to attack by the fungus. As a prelude to testing synergy with *Beauveria*, the effects of the various compounds on germination of *Beauveria* conidia was assessed. Results from these tests are presented here.

Materials and Methods

I. Field plots at Rothesay Fire Hall

On July 26, 2006 test plots were established at the Rothesay fire hall, Rothesay N.B. This site was chosen since preliminary inspections showed that it had a consistently high population of chinch bugs throughout the 2005 season. The turf here is very badly damaged most of the grass species, other than fescues, having been killed (see Fig. 1). The plots were placed on the south facing slope, running down to the road in front of the fire hall. A total of 45 plots were established with 15 plots across the hill and 3 plots running down it. (i.e. a 3 x 15 plot array). Each plot was 1m wide and 2 m long, with a 0.5 m inter-plot distance. Treatments were assigned using random numbers, for a completely random (no blocking) design. The corners of each plot were marked with spikes going through small coloured rectangular plastic markers.

On July 28 some of treatments were applied. Others were applied later, as indicated below. There were 3 replicates for each treatment, except for the controls where there were 8. The treatments, with their letter designations are described below.

A. Control. No additions or changes were made to the plots.

B. Sevin (Chemical insecticide). This was applied on Aug. 3 by Urban Landscaping, as per product label.

C. Soil and turf replaced with Kentucky blue grass. For this treatment, the top 6" of soil from a 16" x 48" area in the middle of the plot was removed on July 28 and replaced with manufactured topsoil, produced by the city of Fredericton. Kentucky blue grass sod,

donated by New Brunswick Quality Turf, Maugherville N.B. was planted on top of this. These plots were watered heavily at planting, and approximately twice per week for the 3 weeks following planting. A goal was to determine if the chinch bugs would rapidly migrate into the plot from the surrounding area.

D. Turf replaced with Kentucky Blue Grass. These plots were similar to C, except the soil was not replaced. i.e. The resident fescues were removed and replaced with Kentucky blue grass sod. Here too, we wanted to determine if chinch would move rapidly into healthy Kentucky Blue Grass sod.

E. Turf replaced with Angelview sod and soil. For these, we cut and lifted sods with the associated underlying 6" of soil from Angelview Park, Fredericton, this site being notorious for its lack of chinch bugs. The sod and soil were transplanted into the middle of plots, using the same procedures as for B.

F. Decreased water stress. These plots were watered twice per week with 20 liters (1 cm) of water each time. Watering started the week of August 8th.

G. Light reduced to ¼ intensity. On August 10th, wooden frames, covered with two layers of black horticultural shade cloth (Halifax Seed, Saint John N.B.) were positioned 30 cm above the plots on legs at the corners of the plots. Measurements taken with a light meter showed the cloth decreased the light reaching the turf by 75%.

H. Nutrient stress reduced with soluble fertilizer. A total of 25 g.m⁻² (50 g per plot) Plant ProdTM 20:20:20 All Purpose Fertilizer (Halifax Seed) was applied to plots. Half of this was applied in 8 l water on July 28, with the other half being applied two weeks later. The total amount gave 10 g mineral N per plot, this being equivalent to the organic N (mainly protein) applied in treatment I or K, below.

I. Nutrient stress reduced with Organic fertilizer. For these replicates we used NutriteTM Organic 100% Natural Lawn Food with corn gluten, this having a analysis of 8:2:4. It was applied on August 3rd at 62.5 g.m⁻² (125 g per plot), giving a N application equivalent to that in H or K.

J. Low level of a mustard-derived product. The primary purpose of treatments J, K and L was to ascertain if a mustard-derived product would kill chinch bugs (See Brown and Morra, 2005 for background). The product has about 5% N and other nutrients in it, so it is an organic fertilizer. Fifty g.m⁻² (200 g/plot) of the product was spread evenly onto the plot on Aug 3rd and watered with ca 1.5 cm water applied intermittently over a 20 min period so it would soak into the soil.

K. Intermediate level of the mustard product. Here, we applied 100 g.m⁻² of the product on Aug 3rd, following the methods in J.

L. High level of the mustard product. Here we applied 150 g.m⁻² of the product on Aug 3rd following methods in J.

M. Top dressed with compost. In this treatment, 1 cm of compost was evenly spread onto the plot on Aug 10th. This was produced at Fredericton's compost manufacturing facility. It was passed through a 1 cm mesh sieve immediately before use.

Sampling. At the indicated dates chinch bugs were vacuum sampled from a sub-plot of the turf that was enclosed by a 20 cm x 50 cm (1/10 m²) quadrat (see Figure 2). The quadrat was placed on the plot in a different location for each sampling date. The vacuum sampler was made by covering the inlet pipe of a Makita gas powered vacuum with fine mesh (0.1 mm diameter) nylon fabric, forming a small sac in which the insects and organic debris (thatch, leaf clippings, etc.) from the sub-plot were retained. The vacuum was used at full power for about 10 seconds on each sub-plot, and the material retained on the mesh was then emptied onto a white tray, where the insects were readily visible. Insects in each age class were counted. After counting, the insects and debris were returned to the sub-plot.

At the end of the season, we measured volumetric soil moisture of the soil in plots that had (treatment F) and had not (controls) been watered using a Theta Probe Type ML2, Delta T instruments, borrowed from Pat Toner, NBDFAFA. These measures were made both 30 minutes and 6 days after the test plots were watered.

In addition, we assessed plant vigour on a subjective scale of 0 (all plants dead) to 5 (green, luxuriant growth, healthy grass) and made assessments of the incidence of disease.



Figure 1. On the left is a general overview of the Rothsay Fire Hall site, the three shaded plots being evident. On the right is one of the plots soon after Kentucky Blue grass was planted in it. The poor health of the surrounding plots is evident. In the background is a patch of white clover, this appearing relatively green.



Figure 2. Vacuum sampling for chinch bugs.

II. Testing and developing *Beauveria* for use against chinch bugs.

A. Microplots. These are located outside MMBI's lab. They were constructed and seeded in 2005. Details of the construction methods (soil types, seeding, watering regimes, fertilizer applications, etc.) are presented in the year 2 final report. A picture of the plot, with the roof open, is in Figure 3.

Due to the delayed project start, we abandoned some of the objectives for these plots and focussed on assessing the effects of *Beauveria* inoculation on chinch survival. Briefly, in August we set up a completely randomized plot design to look at the effects of a) *Beauveria* inoculation (ca 10^7 conidia per gram vs 0 conidia in the controls) b) soil type ("A" vs "B") and c) watering regime ("high" vs "low") on chinch survival.



Figure 3. The microplots at Maritime MicroBiologicals Inc. The roof that keeps rain off the plots facilitating moisture control, is in the open position. Fine mesh is over the plots.

In October of 2005 the plots were heavily watered and mulch was packed around the buckets for insulation over the winter.

In early May of 2006, a lot of variation in over-winter survival of the plots was noticed. Until the contract was secured in August, the plots were managed with the view to decreasing this variability, and improving turf vigour. To this end, the fine mesh screen that had been over them during the winter was removed to improve ventilation. (Some mould had grown on some plots during the spring). All the plots were watered to about field capacity and the grass in them was “mowed” to 2” above the crown using the high speed rotary cutter described in last year’s report. For the duration of the project mowing was repeated when the grass in the fastest growing plots reached a height of about 6”.

In early June, since there were still many bare patches in some plots, they were over-seeded with 1 g of CIL Golf Green mix (5% Premium chewings fescue, 55% Creeping Red Fescue, 40% Turf-type, Perennial Rye grass). The soil surface was lightly scarified to cover the seeds, and the plots were watered. After a week, many of the new seeds had germinated, and all the plots were fertilized with 1g of soluble 15:30:15 fertilizer (“Miricle Gro”). In subsequent weeks, plots were watered twice a week with a moderate amount (1 l) of water, which was sufficient to keep the soil moist at all times. At the middle and end of June the plots were re-fertilized.

Watering was continued until July 20, when 12 plots that contained soil A (Nashwaak River Loam), and 12 that contained soil B (Manufactured topsoil from Halifax) were selected on the basis of having relatively healthy-looking sod. These plots were then randomly assigned to either “high” or “low” watering regimes, these receiving 2 l or ½ liter water, twice per week respectively, until the end of the experiment.

On August 19, 2006 the first dose of *Beauveria* was applied to half of the plots (i.e. 3 of soil A, high water, 3 of soil A low water, 3 of soil B high water and 3 of soil B low water) in each of the 4 treatments, the other plots (controls) receiving dead inoculum. . The inoculum consisted of rice grains upon which *Beauveria* had been grown for 3 weeks. A 1:1 mixture of grains inoculated with the *Beauveria* strain used in Botinigard™ (GHA), and grains inoculated with strain B3 (the strain that performed well in the lab tests reported in the Year 2 report) was used. Fifty g of this mix was spread onto the soil surface, and 1 L of water was sprinkled on to wash the conidia off of the rice onto the soil surface. A dilution plate count of the inoculum showed this gave about 1.2×10^{10} conidia per plot, or about 1×10^7 conidia per gram soil, assuming the conidia dispersed through the soil in the plot. (In actuality, it seems likely the conidia would have been at a higher concentration in the surface soil layers, where the chinch bugs are often located).

The control plots were also inoculated using the same inoculum, after this had been steam-sterilized.

On August 20, one day after the inoculum was added, 50 chinch bugs were added to each of the 24 plots. About 80% of these were adults, the others being 5th stage instars. These insects had been collected three days previously from various locations around the Rothesay Fire Hall using the vacuum sampler mentioned above. The plots were re-closed with fine mesh to retain the insects. This was secured around the top of the container using two elastic bands. In addition, a 5 cm-wide band of Vaseline was spread around

the upper part of the container's wall, under the mesh. Previous experience had shown that chinch bugs do not readily cross such a band, and do not get stuck in it.

At some of the subsequent cutting and/or watering times, the number of chinch bugs in the plots was assessed by spreading the grass and inspecting the soil surface. Only the results obtained on September 12 are presented, but the trends (no effect of the inoculation) on the other dates were similar, so the data is not presented. Note was also sometimes made of the amount of growth that had occurred since the last cutting, and of general turf vigour. This was subjectively assessed on a 0 (dead) to 5 scale.

