ABSTRACT

LAWRENCE, JESSICA L. Damage Relationships and Control of the Tobacco Splitworm (GELECHIIDAE: *Phthorimaea operculella)* in Flue-cured Tobacco. (Under the Direction of Dr. Clyde Sorenson).

Phthorimaea operculella (Zeller) is an oligophagous feeder commonly known as the potato tuberworm. While it is a pest of several Solanaceous crops, little is understood about this pest when it attacks tobacco because the biology and ecology of this insect have not been researched sufficiently. The damage and economic losses associated with this insect in tobacco are not well understood, and there is not adequate insecticidal control available if there is an outbreak in a field. The objectives of this study were to understand the damage relationships between *P. operculella* and tobacco, and to identify possible insecticides that could be useful for splitworm control. Tobacco plots were inoculated with splitworm larvae and monitored for damage. Damage in three of five trials was significant, including two early season trials and a late season trial. Early season infestations were less harmful than late season infestations because the quality for the early season was slightly higher at higher infestation levels. Late season infestations produced a decline in grade that correlated with infestation level. Efficacy trials were conducted using four different insecticides over the course of four years. Two insecticides, chlorantraniliprole and novaluron, were effective in controlling the splitworm.

Damage Relationships and Control of the Tobacco Splitworm (GELECHIIDAE: *Phthorimaea operculella)* in Flue-cured Tobacco

by
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Chair of Advisory Committee

BIOGRAPHY

Jessica Lucy Lawrence was born to Joyce and Donnie Lawrence on August 2, 1984 in Winston-Salem, North Carolina. Raised in a small tobacco-oriented community called Francisco that was miles from any gas station, Jessica developed a love of all animals, plants, and driving unhindered on the left side of the road (even when paved). After the tragic death of her father when she was three years old, Jessica was raised through a group effort by her community and extended family on her mother's side (and her mother, of course!).

After much guidance by a brilliant but underpaid high school teacher, Mr. Hilton Webb, Jessica was steered on an agricultural course towards N.C. State. She graduated with a Bachelor of Science in Botany and a minor in Spanish in May 2006, and immediately took up an open position in Tobacco Entomology under the direction of Dr. Clyde Sorenson. During her time in the Entomology Department, Jessica not only made some of the best friends of her life, but discovered herself in her life partner Lauren Moeller, as well as the responsibilities that come with four-legged parenthood of the pit-bull sisters Atlantis and Athena, and the future prospect of adopting two-legged children.

While completing final preparations for her Master's degree, Jessica began working with the NCDA on a project to control Hemlock Woolly Adelgids with biocontrol methods. She then accepted a permanent position as the Entomologist for Eurofins Agroscience Services in Mebane, NC, where she currently resides with her family.

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The position wouldn't have been available without the monetary support of the North Carolina Tobacco Research Commission, so my immense gratitude goes to them. I also could not have done my research without help from the Central Crops Research Station in Clayton, NC. The guys out there went out of their way to provide field help, encouragement and some good advice when I needed it. I couldn't have gotten through the data analysis without the statistical guidance of Hannah Burrack, and she gets my undying gratitude as well.

My lab deserves a multitude of acknowledgements. First there was Alan Stephenson, who went out of his way to help me figure things out, build things that sounded crazy without hesitation, and provided a little bit of gun control instruction, skeet shooting, quail hunting, and dog advice. Mariah Bock and Anirudh Dhammi were sources of dependable and reliable field help, and without them I could have never gotten through all of the harvests and inoculations!

I'd also like to thank the friends I have made in the past three years as a graduate student. There are my first friends in the department, Micah Gardner and Whitney Swink,

who have a life bond with me involving Dungeons & Dragons and a tattoo…Then there is Eleanor Spicer, my ginger snap love, who has an amazing sense of humor and always a kind word about everyone...and probably would not ever go to a tattoo shop with me again…There are also those people who made my life more interesting in grad school, even though I no longer talk to them. I would not have had as nearly of an…exciting… time in grad school if it hadn't been for Emily Bone, Dylan Liverman, and Jeremy Salter. If it wasn't for these people, I wouldn't have so many interesting stories to tell everyone else!

I'd also like to mention the people who have been in my life since the end of classes. Kathy Kidd at the NCDA gave me a great job and the opportunity to work with great people. Then I started working for one of the most kind-hearted people I know, Lisa Darmo at Eurofins, who took a chance and believed in me before I finished my degree.

My last thanks goes to the people I consider to be part of my family. Kathryn Cherry has been around since Botany classes, and proved the worth of our friendship by securing a roach in her freezer for my ENT 502 collection although she is deathly afraid of insects! Joseph Douglas is one of the most intelligent, frustrating, and amazing people I have ever known, and gets props for letting me finish my thesis at his house on Halloween. Then there is my shey-kreth-ashke Lauren Moeller, because without her love, support, and taking the girls out to the bathroom when I got up late, I probably would never have had time for anything else!

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Damage Relationships and Control of the Tobacco Splitworm (GELECHIIDAE: *Phthorimaea operculella)* in Flue-cured Tobacco: An introduction

The potato tuberworm, (*Phthorimaea operculella* Zell.) is the most serious of all potato (*Solanum tuberosum* L.) pests worldwide (Fenemore 1988; Saur 2004; Kroschel 1995). It also goes by older synonyms, *Bryotropha solanella*, and *Gelechia solanella* (Meyrick, 1885). In tobacco, the insect is known as the tobacco splitworm, and can be found in most tropical and subtropical areas worldwide. It was first detected in the U.S. in 1856 in California (Graf 1917).

Biology

The tobacco splitworm is an oliphagous insect confined to species in the family Solanaceae, including both cultivated and feral plants (Broodryk, 1971, Fenemore 1988). The cash crops attacked by *P. operculella* include *Solanum tuberosum*, *Nicotiana tabacum*, *Solanum melongena,* and *Solanum lycopersicum* (Fenemore, 1988), while some of its weed host species include *Solanum dulcamara*, *S. rostratum,* and *Datura stramonium* (Potato Association, 2008). The moth is distinguished from other solanaceous-feeding moths by a costal hairpencil on the hind wings of the male (Meyrick, 1885).

