ABSTRACT

MCPHIE, DOUGLAS ROY. Impacts of *Anthonomus signatus* (Curculionidae:Coleoptera) on annual plasticulture strawberries (*Fragaria* × *ananassa*)**.** (Under the direction of Hannah Burrack.)

The strawberry bud weevil (*Anthonmus signatus*) is a univoltine beetle that feeds upon developing buds of spring-fruiting plants, including strawberry (*Fragaria* × *ananassa*). The timing and extent of *A. signatus* damage to strawberries grown in southeastern annual plasticulture strawberry production was unknown. We conducted a two year survey of commercial strawberry farms throughout North Carolina and one location in Virginia and observed bud damage ranging from zero to 42 percent. Physical inspection of plants for damage proved a more effective way of monitoring *A. signatus* as compared to yellow sticky card traps. The timing of first injury by *A. signatus* after emergence from overwintering sites ranged from 9-18 April in 2014 and 2-10 April in 2015. A comparative degree day recommendation for scouting was developed with a base temperature of 0°C and a biofix date of 1 January. This degree day model predicts that damage to spring host crops will begin at 509.47 degree days, and that the summer generation will move to overwintering habitat at 2251.43 degree days.

The ability of strawberries grown in annual plasticulture to compensate for bud loss caused by *A. signatus* ovipostion was studied through a two year field experiment. In 2014, the response of the cultivar Camarosa to simulated *A. signatus* injury was measured, and there was no significant difference in total yield across the treatments and control plots. During 2015, total yield of five cultivars (Albion, Benicia, Camarosa, Chandler, and Sweet Charlie) was also unaffected by simulated *A. signatus* injury, although yield varied between

cultivars. Harvest timing did vary between treatments, depending on the hierarchal order of buds removed. In the 2015, Albion, the only day neutral cultivar, had a low number of buds during the period that *A. signatus* injury was expected to occur. No significant differences in berry size were detected between treatments of any cultivar within the same week.

Historically, *A. signatus* has been managed through use of broad spectrum insecticides, often applied as preventative treatments. The efficacy of reduced risk materials and materials acceptable for use in organic production were tested against *A. signatus* in semi-field bioassays in 2014 and 2015. None of the materials assessed during 2014 resulted in higher *A. signatus* mortality than the untreated control, but two materials compared during 2015 did produce significantly higher mortality than the untreated control. Spinosad had similar mortality to the positive control, bifentherin, and acetemiprid had higher mortality than the untreated control but lower mortality than the bifentherin.

Our results suggest that the current recommended economic thresholds for *A. signatus*, of two clipped buds per meter, are very conservative and that compensation appears common in cultivars grown in annual plasticulture. Monitoring for *A. signatus* is best done through physical examination of plants for signs of injury. Degree days may be useful to begin monitoring for presence and abundance of injury. If treatment is needed, some reduced-risk materials and Organic Material Review Institute (OMRI) listed materials may be preferable to currently used broad spectrum materials for controlling populations of *A. signatus*.

Impacts of *Anthonomus signatus* (Curculionidae:Coleoptera) on Annual Plasticulture Strawberries (*Fragaria* × *ananassa*)

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DEDICATION

To Jenny and the pets

BIOGRAPHY

Douglas was born and raised in Wheaton Maryland, a suburb of Washington DC. He severed honorably in the United States Army where he was an electronic missile systems technician. After the military he was a theater technician at venues throughout the country. In 2009 a chance move to North Carolina allowed him find a renewed interest in the sciences while taking classes at Durham Technical Community College. From there he proceeded to complete his Bachelors of Science at North Carolina State University. While working in the Small Fruit and Specialty Crops lab as an undergraduate he became very interested in the discipline of entomology.

ACKNOWLEDGEMENTS

Douglas wishes to express his thanks to all that help his thesis project come together. His wife, Jenny, spent countless hours listening to his stories about strawberries and insects. All the students and workers that helped in planting and picking strawberries; Linda Della Rosa, Aurora Toennisson, Peter Cajamarca, Melissa Somody, Connor Thomas, Yanan Zheng, Cameron McLamb, Pete Nelson, Katie Swoboda, Alejandro Merchan, and Lauren Diepenbrock. All of the participating farms, the agencies that help to fund this research; The North Carolina Strawberry Association, the Virginia Strawberry Association and The Southern Region Small Fruits Consortium. Also, committee members who were always there when guidance was needed; Dominic Reisig, Gina Fernandez, and advisor Hannah Burrack.

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Introduction and Objectives

The strawberry (*Fragaria* × *ananassa*) is an economically important high value fruit crop grown throughout the southeastern United States. Strawberry production in this region is decentralized, consisting of small (less than 4 ha) pick-your-own operations (Safley et al. 2004). In North Carolina there are over 150 pick-your-own strawberry locations located throughout the state with the majority of locations in the eastern Piedmont and Coastal regions. *Anthonomus signatus* Say (Curculionidae: Coleoptera), the strawberry bud weevil (SBW), is considered a key pest in eastern North America strawberry production (Clarke and Howitt 1975, Schaefers 1978). Adults emerge from overwintering locations in early spring and move to host plants with early season bud development (Schaefers 1978). After depositing an egg by chewing through the bud, the female then girdle or "clip" the buds from the plant at the pedicel. This causes the flower bud to droop and then fall of the plant completely (Chittenden 1908, Headlee 1916). Because of this behavior of the female, strawberry bud weevils are often referred to as "clippers".

The ability of some common strawberry cultivars grown in perennial matted row production systems to compensate for potential lost yield due to *A. signatus* activity is well documented (Clarke and Howitt 1975, Bostanian et al. 1999, English-Loeb et al. 1999, Kovach et al. 1999). Some of cultivars that have been evaluated (Mohawk, Mira) have shown tolerance for *A. signatus* injury by increasing weight of remaining fruit while other cultivars (Jewel, Seneca) compensated for yield loss through the production of increased

numbers of higher ordered floral buds which in turn increased number of available fruit. A few cultivars (Northeaster, Honeoye) showed no tolerance (Pritts et al. 1999).

Southeastern states adopted the annual plasticulture strawberry growing system in the early 1980s. This method of production is cost and input intense, but in return, plasticulture strawberries can be 2.5 times as productive as matted row planting (Poling et al. 2005). The higher monetary cost of the plasticulture production system increases the risk of losses due to insect pests and disease. To date there has not been a comprehensive investigation into the cultivars commonly grown in plasticulture production in this region to determine their response to *A. signatus* injury.