On September 3, two weeks after the chinch bugs were added, the *Beauveria* treatments were re-applied, following the procedures indicated above; i.e. Another 1.2×10^{10} conidia on rice were applied to the experimental plots, with autoclaved rice being applied to the controls.

The percent moisture of the soil in the plots was measured on October 3, four days after the plots had last been watered, using the Theta probe. The plant vigour was also assessed (subjective measurement on a 0-5 scale, see last year's report).

On October 13 the soils from the treatments were sampled and assayed for *Beauveria* using a dilution plate count with selective agar media. (EEA, see last year's report).

On October 14th, the number of insects in the plots from the various treatments was measured using the floatation assay. For this, the bucket from each plot was placed into a larger bucket that contained warm (ca 30C) water. This percolated up through the drainage holes and through the soil and sod so that the chinch bugs were forced to the surface, where they could be counted (fig 4).



Figure 4. Chinch bugs on grass blades during floatation assay of microplots.

B. Effects of Beauveria on chinch bugs on grass growing in pots.

The goal here was to determine if the *Beauveria* would kill chinch bugs on Kentucky Blue grass growing in pots under lab conditions. To this end, on September 5th, pieces of sod (donated by Wetmore's) were cut and planted on Myke™ potting mix in 2 ½ inch diameter square pots. The pots were kept under the roof of the microplot area at Maritime MicroBiologicals, being watered three times per week and cut to a height of about two inches once per week. After 4 weeks, the pots were brought into the lab and placed into clear plastic bags equipped with microporous patches for ventilation. (These bags are used for mushroom spawn production at Maritime MicroBiologicals Inc. and are from Unicorn Bags in Texas). Prior to sealing the bags, they were inoculated with either 10 g of rice grains colonized with *Beauveria bassiana* isolate 2 (ca 1×10^9 conidia per pot) or 5 mL of Botinigard ES (Emerald Agriculture), this being diluted 1:10 with water. The rice grains or liquid inoculum were sprinkled onto the sod surface in the pots. The Botinigard ES that was used in this test was old (Expiry 5/31/05) but plate count assays showed that the conidia in it were still viable, over 90% of them germinating. About 2×10^9 conidia were added per pot. It should be noted that the isolate 2 is the same strain (GHA) that is used in Botinigard.

Control treatments received a mixture of the rice and the Botinigard, after this had been autoclaved to kill the fungus. After inoculation, 15 adult chinch bugs, provided by Nancy Hudson, St. John's Newfoundland, were added to the pots, the bags were sealed, and moved to a controlled environment chamber (ca 25°C, 16 hr photoperiod). There were four pots (replicates) in each treatment.

The chinch bugs were sometimes seen feeding. Other times they were on the plastic wall of the bag or on the soil surface. Others could not be seen, possibly being below the soil surface. On two occasions they were counted.

After three weeks, the grass in the pots had grown considerably, making it necessary to terminate the experiment. To count the chinch bugs, the bags were opened and filled to a height of about 6" with 25°C water. This percolated up through the pots' drainage holes, displacing the live bugs from the soil, plastic bag surfaces, leaf surfaces, etc. After 10 minutes, the bugs were counted. This procedure recovered the living chinch bugs.

Figure 5. Pot system for assessing the effect of *Beauveria* on chinch on grass. This picture was taken at the end of the assay period, as is evident from the over-grown grass.



C. Testing *Beauveria* against various nymphal stages of chinch bugs.

The literature indicates that *Beauveria* can be more active against certain developmental stages of insects. We assessed this using insects collected with the vacuum sampler from the Rothesay Firehall site on August 10. These were brought back to the lab where they were sorted into age classes. For this, the insects were first separated from the debris using sieves. They were then transferred onto a white tray to make them more readily seen. Insects from each age class were collected into separate glass vials using an aspirator (provided by Garth Nickerson) attached to a vacuum line. The insects were kept in a refrigerator overnight, and then placed into the multi-well assay system (Figure 6).



Figure 6. The assay system used to assess the effects of *Beauveria* on various chinch developmental stages.

This was made from three pieces of 7 cm x 9 cm x 3 mm thick clear plexiglass, held together as a “sandwich” using spring clips. The middle piece had a 3 x 4 array of 8 mm diameter holes drilled in it, thereby creating 12 separate wells.

For the assay, the top plexiglass sheet was removed, thereby opening the wells. Four or five insects were placed into each well. To facilitate handling, the insects and the chamber were cooled to about 5°C on a cold pack. *Beauveria*-treated insects were in the wells at one end of the chamber with controls (dead *Beauveria*) at the other end. Replicates were made using four separate multi-well assay systems for a total of about 20 insects in each treatment.

The inoculum was *Beauveria bassiana* 2 (e.g. isolate GSA from Botinigard), this having been grown for 3 weeks on rice. A single *Beauveria*-colonized rice grain, along with associated conidia was placed in each well, autoclaved grains being used for the controls. Dilution plate counts made on this inoculum showed there were about 10⁸ conidia per rice grain.

The chambers were kept in a polyethylene bag containing a wet paper towel (i.e. high humidity). This was maintained in a dark, 22°C room. Once per day the chambers were assessed under ca 10X magnification attained using a video camera - monitor with a macro lens. Insects were considered dead when they showed no signs of movement, even when subjected to high frequency vibration. (For this, we touched the floor of the assay well with the body of an electric toothbrush).

D. Testing compatability of Beauveria with potential synergists.

To assess compatability of *Beauveria* with Safer’s insecticidal soap (Safer Limited. Scarborough Ontario) conidia were mixed into various concentrations of the product to give a conidial concentration of about 10⁷ per mL. After either 10 minutes or 24 hours storage at 4°C, the percentage of conidia in the suspension that were viable was determined by plating an aliquot onto *Beauveria*-selective agar (EEA, see year 2 report). After 5 days the percentage of applied conidia that formed *Beauveria* colonies on the agar was calculated. This experiment was first done using soap concentrations ranging up to 100%. It was then repeated, focus being on concentrations up to 12%, this being more relevant from an applied vantage.

Similar methods were used to test conidia viability after either short or longer term exposure to other surfactants including Tween 20, Triton X 100 (both used in widely in research and industry) and in Sunlight Lemon Fresh™ dish detergent.

Conidial survival in Muscle™ (Soil Technologies Corp., 2103 185th St., Fairfield, IA 52556 USA, 800-221-7645) was also assessed using the same methods. This product contains both essential oils (25% eugenol and 1.25% peppermint oil) and also 15% sodium laury sulphate. The latter is a detergent (wetting agent) so might be expected to work like an insecticidal soap. The label claims that it kills chinch bugs. It is EPA registration exempt. Steve Nichols, who is responsible for sales and development of the

product, says it has shown good potential for chinch control in tests made in Florida. Normal practice is to apply a 1:20 dilution as a spray. Similar methods were used to assess conidial survival in the two essential oil components (eugenol and peppermint oil) of the product.

We also assessed survival of *Beauveria* conidia in compost 'tea'. This was made by mixing 10 g of mature compost from the city of Fredericton (the same compost that was used on the Rothesay plots) with 100 ml of distilled water. This placed for 18 hours on a magnetic stirrer run at high speed (ca 100 rpm) so that a collapsing vortex formed. This would presumably have kept the tea aerobic. According to Dr. Elaine Ingram (2001) compost tea's should be aerobic.

III. Characterization of Soils

A. Characterizations done by A&L Canada Laboratories Inc.

The chemical characterization of the soils was done by A&L Canada Laboratories Inc. in London Ontario. Both their standard analysis and a total Kjeldahl nitrogen assay were included. The latter includes the organic N in the soil, and also the ammonia, but not the nitrate. The sample codes for the lab with their corresponding letter designation of the field plot treatment (see last report are: #1 (A) = the control plots. #2 (I) = organic fertilizer. #3 (H) = Soluble fertilizer #4 (L) = the high level of mustard product. #5 is the soil sample from Angelview park. Samples were composites of 3 sub-samples taken from the top 15 – 20 cm.

B. Characterizations done by Soil Foodweb Canada East Ltd.

These biological analysis were made on composite samples from the Rothesay Firehall control plots, Angelview park and manufactured Lawn Soil and Garden Soil (provided by Urban Landscaping). The analysis were made within about a week of sampling.

The "complete" Soil Foodweb analytical package was done, this including active bacteria, total bacteria, active fungi, total fungi, protozoa, nematodes and (for soils with roots in them) mycorrhizal fungi. The details of these assays were not disclosed, but basically most involved microscopic analysis, using the vital stain fluorescein diacetate (FDA) to differentiate active from quiescent or dead organisms. The mycorrhizal fungus assay involved quantifying colonization in root fragments recovered from the soil sample. (Mycorrhizae in the manufactured topsoil samples was not assessed since they did not contain roots).

C. Characterizations done by Maritime MicroBiologicals Inc.

The respiratory activity is an index of the total microbial activity in the soil. For this, 30g of the moistened soil was placed into a closed mason jar. The carbon dioxide concentration in the headspace was then periodically measured using an infra-red gas analyser (Gas Tech, model R1 41AA).

The fluorescein diacetate hydrolysing activity (FDA) of the soil was also measured. Like the respiratory activity, this is a measure of total microbial activity in the soil (Boyle and Kropp, 1992). Briefly, a soil sample was mixed with 20 mM pH 7.2 phosphate buffer

containing fluorescein diacetate. This substrate (which is colourless) is hydrolysed to fluorescein (which is bright green) by many enzymes that most microbes have, so a high rate of hydrolysis is indicative of high microbial activity. To measure fluorescein formation, the samples were centrifuged, and the optical density of the supernatant at 490nm (absorbtion max) was measured.

The water holding capacity of the soil was determined by slowly adding water to the soil until it was saturated. The soil was then weighed, dried and re-weighed so the water held per gram dry soil could be calculated.