Females lay 60-200 eggs in as little as four days, and eggs hatch within three to six days. Eggs are typically laid on green foliage in potato, as larvae prefer to be leaf and stem miners rather than tuber miners, but can be laid in soil or on stems (Potato Assn, 2008). Larvae will pupate in a variety of sites, including walls, windows, screens, plant debris, leaves, soil, ect. (Ferro 1993). Adults usually emerge within a week. In New Zealand, the pupal stage overwinters (Potato Assn, 2008). In the Pacific Northwest, it is possible that all stages but the adult are able to overwinter, although the overwintering behavior is not understood as well in the United States (Potato Assn 2008).

Pest Status

As a pest of potatoes, the tobacco splitworm not only does minor to moderate damage in the field through larval leaf mining, but can cause severe crop loss in stored potatoes through larval tuber mining (Das 1995). There is zero tolerance for tuberworm in fresh potatoes (Potato Assn 2008). Control methods in potatoes are mostly cultural, such as deep planting, sealing soil cracks, covering tubers with soil, eliminating waste products and maintaining a clean environment, as well as keeping a close eye on stored product (Ali 1993; Coll et al. 2000; Hanafi 1999; Potato Assn 2008). Other practices include using insecticides, mass trapping with pheromone traps, microbial control through the PoGV Granulosis Virus (*Baculoviridae*) and the bacterium *Bacillus thuringiensis*, and biological control through the Braconid wasps *Apantelels subandinus* and *Braconi greeni*, and the Encyrtid egg parasitoid wasp *Copidosoma koehleri* (Baggen 1998; Hanafi 1999; Mohammed et al. 2000).

Little is known about *Phthorimaea operculella* as a tobacco pest since most previous research has been in the context of its status as a potato pest. In tobacco, *P. operculella* is a leaf miner, producing translucent patch mines in tobacco leaves, reducing the value of the leaves. Frass ensnared in these mines also contaminates harvested leaves. Large populations can destroy leaves and plants. The feeding site for this leaf miner makes it difficult to control. Applied insecticides must be systemic to affect the larvae which are protected by the leaf lamina. There are no economic thresholds for the tobacco splitworm, nor have there been any assessments of damage relationships prior to this study. Insecticidal control with currently registered materials has largely been ineffective which occasionally results in season-long outbreaks. Often populations will reoccur in the same field over multiple years, as seen in potato fields (Hanafi 1999).

The incidence of tobacco splitworm damage has increased in recent years, creating a need to identify efficacious insecticides (P. Semtner, personal communication)[1]. Infestations can be patchy and sporadic, and range from mild to severe, depending on the year. This insect can be a serious problem for tobacco farmers with no tools to deal with an unfamiliar pest.

The tuberworm can rapidly increase to uncontrollable populations because of its high fecundity, short generation time, and because it appears to be favored by dry weather. The insect's cryptic nature often allows several generations to cycle undetected, and then the insect suddenly appears in overwhelming numbers. Splitworm populations appear to persist from year to year in previously infested fields, depending on the summer weather and crop rotation. Tobacco splitworm populations are generally higher in eastern North Carolina, where the climate is warmer than the western areas of the state. Eastern North Carolina is in

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[1] Semtner, 2008. Personal Correspondence. Virginia Polytechnic Institute.

plant hardiness zones 7b and 8a, while North Carolina’s overall range is 6b-8a (US Nat. Arboretum, 2003).

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Damage Relationships for the Tobacco Splitworm, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) on Flue-Cured Tobacco, *Nicotiana tabacum* L.

I. Introduction

The potato tuberworm, (*Phthorimaea operculella* Zell.) is the most serious potato (*Solanaum tuberosum* L.) pest worldwide (Fenemore 1988; Saur 2004; Kroschel 1995); the larvae not only cause minor to moderate damage in the field by leaf mining, but can cause severe crop loss in stored potatoes by mining tubers (Das 1995). This insect can be found in tropical or subtropical areas worldwide, and also occurs in temperate areas where potato is grown. *P. operculella* is an oliphagous insect confined to a few species in the family Solanaceae, in both cultivated and feral plants (Broodryk, 1971, Fenemore 1988). It was first detected in the U.S. in 1856 in California (Graf 1917). In tobacco, where it is known as the tobacco splitworm, it is a leaf miner, producing translucent patch mines in the tobacco leaves. The mines and resulting dead leaf tissue reduce the yield and quality of harvested tobacco. Frass entrained in these mines also contaminates harvested leaf. Large populations can destroy leaves and whole plants. The feeding site for this leaf miner makes it difficult to control. Foliar applied insecticides must be systemic to affect the larvae which are protected by the leaf lamina.

Little is known about *Phthorimaea operculella* as a tobacco pest; most previous research has been in the context of its status as a potato pest. There are no economic thresholds for the tobacco splitworm, nor any assessments of damage relationships. Incidence of damage from tobacco splitworm has risen greatly in North Carolina and Virginia in the recent years, generating an increasing need for efficacious insecticides. (P. Semtner, personal communication)[2].

The tuberworm can rapidly increase to uncontrollable populations because of high fecundity and short generation times, and because it is favored by droughty weather (Potato Assn, 2008). The insect's cryptic nature often allows several generations to cycle undetected, and then suddenly appear in overwhelming numbers. Splitworm populations appear to persist from year to year in previously infested fields, depending on the environmental conditions and crop rotation. Fields associated with the tobacco splitworm are generally in the eastern region of North Carolina, where the climate is milder overall than western areas.

The ecology, biology, and plant interactions of splitworms are poorly understood. In particular, we do not currently understand how much damage, measured as yield and quality loss, actually occurs due to splitworm populations in a tobacco field. Since there have been no previous studies of damage relationships for the tobacco splitworm in North America, field trials were conducted to establish such relationships using introduced populations of larvae.

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[2] P. Semtner, 2008. Personal Correspondence. Virginia Polytechnic Institute.

II. Materials & Methods

Tobacco Splitworm Colony Maintenance

A colony was maintained in the NCSU Insectary. The generation time for this colony was approximately 30 days. There were approximately 30 generations maintained under the following laboratory conditions. Each summer, genetic diversity in the colony was enhanced by augmenting the colony with individuals collected from feral populations in local tobacco fields. Fields with recurring populations were scouted, and larvae were collected from leaves. Larvae were reared separately until adulthood to reduce the likelihood of introducing parasitoids and pathogens to the colony.

