Striped Cucumber Beetle Management with Plant and Microbial Metabolites

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The striped cucumber beetle is considered a key pest of cucurbits in the northeastern United States. Adults act as a competent vector for the bacterial pathogen *Erwinia trachaeiphila,* the causal agent of a bacterial wilt disease; once infected a plant is infected with bacterial wilt, it is impossible to halt disease progression. Thus, disease management currently focuses primarily on management of its beetle vector, typically through widespread use of synthetic insecticide products like pyrethroids and neonicotinoids.

A number of microbial metabolites have been formulated into biorational insecticides. For example, spinosynss, derived from the soil bacterium *Saccharopolyspora spinosa,* are broad-spectrum, foliar insecticides that act primarily through oral ingestion. Common commercial formulations include SpinTor, Radiant, and Entrust Naturalyte (a wettable powder) and Entrust SC, the latter two being formulations allowed in certified-organic production systems.

Various formulations of spinosyns have been shown to provide effective control for beetles closely related to SCB, including the Colorado potato beetle. However, efforts to manage striped cucumber beetles using this product have failed to date. It is possible this failure of control stems from insufficient SCB ingestion of insecticide material.

We attempted to improve Entrust's efficacy by increasing beetle consumption of the insecticide material. Cidetrak-D is an plant extract, made from the roots of buffalo gourd (*Cucurbita foetidissima*), and is marketed as a feeding stimulant for SCB and other closely related beetles. Its active ingredient, cucurbitacin, is a naturally occurring molecule known to induce compulsive feeding behavior in SCB. By adding Cidetrak to an Entrust mixture, we aim to enhance Entrust's efficacy for SCB control.

Objectives of this study:

1. Determine if the addition of cucurbitacin induces compulsive SCB feeding of Entrust droplets through laboratory bioassays
2. Evaluate three different rates of spinosad applications in combination with Cidetrak to determine if we can improve field control for SCB

Objective 1: Laboratory bioassays were conducted to generate a dose response curve for striped cucumber beetle mortality to Entrust SC (a mixture of spinosyns A and D, comprising 22.5% of the formulation, which is 2 lbs ai/gallon), both with and without Cidetrak added to the mixture. Both water and Cidetrak alone were used as controls. Beetles were exposed to five different concentrations of Entrust, which were obtained through half-fold serial dilutions: 0.025, 0.0125 (maximum labeled rate), 0.00625, 0.00325, or 0.0015625 ml Entrust SC per ml of water. Cidetrak was added at a rate equivalent to 3.1 oz/acre with a 2 gallon spray volume.

Striped cucumber beetles were collected from an unmanaged pumpkin patch at the Rock Springs research farm in Pennsylvania, and held in lab for at least one week before use in assays. While

in lab, they received a steady supply of cucumber leaves and fruit. Early assays and preliminary work was done using beetles that were reared for several generations. Beetles were starved 24 hours prior to starting an assay to ensure that they consumed insecticide material.

Assay Development: Preliminary assays were conducted to determine the range of concentrations needed to get a dose response curve that included both low and high mortality. We ran three different types of bioassays, due to having difficulty finding a method that produced clean, consistent results. We started by dipping pre-cut leaf disks into a given solution. Leaf disks were left to dry for 45 minutes before being transferred into an individual plastic container along with one starved beetle. Mortality measurements were taken 24, 48, and 72 hours after exposure; only beetles that were completely still were considered dead. Mortality was unexpectedly high, even at low concentrations. We suspect this was due to the Entrust entering the leaf tissue via the wound created when cutting a disk. Moreover, the amount of insecticide absorbed by the leaf was dependent on the freshness of the wound, a factor not controlled for.

We then switched to a filter paper bioassay. Uniform squares of filter paper were dipped into solution, left to dry for 30 minutes, and transferred to beetle containers. Using the filter paper, we obtained a nice dose response curve for Entrust alone. However, when the Cidetrak was added, mortality dropped significantly, the opposite what we hypothesized. For Entrust alone, the LC 50 was 0.007065 ml Entrust/1 ml water. With Cidetrak, the LC 50 dropped to 0.0002372. We are not entirely sure why the Cidetrak affected mortality this way. One possibility is that the presence of Cidetrak interfered with Entrust's absorption into the filter paper due to a difference in polarities. In any case, we decided to try a third method to see if the unexpected results were an artifact of methodology.

Final Bioassays. An entire cucumber leaf was dipped into solution and left to dry for 45 minutes. One mm disks were then cut from the dipped leaf and placed into individual plastic containers with a single beetle. We ran three replicates for this assay, with ten beetles receiving each treatment. Gender and mortality were recorded 24, 48 and 72 hours post exposure. However, we only analyzed the 72 hour data, because it provided a curve that covered the widest range of mortality. Very low mortality was observed in the control beetles, and Abbots correction was applied to the data to account for mortality in the control.