The soils' abilities to support seed germination and grass growth was assessed by planting 100 seeds of Kentucky blue grass on a 2" deep layer (ca 120 g fresh weight) of the soil, held in a 7 cm diameter square plastic flower pot. There was a 1" layer of gravel in the bottom of the pot, and the pots were sitting in a tray containing wet perlite. The pots were watered bi-weekly, but not fertilized. For purposes of comparison, a pot containing Myke Potting mix (Premiere Peat, Riviere de Loup) was included.

The soils were assessed for the entomopathogenic fungi *Beauveria* and *Metarhizium* as discussed in the year 1 up-dated report (March 30, 2006).

The number of mycorrhizal propagules in the soils we measured. These beneficial fungi are obligately symbiotic (ie can not grow independently) so to measure them, we used white clover as "bait". This was planted in 10 g of the soil in small pots, or in 1:10 or 1:100 dilutions of the soil made with sterile (no mycorrhizal propagules) carrier soil. After 6 weeks, the clover roots were cleared in KOH and stained in trypan blue as described at <http://invam.caf.wvu.edu/methods/mycorrhizae/staining.htm>. If any mycorrhizae formed on the clover from a pot, it was registered as "positive". (Mycorrhizae can be recognized by the formation of characteristic vesicles, arbuscules and hyphae). The number of mycorrhizal propagules (i.e. spores, mycorrhizal root fragments or other entities that are capable of initiating a new mycorrhizal infection) was calculated by the most probable number method (MPN) with 3 replicates of each dilution. See e.g. (<http://www.jlindquist.net/generalmicro/102dil3.html>.)

Results and Discussion.

I. Field plots at Rothesay Fire Hall

On all the assay dates chinch numbers were lower and more variable (relatively high standard deviation) than anticipated (Table 1). To confirm that this was not an artefact stemming from our use of our vacuum sampling method, we assayed some plots using the more conventional “scrabble method” (close visual inspection). Insect numbers were lower and more variable than with the vacuum sampler (data not presented), so we decided to do all our assays using the vacuum sampler. Last year, when we did ‘informal’ scrabble counts at this site, counts were much higher than this year. Unfortunately, from the research point of view, it seems that the chinch bug problem has at least temporarily abated at this site.

When the first assay was made on August 3, focus was on the plots that had been treated with the mustard product which had been applied a few days previously. It was clear that this had not killed the chinch bugs at any of the application rates tested. To the contrary, at the highest application rate the number of insects present (203 +/- 11 per square meter) was considerably higher than in the control plots. It was clear that this organic product had actually *attracted* chinch.

On subsequent dates, the other treatments were also assayed. On August 10 the number of insects on the control plots had not changed appreciably from August 3, still being low and variable. Where Sevin had been applied, about 75% of the insects had died, a few live bugs being recovered from some plots. There were also some live ants and spiders. By August 17 the Sevin had killed all the chinch bugs, only dead bugs being found in the vacuumed sample. Some live ants and spiders were still present, but no live insects were seen. The Sevin was active against the chinch, but was not very selective. Interestingly, by September 1st, live chinch were recovered from some of the Sevin treated plots. These had presumably migrated in from the untreated border zone or adjacent plots. This indicates that Sevin does not have significant residual activity. Chinch suppression might have lasted longer if the treated area had been larger, but the currently prevailing IPM techniques favors spot treatments.

By August 10, chinch numbers had decreased in the shaded plots almost as much as in those that were treated with Sevin. Later in the season, some chinch bugs were recovered from the shaded plots, but numbers remained relatively low. The grass in these plots looked greener and more vigorous than most other plots, although this should not be attributed to decreased chinch activity. It could be that the direct sunlight on the other plots simply over-stressed the plants, possibly bleaching their chlorophyll.

No chinch bugs were recovered from any of the plots with transplanted sod until August 17, and at this time and subsequently chinch numbers were lower than in the controls. We had speculated that the chinch bugs might *rapidly* migrate into these plots to feed on the healthy grass (including the Kentucky blue grass), but this did not happen. Numbers

were similar in the Kentucky Blue grass sod, the KBG sod where the soil under it had been replaced, and the sod taken from Angelview park. The finding of *some* chinch in the Angelview sod shows that this sod/soil is not completely chinch resistant. Some factor other than the sod type or top soil composition must be responsible for the complete absence of chinch bugs in Angelview park.

The chinch counts in the mustard product-treated plots remained higher than the other plots until the end of the season, confirming that this product, applied in this way did not control chinch bugs. We speculated that the heavy watering might have rinsed potentially insecticidal compounds out of the mustard, leaving an insect-attracting organic material. A chinch attractant could be of value if, e.g. it was combined with a chinch-killing agent. It would be interesting to have the active attractant identified. Despite the higher chinch numbers, these plots looked greener than the controls, this presumably being due to the added nutrients.

The other organic fertilizer did not attract or repel the chinch bugs, chinch numbers being similar to those in the controls. Towards the end of the season these plots also looked greener than the controls, probably due added nutrients.

Interestingly, chinch numbers were relatively low in the plots that were treated with the soluble fertilizer. The amount of fertilizer applied was chosen to give an amount of N approximately equal to that applied with the organic fertilizer. Possibly some other factor (PO₄? K?, mineral salts?) associated with the mineral fertilizer repelled the chinch bugs. Within a few weeks of adding this fertilizer, growth of the sod increased. This finding suggests that for chinch control, use of non-organic fertilizer might be advantageous.

Of all the treatments, the compost top dressing one was of particular interest in that it was the only case where no chinch bugs were seen. We did not see any dead chinch bugs in the samples, suggesting that the insects had been repelled, not killed, as they had been with Sevin. Others (e.g. Rob Teale, Ecology Action Centre, Halifax and Tim Livingston at Jolly Farmer, Woodstock, personal communication) have also had encouraging results with compost. Compost tea has also shown some potential. These possibilities should be explored further.

Table 1. Chinch bug numbers in the plots at the Rothesay Fire Hall*.

	Aug. 3	Aug 10	Aug. 17	Sept 1	Sept 28
A. Control	48 (41)	48 (38)	30 (28)	10 (19)	0(0)
B. Sevin	ND	13 (15)	0 (0)	13 (23)	0(0)
C. Soil + KBG	ND**	0 (0)	13 (11)	7 (6)	0(0)
D.KBG	ND	0(0)	7 (6)	3 (5)	3 (3)
E. Angelview Soil and sod	ND	0(0)	13 (6)	0 (0)	0(0)
F. Watered	ND	ND	66 (49)	0 (0)	0(0)
G. Shade		16 (15)	23 (12)	0 (0)	0(0)
H. Soluble Fert.	ND	ND	7 (12)	0(0)	0(0)
I. Organic Fert.	ND	ND	37 (23)	7 (12)	0(0)
J. Must. 1.	60 (40)	45 (7)	60 (0)	10 (0)	0(0)
K. Must. 2	43 (30)	40 (42)	130 (150)	73 (94)	0(0)
L. Must. 3	203 (11)	203 (170)	660 (660)	50 (63)	0(0)
M. Compost Topdress	ND	ND	0 (0)	0(0)	0(0)

*Values are the mean number of insects per m² with the standard deviation (sd) in parenthesis.

N=3, except for the control where it was 6 or 8.

** . ND = not determined.

At each assay date, we made note of the developmental stages of the insects in each plot. There did not seem to be any obvious differences between plots from the various treatments, but the number of insects recovered was too small to assert this. The distribution of developmental stages did obviously change from one assay date to the next (Figure 7). On August 3, 4th instar insects predominated, although significant numbers of 3rd and 5th instar ones, as well as a very small number of 1st and 2nd instars and adults were also seen. On August 10th, most of the insects were 5th instar, with some 4th instars and adults also present. On September 1, essentially all the insects were adults. On September 28th, the few insects that were seen were adults. There was no evidence for a second generation of insects.

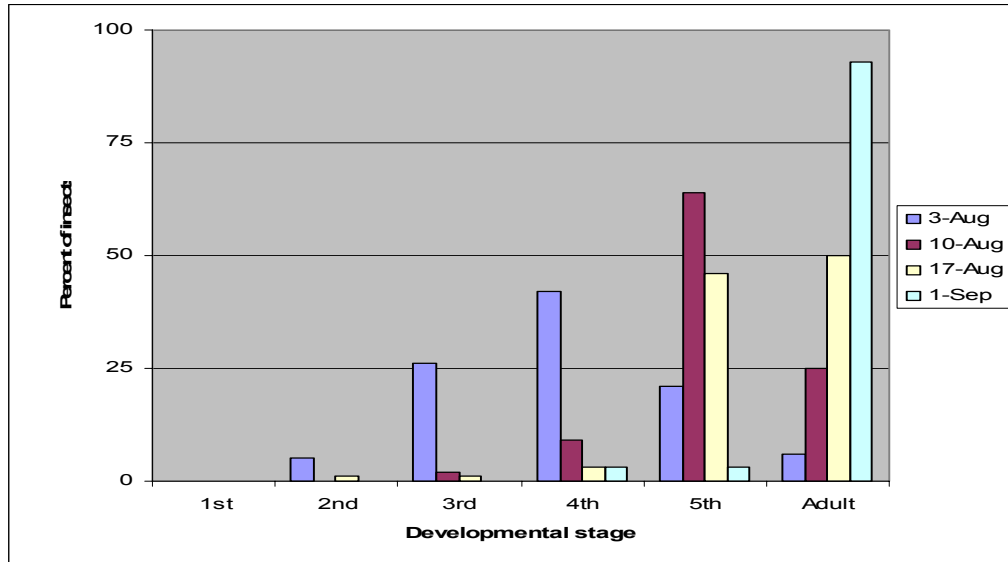


Figure 7. Chinch developmental stage at the various assay dates.

At the last assay time (September 28) very few chinch bugs were found in any of the plots and it was decided to terminate the experiment. A subjective assay of grass vigour was made by visually rating the plots on a 1 (very unhealthy) to 5 (healthy) scale. None of the plots had deteriorated noticeably during the season. None looked great. All (except those with transplanted sod) were composed of clumps of fescues with dead plant material in between, but some treatments were clearly more vigorous-looking than the controls (Table 2). Specifically, the shaded plots, and all of the plots that had been fertilized with inorganic fertilizer, organic fertilizer or mustard were better than the controls. The plots that had been top dressed with compost did not look significantly different than the controls - The compost was no longer visible, but the grass did not appear to be healthier. Observations made after a longer period might serve to show the improved growth that might be expected from compost application.