The colony was maintained as described by Platner and Oatman (1968) and Getz and Chi (1988). Adults were kept in 946mL Ball Mason jars, with mesh screen lids held in place by the metal jar rims. Paper towels were cut to match the jar mouth, and were weighted with 7mm steel nuts. Each screen had a hole cut to fit the mouth of a 1.848 ml vial (Bioquip, www.bioquip.com). Vials were filled with sugar water (prepared by dissolving 25 grams of cane sucrose in 500mL water), and stoppered with a cotton ball. Adult females oviposited on the paper towel sheets. Sheets were collected daily and placed into small plastic containers until hatching. After hatching began, egg sheets were placed in 3.785-liter plastic containers with lids that had been modified with a mesh screen covering. Potatoes were prepared to be placed in containers by rinsing in cool water and were then punctured by rolling them across a board with 25 8mm tacks nailed at $0.25cm^2$ intervals in a grid pattern; these punctures facilitate establishment of neonates. After five days in the buckets, when larvae had successfully established in potatoes, the infested potatoes were transferred to a baker's rack with wire shelves (Fig. 1). As larvae emerged to pupate, they fell to a drawer in the bottom of the rack. The drawer had a medium-density fiberboard panel on the bottom covered with a thin layer of sand. Pupae were collected from the sand every other day and put at the top of the rack to develop. After 4-5 days, boards were scraped with a putty knife and sand was sifted out. Pupae were placed in a chamber (Fig. 2) where they were held until eclosion. After emergence, moths were anesthetized with CO_2 and collected at the bottom of the chamber in a funnel and then transferred to the mason jars.

Damage Relationship Studies

Damage relationship experiments were conducted during the summers of 2006, 2007, and 2008. The experimental design was a randomized complete block with number of tobacco splitworm larvae introduced per plant as the treatment; each plot consisted of five consecutive plants in a row. Two buffer plants were left between plots within rows, and a buffer row of plants was left between plot rows. Tobacco was managed according to North Carolina Cooperative Extension recommendations, except that no insecticides with activity against Lepidoptera were used. All tobacco was planted at the Central Crops Research Station in Clayton, NC. In 2006, tobacco was planted in field F3 on April 19, with the NC71 variety. In 2007, fields S1 and S2 were planted with variety NC71 on April 24. In 2008, Field D4 was planted with variety NC70 on April 24.

Neonates were released on the upper-third portion of each plant with a size 2 Folk Art® brand paintbrush during the early morning, generally before 10AM to avoid windy conditions. Applying the larvae early in the morning provided time for the insects to acclimate to field conditions before the daytime temperatures increased significantly. Rainfall, which might dislodge larvae before they established, was also less likely early in the day. In feral populations, splitworms are found on the bottom leaves of tobacco. Larvae were introduced in this study only on the upper leaves to ensure that introduced insects were accessible to the insecticide application equipment used to terminate populations before the turn of the next generation, to reduce the confounding influence of subsequent, unregulated and unknown splitworm populations.

Plants were monitored daily for mine formation. Mines were surveyed approximately a week after the larvae were released on the plant. Mines were not counted unless they exceeded one cm^2 in area. After larvae exhibited signs of maturation based on on mine size, usually after about 10 days, all plants were sprayed with insecticide in an attempt to terminate the population, reduce spread of splitworms to adjacent areas of the field, and to maintain non-infested plots for later field tests. In 2006 and in 2007 we applied acetamiprid at a rate of 0.247 kg/h, and with novaluron in 2008 at a rate of 0.064 kg/h. All insecticides were applied using a CO_2 - powered backpack sprayer with a TeeJet D2-33 Full Cone spring system nozzle that had an output of 107 liters per hectare at a pressure of 4.22 kg/cm.

Flue-cured tobacco typically has 20-25 harvestable leaves and is harvested multiple times from the bottom up, at about 2 -3 week intervals. In our studies, we took 5-7 leaves per harvest for three or four harvests. The last harvest included all remaining leaves on the stalk. The tobacco removed from each plant at each harvest was weighed to the nearest milligram with an electronic balance, bundled together by plot with a cotton string, and cured. Cured tobacco generally leaves about 14-18% moisture. Cured leaf yield was recorded in grams per plot.

When two trials were done in a season, they were considered either “early” or “late” season, depending on the time of neonate introduction. If larvae were introduced before topping, it was considered early season. If larvae were introduced after topping, it was considered late season.

The treatments in 2006 trial consisted of four infestation levels: 0, 15, 30, or 60 splitworms per plant. Tobacco was harvested on July 20, August 3, August 17, and September 8.

The 2007 early season trial had seven treatments ranging from 0-60 splitworms per plant in increments of 10. The splitworms were applied on June 13, 2007. Tobacco was harvested on August 2, 22, September 8, and 14.

The 2007 late-season trial had four treatments: 0, 30, 60, or 90 splitworms per plant. Splitworms were applied on July 20, 2007. Tobacco was harvested on August 22, September 8, and 14.

The 2008 early season trial had four treatments: 0, 15, 30, or 60 larvae per plant. Splitworms were applied on June 18, 2008. Tobacco was on August 12, August 25, September 16, and September 30, 2008.

The 2008 late season trial had three treatments: 0, 60, or 120 splitworms per plant. Splitworms were applied on July 29, 2008. Plots were examined on August 6th for mines Tobacco was harvested on August 12th, 25th, September 16th, and 30th.

Statistical Analysis

Green and cured weights were subjected to regression analysis using PROC REG in SAS (SAS Institute 2003). The analysis was run by using the actual mine counts from each plot, rather than the number of splitworms applied to the plots. All weights are by plot per harvest rather than by plant because the curing method required plots to be bundled, rendering individual plant weights impossible to separate.

III. Results

No significant relationships were observed between infestation levels and cured weights in 2006. However, in the 2007 field trials, a significant decrease in cured weight with increasing larval densities was observed at $\alpha = 0.05$. There was a negative correlation between the cured weight and the splitworm infestation for each trial. The early 2007 average weight in non-infested plots was 1176.88 grams per plot, and weights declined about 3.4 grams per plot for every 10 splitworms found. In the late 2007 season, the average non-infested weight was 1073 grams per plot, and lost approximately 6.1 grams per plot for every 10 splitworms. The R^2 of the early season was 0.12 (Figure 1) and an R^2 of 0.33 in the late 2007 season (Figure 2).