With the southeastern strawberry industry consisting of a large number of small pick-your-own operations, it is also important to determine how widespread *A. signatus* is throughout the production region. *A. signatus* is believed to move into fields from wooded edge, and plants at field edges are thought to be at greatest risk of damage. In matted row planted strawberries, ovipostion activity of female *A. signatus* was observed to move into fields at a rate of 6-14 meters per year over three years (Kovach et al. 1999). Smaller field sizes may be easier for *A. signatus* to move across, and damage may be more widely distributed as compared to large fields. A clearer understanding of the distribution, timing and behavior of *A. signatus* combined with a characterization of tolerance, through yield compensation, of cultivars grown in the plasticulture system will allow growers to make better management decisions about the need for, and timing of management to prevent significant yield loss. The research objectives of the study are:

1. Determine *A. signatus* activity timing at farms throughout North Carolina and distribution on local and statewide levels.
2. To quantify the impact of potential damage *A. signatus* at different rates and timing on yield of five strawberry cultivars popular in the plasticulture system.
3. To identify and screen reduced risk insecticide materials for activity against *A. signatus* in the event treatments are necessary.

Literature Review

Strawberry bud weevil (*Antonomus signatus* Say)

The Strawberry bud weevil (SBW), *Anthonomus signatus* Say is a small, univoltine beetle in the family Curculionidae. *A. signatus* utilizes a wide taxonomic range of plants including caneberries (*Rubus* spp.*)*, red bud (*Cercis* spp.*)*, and strawberries (Fragaria × ananassa) (Kovach et al. 1999). An adult *A. signatus* is approximately 2.54 mm long with color ranging from reddish-brown to an almost black (Figure 1.1a). On each elytron there is a distinctive dark spot surrounded by white setae. The rostrum or "snout" is elongated with the mouth parts at the end to enable the weevil to chew through the unopened flower bud for pollen feeding. Female beetles lay a single egg and girdle or "clip" buds from the plant at the pedicel, causing the loss of a potential fruit (Headlee 1916)(Figure 1.1c). The clipping behavior prevents the bud from opening, and provides a protected environment for egg to hatch and the larvae to develop. Each adult female can produce upwards of 75 eggs within their lifetime (Pritts et al. 1999). Eggs are 0.5mm in length, white, and are placed around the anthers inside of the developing flower bud (Figure 1.1b). A legless white larva hatches from the egg in approximately seven days. Larvae turn grey as they develop and mature over the course of three to four weeks, growing up to 3 mm in length (Figure 1.1d). There are three larval instars, with stadia lasting two, three, and nine days each at a constant temperature of 21.1° C (Clarke and Howitt 1975). *A. signatus* pupates within the flower bud, and pupal duration is approximately 10 days (Figure 1.1e).

The sex ratio has been determined to be maintained at 1:1 year-round in both fields and overwintering locations with very small variability from year to year (Mailloux and Bostanian 1993). Newly emerged adult *A. signatus* spend some time feeding on pollen before returning to overwintering habitat during mid-summer (Headlee 1916). *A. signatus* overwinter in leaf litter and soil at wooded edges, out buildings, and fences near host plants. In perennial matted row strawberry plantings, *A. signatus* may overwinter within fields as observed through soil core samples extracted from strawberry growing locations during aestivation and diapause periods (Mailloux and Bostanian 1993).

Overwintered adults migrate to the field when temperatures reach approximately 16°C at some level of consistency (Schaefers 1978). A degree day model has been developed for Canadian *A. signatus* populations with a base temperature of 0°C and biofix of 1 April. This model predicts migration of *A. signatus* into host plants between 305 to 357 DD and spring population at maximum abundance at 500 to 670 DD (Bostanian et al. 1999).

In an attempt to limit the usage of pesticides, integrated pest management (IPM) programs recommend applying treatment only after a pest has reached an economic threshold at which the cost of treatment is less than the cost of damage. IPM is most successful when sound economic thresholds have been established based on pest population size and host plant response to damage or injury (Stern et al. 1959). Injury levels on strawberries from *A. signatus* vary from year to year at any single location, making monitoring for insect presence and injury necessary. The current economic threshold for *A. signatus* injury is two buds clipped per meter (Sprangler et al. 1988). This threshold was calculated based on the

assumption that one clipped bud is equal to the loss of on average sized berry. However, there has been little correlation observed between yield and number of damaged buds (Popov 1996, English-Loeb et al. 1999). Research conducted in perennial matted row strawberry plantings suggests that, dependent on an individual cultivar's ability to tolerate damage, the management action threshold could be as high as 20 clipped buds per meter (Cross and Burgess 1998, Pritts et al. 1999). For cultivars with little or no tolerance, a lower economic threshold of five clipped buds per meter has been suggested (English-Loeb et al. 1999, Pritts et al. 1999).

A. signatus populations have been managed primarily with broad spectrum insecticides, including organochlorides, organophosphates, and pyrethroids (Clarke and Howitt 1975). All of these materials can have negative effects on predators, pollinators, and the environment. The use of organophosphates and pyrethroids can lead to further treatments being needed to control twospotted sider mite populations later in the season (Louws et al. 2015).

The strawberry plant (*Fragaria* × *ananassa)*

The strawberry plant (*Fragaria* × *ananassa)* is a perennial, with shortens modified stems called crowns, white hermaphroditic flowers. The large red fruit produced is not a true berry but an aggregate compound fruit (Lim 2012). The flower clusters, or inflorescence, of the strawberry plant produce a single primary, at least two secondary, and multiple tertiary

flowers, and some plants can produce higher ordered flowers. For optimal yields, a minimum of three crown per plant and a maximum of six are recommended, and each crown produces its own inflorescences (Poling 2012).

The first cultivation of the strawberry occurred by the 14th century in France where the wood strawberry, *Fragaria vesca*, was transplanted into gardens from the wild (Darrow 1966). Modern day strawberries were first hybridized in France in the 1750's by crossing *Fragaria virginiana* from North America and *Fragaria chiloesnsis* that was brought to Europe from Chile in the early 18th century. The hybridization of these two species resulted in the synthesis of *Fragaria x ananassa*, and strawberries of this human made cross produced larger fruit of better quality than any of the wild type *Fragaria* species and is the most economically important of the strawberries (Hummer and Hancock 2009). The introduction of day neutral cultivars came with the arrival of wild genotype *F. virginiana glauca* to the California breeding programs in 1960's allowing for longer production seasons each year. The high variability and range of desirable characteristic found across current cultivars is due to the octoploid chromosome number (Darrow 1966).