Table 2. Plant vigour, subjectively assessed on September 1, 2006*

Treatment	Vigour, 0-5, Subjective
A. Control	2.4
B. Sevin	2.3
C. Soil + KBG	na
D.KBG	na
E. Angelview Soil and sod	na
F. Watered	2.3
G. Shade	3.6
H. Soluble Fert.	3.6
I. Organic Fert.	3.0
J. Must. 1.	3.0
K. Must. 2	3.6
L. Must. 3	4.0
M. Compost Topdress	2.4

*Low values assigned to plots with low vigour. N=3, except for the controls where N=8. Na = not applicable, since implanted sod.

At the end of the growing season we noticed some fungal disease on the plots. All of the transplanted sods had rust on the Kentucky Blue grass. Rust was apparently very common around Rothesay this year (John Smith, Pers. comm.). We also noticed some Red thread on the plots (fig. 8) although there was no clear relationship to treatment. For example, it was present on the plots that had or had not been fertilized, although various references (e.g. <http://www.uri.edu/ce/factsheets/sheets/turffungaldis.html>) cite low fertility as being a ‘cause’ of this turf disease.



*Figure 8 . Red thread, probably caused by *Laetisaria fuciformis*. Dried, red-coloured hyphae are clumped onto dead, bleached blades of grass.*

We also measured soil moisture in the control and watered plots using the Theta Probe (see methods). The probe had not been calibrated for this particular soil, so the measurements may not be precise, but they do serve to demonstrate relative values. In the control soil, the moisture content was 17.3%. The moisture content of the watered plots was 24.9% 30 minutes after watering (i.e. it increased by 7.6%), but was only 18.9% (i.e. no significant increase) 5 days after watering (data not presented). This indicates that this soil does not hold much water, draining rapidly. During watering, it was noticed that unless the water was applied very slowly, it would run off the plot, the plot surface (thatch) being quite hydrophobic. In conjunction with the low moisture holding properties of the soil and the rocky sub-soil, this undoubtedly would contribute to plant water stress. This may be a “cause” of the chinch problem at this site.

II. Testing and developing *Beauveria* for use against chinch bugs.

A. Microplots at Maritime MicroBiologicals.

The main focus of this experiment was to determine if inoculation with *Beauveria* would kill chinch bugs on sod growing in either soil A or soil B when these were maintained under either low or high water conditions. The results (Table 3) were disappointing, in that there was no clear cut effect of the *Beauveria*. In all cases at least some chinch bugs were recovered so the fungus was obviously not 100% effective.

Table 3. Results from Microplots at Maritime MicroBiologicals *

Soil	Water	Inoc'd	<i>Beauveria</i> (cfu) ^a	moisture % ^b	Vigour (0-5)	Chinch (scrabble)	Chinch Float'n
A	Low	Yes	10 ⁷	35 (3)	3 (0)	2 (1)	3.3 (2.1)
	Low	No	< 10 ⁴	32 (5)	3(0)	1 (1)	4.3 (1.5)
	High	Yes	10 ⁷	40 (4)	3.3 (0.6)	2 (3)	1.7 (2.9)
	High	No	< 10 ⁴	45 (2)	3.6 (0.5)	1 (1)	1.6 (1.1)
B	Low	Yes	10 ⁷	19 (2)	3.3 (1.1)	0 (0)	8.5 (8.5)
	Low	No	< 10 ⁴	22 (4)	2.3 (0.6)	3 (3)	8.8 (7.9)
	High	Yes	10 ⁷	34 (2)	4.0 (0)	2 (2)	2.0 (3.5)
	High	No	< 10 ⁴	34 (2)	3.0 (0.6)	1 (1)	3.0 (0.0)

*Values are means with the standard deviation (sd) in parenthesis. N=3.

a) colony forming units (cfu) per gram soil. Detection limit ca 10⁴ conidia/g.

b) Theta probe measurements made 4 days after watering.

The scrabble counts (second to last column) showed the average number of chinch bugs ranged from 1 to 3 per pot for the various treatments. The floatation assay (last column) gave higher numbers (average ranging from about 2 to 9). However in neither case was there a relationship to *Beauveria* inoculation. It should however be emphasized that, regardless of whether the scrabble or floatation data is considered, there is a lot of room for a “false negative” (no observed effect when in actuality there is an effect) since the percentage of the 50 added insects that were detected was low, and the variability between replicates was high.

The disappointing results were not attributable to poor *Beauveria* survival since the plate counts showed there were many viable conidia present in the soil at the end of the experiment. A total of about 4 x 10⁷ conidia per gram soil were initially added to the plots. About this number were recovered, with less than 10⁴ (the detection limit for this plate count method) being in the control plots. It seems likely that both *Beauveria* strains would have persisted, but we could not assess this with the plate count method we used since it does not differentiate between strains. In any event, both the GHA and the isolate 3 showed similar virulence towards the chinch bugs in the lab assays. In short, the lack of effect of *Beauveria* on chinch recovery can not be attributed to absence of propagules of a virulent strain. The results are consistent with those obtained in the trial presented in the Year 2 report.

There was a tendency for the numbers of chinch bugs to be higher in the Soil B (manufactured topsoil) treatments that had been maintained under the low water regime. This may be of significance since others (e.g. Gregor MacAskill, Ecology Action Centre, Halifax, pers. comm..) have noted that chinch problems are often worse on manufactured topsoils, particularly where these have soil moisture problems. More chinch bugs were also recovered from the manufactured topsoil treatments in last years tests.

The 'high' and 'low' watering regimes did result in plots with higher and lower soil moisture respectively for both Soil A (Nashwaak River loam) and Soil B (manufactured topsoil from Halifax). In both cases soil A had a higher moisture content than Soil B, attesting to its higher water holding capacity. There was however only weak evidence that these watering regimes resulted in differing plant growth. On soil A, the subjective assay showed marginally better growth with more water. On soil B, results were too variable to detect any differences. There was no relationship between the watering regime and chinch numbers, substantiating last year's results and those from the Rothesay firehall plots.

Overall, the results from the microplots were disappointing. If this experiment were to be done again there is room for improvement. First and foremost, it should be started earlier in the season. As will be reported later, the younger chinch bugs (nymphal stages 1-3) are more susceptible to *Beauveria*. More insects, representing a wider range of age classes should be added so statistically-valid data can be obtained. To this end, the floatation assay should probably be done within a few weeks of adding the *Beauveria* so that more live insects are recovered. However, this would preclude assessing possible effects on insect reproduction. The watering regime should also be altered making the 'low' lower, thereby so it gives detectable plant stress, including e.g. decreased growth. This is surprisingly difficult to do, without over-stressing the grass and killing it.

A factor that may have limited *Beauveria*'s virulence was soil temperature, this having been cited as a principle factor that limits efficacy of entomopathogenic fungi (Fargues et al. 1997, Glare et al. 1994). When the experiment was done, the temperature, particularly during the night had started dropping. This might have both limited activity of both the insects and the fungi that might have been infecting them.

B. Effects of Beauveria on Chinch bugs on grasses growing in pots.

This method (pots of sod in microporous patch bags, see figure 5) showed distinct advantages over the outdoor microplot approach, and was certainly less laborious. It gave some interesting results, supporting the idea that *Beauveria* can control chinch bugs on sod.

At least some of the chinch bugs could be seen through the plastic wall of the bags that enclosed the turf in the pots. The number of chinch seen varied with the time of day, generally being highest in the afternoon. On the afternoon of day 7, some *Beauveria*-killed insects were seen on the grass blades in some of the Botinigard-treated bags so we

counted all the remaining live insects in the bags. The results (Table 4) showed quite clearly that there were fewer live chinch bugs visible in the Botinigard treated replicates than in the controls. Chinch numbers were also somewhat lower in the replicates inoculated with *Beauveria* isolate 2.

After an additional week, the bags were opened and the chinch bugs were counted by the more rigorous floatation method. The results confirmed that the Botinigard, and to a lesser degree the *Beauveria* Isolate 2 killed many of the chinch bugs. With Botinigard, on the average, only 9% of the original 15 chinch bugs per bag remained alive. With the *Beauveria* Isolate 2 this number was 35%. With the controls, 52% remained alive. It is interesting that the results with the Botinigard and the *Beauveria* isolate 2 differed from each other, since they were both the same fungal isolate.

The higher activity of the Botinigard may be due to it delivering about twice the number of conidia. Alternatively, the difference may be attributed to differences in the formulation methods (rice grains vs proprietary oil-based formulation) that were used. In any event, the results contrasted to those obtained with the microplots in that they clearly showed that some *Beauveria* preparations can kill chinch bugs on grass growing on soil.

Table 4. Effects of *Beauveria* on Chinch bugs in on grasses growing in pots*.

Treatment	Chinch seen in bags 7 days after <i>Beauveria</i> added	Chinch recovered by floatation 20 days after <i>Beauveria</i> added. The lower value is the percentage of insects presumed dead.
Control	2.8 (1.7)	7.8 (1.3) 48%
<i>Beauveria</i> Isolate 2	2.0 (1.4)	5.3 (2.2) 65%
Botinigard TM	0.8 (0.9)	1.3 (1.5) 91%

*Values are the mean with the sd in parenthesis. N=4. For the floatation assay, the insects that were not recovered (i.e. 15 – number recovered) were presumed dead. This is presented as a percentage of the added insects.

C. Effect of *Beauveria* on Nymphal Stages of Chinch Bugs.

On August 10th we obtained a limited number of insects from each age class which we exposed to the *Beauveria* in the plexiglass test chamber. The results (Table 5a) showed quite clearly that the earlier developmental stages (1st, 2nd, 3rd stage nymphs) were more sensitive to the *Beauveria* than the 4th or 5th stage nymphs or the adults. In Table 5A, the upper value (a ratio) is the number of live insects/total number of insects. It is the sum (a composite) for the four replicate chambers. The lower value is the percentage of insects that were dead in the four replicate chambers. The difference between these two values can be attributed to effects of the fungi (Abbott 1925). In other words, for the 1st, 2nd, 3rd, 4th, 5th instars and adults respectively, the *Beauveria* was responsible for killing 74%,

75%, 89%, 21%, 27% and 14% of the insects ... within *three* days of the insects being exposed to the fungus. It is clear that *Beauveria* rapidly killed a large percentage of the immature insects.