Significant, negative regressions between mine number and cured weight were obtained for both the early and late season trials in 2008. Yield in the non-infested plots in the early season trial average 1216.65 grams per plot, and yields decreased approximately 10 grams per plot for every 10 splitworms. The late season trial had an average non-infested weight of 1289.1 grams per plot, and lost about 8 grams for every 10 splitworms in the plot. The early season 2008 had an R^2 of 0.41 (Figure 3) and the late 2008 season had an R^2 of 0.26 (figure 4).

IV. Discussion

Growing conditions during the 2006 season were conducive to good tobacco growth. This coupled with the low preliminary larval establishment numbers resulted in the absence of a relationship. Green weights collected in 2006 show a steady decline in weight in relation to the initial splitworm population, but our inability to identify a significant relationship was most likely due to plant compensation for the low numbers of larva introduction introduced.

The 2007 season trials produced a statistically significant relationship between yield and infestation. The 2007 season was droughty, but the effects of the drought on crop performance were not pronounced until later in the season; since the first trial started in June, it avoided the more severe effects during the introduction of the splitworm larvae, and may have made the plants less susceptible to splitworm damage. The R^2 of 0.12 suggests that the splitworm density affects only a small portion of the weight change in the early 2007 season, when the plants had more time to adjust for damage and compensate. Also, with an early season infestation, mines and frass would have time to fall out, causing less noticeable damage on the leaves. Based on the non-infested weight for early 2007, we would expect to get 1412.3 kg yield per hectare. Based on the average price per stalk position, this would represent a value of approximately \$4060 per hectare from the early 2007 tobacco. With an infestation of 10 splitworms per plant, the expected value would be about \$4050; a loss of approximately \$10 per hectare. In late 2007, an R^2 of 0.33 suggests a stronger negative between splitworm densities on the tobacco and yield. Based on weights of in the non-infested plots, it the estimated total weight per hectare would be 1302.8 kilograms. Based on average price per stalk position, the value of tobacco from the late 2007 season would be about $3700 per hectare. With an infestation of 10 splitworms per plant, the estimated loss in value would be about $27 per hectare.

We also do not know the effects of splitworm infestations on a plant's physiology, or how it may affect normal harvesting and curing outcomes. Studies on wound responses, particularly in solanaceous plants, have shown that plants quickly produce chemicals to ward off their attacker. In the case of tomatoes and potatoes, it is a proteinase inhibitor that causes feeding cessation in insects (Green, 1972). Changes in chemicals in the leaves may change the way the tobacco tastes, so even if it does not show a weight loss, it may still be commercially unacceptable.

The 2008 early-season trial produced a significant relationship between cured weight and splitworm populations, as well as cured weights between harvests. There was a higher cured weight loss during the early season trial. In this trial, the R^2 of 0.41 suggests a stronger negative correlation between splitworm density and tobacco yield. Based on an average yield in the non-infested plots a yield of 1459.8 kilograms could be expected per hectare. From this, the early 2008 tobacco could be expected to produce a value of about $5050 per hectare, based on stalk position, but with 10 splitworms per plant, there would be an estimated loss of about $48 per hectare.

The late 2008 season trial also produced a significant relationship. The larval introduction in this trial occurred barely two weeks before the first harvest took place, leaving the plants little time to compensate for damage due to the artificial infestation. The late season trial had an R^2 of 0.26, with splitworm infestation level accounting for about a quarter of the negative total variation in yield. Based on the initial weights in non-infested plots, the expected yield would be about 1545 kilograms per hectare, which would have an expected value of about $5335 per hectare. With an infestation of 10 splitworms per plant, a loss of about $25 per hectare would be expected.

The results from the three tests indicate that splitworm infestations in a field can reduce a tobacco farmer’s income. In 2007, the summer was very dry and the plots were not irrigated, so both weights are lower than the 2008 season. Results from the 2008 season demonstrate that the potential for yield loss is still significant even under favorable growing conditions. There was also less weight loss during the 2007 seasons than in the 2008 seasons. Since yields in the absence of splitworms were lower in 2007, and the plants were already stressed from drought, the infestations may not have been able to impact the yield as much as when the growing conditions were good. The early trials show that when an infestation occurs earlier in the season, even if insecticide is used after the mines are detected; the overall weight of the leaves can be reduced, even if no mines are present on the harvested portions. The mining holes at this point were only on the lower portion of leaves, so there was no damage on the last harvest. The late-season infestation also proved to be more aesthetically damaging than the early season infestation because of the presence of mines and frass. The most important thing in farming is the profit. Even though there was more weight loss with early season infestations, it provided higher quality leaves than the late season infestations. If a farmer recognizes splitworm damage in his field, it would be even more important to stop it quickly in the later season than the early season because the plant will not have time to compensate, and contamination by frass and insects will be greater. While the overall loss numbers may not have seemed substantial, the price loss estimates are based solely on the weight reduction, and do not include unquantified aspects such as reductions in grade or potential changes in flavor from chemical changes in the plant.

The third harvest was the most affected overall by splitworm density, except in the early 2008 season, when both the third and fourth harvest were similar in their yield loss. While the starting non-infested weight of the third harvest is two-thirds less than the starting non-infested weight of the fourth harvests (except in late 2007 when there were only 3 harvests, so a third of the weight), the yield loss from the third harvest accounts for a little less than half of the overall loss for every season.