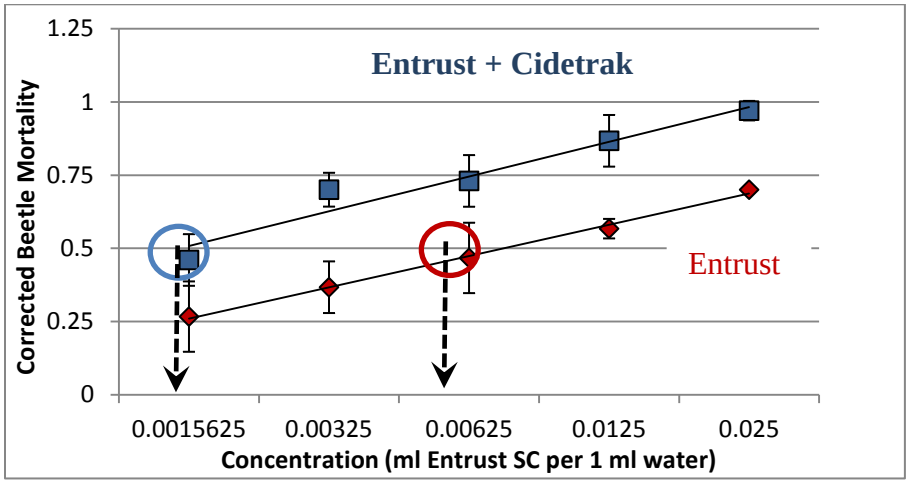

Figure 1: Average beetle mortality with Abbots Correction at each concentration, with standard error. The arrows indicate the LC 50 values derived from each curve; there is a significiant (74%) drop in LC 50 ($p<0.05$) with Cidetrak

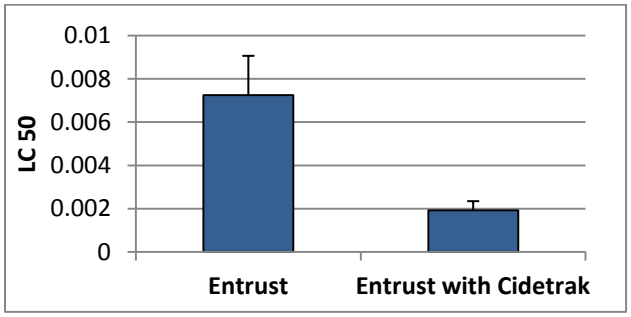

Figure 2: LC 50 values for Entrust and Entrust with Cidetrak

These assays indicate that under lab conditions, addition of Cidetrak significantly improves Entrust's efficacy. With addition of Cidetrak, the LC 50 dropped from 0.00725 ml Entrust/1 ml H20 to 0.00192 ml Entrust per 1 ml water.

Objective 2: Field trials were conducted over a four week period at the Southeast Agricultural Research and Extension Center in Landisville, PA to test the following treatments for field control of striped cucumber beetle:

Treatment	
1	Water (control)
2	Warrior at 1.5 fluiz oz/acre
3	Cidetrak-D at 3.1 fluid oz/acre + Entrust at 8 fluid oz/acre (max rate)
4	Cidetrak-D at 3.1 fluid oz/acre + Entrust at 16 fluid oz/acre
5	Cidetrak-D at 3.1 fluid oz/acre + Entrust at 32 fluid oz/acre

Table 1: Treatments used for 2014 field trials

The experiment was arranged as a complete randomized block design with six replicates; a single plot consisted of one thirty foot row of cucumbers, with one buffer row planted between plots. Treatments were applied once a week using a ground sprayer, beginning June 5th and ending July 1st. Plots were then scouted for both cucumber beetles and bacterial wilt. For the first three weeks, we randomly scouted five plants per plot; on the fourth week, we switched to scouting two one-meter sections per plot as vines had begun to intertwine.

We scouted 0, 1, 3 and 7 days post spray. Plots were scouted Tuesday morning, and sprayed immediately after. Counts were then taken on Wednesday and Friday. Scouting on the following Tuesday was considered both the 7 day count and the 0 day count for the upcoming week.

The first week of scouting and sprays started as soon as beetles immigrated into the field. During this time (Figure 3), beetle counts were low and there were no treatment effects.