When the chambers were re-assessed after an additional two days, over 95% of the insects younger than 5th nymphal stage in the *Beauveria*-treatments had died (Table 1B). However, a lot of the insects in the control group had also died. This was particularly the case for the first stage nymphs (74% dead) suggesting that these were relatively sensitive to the stresses imposed by the test conditions, regardless of whether *Beauveria* was present.

Table 5a. Effect of *Beauveria* on chinch bugs of various developmental stages. Day 3.

Stage	1 st	2 nd	3 rd	4 th	5 th	Adults
Control	12/15 (20%)	20/21 (5%)	16/17 (6%)	14/19 (26%)	15/18 (17%)	13/17 (24%)
<i>Beauveria</i>	1/17 (94%)	3/15 (80%)	1/19 (95%)	10/19 (47%)	10/18 (44%)	14/20 (30%)

Table 5b. Effect of *Beauveria* on chinch bugs of various developmental stages. Day 5.

Stage	1 st	2 nd	3 rd	4 th	5 th	Adults
Control	74%	43%	41%	63%	50%	41%
<i>Beauveria</i>	100%	95%	100%	95%	83%	75%

We repeated this experiment on August 24th, using only 4th and 5th stage nymphs, since these were the only ones available. The results (not presented) confirmed that the younger insects were more susceptible to the *Beauveria*. However, on this occasion survival of the insects was not good in the test chambers, even when *Beauveria* was not present. Most of the control insects died soon after the *Beauveria*-treated ones did.

We did not assess the virulence of the *Beauveria* towards chinch eggs. Others (Samuels et al. 2002) have compared virulence of fungi towards eggs of *Blissus antillus* (a chinch bug found in Brazil) and reported that the eggs were more susceptible to colonization by the entomopathogenic fungus *Metarhizium anisopliae* than *Beauveria bassiana*. Interestingly, *M. anisopliae* was noted in the soils from Angelview Park, but not those from Rothesay. (See Year 1 report, this project).

Taken together, results from this part of the project confirm that *Beauveria* can kill chinch bugs, the early stage nymphs being more sensitive than the latter stage ones, or than the adults. In the past, our efforts have focussed on the adults (see Year 2 report) but this may have been mis-guided. It seems probable that application of *Beauveria*-based products for chinch control should take place relatively early in the season, when the early stage nymphs are present. The results of Wellwood Nickerson and Wetmore (2003)

suggest that mid July (or possibly earlier) would be appropriate for the region around Rothesay.

As mentioned above, a confounding factor in the field may be temperature. Although *Beauveria* spores can germinate and grow at temperatures as low as ca 8 °C, the maximal growth rate generally occurs between 20 and 30°C (Fargues et al. 1997). It is likely that chinch control by *Beauveria* will involve finding the optimal balance between effects that temperature has on the fungus (germination, growth rate, penetration of host cuticle, etc.) and on the insect (grooming behaviour, movement, metabolic factors).

D. Compatibility of Beauveria With Potential Synergists.

The germination of *Beauveria* conidia was inhibited by Safer's Soap, but only when the concentration was high (Figure 10). Inhibition was also greater when the spores were soaked for longer (24h) periods than for short (10 min) ones. The product label recommends using it at a 2% concentration where there was minimal effect on conidial germination, even after the 24 hours exposure. In short, the results leave open the possibility that *Beauveria* and Safers Insecticidal Soap might be applied together.

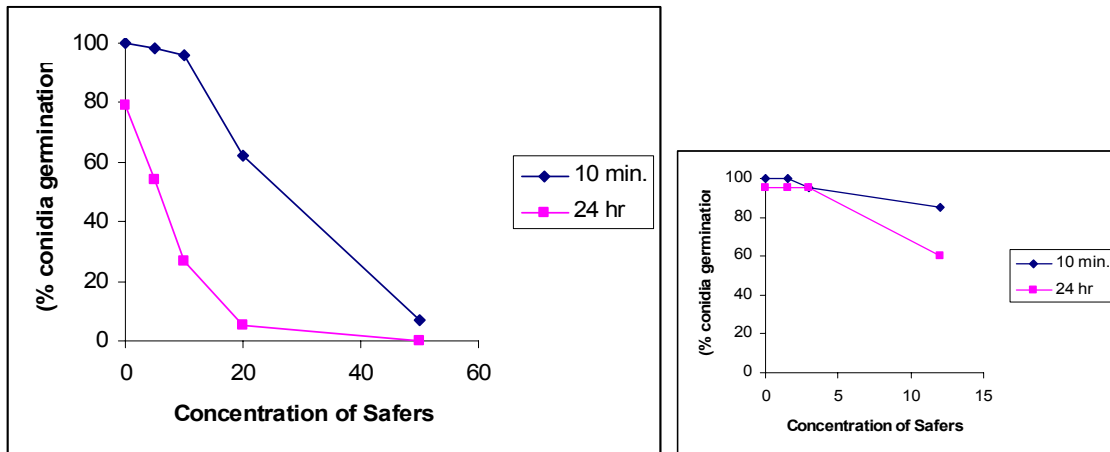


Figure 10. The effect of Safer's Insecticidal soap on germination of *Beauveria* conidia.

The effects of a few other surfactants on germination of *Beauveria* and *Metarhizium* spores was also investigated following the same methods as used for the Safer's soap. The results (not presented) showed that the spores survived in Tween 20 and Triton X (two common surfactants used in research and industry) but they did not survive well in Sunlight Lemon Fresh™ dish detergent. This suggests that using dish detergent to control chinch could be counter-productive, since it might kill entomopathogenic fungi that might otherwise have kept the chinch (and other insects) in control.

Safers soap is a mix of potassium salts of various fatty acids. Its mode of action (and that of other soaps) is through lowering the surface tension of the water so that it enters the spiracles of the insect. Our preliminary lab tests have shown that it, and also the other surfactants mentioned above kill chinch bugs, if the insects are immersed in dilute

aqueous solutions of them. It seems likely that any surfactant might serve to stress (or kill) insects. Insects that survived the surfactant might become more susceptible to attack by the fungus. We intend to explore this idea when insects become available.

Muscle™ is a mix of biologically active essential oils (eugenol, peppermint oil) and surface active compounds (sodium lauryl sulphate). The manufacturer claims it is active against chinch bugs. This should be investigated. It, like insecticidal soap might stress the insects, making them more susceptible to control by *Beauveria*. As a preliminary to testing this possibility, we investigated Muscle™ 's effect on germination of *Beauveria*'s conidia.

The results (Table 9) showed that the product completely inhibited spore germination at concentrations over about 1%. This was not too surprising since many essential oils, including these two have been shown to be toxic to a variety of fungi (Boyle and Lonergan, 2001. In that the manufacturer recommends using it at 1:20 mixed with water (ie. 5%) it could not be used as a carrier for the conidia. However, at considerably lower doses (e.g. 0.1%) it did not inhibit spore germination. This dose might not kill the chinch bugs, but it might stress them, making them more susceptible to e.g. *Beauveria*.

Table 9. The effect of Muscle™ on germination of *Beauveria* spores.

Concentration	Time spores soaked	
	1 hr	24 hrs
0	100%	100
0.1	100	100
1.0	0	0
10	0	0
100	0	0

The results with the compost tea were interesting (Table 10). The conidia did not germinate in the tea, irrespective of whether this had been centrifuged to remove the particulates or autoclaved to eliminate competing microbes. It seemed there was something in the tea that inhibited the fungus. When the spores that had soaked for 24 hours in either autoclaved or non-autoclaved tea were put onto Sabouroud medium, they *did* germinate, showing that the tea had not killed the spores, but simply did not support their germination (data not presented). It is well known that compost tea can have antifungal activity (Scheuerell and Mahaffee, 2004) and this may have temporarily inhibited conidial germination.

Table 10. The effects of compost tea on germination of *Beauveria* conidia

<u>Liquid phase</u>	<u>Conidial germination (%)</u>
Compost tea	0
Centrifuged tea	0
Autoclaved tea	<2
Sabouroud liquid medium	>90

From a practical viewpoint, these results suggest that it should be feasible to use compost tea as a carrier for the *Beauveria* conidia. There are many types of compost and many ways of making tea, but these preliminary results are encouraging. It might be worthwhile to test the virulence towards chinch bugs of *Beauveria* conidial suspensions in various types of tea. Dr. Elaine Ingham (personal communication) indicated that her company sometimes adds *Beauveria* conidia to their teas to get insect control. Unfortunately, no data appears to be available about efficacy of these preparations.

III. Characterization of Soils.

A. A&L labs. Chemical Characterization

The data from A&L labs (Table 6) showed that the four Rothesay Firehall soil samples were similar. This was not surprising, since they only differed in the type of fertilizer they received. The organic matter, measured as loss on ignition at 350 °C, values were not affected appreciably by any of the fertilizers. The values were within “acceptable” range (Wetmore and Brown, 2003).

Surprisingly, the Kjeldahl N values were also all similar, except with addition of the high level of mustard. The soluble fertilizer may have leached away, but it is not clear why the organic fertilizer did not affect the N. The increased value for N (1113 to 1400 or 286 micrograms per gram) is less than what would be expected from the addition of 150 g of product with 5% N in sample 4. A rough calculation shows that this application rate would have given about 750 micrograms N per cm². The surface area that was sampled was probably about 20 cm².

It is clear that a substantial amount of the applied N disappeared from all the N treatments, either by being assimilated, leached, washed away or volatilized. In that all the fertilized plots were greener and generally more vigorous (see Table 2) it seems probable that at least some of the N was assimilated.