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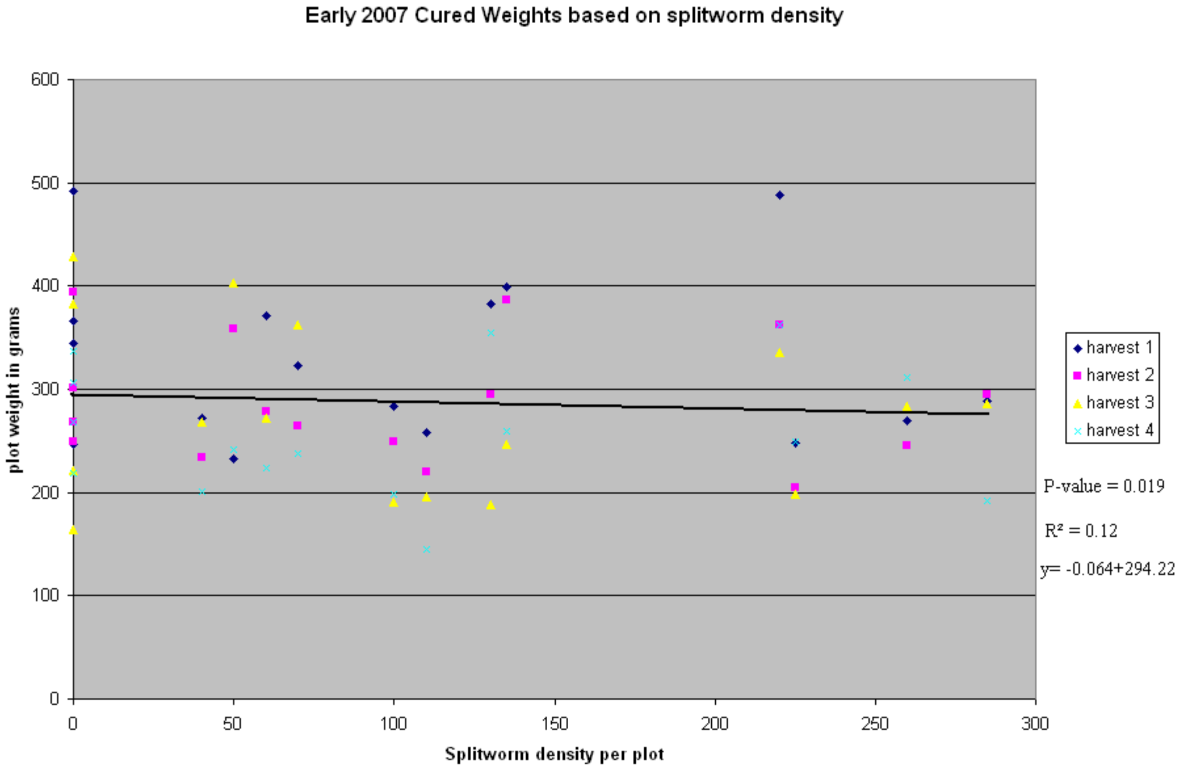