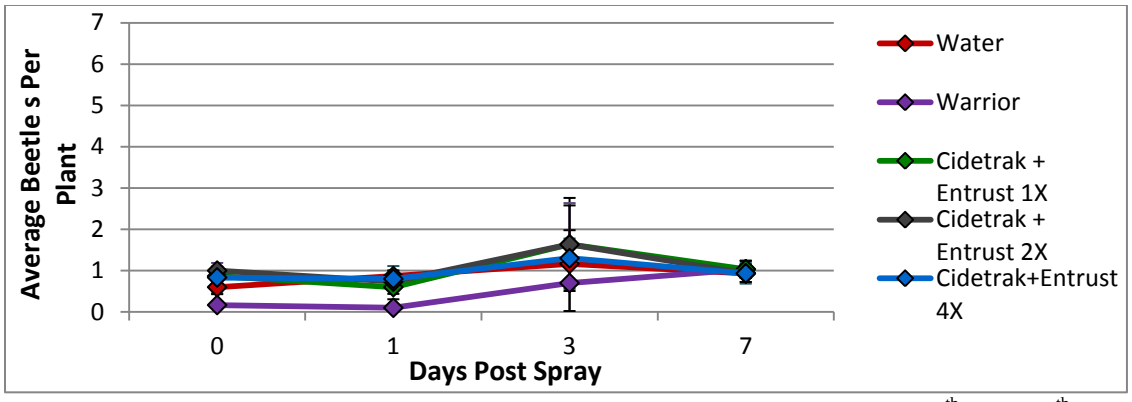

Figure 3: Average striped cucumber beetles per plant for first week of scouting (June 10th – Jun 17th)

By the second week (Figure 4), beetle pressure began to build up in the untreated control plots (water). One day after spray, there was a significant decrease in beetle numbers in the Warrior plots but no response in any of the Entrust plots. However, by three days post spray, we observed a reduction in beetle numbers in all four insecticide treatments. Entrust is an oral insecticide, which might explain the slow response in the Entrust/Cidetrak plots, since beetles need time to ingest the material. In contrast, Warrior is a contact insect. By day 7, both the Entrust and Warrior treatments appeared to have lost their effect.

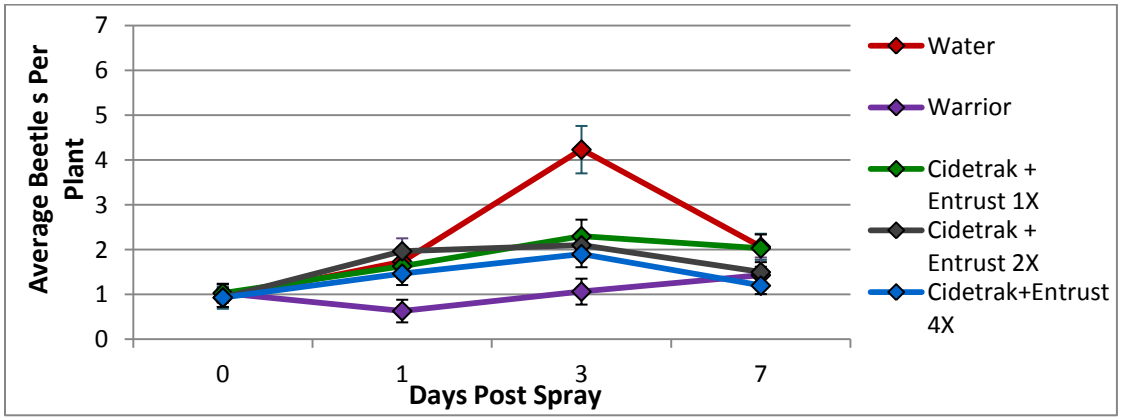

Figure 4: Average striped cucumber beetles per plant for second week of scouting (June 17th- June 24th)

Beetle pressure in the untreated control remained high for both weeks 3 (Figure 5) and 4 (Figure 6). Warrior consistently suppressed their population one and three days post spray (again seeming to wear off by day seven). Additionally, the highest rate of Entrust (4X the max rate) also appeared to reduced beetle numbers. The lower two rates of Entrust (1X and 2X the max rate) provided some amount of suppression, though overall they were less effective than the highest rate of Entrust.