In contrast, all the fertilizers did influence the value of PO₄, raising it from a value of 5 ppm (very low) to over 10(low). This may also have contributed to the improved plant growth. The other parameters were not influenced in any dramatic or consistent way by the fertilizers. The small differences may be due to sampling or measurement error.

Comparing the Rothesay soils to the Angelview soils shows a number of interesting differences. The pH is clearly higher in the Angelview soil, as is the Ca and Mg. This is consistent with the Angelview site having been limed. The liming may also be responsible for lowering the Al, since this becomes less available (extractable) at neutral pH.

The organic matter content of the two soils is similar, both being reasonably high. However, it seems likely that the quality of the organic matter may differ, since the Firehall soil contains residual organic matter that reflects its origin from a coniferous forest.

The concentrations of the major nutrients required for good plant growth (N, P, K, Mg, Ca) are all much higher in the Angelview soil. The nitrogen content of Angelview soil is more than twice as high as that of the Firehall soil, even after the latter received the high level of 5% N mustard fertilizer. Even more striking is the phosphate, which is 8X lower in the Firehall soil, and remains that way even after fertilization. The numbers in Table 6 were obtained using the Bray extractant, but were similar using a bicarbonate extraction (data not presented).

The higher pH at Angelview, in conjunction with its better water holding capacity and higher nutrient levels may explain why the grass at this site is healthier, and more chinch resistant. (It may also explain why others find the grass does not respond much to fertilizer at Angelview).

Table 6. Chemical characterization of soils. (A&L Labs)*.

Sample	pH	%Org Matter	Kjeld N (ug.g-1)	PO4 (ppm,Bray)	K (ppm)	Mg (ppm)	Ca (ppm)	CEC (meq/100 g)	Al (ppm)
Rothesay Control	5.8	7.2	1114	5 VL	82 M	35	420 VL	15.8	2357
Rothesay + Org	5.5	9.4	1074	10 L	74 L	30	450 VL	18.4	2494
Rothesay +Soluble Fert	5.5	9.3	1058	10 L	88 M	30	520VL	18.7	2570
Rothesay +Hi Must.	5.8	7.3	1400	13 L	79 M	35	520 VL	15.1	2404
Angelview	7.0	5.3	2980	44 H	120 M	235 H	1670 M	12.2	797

*The original data from the nutrient analysis by A&L Labs are in the Appendix. VL = very low. L = low. M = medium. H = high.

B. Characterization by *Maritime MicroBiologicals Inc.*

The characterizations by Maritime MicroBiologicals showed some interesting trends (Table 7). It will be noted that the Angelview and Firehall soils from above were assessed, but in addition, Garden Soil and Lawn Soil provided by Urban Lanscaping, and Myke Potting soil were also included.

The pH measured for the Angelview soil was considerably lower than that reported by A&L. We re-measured ours with the same result. A&L re-measured theirs, and adjusted their value down to 6.5. (Three stars for us!!!). In any event, both labs found the Angelview soil pH to be considerably higher than that of the Fire hall soil. The latter's pH is typical for a soil in this region, derived from Coniferous woods. The other soils were less acidic. The Myke soil was also acidic, probably due to it containing a lot of peat moss. Despite this, it gave much better growth than any of the other soils that were tested (Figure 7).

The water holding capacity of the soils varied greatly. Angelview soil held more water than the Fire hall soil. The low capacity of the latter was mentioned above as a possible problem that might cause plant stress and chinch problems. The compost and the Myke Mix both held much more water, as would be expected from their high organic matter

content (Myke is almost all peatmoss). The two manufactured topsoils held moderate amounts of water, probably in proportion to the amount of compost they contained.

The respiratory activity of the Angelview soil was higher than that of the Firehall soil. This is probably a reflection of the greater amount of sugars and amino acids that leaked from the healthy, actively photosynthesizing turf at Angelview. (These compounds support the activity of the microbes in the rhizosphere of grasses). Consistent with this is the observation that the respiratory activity of the lawn and garden soils, both of which are made with mature compost where the readily metabolizable materials have been degraded, were low. Respiration of the Fredericton City compost sample may have been elevated since it still had un-degraded wood particles in it.

The FDA values exhibited a similar pattern to the respiratory values. This is not surprising, since FDA, like respiration, can be considered as an index of total microbial activity. (Note that Soil Food web also uses the FDA in their microscopy to differentiate active from non-active microbial cells). Both measures showed that the Angelview soil was relatively active microbiologically, only compost having higher values. In contrast, the Firehall soil had very low activity. The manufactured topsoils had intermediate activity, the garden soil being slightly higher than the lawn soil. Again, this probably reflects their compost contents.

Many of the results for the *Beauveria* and *Metarhizium* assays were presented in last year's report, but they are collated and re-presented in Table 7. The indicated value is the number of colonies of the particular entomopathogen (*Beauveria* or *Metarhizium*) that was detected in the plate count assay. The "<" sign means there were *fewer* than the indicated number, this being the limit of detection for the assay - It is possible there were zero. The "+" or "-" indicates if we detected the fungus with the more sensitive *Galleria* bioassay.

By way of example, the Angelview soil had about 2.5×10^3 propagules of *Beauveria* per gram in it. It also gave a "+" for this species in the *Galleria* assay. It had 1×10^4 propagules of *Metarhizium* in it, and gave a "+" for this species too. In contrast, for the Firehall soil, *Beauveria* was not detected by dilution plate count where the detection limit was 1×10^3 propagules per gram. It was however detected with the *Galleria* assay. No *Metarhizium* was detected in the Firehall soil with either assay.

As mentioned in the year 2 final report, it seems unlikely that propagule numbers of *Beauveria* as low as these would rapidly kill *adult* chinch bugs. The lab results showed the time for insect death to occur was proportional to the number of propagules present, with 10^7 spores taking about a week. The spore numbers presented here are 1000 X lower. However, it is possible that activity against juvenile insects, or against eggs, or against stressed insects might be considerably higher. We intend to assess these possibilities as soon as suitable insects become available.

Angelview soil was interesting in that both *Beauveria* and *Metarhizium* were detected in it. The literature (Samuels et al. 2003) indicates that *Metarhizium* is more active against

chinch (*Blissus antillis*) eggs. We have not looked at infection of eggs, but perhaps should. In short, the results comparing entomopathogenic fungi in the Angelview and Firehall soils do not prove anything, but the higher incidence of the fungi in the Angelview soil are consistent with them playing a role in chinch control.

A small amount of *Beauveria* was detected in the manufactured lawn soil, but not in the garden soil. It seems likely that *Beauveria* spores could have entered the soil as wind-borne spores. Spores of these and many other entomopathogenic fungi can become air borne. Neither fungus was detected in the compost. This was not surprising, since the elevated temperatures that occur during compost production would kill the fungi, both of which have upper limits of about 35°C.

With respect to assessing the soils' abilities to support good growth of grass, the most straight-forward assays were those that simply looked at grass growth in pots of the soils (See figure 9). Seed germination was similar (20 to 27%) on all the soils, although it occurred more rapidly on the Myke potting mix. The subsequent plant growth was also much better on the Myke potting mix than on the other soils.

It is not clear why the growth was so good on Myke soil relative to the other soils. Its pH is relatively low. The Angelview soil had high nutrient content, at least relative to the Firehall soil, but grass growth on it was not much better than on the Firehall soil, and was much worse than on Myke. It may be that the nutrients in the Myke Potting Mix were more available. This may relate to the Myke's main component being peatmoss, which does not have a high exchange capacity.

One factor that *might* explain the growth differences between the Myke mix and the other soils would be mycorrhizal fungi. There are thousands of research articles, books, web sites, etc. attesting to the ability of these fungi to improve nutrient uptake especially phosphate, by plants (Pfleger and Linderman, 1996). The Myke potting soil package claims it to contain 1 propagule of the fungus *Glomus intraradices*.

To assess this possibility, mycorrhizal propagule numbers were estimated for the soils using a MPN assay. The results (Table 3) showed that the Angelview soil had the highest propagule number (2.3 per gram soil). Interestingly, the Myke mix only had 0.1 propagule per gram, although the manufacturers said it had at least 1. The Firehall soil and the lawn soil had some propagules too, although the numbers were relatively low.

The MPN assay does not differentiate between different mycorrhizal species, of which there are many (Pfleger and R.G. Linderman, 1996). It is probable that different species differ in their effect upon different grass species, and that they perform differently on different soils (see e.g. Gollotte et al. 2004). Getting the "right" species for a given plant-soil combination can be a challenge. From a practical point of view, having a soil with a mixture of different mycorrhizal species would probably be preferred since there is some evidence that, teleologically speaking, plants "select" the fungus that suits their "needs" (Boyle and Robertson 1985).

Table 7. Soil characterizations by Maritime MicroBiologicals

Sample Name	pH	Water at Field Cap.(%)	Resp. (nmolCO ₂ g ⁻¹ h ⁻¹)	FDA (OD /h)	<i>Beauveria</i>	<i>Metarhizium</i>	Mycorr fungi. MPN (prog/gr)	Seed germ (%)	Grass growth (0-5*)
Angelview	5.50	62.56	18	0.31	2.5 x10 ³ (+)	1 x 10 ⁴ ++	2.3	21.00	2.00
Lawn	6.00	57.18	7	0.17	< 1 x 10 ³ (-)	1 x 10 ³ +	0	27.00	2.00
Garden	6.20	86.23	9	0.20	nd	nd	0.2	20.00	2.00
Fire Hall	4.60	51.54	13	0.12	< 1 x10 ³ (+)	< 1 x10 ³ (-)	0.2	23.00	2.00
Compost	7.20	164.15	28	0.32	< 1 x 10 ³ (-)	< 1 x10 ³ (-)	0	nd	nd
Myke Mix	5.0	375	nd	nd	nd	nd	0.1	30.00	5.00

nd = not determined. *See photo below.



Figure 9. Growth of Kentucky blue grass on the various soils from Table 3.

C. Characterization of Soils by Soil Food Web.

Dr. Hofman and Glen Monroe of Soil Foodweb East were asked to interpret their data, putting particular emphasis on factors that might pertain to chinch bugs. They were also asked about factors that might have bearing on the soils' quality, and or ability to support growth of healthy turf grasses. (One hopes these are related). I have summarized the data from their reports in Table 8, collating that for the 4 soils in one table. Scanned copies of the original documents are in the Appendix.