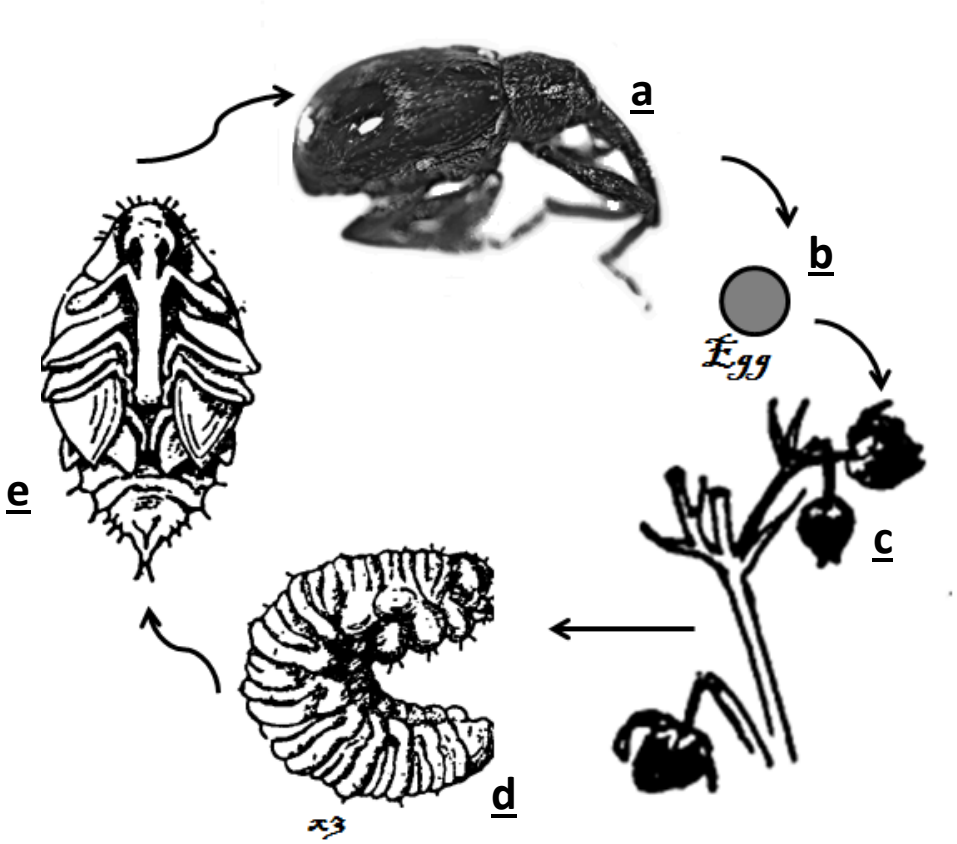
Production of strawberries in the southeastern United States is of significant economic importance. North Carolina is the fourth largest strawberry producing state behind California, Florida, and Oregon. In North Carolina, there are approximately 1600 acres producing over 20 million pounds, worth $29 million dollars annually. The demand for fresh strawberries has increased steadily, and strawberries are now the fifth most popular fruit with North American consumers (Perez and Plattner 2013).

Plasticulture strawberry production

Although they are a perennial plant, most commercial strawberries in southeastern states are grown in annual plasticulture systems. In these systems, strawberries are transplanted in fall as plugs or bare root plants into plastic mulch-covered raised beds. Beds are typically fumigated during formation to control soil-borne pathogens and insects. Harvest begins about seven months after planting and lasts for approximately four to six weeks depending on temperature and cultivar (Poling et al. 2005). Plasticulture production was first developed in the 1950's by Dr. Emery M. Emmert at the University of Kentucky in an effort to replace glass greenhouses. It was later adapted in 1958 at the University of California and combined with drip irrigation in strawberry production (Voth and Bringhurst 1990). During the late 1980s, North Carolina began plasticulture strawberry trials and quickly adopted the practice as yield, fruit size, and marketability were greatly improved from those in matted row production.

The higher marketable yield in plasticulture systems comes with higher levels of risk since the initial costs of production per acre is significantly greater than the production cost of perennial matted row production. Estimated cost per acre averages $4,000 and $13,550 for matted row and annual plasticulture, respectively (Sydorovych et al. 2006, Walh et al. 2014).The return on an acre of annual plasticulture production can be 2.5 times the amount of first and second year matted row production, and up to 5 times for any additional years of matted row plantings.

Figure 1.1. Life cycle of *Anthonomus signatus,* adult weevils (a) emerge from overwintering locations, oviposit an egg (b) inside of flower bud (c), the egg develops through three larval stages (d), and pupates (e) protected inside of the clipped flower bud. Images c,d,e (Chittenden 1908)



***Anthonomus signatus* (Curculionidae:Coleoptera) in annual plasticulture strawberries (*Fragaria* × *ananassa*)**

Abstract

Anthonomus signatus, the strawberry bud weevil, damages plants in early spring when female egg laying begins. Female *A. signatus* deposit a single egg inside an unopened flower bud and then girdle or "clip" the buds from the plant at the pedicel. Each female is capable of laying up to 75 eggs in their life span, so injury levels may increase rapidly over a short period of time. For accurate management decisions it is important to understand the best method and time for monitoring to occur. Previous research has determined monitoring plants for injury is the best method for scouting, and that degree days are a good indicator for when to begin monitoring. Ten strawberry farms in 2014 and nine in 2015 across North Carolina and Virginia were monitored weekly for *A. signatus* presence and injury. Detectable populations were identified at two-thirds of the locations during at least one year of monitoring. To determine an optimal scouting time, a degree day recommendation with a base temperature 0° C and a biofix date of 1 January is suggested for North Carolina and Virginia strawberry growers.