Figure 1. Early 2007 cured weights by harvest

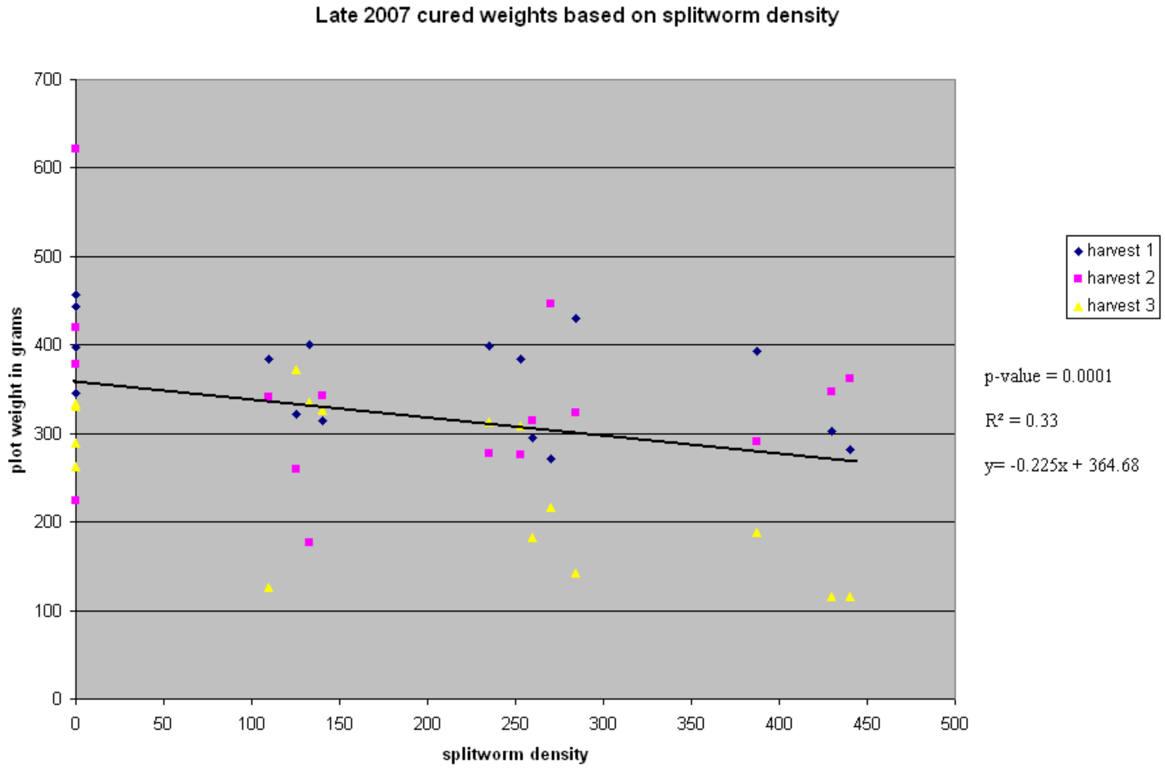

Figure 2. Late 2007 cured weights by harvest

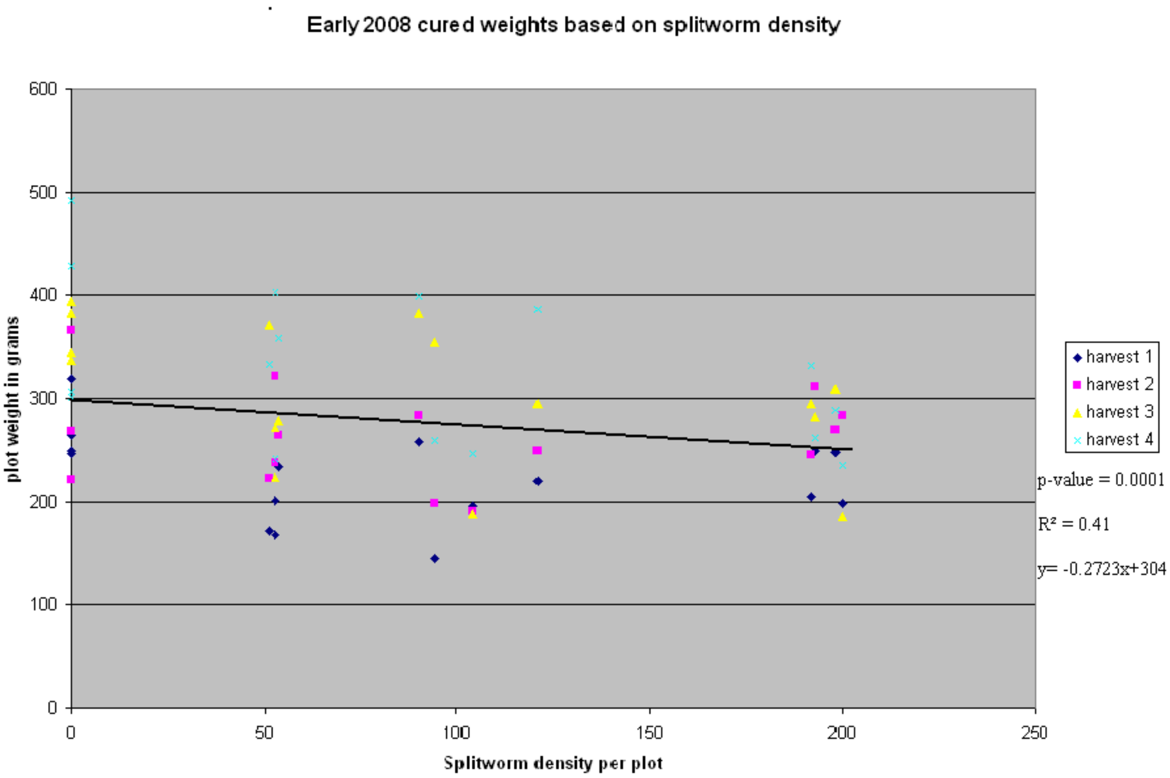

Figure 3. Early 2008 cured weights by harvest

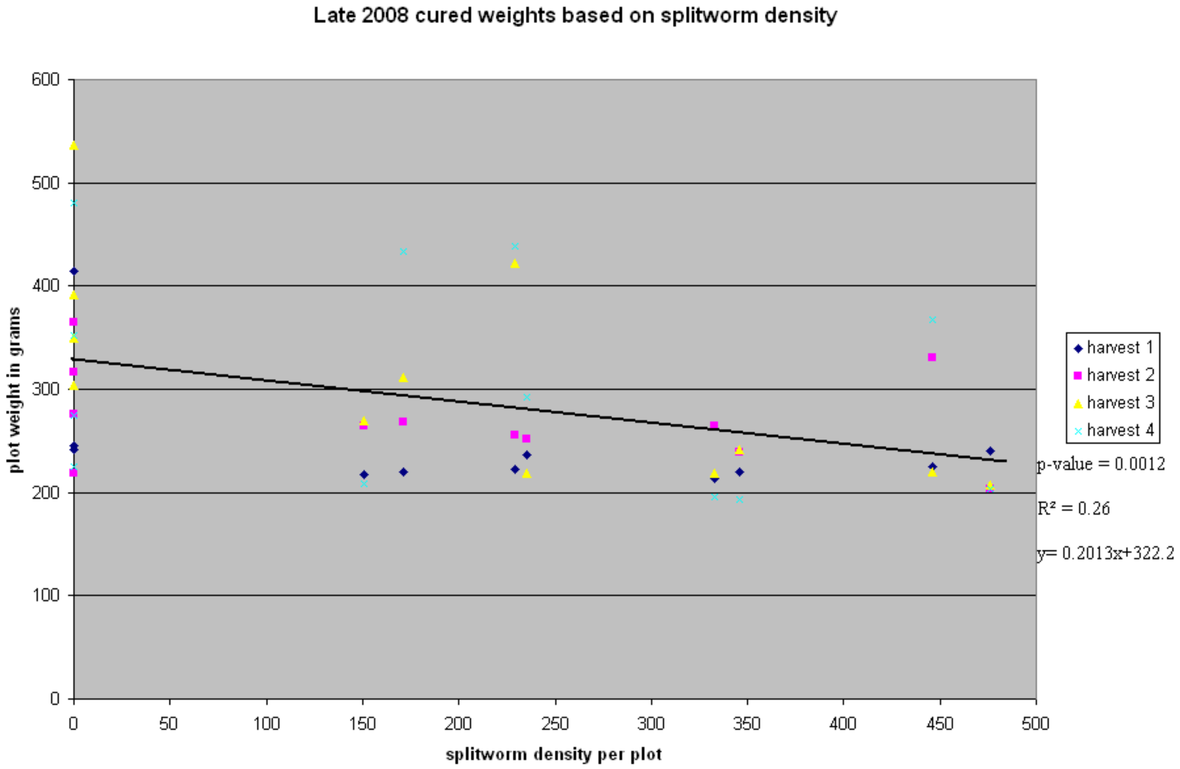

Figure 4. Late 2008 cured weights by harvest

Figure 5. Baker’s rack used to store infested potatoes.

Figure 6. Carbon Dioxide chamber used to capture newly emerged moths.

Control of the Tobacco Splitworm, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) on Flue-Cured Tobacco

I. Introduction

The potato tuberworm, (*Phthorimaea operculella* Zell.) is the most serious potato (*Solanum tuberosum* L.) pest worldwide (Fenemore 1988; Saur 2004; Kroschel 1995); it not only does minor to moderate damage in the field by larval leaf mining, but can cause severe crop loss in stored potatoes by larval tuber mining (Das 1995). This insect can be found in most tropical or subtropical areas worldwide, and also occurs in the temperate areas where potato is grown. *P. operculella* is an oliphagous insect confined to a few species in the Solanaceae family, in both cultivated and wild plants (Broodryk, 1971) (Fenemore 1988). In tobacco, where it is known as the tobacco splitworm, it is a leaf miner. It reduces leaf weight and quality by producing translucent patch mines in the tobacco leaves. Resultant frass also lowers leaf quality. Large populations can destroy leaves and plants. Topical insecticide sprays must be systemic to affect the larvae because they are protected by the leaf lamina.