Table 8. Synopsis of Data from Soil Food Web

	Act. Bact.	Tot. Bact.	Act. Fungi	Tot. Fungi	Hyphal Diam	Flag el tes	Protoz. Amoeb	Cili-ates	Nem atod es	VAM	TF/ TB	AF/ TF	AB/ TB	AF/ AB
Soil	µg per g dry soil				µm	Number per gram soil				%				
Angelview	45.1	4553	24.6	109	3.5	118	1947	389	11.9	24%	0.02	0.23	0.01	0.55
Firehall	30.2	1047	0	5.0	2.5	397	1945	8	1.93	12%	0	0	0.03	0.17
Lawn Soil	87.8	635	88.0	211	3.0	50	19586	64	2.49	nd	0.33	0.42	0.14	1.00
Garden Soil	60.4	636	57.6	135	2.5	740	6863	93	2.9	nd	0.21	0.43	0.10	0.95

Interpretation of the results is made complex by the quantity of the data, however, certain features were identified as being significant. The interpretations are based on a data base for soils from a region similar to that of Maritime Canada that Dr. Ingram makes available. (They said it was for upper state New York).

The main feature of the Firehall soil is its low content of fungi. The Foodweb report shows there are no active fungi (those that show FDA activity in their hyphae) in this soil, and a relatively low amount of total fungi. This finding is compatible with *Maritime MicroBiologicals* reporting the low levels of *Metarhizium*, *Beauveria* and mycorrhizal fungi, and also the low FDA and respiratory rate of this soil. In addition, the diameter of the hyphae that are present is, on the average small (2.5 µm). According to Dr. Ingram (and Hofman and Monroe) wide diameter hyphae are generally preferable. (Many mycologists, including myself, would say this is a serious oversimplification).

The Soil Foodweb report also indicated that the nematode numbers in this soil are very low. Hofman had an analysis of the nematode species done (data presented in the appendix) which showed many of the nematodes were root feeders. These are considered “bad”. The data did not give much information about e.g. entomopathogenic nematodes which might have a more direct bearing on the chinch bug problem. Hofman and Monroe said they would try to get more information about this.

Their recommendations for improving this soil are to increase prevalence of active fungi in it. They suggested this could be done by adding fungi-dominated compost, and/or fungi-dominated compost tea. To increase the numbers of beneficial nematodes in the soil, the goal would be to increase populations of beneficial bacteria and fungi that the nematodes feed on. Adding the fungal-dominated compost or tea would allegedly do this, at least in part by opening up the soil, allowing the larger, generally beneficial nematodes to penetrate into it.

Their comments about the garden and lawn soils were similar. Here too, there seems to be a shortage of both total and active fungi. Other factors seemed to be good. To improve the soil, their recommendation is to increase the fungal populations in it. They say this could be done by using fungal dominated compost and compost tea. This could be tested.

The soil foodweb analysis also included mycorrhizal fungi. These beneficial fungi were detected in roots collected from both the Angelview and the Firehall soils, confirming that at least some of these fungi are present. The value for the Angelview soil was higher than for the Firehall soil, since more of the roots recovered from the

former were colonized. It should be emphasized that this does not mean there were more propagules in the soil – it simply means that the plants from which the particular roots came were more heavily colonized. After discussing this data with Hofman and Monroe, we agreed that little can be concluded from the data, other than that at least *some* mycorrhizal fungi are present. The data from Maritime MicroBiologicals (see above) does however indicate that mycorrhizal propagule numbers are higher in the Angelview soil.

Taken together, the data from A&L labs, Maritime MicroBiologicals Inc and Soil Foodweb do not point at any one factor as being the “cause” of the chinch bug problem at the Firehall site. A more tenable hypothesis is that the chinch problem results from interaction of poor physico-chemical characteristics (e.g. low water holding capacity, low nutrient status) in conjunction with imbalances in the soil microbiology (poorly developed populations of various fungi, including e.g. entomopathogenic fungi and mycorrhizae). It would seem that a “cure” for the problem (as opposed to a symptomatic fix) would be to address soil quality problems. One way of doing this, short of replacing the soil, might be through addition of good quality, nutrient rich compost. Soil Foodweb would suggest that this should be a fungus dominated compost.

Conclusions from Year 3

Results from the field plot trial in conjunction with the soil analysis suggest some methods that might be used to control chinch bugs, or at least lessen damage from them. In general, it seems that the chinch bug problem correlates with poor grass growth, which in turn often results from poor quality of the underlying soil. Correlation can never prove relationships between variables, but it seems likely that having a good soil under a lawn will go a long way towards solving the chinch problem. What is a good soil? Much information about this is available from Wetmore and Brown (2003).

As an outgrowth of twentieth century chemistry, the *chemical* attributes of a “good” soil became clear, and nutrient concentration ranges that were optimal for the major and minor nutrients were delineated. Chemical analysis serves to show that at least some soils that have chinch problems (e.g. the Rothesay firehall soil) suffer from significant nutrient shortages, while nutrients in the soil from the chinch-free site were much more aligned with the ideal. Water is also a key requirement for healthy plant growth, and here too, the water holding capacity of the firehall soil were not very good compared to the Angelview soil.

Although the chemical and physical properties of soil are certainly important, it is becoming clear that micro-organisms are also important, and inter-relate with the chemistry. Mycorrhizal fungi are one of the best known and thoroughly studied group of beneficial microbes. These symbiotic fungi can enable plants to obtain phosphorous from sources that are not available to mycorrhizae-free plants. The relationship between plants and mycorrhizae has co-evolved so the photosynthetic partner (e.g. the grass) provides nutrients (e.g. carbohydrates, amino acids) while the heterotrophic fungus

provides mineral nutrients, or other ‘benefits’ that might otherwise not be available to the plant (Pfleger, and Linderman, 1996). Other microbes, including protozoans, nematodes, fungi, bacteria, etc. may also form mutually-beneficial arrangements with the plant. These can be complex, and are the subject of much current and past research.

The efforts of many scientists over the last few centuries has generated a large literature concerning the importance of microbes for the functioning of healthy soil. The recent, well-justified concern with the environmental problems that result from the chemical industry having ignored this information has prompted development of companies like Soil Foodweb East (www.sfwe.ca), to fill the gap. There is much value to their approach, although proponents of it sometimes oversimplify and discount the validity of results other than their own. Their basic tenet however, that a “good” soil is actually a complex “food web” of interacting organisms, one participant being the plant (e.g. grass) itself is not debatable. Part of the grass’s ecological role in this web is provision of carbohydrates and some amino acids, vitamins, etc., while the microbial participants may provide nutrients, allow the plant to gain access to soil nutrient, protect against disease organisms, etc., all of this leading to improved plant vigour. By understanding and manipulating these interactions, it may be possible to devise non-chemical ‘fixes’ to ‘problems’ like chinch bugs.

Unfortunately, nature’s “design” does not always result in the ‘soil-food web’ supporting the healthy green monocultures of grass that some lawn owners demand. The problem is that in the “eat and be eaten” world of nature, the grass supports a variety of herbivores whose interests are not entirely altruistic, and are not aligned with those of the lawn owner. Chinch bugs are one such organism. Fortunately for lawn lovers, in a healthy soil, if the chinch bug population rises too high, they become a good potential nutrient source for other organisms like entomopathogenic fungi, including *Beauveria bassiana* (Goettel et al, 2005). These fungi may then keep the insect population in check, possibly by attaching and killing young stages of the insect.

Entomopathogenic fungi should be present in healthy soil, and indeed are more prevalent in the chinch resistant Angelview soil than in the Firehall soil. Our earlier work (see report for year 1) also showed differences in the types (i.e. strains) of *Beauveria* from the two soils. These qualitative differences may be as important as the quantitative ones. Our inoculation tests did demonstrate that some isolates of *Beauveria* (e.g. those from dead chinch bugs) can be much more active than others. Interestingly, entomopathogenic fungi seem to be rare or absent in manufactured topsoils, and this may be at least part of the reason why lawns on these soils can have chinch problems. Adding the right strain of e.g. *Beauveria* to a soil might improve its quality.

The tests in which chinch-containing outdoor microplots were inoculated with *Beauveria* were disappointing, but there are possible explanations for this. Subsequent testing showed that *Beauveria* is much more active against younger insects. All of the chinch that were added to the microplots in our tests were adults. It is also known that *Beauveria*’s growth rate, and presumably its virulence, decreases if the temperature drops below about 25C (Fargues et al. 1997) . When the *Beauveria* was added in early

September, the temperature would often have been much lower than this, particularly during the night. It is encouraging that the chinch bugs did die in the analogous tests that were made in the pots in bags under warmer temperatures. This suggests that if the microplot tests had been done earlier when the soil temperature was higher and the insects were younger, the results would have been better. We plan to verify this next year.

Chemical insecticides have come into favour in part because they rapidly kill chinch bugs, giving ‘instant gratification’. However, our field plot results show that the insects will soon re-enter a Sevin-treated zone, and if conditions are favourable they would presumably reproduce. Migration into smaller treated areas would be more rapid than into larger ones. This could be used as an argument for treating larger areas, but this would be counter to IPM principles. This conflict should be resolved.

Beauveria might not give the rapid kill that chemical insecticides give, but this is not its mode of action – It is rather to establish a sustained ecological balance in which the population birth rate and death rate are brought into balance. This might be attained by lowering the insects fecundity, or by killing a proportion of any of the instars, or of the adults. The in-vitro test results from last year show that select strains of *Beauveria* can decrease the life span of adult chinch considerably, killing all the insects within a week. This year’s results show that it is even more active against the younger insects, killing a substantial fraction of them within a few days. The literature suggests that some entomopathogenic fungi (e.g. *Metarhizium*) can be very virulent towards some species of chinch bug eggs (Samuels et al. 2003). Their work looked at *Blissus antilles*, but this insect is closely related to the Hairy Chinch bug. Activity of *Beauveria* and or *Metarhizium* against Chinch egg survival should also be investigated. If control were attained, it would seem likely it would persist for longer than control with chemical insecticides, since our test results showed the spores remained viable in the soil for extended periods. In order to determine if inoculation with entomopathogenic fungi can control chinch bugs, it will be necessary to monitor the insects for at least a generation. Until this is done, results from the in-vitro tests against the various age insects and those done in the plant-containing pots should be viewed as very encouraging.