Keywords: *Anothomus signatus*, scouting, degree days, strawberry

Introduction

The Strawberry Bud Weevil (SBW), *Anthonomus signatus* Say, is considered a key pest in cultivated strawberry production. In early spring when daytime temperatures reach 16° C with some consistency, adults emerge from overwintering locations and move into host crops (Schaefers 1978). After a short period of time spent feeding on pollen and mating,

female *A. signatus* begin to chew through unopened flower buds to deposit eggs, on per bud. Once the egg is placed inside the unopened bud near the anthers the adult female girdle or "clip" the buds at the pedicel and causes the bud to hang by a small piece of plant tissue or drop off the plant (Headlee 1916). Inside the dried unopened bud, the egg hatches, and the larva feeds on immature pollen, develops through three instars and then pupates protected inside the dried unopened bud (Clarke and Howitt 1975, Mailloux and Bostanian 1993). If the buds have opened, they are no longer a suitable location for oviposition and escape injury (Headlee 1916, English-Loeb et al. 1999).

The existing economic threshold for *A. signatus* was established for matted row plantings of strawberries, and under the assumption that one clipped bud was equal to the loss of one average sized berry. This has led to a very low economic threshold of two clipped buds per row meter. Previous research conducted in matted row plantings of strawberries have suggested that the economic threshold be raised dependent on a cultivars ability to compensate for damage(English-Loeb et al. 1999, Pritts et al. 1999).

Efforts to determine the most efficient method to detect and quantify *A. signatus* population and damage potential have concluded that visual inspection provides the best method of detection in both strawberries and raspberries (Bostanian et al. 1999, Howard 2007). Although pheromone baited traps containing blends of the four Grandlure pheromones (I, II, III, IV) with 1,4-dimethoxybenzene , a plant volatile from the strawberry plant have been developed for the closely related *Anthonomus rubi*, a pest in European strawberry production (Innocenzi et al. 2001), no baited traps have been developed for *A.*

signatus, and baits for congeneric species are not effective (McPhie, personal observation). Large numbers of *A. signatus* can be present in a single location over a very short period of time, making timely detection methods and economic thresholds a necessary management tool.

Many growers use predetermined calendar dates to manage pests, such as *A. signatus*. This method is unreliable due to fluctuations of weather conditions between years and locations (Herms 2004). Identifying areas of high *A. signatus* activity at both the field and regional level and emergence timing of overwintering adults will help to make more accurate management decisions. The use of accumulated degree days is commonly used to predict timing of crop plant stages and insect development events (Pruess 1983). Degree day models provide a more accurate determination for timed agricultural practices and have been recommended for use integrated pest management (IPM) programs (Ascerno 1991).

A degrees day model for activity of *A. signatus* would benefit strawberry growers in the region to help determine when to begin monitoring based off of temperature instead of the use of calendar days. This would help in management decisions and reduce prophylactic treatments that are often mistimed and require additional treatments later in the season. Understanding the complete biology and life cycle of *A. signatus* would help to better control the summer generation. The timing of the summers generations return to overwintering habitat would help to target non-crop locations.

We found that populations of *A. signatus* in the southeastern plasticulture system have the ability to be highly mobile and whole field infestations could occur over one season

and that limited distance of travel in to the host crop field was not detected at the range of the monitored area. Different from perennial matted row strawberry plantings where populations can overwinter in host crop and a single season increase in distance of dispersal into the field can be detected, annual plasticulture fields are fumigated prior to planting and no populations are able to overwinter in the host crop.

The objectives of this study were to determine where and when *A. signatus* is active in North Carolina annual strawberry fields, particularly when they migrate into fields from overwintering locations and when they return. In addition, we wanted to determine if accumulated degree day were a good predictor and monitoring tool.

Methods and Materials

We monitored for *A. signatus* populations and associated damage at ten commercial you-pick strawberry farms in 2014 and nine farms in 2015 (Table 1.1). Observations began during the second week of March in both years, just before flowering, and continued through the start of harvest. Four transects were established into the field from a potential *A. signatus* overwintering habitat (i.e. wood line, fences) (Figure 1.2). Yellow sticky traps (Pherocon® AM-no bait traps, Trécé, Inc. Adair, OK) were placed every 20 meters down the row, and transects were established every sixth row, approximately ten meters apart. Each location was visited weekly during which traps were checked and the four plants surrounding each trap were observed for clipped buds and total undamaged buds. After strawberry plants were

removed from fields, locations where beetles were observed were visited weekly from 18 June through 22 Aug, 2014 and 02 July through 31 July 2015. Each week four samples of leaf litter and soil were collected per location from the edge of wood lines adjacent to locations of transects monitored during the season in 3.79L sealable plastic bags. The samples were processed in a Berlese funnel with 40 watt lamp and wire mesh with 4mm² openings for five days. Samples were captured in 100 ml of 70% EtOH and *A. signatus* specimens were identified with an Olympus SZX10 stereoscope (Olympus Corporation, Center Valley, PA).

Degree days were calculated using the rectangle method, where the mean daily temperature is calculated and the minimum developmental threshold or base temperature is subtracted (Higley and Haskell 2001). Degree days were determined using base temperatures of 0, 4, 8, 10° C, biofix dates of 1 January, 1 February, 1 March and no upper developmental limit. Total degree days were calculated for each location within a year beginning at the selected biofix and ending when *A. signatus* injury was first observed. Degree days were also calculated from 1 January through the date when *A. signatus* were first observed in Berlese trap samples for a given location.

Weather data obtained from North Carolina Climate Retrieval and Observations Network of the South East Database (CRONOS Database, climate.ncsu.edu/cronos). Weather stations closest to the monitoring location were selected through CRONOS, and distance from locations monitored for *A. signatus* ranged from 5 to 22km (Table 1.2).

Data Analysis. Field monitoring data for the four weeks of peak *A. signatus* activity from each year were analyzed using SAS (v.9.4; Cary NC) Analysis of Variance (ANOVA) in Proc Mixed to test whether the proportion of total buds clipped per plant were differed based on distance into the field, transect, and between location. The proportion of clipped buds was the dependent variable, and the independent variables were distance down the row from the field edge and distance of the row from the field edge. The week of the year was a repeated measure. The variable of year nested in site was included in the model as a random effect, as all locations were not monitored for both years. Variance components (VC) covariance structure for repeated effects was the best fit for the model. Total degree days accumulated for locations using each biofix and lower development threshold were compared via Proc Mixed. Accumulated degree days were compared between years, and location was included in models as a random variable because not all locations had detectable *A. signatus* populations during both years. Best fit base temperature and biofix date were determined by selecting the analysis results with the highest R^2 value and lowest coefficient of variance (CV) value.