There is little research into the management of field populations of *Phthorimaea operculella* in tobacco. Insecticidal control with currently registered materials has largely been ineffective; this poor efficacy occasionally results in season-long outbreaks. Control methods in potatoes have been mostly cultural, especially in areas where *P. operculella* has been more of a problem, such as African and Middle Eastern areas. These methods involve labor-intensive work, such as sealing soil cracks, covering tubers with soil, destroying leftover waste, as well as keeping a close eye on stored product for infestation. (Ali 1993; Coll et al. 2000; Hanafi 1999; Potato Assn 2008). Often populations will reoccur in the same field multiple years. Since this insect is fecund and has short generation times, and because it is favored by droughty weather (Potato Assn., 2008), the tuberworm can rapidly increase to uncontrollable populations under favorable conditions.

Little is known of the effects of currently registered insecticides on tobacco splitworm in North America; anecdotal evidence suggests that few have any appreciable effect on this insect. We conducted efficacy trials to identify potential control agents for this pest. Trials were conducted with acephate, acetamiprid, novaluron, and chlorantraniliprole, as each showed promise in splitworm control through their mode of action and/or by their potential for systemic activity.

Acephate is a water soluble organophosphate insecticide with demonstrated residual systemic activity of approximately 10-15 days (PMEP 1995). It is used to control leaf miners, as well as foliar feeding Lepidopterous larvae. It is also toxic to *Heliothis* spp and aphids that are otherwise resistant to other organophosphates. This compound has been registered for use for tobacco since 1982, and is normally used as aphid control. Acephate has some negative side effects to humans, is toxic to many beneficial insects and moderately toxic to game birds, but dissipates rapidly in the environment with little residual (PMEP 1995).

Acetamiprid was selected because it was already registered for use on tobacco for aphids as well as other Lepidoptera pests, such as the tobacco hornworm and budworm. It is a neonicotinoid with mild to moderate ecological effects, being somewhat toxic to *Apis mellifera*, but considered a reduced risk insecticide overall (EPA 2002, 2005). It is an agonist to nicotinic acetylcholine receptors (EPA, 2002). Acetamiprid is transported through the xylem and has low hydrophobicity, resulting in effective systemic and translaminar activity.

Novaluron is an insect growth regulator that disrupts growth and development of larvae, killing them over a period of days. It is considered effective against leaf miners. Novaluron displays contact activity as well as demonstrating insecticidal activity by ingestion, but is not systemic. It is considered a safe insecticide to mammals, beneficial insects and the environment, making it useful in integrated pest management programs (EPA 2001).

Chlorantraniliprole is in a new insecticide class called anthranilic diamides. It is effective against nearly all economically important Lepidoptera larvae, and induces rapid feeding cessation (including *P. operculella* as a potato pest) (Dupont 2007). Chlorantraniliprole is a ryanodine receptor modulator, binding to the receptor and disrupting muscle contractions, but has no systemic activity. (Silcox, 2008). It also has virtually no mammalian toxicity, and low impact on beneficial insects and the environment, making it a good candidate for integrated pest management as well.

Materials & Methods

Naturally occurring infestations of the tobacco splitworm are difficult to predict in space and time, so all efficacy trials were conducted with introduced populations from a colony maintained in the NCSU Insectary. The colony was reared as described in the previous chapter (Lawrence, unpublished).

All studies were conducted at North Carolina State University's Central Crops Research Station (CCRS) in Clayton, North Carolina in late summer of 2006, 2007, and 2008. In 2006, tobacco was planted in field F3 on April 19, with the NC71 variety. In 2007, fields S1 and S2 were planted with variety NC71 on April 24. In 2008, Field D4 was planted with variety NC70 on April 24. The tests were set up in a randomized block design with four replicates per treatment; each plot consisted of five plants, with two buffer plants between plots within rows and a buffer row between each experimental row. Infestation levels were consistent across treatments within individual experiments as described below. Neonate larvae were applied to the plots on the upper-third portion of each plant with a size 3 paintbrush during the early morning. This gave the larvae time to acclimate and adjust to field conditions before the temperature increased significantly, and to avoid rainfall later in the day.

All insecticides were sprayed using a backpack sprayer using a Tee Jet D2-33 Full Cone spring system nozzle that had an output of 107 liters per hectare at 3.5 kg/cm.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) through PROC GLM in SAS (SAS Institute 2003). Means separation was accomplished through a Tukey-Kramer adjusted p-value test. The analysis was run on the actual mine count from each plot after spraying, and compared to the other treatments as well as the controls to determine which treatments were statistically different from the others.

2006 Trials

Five treatments were examined in this first experiment; in addition to the control, acephate and acetamiprid were applied at either one day or four days after introduction of the larvae. Fifteen larvae per plant were applied to the upper third of the plants, on July 23, 2006. On July 24, 2006, foliar applications of acetamiprid were applied with a backpack sprayer at 0.247 kg/h, and acephate at 0.741 kg/h, to appropriate plots. The 4-day spray took place on July 28, 2006, with acephate and acetamiprid at the same application rates as earlier applications. Mines were examined and tallied on August 4, 2006.

2007 Trials

This trial consisted of five treatments; in addition to the untreated control, novaluron and acetamiprid were applied either one day or four days after introduction of larvae. Twenty splitworms were applied to upper leaves on each plant within each plot, to allow for spray contact. The control consisted of two plots; all other treatments contained four plots. Acetamiprid was applied at 0.247 kg/h, while novaluron was applied at a rate of of 0.064 kg/h; all insecticides were applied with a backpack sprayer as described above. The splitworms were applied on August 6, 2007. Day one applications were made on August 7, 2007, and day four applications were made on August 10, 2007. Plots were examined on August 10, 14, and 20 for mines and mines tallied.

2008 Trials

Two separate trials were conducted in 2008. Acetamiprid trials were done separately. Twenty splitworms were applied to the top third of each plant on July 21, 2008. Acetamiprid was applied with a backpack sprayer at a rate of 0.247 kg/h on July 22, 2008, July 25, 2008, and July 28, 2008 on four plots each. Observations were taken on August 6, 2008.

The second trial was conducted with novaluron and chlorantraniliprole. Treatments included 1- or 3-day sprays of novaluron at a rate of 0.064 kg/h and of chlorantraniliprole at a rate of 0.073 kg/h in addition to an untreated control. Twenty splitworms were applied to the top third of each plant in plots for 3-day applications and controls on August 12, 2008. Twenty splitworms were applied to the top third of each plant in plots for 1-day applications on August 14, 2008. All applications were conducted on August 15, 2008 with a backpack sprayer. Efficacy data was collected on August 28, 2008.