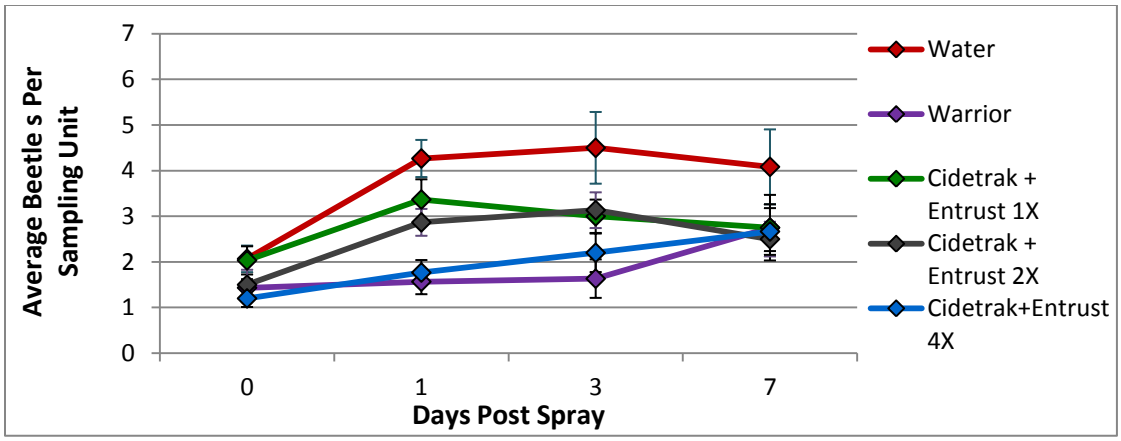

Figure 5: Average striped cucumber beetles per sampling unit for the third week (June 24th-July 1st). On 0, 1, and 3 days post spray, we scouted individual plants. On day seven, we switched to scouting 1 meter sections

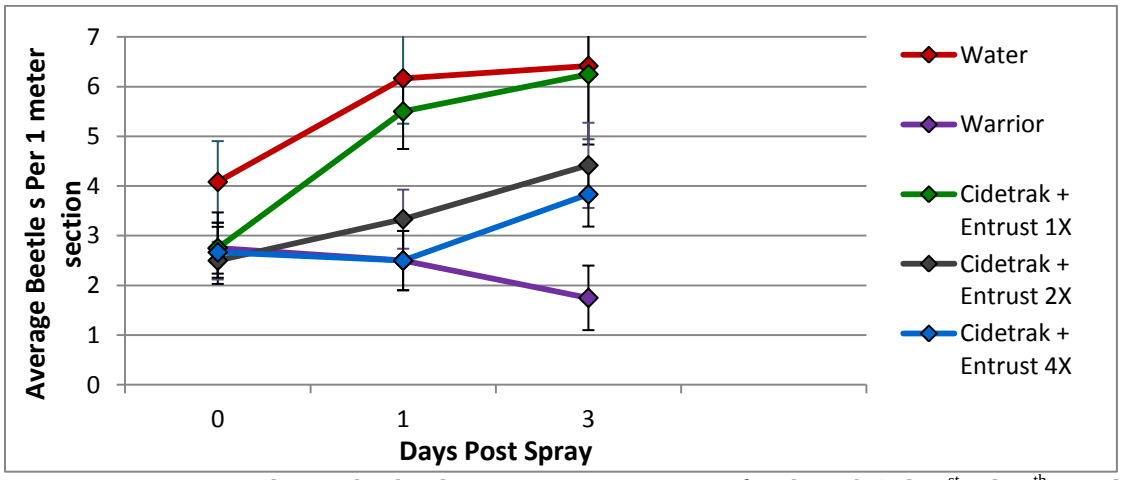

Figure 6: Average striped cucumber beetles per one meter section in fourth week (July 1st-July 4th). Trial ended on July 4th, so no measurements were taken 7 days post spray

Incidences of bacterial wilt were high by the end of the trial. We conducted wilt surveys on July 4th and 11th, but only observed a significant reduction ($p < 0.05$) in wilt severity in the Warrior plots. There was a slight reduction in wilt severity in treatment 5, the highest rate of Entrust.

Trt	7/4/2014	7/11/2014
1	2.37 a	3.60 a
2	1.06 b	2.50 b
3	1.80 a	3.48 a
4	2.23 a	3.80 a
5	1.70 ab	3.24 ab

Table 2 – Average bacterial wilt score per one meter section by treatment.
Means within a column with the same letter are not significantly different by Turkeys HSD at p=0.05 level

Discussion: Scale was a confounding factor in these trials; our plots were very small, allowing for considerable beetle movement between treatments. It is likely that if this experiment was repeated on a larger scale, differences between treatments would be more pronounced. Even with this scale, Entrust at 4X the labeled rate did suppress beetle populations relative to the untreated control. On some weeks, the reduction in beetle numbers was on-par with what we observed using Warrior. The two lower rates of Entrust (1X and 2X) also provided some beetle suppression, though not as effective as what was seen using Entrust at 4X. This is consistent with our hypothesis that ingestion of insecticide material is a limiting factor when using Entrust to control striped cucumber beetle.

We did not include an Entrust only treatment in field, and thus cannot clearly determine the contribution from Cidetrak in suppressing beetle populations in Entrust plots from our field trial. However, in lab bioassays, we were able to significantly reduce the LC50 of Entrust by mixing in Cidetrak, a cucurbitacin based feeding stimulant. This suggests that feeding stimulants such as Cidetrak are effective at increasing beetle ingestion of insecticide material and may be an important component for effectively developing control measures for striped cucumber beetle in cucurbit crops. This may be especially relevant for developing materials that require ingestion, and may help for incorporating Entrust into an organic production system.