Results

In 2014, seven of the ten locations in North Carolina and the location monitored in Virginia were positive for injury by *A. signatus*, and during the 2015 season five of the eight locations in North Carolina and the location Virginia were positive for injury. Trapping with yellow sticky traps was not as effective as physical examination of plants for injury in

determination of timing of presence and abundance of *A.signatus* as traps did not consistently capture beetles before damage was observed (Table 1.3). Within one week of first detection of damage (through physical examination of plants) most locations monitored that had an observable *A. signatus* population that surpassed the existing economic threshold of two clipped buds per meter at multiple locations within the field. Trap captures were also poor predictors of the intensity of *A.signatus* damage (R^2= 0.0074, p= 0.8900).

The observed number of clipped buds per plant was highly variable by week and location. Although no statistical analysis were performed, differences between locations and year were observed in mean proportion of buds clipped per plant (Figure 1.3) and mean clipped bud counts ranged from zero to 10.5 clipped buds per plant for one location in a single week (Figure 1.4). Damage rates did not differ as sampling points within transects were located further into the field from the edge (F=0.69, df= 4, 704, p=0.5993) (Table 1.4) nor across transects (F= 0.37, df= 3, 704, p=0.777) (Table 1.5).

For 2014 the first injury was recorded in monitored fields on 9 April and in 2015 first injury was recorded 2 April. Movement of the summer generation into overwintering habitat was detected in leaf litter samples at three of the four monitored locations for both 2014 and 2015. In 2014 samples from overwintering locations were first recorded on 7 August and in 2015 on 16 July.

The best fit for biofix date to start degree day calculations was 1 January with a base temperature of 0°C (R^2=0.887, CV=6.812). The lower 95% confidence interval for the selected model of the two years of monitoring was 509.47 accumulated degree days Celsius

(Table 1.6), which is a conservative estimate of the injury occurrence. Degree days were also calculated for first incident of capture in overwinter habitat after the end of the growing season with a mean of 3235.1 degree days Celsius (Table 7).

Discussion

Across the North Carolina and Virginia *A. signatus* populations were best detected through direct monitoring of the plants for injury. The indirect monitoring method using yellow sticky traps were not a good predictor of the timing of migration into the field (Table 1.3), and were not correlated to the amount of clipped buds observed.

We found that populations of *A. signatus* in the southeastern plasticulture system have the ability to be highly mobile and whole field infestations could occur over one season and that limited distance of travel in to the host crop field was not detected at the range of the monitored area. Different from perennial matted row strawberry plantings where populations can overwinter in host crop and a single season increase in distance of dispersal into the field can be detected, annual plasticulture fields are fumigated prior to planting and no populations are able to overwinter in the host crop.

At all location with populations of *A. signatus* showed activity at a minimum of 30 meters (Table 1.5) from the edge of the field and significantly further distances from overwintering habitat detected through Berlese funnel sampling of soil and leaf litter where the summer generation had returned. At moderately and highly infested field locations the

entire monitored sections of the field showed the presence of *A. signatus* after three weeks of first detection. A field wide monitoring trial would better determine if there is an upper limit of the distance that *A. signatus* can travel in the course of one season.

At most locations monitored with detectable populations of *A. signatus* the existing economic threshold of two clipped buds per meter was surpassed within one week of first detection at multiple locations within the field. However, an elevated economic threshold dependent on cultivar caused fewer locations to exceed levels of clipped buds that exceeded damage beyond proven tolerance (McPhie, personal observation) (Table 1.6). Timing from first detection to the elevated threshold was approximately three weeks. The additional two weeks from first detection allows growers to make better management decisions with more time to assess the activity while lowering the level of urgency of management. It is important to continue to assess new cultivars as well as the cultivars that are commonly grown in the plasticulture system in order to determine the economic threshold for *A. signatus*. Determination of each cultivars economic threshold will allow growers to accurately manage fields through cultivar selection and proper timing of necessary treatments.

The degree day model for the recommended time to begin scouting for the presence of *A. signatus* remains to be validated. Additionally, the time lapse between the month between strawberry plants being removed and the return of *A. signatus* to a suitable overwintering location, as recorded through leaf and soil samples, raises questions concerning where the summer generation goes. Identifying post strawberry season hosts that

are used as a food source for the summer generation could provide options for late season trap crops.

Tables and Figures

Table 1.1. Monitored locations during the two year survey with geographical coordinates, an (*) after the year identifies locations with positive identification of *A.signatus.*

Location (county)	Longitude	Latitude	Year Monitored
Durham	N35.991904	W-78.992194	2014(*) , 2015(*)
Forsyth I	N36.214933	W-80.082460	2014(*) , 2015(*)
Forsyth II	N36.144059	W-80.048551	2014 (*) , 2015
Franklin	N35.945038	W-78.221413	2014
Granville	N36.152868	W-79.734304	2015(*)
Lee	N35.403810	W-79.182585	2014(*) , 2015(*)
Moore	N35.291454	W-79.658966	2014
Nash	N35.850085	W-77.903118	2014
Orange	N36.097339	W-79.179024	2014(*) , 2015(*)
Pender	N34.403068	W-77.916251	2015
Wake	N35.918818	W-78.765697	2014(*) , 2015
Virginia Beach	N36.672670	W-76.026160	2014(*) , 2015(*)

Figure 1.2. Example of transects at you-pick locations for monitoring damage and yellow sticky traps during the 2014 and 2015 preharvest seasons.

Table 1.2. Weather station identification, and coordinates information from North Carolina Climate Retrieval and Observations Network of the South East Database (CRONOS Database, climate.ncsu.edu/cronos), and distance of weather station location from location monitored for *A. signatus* activity.