III. Results

2006

Acephate was only tested in 2006 (table 1). Acephate provided no control of the tobacco splitworm when compared to the non-treated plots for either the one-day application (t = -0.58, df = 14, P = 0.9911) nor the three-day spray (t = -0.78, df = 14, P = 0.9661). , Acetamiprid did not significantly reduce splitworm numbers compared to the control (Table 1) in the one-day (t = -0.42, df = 14, P = 0.998) or the three-day (t = -0.75, df = 14, P = -0.9724) applications.

2007

Acetamiprid proved to be significantly different from the control in 2007 (table 2) in the one-day (t = -9.49, df = 13, P = <0.0001) and 4-day (t = -8.53, df = 13, P = <0.0001) applications. Novaluron was first tested in 2007 in conjunction with acetamiprid. It significantly reduced splitworm numbers compared to the control (figure 4) in the one-day (t = 8.96, df = 13, P = <0.0001) and the four-day (t = 9.27, df = 13, P = <0.0001) treatments.

2008

Acetamiprid was tested alone in 2008 with multiple residual periods. It proved to be no different from the control (table 4) in the one-day (t = 0.48, df = 11, P = 0.9875), the four-day (t = 0.2, df = 11, P = 0.9995), or the seven-day (t = 1.55, df = 11, P = 0.5535) treatments. Novaluron was tested alongside chlorantraniliprole during the late season. Novaluron proved to be significantly different from the control (Table 3) in the one-day (t = -23.18, df = 15, P = <0.0001) and in the three-day (t = -23.92, df = 15, P = <0.0001). Chlorantraniliprole proved to be different from the control at both one-day (t = -25.67, df = 15, P = <0.0001) and three-day (t = -25.27, df = 15, P = <0.0001) timings (Table 3).

IV. Discussion

2006

Both Acephate and Acetamiprid were ineffective. There were low mine counts even among the control plants, and populations allowed only one count to be taken. This test was interrupted shortly after the first count, so the data was not very representative of a natural infestation. Possibly the mine counts would have been different after a second assessment. The resolution of this trial was probably also compromised by the low introduction rate.

2007

This efficacy trial was conducted at the end of the tobacco season. The drought that took place during the summer had taken its toll on the field, stressing the tobacco and helping an explosive splitworm population to dominate the area. Non-treated plots were obviously affected by splitworm, ending with an average of 30 splitworms per plot (of a possible 100), while the highest population recorded from any of the four treated plots was less than 2.5 splitworms per plot. Some damage had been recorded prior to spraying on the four-day trials, but no mines were observed that showed continued growth of a splitworm after spraying. However, the surrounding treated plants could have kept some splitworms away, keeping the control plots from becoming overloaded with wild populations. The few mines found on applied plots could have been remnants from the original introduction, or new larvae that came in later, when the insecticide efficacy was fading, or was never really there. This occurrence led to the repeat trials of both insecticides.

2008

Both insecticides from 2007 were examined, and a new insecticide was introduced. Acetamiprid had essentially no efficacy in 2008. In some cases, acetamiprid plots had higher mine counts than control plots. The field populations were held under strict control throughout the tobacco season, so there was no outside interference in any of the trials. This experiment leads to questions of the efficacy of acetamiprid against tobacco splitworm infestations.

Novaluron was efficacious at both the 1-day and 3-day timings. Efficacy at three days is particularly important, since it indicates that the insecticide is effective against larvae which have established in the lamina. Field populations of splitworms are not uniform; they typically consist of a range of larval ages, with most inhabiting the lamina. Furthermore, growers typically will not detect damaging populations until a substantial proportion of the insects are ensconced in the leaf.

Chlorantraniliprole, the newest insecticide, provided control comparable to that of novaluron. It’s efficacy at both the 1-day and 3-day application timings suggests it will be useful as a larvicide against this insect, so it can also be applied with a delay and efficiently control mining. The systemic activity of this insecticide is important because it is also effective against established larvae in the lamina. It is unlikely that growers will distinguish signs of infestation until numbers have risen in the field significantly, so being able to spray later in the life cycle is imperative for effective control. Unfortunately, chlorantraniliprole did not pass the smoke test in 2008, rendering it unsuitable for use on tobacco because it changes the taste of the tobacco.

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Table 1. SAS data outputs for 2006 of acephate and Acetamiprid, using PROC MIXED for an LSD with a Tukey-Kramer adjustment.

Treatment	Rate	surviving splitworms per plot %
Acephate 1-day	0.741 kg/h	30a
Acephate 3-day	0.741 kg/h	17.5a
Acetamiprid 1-day	0.247 kg/h	32.5a
Acetamiprid 3-day	0.247 kg/h	15a
Control		50a

Table 2. SAS data outputs for 2007 of Acetamiprid and Novaluron, using PROC MIXED for an LSD with a Tukey-Kramer adjustment.

Treatment	Rate	surviving splitworms per plot %
Acetamiprid 1-day	0.247 kg/h	0a
Acetamiprid 4-day	0.247 kg/h	11.25b
Novaluron 1-day	0.064 kg/h	3.75a
Novaluron 4-day	0.064 kg/h	1.25a
Control		30c
*control error was 0.3909		

Table 3. SAS data outputs for 2008 of Chlorantraniliprole and Novaluron, using PROC MIXED for an LSD with a Tukey-Kramer adjustment.

Treatment	Rate	surviving splitworms per plot %
Chlorantraniliprole 1-day	0.073 kg/h	0a
Chlorantraniliprole 4-day	0.073 kg/h	2.5a
Novaluron 1-day	0.064 kg/h	1.75a
Novaluron 4-day	0.064 kg/h	1.25a
Control		59b

Table 4. SAS data outputs for 2008 of Acetamiprid, using PROC MIXED for an LSD with a Tukey-Kramer adjustment.

Treatment	Rate	surviving splitworms per plot %
1-day spray	0.247 kg/h	3.25a
4-day spray	0.247 kg/h	2.2a
7-day spray	0.247 kg/h	11.775a
control		1.3a