Biological control principles aside, *rapid* insect kill like that attained with the chemical insecticides certainly has marketing allure since it shows the customer that something is happening. Rapid kill might be attained through the use of insecticidal soaps and possibly MuscleTM or other essential oil-containing products. Including *Beauveria* spores in these could give both rapid kill and longer-term control.

The field plot results with the compost were interesting, in that no chinch bugs were seen in these plots. The tests were limited in scope (3 replicates) and the trial only lasted a few months, but in conjunction with the reports of others (Ecology Action Centre, Tim Livingston, Jolly Farmer) they suggest that compost can control chinch bugs. This warrants further study. If the factors in the compost that deterred the chinch bugs were soluble, a compost tea might also be effective. The findings that *Beauveria* conidia remain viable in compost tea suggest this could be used as a carrier for the fungus. In

other work we have found that *Beauveria* also persists in compost. Possibly compost or compost tea mixed with *Beauveria* conidia would give an effective, long lasting chinch control.

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Literature Cited.

Abbot, W.S. 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology*. 18:265-267.

Boyle, C. D. and B. R. Kropp. 1992. Development and comparison of methods for measuring growth of filamentous fungi on wood. *Can. J. Microbiol.* 38: 1053-1060.

Boyle, D. and G. Lonergan. 2001. Controlling *Phytophthora infestans* and other field and storage pathogens of potato using novel formulations of essential oils. Final Report for NB Dept. of Agriculture, Fisheries and Aquaculture's TDF program. NBDARD and 3M Canada.

Boyle, C. D. and W. J. Robertson. 1985. Assessment of potential for improving alfalfa yields in maritime soils by rhizobium-mycorrhizae inoculation. Final report, DSS Contract ISZ82-00088 (Agriculture Canada).

Fargues, J., M.S. Goettel, N Smits, A. Ouedraogo, M. Rougier. 1997. Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. *Mycologia*. 89(3) 383-392.

Glare, T.R., R.J. Townsend and S.D. Young. 1994. Temperature limitations on field effectiveness of *Metarhizium anisopliae* against *Costelytra zealandica* in Canterbury. pp. 266-270 in Proceedings of the NZ Plant Protection Conference

Goettel, M.S., Ellenberg, J and T. Glare. 2005. Entomopathogenic fungi and their role in regulation of insect populations. In: Comprehensive molecular Insect Science. Vol. 6. L.I.Gilbert, K. Iatrou and S.S. Gill, Eds. Elsevier. Pp 361-405.

Gollotte, A., Tuinen, D. and D. Atkinson. 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhizae*. 14: 111-117

Ingham, E. 2001. The Compost Tea Brewing Manual. Second edition. Soil Foodweb Inc. Oregon.

Patriquin, Dr. David. (Dalhousie University). Website at <http://versicolor.ca/lawns/docs/SoapFeb05/soapNEW.html#Overview>

Pfleger, F.L. and R.G. Linderman (eds). Mycorrhizae and Plant Health. 1996. The American Phytopathological Society. 344 pps.

Samuels, R. I., D.L.A. Coracini, C.A. Martins dos Santos and C.A.T. Gava. 2003. Infection of *Blissus antillus* (Hemiptera : :Lygaeidae) eggs by the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. Biological Control. 23:269-273.

Scheuerell, S. J., and Mahaffee, W. F. 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. Phytopathology 94:1156-1163.

Wellwood A, Nickerson G. and Wetmore J. 2003. Hairy chinch bug survey, demonstration and monitoring in New Brunswick, 2002. New Brunswick Horticultural Trades Association (<http://www.nbhta.ca/index.htm>), Spons. Document posted at http://www.nbhta.ca/Chinch_Bug_Report.pdf

Wetmore, J. and K. Brown. 2003. Sustainable Turf. Establishment, Maintenance and IPM Guidelines for Turf in Atlantic Canada. First Edition. New Brunswick Horticultural Trades Association.

Appendix.
Results from Soil Foodweb.



Soil Foodweb Analysis

Report prepared for:
 Prithvi Microbiologicals, Inc.
 3000 Midway Blvd
 3000 Saunders Street
 Fredericton, New Brunswick E3B

Report Sent: 12/14/2006
 Sample#: 11-000006 | Submission: 11-000006
 Unique ID: Rothesay Firestation
 Plant: Not Indicated
 Invoice Number: 0
 Sample Received: 11/24/2006

For interpretation of this report please contact:
 Soil Foodweb Canada East Ltd.
 info@sfce.ca
 (902)421-5696
 Consulting fees may apply

Organism Biomass Data	Dry Weight	Active Bacterial (µg/g)	Total Bacterial (µg/g)	Active Fungal (µg/g)	Total Fungal (µg/g)	Hyphal Diameter (µm)
Results	0.710	30.2	1047	5.06	0	2.5
Comments	Too Dry	Excellent	Excellent	Excellent	Excellent	
Expected Range	0.45 - 0.85	01 - 05	0175 - 0300	01 - 05	0175 - 0300	
Protozoa						
Flagellates		Numbers/g	Ciliates	Total Nematodes #/g		Percent Mycorrhizal Colonization
Low	High	Amoebae		ENDO	ECTO	
397	High	1945	8	1.93	0%	
0	Low	±	High	High	Low	
5000+	Low	±	± 50	± 10	40%	
5000+	High	5000+	± 100	± 20	80%	
Plant Available N Supply (kgs/hectare)						
Organism Biomass Ratios	Total Fungal to Total Bacterial	Active to Total Fungal	Active to Total Bacterial	Active Fungal to Active Bacterial	Plant Available N Supply	
Results	0	0	0.03	0.17	75-100	
Comments	High	Low	Low	Low		
Expected Range	0	0.15	0.15	0.75		

Nematodes per Gram of Soil

6333 6334 report

Arthropods Field

Bacterial Feeders

Actinomadora 0.08
 Acrobeles
 Butlerius 0.31
 Cephalobus 0.16
 Heterocephalobus
 Montysetera
 Prototrunculus
 Rhabditidae 0.20
 Apoccelaimus 2.35
 Epidoryaimus
 Eudoryaimus
 Lainydorus
 Pungentus
 Thonus
 Thomsia

Nematodes 18 ml

Fungal/Root Feeders

Aphelenchoides
 Filanchus 0.16
 Merinilus
 Pstlenchus
 Tylenchus
 Predatory 1.37 0.08
 Clarkus
 Mononchus 0.12

Subtotal from field for root =

Root Feeders

Cicconemella 0.20
 Glaciacus
 Meloidoclype
 Paratylenchus 1.76 0.12
 Pratylenchus
 Rolylenchus
 Tylenchorhynchus 0.39

Lesions on roots

1169 1.93



Soil Foodweb Analysis

Report prepared for:
 Urban Landscaping
 Leil Pond
 5 Marr Road -
 Tothesay, New Brunswick E2E 3J

Report Sent: 12/14/2006
 Sample#: 11-000028 | Submission: 11-000010
 Unique ID: 0003-Garden Soil
 Plant:
 Invoice Number: 0
 Sample Received: 12/14/2006

For interpretation of this report please contact:
 Soil Foodweb Canada East Ltd.
 info@sfc.ca
 (902)421-5696
 Consulting fees may apply

Organism	Dry Weight	Active Bacterial (µg/g)	Total Bacteria (µg/g)	Active Fungal (µg/g)	Total Fungal (µg/g)	Hypheal Diameter (µm)
Results	0.620	60.4	636	57.6	135	2.5
Comments	Good	Excellent	Excellent	Excellent	Excellent	
Expected Range	Low Hig	0.1 0.5	0.175 0.300	0.1 0.5	0.175 0.300	

Organism	Protozoa Numbers/g	Total Nematodes #/g	Percent Mycorrhizal Colonization
Results	740	2.90	Not Ordered
Comments	High	High Low	Not Ordered
Expected Range	Low Hig	0 50	40% 80%

Organism	Flagellates	Ciliates	Plant Available N Supply (kg/ha/decade)
Results	740	93	75-100
Comments	High Low	High Good	Good
Expected Range	Low Hig	0 100	0.75 1.5

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Soil Foodweb Analysis

Report prepared for:

Urban Landscaping
 Hill Pond
 5 Marr Road -
 Sutherland, New Brunswick E2E 3J

Report Sent: 12/14/2006
 Sample#: 11-000004 | Submission: 11-000005
 Unique ID: Lawn Soil
 Plant: Not Indicated
 Invoice Number: 0
 Sample Received: 11/23/2006

For interpretation of this report please contact:

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Organism Biomass Data	Dry Weight	Active Bacterial (µg/g)	Total Bacterial (µg/g)	Active Fungal (µg/g)	Total Fungal (µg/g)	Hypal Diameter (µm)
Results	0.710	87.8	635	88.0	211	3
Comments	Food Web Gap	Excellent	Excellent	Excellent	Excellent	
Expected Range	Low	0	0	0	0	
	Hig	0	0	0	0	

Organism Biomass Ratios	Flagellates	Protozoa Numbers/g	Amoebae	Ciliates	Total Nematodes #/g	Percent Mycorrhizal Colonization	
						ENDO	ECTO
Results	50	19586	High	64	2.49	Not Ordered	Not Ordered
Comments	High	Low	High	Good	High	Low	Low
Expected Range	Low	0	0	0	0	40%	40%
	Hig	0	0	0	0	80%	80%

Organism Biomass Ratios	Total Fungal to Total Bacterial	Active to Total Fungal	Active to Total Bacterial	Plant Available N Supply (kgs/hectare)
Results	0.33	0.42	0.14	100-150
Comments	High	High	Low	Good
Expected Range	Low	0	0.15	0.75
	Hig	0	0.2	1.5

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