Location	Weather station ID	Longitude	Latitude	Distance(km)
Durham	DURH	N36.02896	W-78.85851	12.71
Forsyth I	KINT	N36.1337222	W-80.222	15.44
Forsyth II	KINT	N36.1337222	W-80.222	15.62
Granville	NCAT	N36.06733	W-79.73447	9.51
Lee	KTTA	N35.5837394	W-79.1007656	21.33
Orange	311429	N35.95333	W-79.23056	16.67
Wake	KRDU	N35.8776389	W-78.7874722	4.98
Virginia Beach	440385	N36.66194	W-75.91139	10.31

Table 1.3. Dates *A. signatus* first observed in monitoring traps or *A. signatus* damage observed on plants. Trap captures inconsistently predated damage. Locations at which *A. signatus* not detected not shown

Location	**Year**	**Week of first trap capture**	**Week of first damage**
Durham	2014	14	15
	2015	14	15
Forsyth I	2014	14	15
	2015	15	15
Forsyth II	2014	15	16
Granville	2015	15	14
Lee	2014	15	15
	2015	16	14
Orange	2014	14	14
	2015	14	14
Wake	2014	15	15
Virginia Beach	2014	15	16
	2015	15	15

Figure 1.3. Mean proportion clipped by location for 2015 for locations that exceed the existing threshold. Different letter indicates significant difference at α=0.05 (F=20.52, *df*=4,388 , *p*<0.0001)

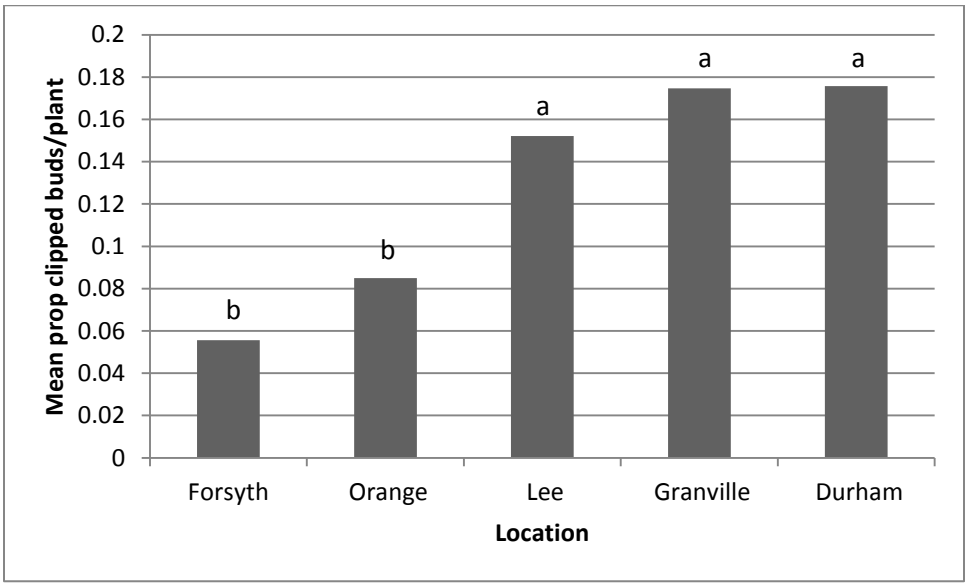
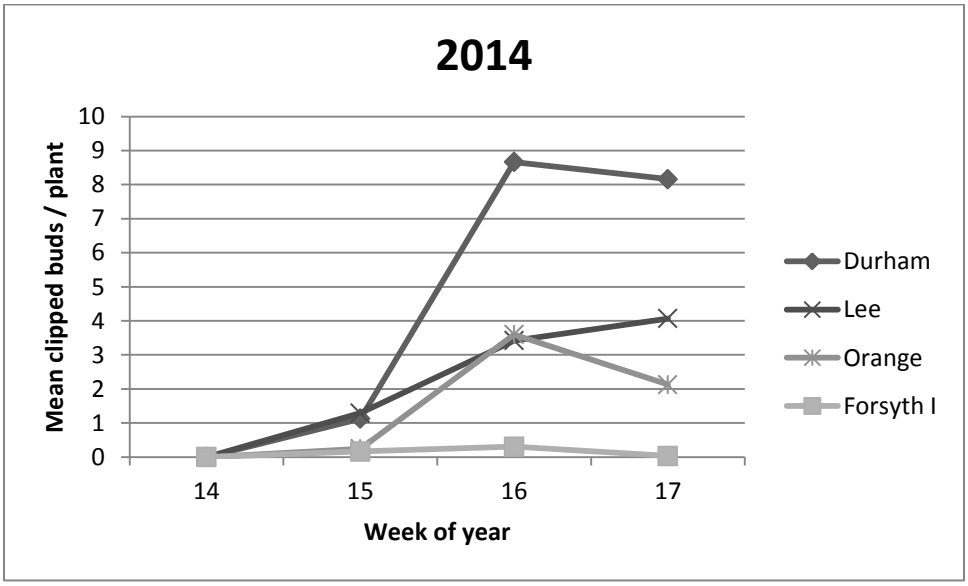


Figure 1.4. Mean clipped buds per plant for locations where injury exceeded the existing threshold of two clipped buds per row meter. Results by week for the 2014 and 2015 seasons.



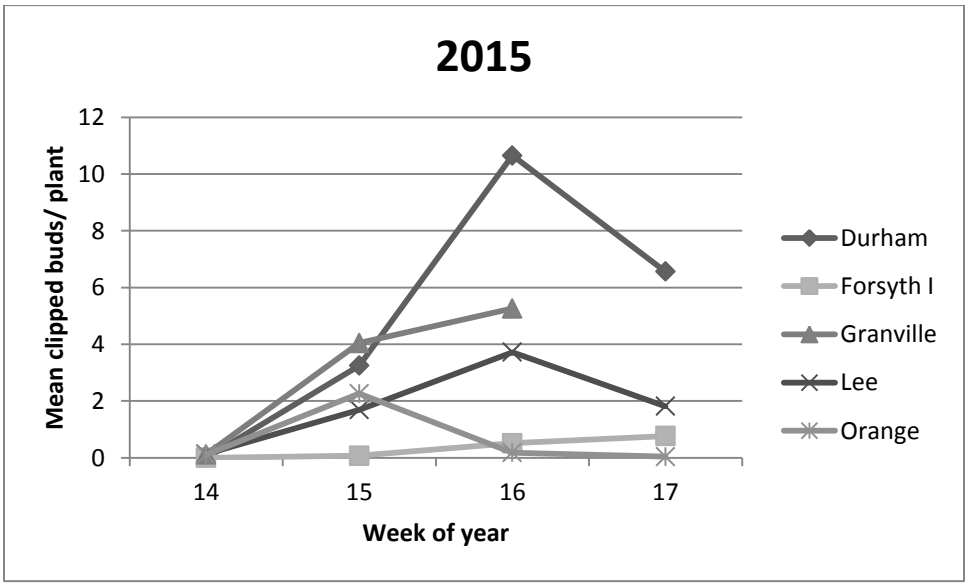


Table 1.4. Mean±SE proportion of clipped buds per plant at different distances away from the field edge during the four weeks of peak *A. signatus* activity. Data presented are for two year North Carolina locations with detectable populations that exceeded existing clipped bud threshold. No significant difference (F=0.69, *df*= 4, 704, *p*=0.5993) for position down the row.

		Distance down row (position) in meters				
Location	Year	0	20	40	60	80
Durham	2014	0.123±0.029	0.160±0.038	0.145±0.038	0.112±0.027	0.100±0.027
	2015	0.282±0.055	0.245±0.051	0.203±0.051	0.211±0.046	0.177±0.038
Forsyth	2014	0.074±0.029	0.075±0.023	0.042±0.015	0.088±0.028	0.032±0.013
	2015	0.053±0.024	0.067±0.037	0.064±0.029	0.026±0.017	0.035±0.021
Granville	2015	0.178±0.045	0.209±0.057	0.202±0.061	0.167±0.054	0.122±0.039
Lee	2014	0.116±0.037	0.136±0.029	0.124±0.029	0.138±0.037	0.166±0.037
	2015	0.173±0.055	0.112±0.038	0.202±0.066	0.133±0.040	0.221±0.059
Orange	2014	0.006±0.003	0.082±0.013	0.101±0.017	0.081±0.015	0.053±0.009
	2015	0.017±0.007	0.044±0.015	0.027±0.013	0.042±0.016	0.031±0.015

Table 1.5. Mean±SE proportion of clipped buds per plant for transects going into the field from the border during the four weeks of peak *A. signatus* activity. Data presented are two year North Carolina locations with detectable populations that exceeded existing clipped bud threshold. No significant difference (F=0.37, *df*=3, 704 *p*=0.7771).

		Distance across rows (transect) in meters			
Location	Year	0	10	20	30
Durham	2014	0.142±0.031	0.150±0.030	0.105±0.025	0.116±0.045
	2015	0.247±0.045	0.227±0.043	0.236±0.049	0.182±0.036
Forsyth	2014	0.068±0.025	0.053±0.016	0.062±0.017	0.065±0.024
	2015	0.056±0.029	0.079±0031	0.049±0.019	0.012±0.006
Granville	2015	0.140±0.035	0.173±0.042	0.191±0.047	0.194±0.059
Lee	2014	0.127±0.028	0.145±0.029	0.163±0.035	0.109±0.028
	2015	0.105±0.044	0.186±0.056	0.151±0.036	0.232±0.047
Orange	2014	0.088±0.013	0.081±0.010	0.054±0.009	0.037±0.005
	2015	0.031±0.013	0.040±0.015	0.036±0.010	0.022±0.010

Table 1.6. Two year accumulated degree days for 10, 8, 4, 0°C at biofix dates of 1 January, 1 February, and 1 March. All R^2 values are greater than 0.85, indicating good model fit. Therefore, the lowest value of coefficient of variance selected the model of a base temperature of 0°C with a biofix date of 1 January.

Biofix date	*Base Degree C*	*Year*	*Mean*	*R^2*	*Co. Var.*	*95 CI lower*	*95 CI upper*	*sig diff (years)*
1-Jan	0	2014	628.8	0.887	6.812	548.56	705.82	
1-Jan	0	2015	579.12			509.47	666.73	
1-Jan	4	2014	333.27	0.865	8.941	278.71	386.91	
1-Jan	4	2015	317.69			270.19	378.39	
1-Jan	8	2014	150.44	0.854	13.31	114.42	186.98	
1-Jan	8	2015	148.55			116.82	189.37	
1-Jan	10	2014	92.19	0.879	15.263	62.825	117.85	
1-Jan	10	2015	89.972			68.3714	123.4	
1-Feb	0	2014	518.83	0.908	7.554	447.12	588.95	*
1-Feb	0	2015	448.62			385.15	526.98	
1-Feb	4	2014	291.19	0.887	9.47	238.38	343.28	
1-Feb	4	2015	270			223.63	328.53	
1-Mar	0	2014	352.77	0.857	9.95	288.2	416.01	
1-Mar	0	2015	374.66			315.84	443.65	
1-Mar	4	2014	242.94	0.869	11.96	166.78	265.04	
1-Mar	4	2015	215.99			198.05	296.31	

Table 1.7. Accumulated degree days for first detection in post season monitoring of overwinter habitat with base degree of 10, 8, 4, 0°C and a biofix date of 1 January. The lowest coefficient of variance and the greatest R^2 value were for a base temperature of 0°C.

Base Degree C	Year	Mean	R^2	Co. Var	95 CI lower	95 CI upper
0	2014	3476.2	0.881	6.34	2733.66	4218.71
0	2015	2994			2251.43	3736.49
4	2014	2661.06	0.861	7.01	2065.06	3257.05
4	2015	2294.34			1698.35	2890.34
8	2014	1959.7	0.846	7.74	1487.14	2432.33
8	2015	1684.5			1211.92	2157.11
10	2014	1644.2	0.843	8.14	1227.41	2061.18
10	2015	1408.3			991.28	1825.32

Effect of Simulated *Anthonomus signatus* Say (Curculionidae: Coleoptera) Injury on Strawberries (*Fragaria* × *ananassa*) Grown in Southeastern Plasticulture Production

Abstract

Female strawberry bud weevils (*Anthonomus signatus*) oviposit in developing flower buds of strawberries (*Fragaria* spp.), caneberries (*Rubus* spp.), and red bud (*Cercis canadensis*). After laying a single egg, weevils will girdle or "clip" the buds at the pedicel, killing the bud and preventing fruit development. This injury is of concern to commercial strawberry growers, who typically assume one clipped bud is the loss of one average sized fruit causing the economic threshold to be set extremely low. There is evidence of compensation in some cultivars of strawberries, but research has previously only been conducted in perennial strawberry production. The majority of strawberries are grown in annual plasticulture systems in the southeastern United States. We assessed the ability of five strawberry cultivars commonly grown in annual plasticulture to compensate for *A. signatus* injury by removing buds at different growth stages. There was no effect of bud removal on total yield in any of the cultivars tested. Harvest timing was affected by simulated *A. signatus* damage in some cultivars, which may an important consideration for direct market strawberry growers.

Key words: *Anthonomus signatus*, strawberry, host plant resistance, HPR, compensation

Introduction

The strawberry bud weevil (SBW), *Anthonomus signatus* Say, is considered a key pest of strawberries in eastern North America. In early spring, overwintered adults emerge and migrate into host crops when temperatures reach 16°C consistently (Schaefers 1978).

Anthonmus signatus has a wide taxonomic range of host plants and has been documented feeding on caneberries (*Rubus* spp.*),* red bud (*Cercis* spp.*),* and strawberries (*Fragaria* × *ananassa*) (Kovach et al. 1999). Damage to the host plant is caused by the female chewing into an unopened flower bud and depositing an egg, then girdling the bud from the plant at the pedicel (Headlee 1916). The bud drops from the plant, preventing the flower from developing and providing a protected environment for the immature to develop. Inside the unopened flower bud, the egg hatches, and the larva feeds on immature pollen in order to develop through three instars and then pupates, still protected inside the dried unopened bud (Clarke and Howitt 1975; Mailloux and Bostanian 1993). Once buds have opened they are no longer a suitable site for oviposition (English-Loeb, Pritts et al. 1999). The assumption that the loss of one bud is equal the loss of an average sized fruit has led to the development of very low *A. signatus* economic threshold of two clipped buds per meter (Schaefers 1978).

Understanding the mechanisms of host plant resistance (HPR) has helped breeding programs in many crop systems to breed for associated traits. Resistant plants can affect insect abundance (antibiosis), insect behavior (antixenosis), or the plant response to insect feeding (tolerance). Tolerant plants can withstand or recover from insect injury and perform similarly to non-resistant plants (Painter 1958). Compensation is one mechanism of tolerance, where by plants can reallocate biomass from damaged portions of the plant. Understanding compensation ability between cultivars is important for determination economic threshold levels (Stern 1973). In cereal crops, some cultivars overcompensate and increase yield occurs in response to attack by the Russian wheat aphid, *Diuraphis noxia*

(Castro, Ramos et al. 2001). In high producing cotton cultivars, heavy early season fruit removal, up to 100 percent, caused no significant loss of yield, however timing of harvest was delayed (Wilson et al. 2003). In response to simulated injury of *A. signatus,* compensation was observed in raspberries (*Rubus idaeus*), but cultivars responded differently based on the amount of damage and order of bud damaged on inflorescence (Howard 2007).

In matted row strawberry systems, compensation for bud loss due to spring frosts and to *A. signatus* oviposition has been observed to some extent (Khanizadeh et al. 1992; English-Loeb et al. 1999; Pritts et al. 1999; Handley et al. 2002). Studies have also assessed strawberry plants ability to compensate from damage caused by the closely related *Anthonomus rubi* (Strawberry blossom weevil) in perennial European strawberry crops (Terrettaz, Antonin et al. 1995; Popov 1996; Cross and Burgess 1998; Faby, Svensson et al. 2003; Aasen and Trandem 2006). Some strawberry cultivars grown in perennial matted row plantings have compensated for damage caused by *A. signatus* at varying levels, and therefore have higher economic thresholds of up to 20 damaged buds per meter (Cross and Burgess 1998; English-Loeb et al. 1999; Pritts et al. 1999). The method of compensation varies, with some cultivars producing a greater number of higher ordered flower buds and other cultivars compensating by an increase in weight of existing fruits (Pritts et al. 1999). The majority of strawberries grown in the United States are grown in annual system on raised beds covered with plastic mulch. This system produces higher yields than perennial systems, and the cultivars grown in the annual system differ from those grown as perennials. To date there have been few studies of the effects of *A. signatus* in annual plasticulture growing systems and the cultivars grown as an annual crop.

The objective of our study was to investigate the abilities of five cultivars of strawberries, grown under the annual plasticulture system, to compensate for bud removal simulating *A. signatus* injury occurring at different phenological stages.

Materials and Methods

We conducted simulated bud removal experiments over the course of two years at the Central Crops Research Station in, Clayton, North Carolina (N 35.6686, W -78.5060). The first year, we measured the effects of bud removal on yield in a single cultivar (*Fragaria ananassa* cv. Camarosa), and in the second year, we expanded our comparison to include multiple cultivars. In the first year of our study, strawberries were planted 15 October 2013. Camarosa was selected for its extended fruiting season, durability of berries, and growing popularity in North Carolina wholesale and you-pick operations. Strawberry plugs were obtained from Cottle Strawberry Nursery (Fasion, NC). Plants were planted in four double-row beds covered in black plastic mulch on 1.53 meter centers with a plant spacing of 0.36 meters. The field was fumigated with Pic Clor 60 at a rate of 134.425 kg/hectare prior to bed formation and mulch application. Plants were grown under standard nutrient and pathogen management strategies (Louws et al. 2015), but no insecticide applications were made. Treatments were randomly assigned to 20 plant plots (0.00036 hectares) within a row, and each row included one replication of each treatment. Treatments were: no bud removal (untreated control), primary bud removal, secondary bud removal, and tertiary or higher bud removal (Figure 1). Buds were removed weekly from 25 March to 29 April 2014 which

corresponded to the period of *A. signatus* injury was observed at field monitoring sites throughout North Carolina. We recorded the number of buds removed from each plot during each week. Plots were harvested twice a week beginning on 23 April 2014, and total berry weight in each plot was recorded. Plants were removed from the plots in mid-June.

We expanded our comparisons to five cultivars during the second field season: Albion, Benicia, Camarosa, Chandler, and Sweet Charlie. Strawberry plugs were obtained from Wrenn's Farm (Zebulon, NC) and were planted 7 October 2014. Plants were planted in four double-row beds covered in black plastic, which had been previously fumigated with Pic Clor 60 at a rate of 134.425 kg/hectare, on 1.53 meter centers in black plastic with a plant spacing of 0.36 meters. Plantings were maintained using the same management strategies as in 2014. Blocks of 80 plants per cultivar were planted in a randomized arrangement within a row, replicated in each row. The main blocks were then separated into four sub plots of 17 plants and randomly assigned one of the four treatments: (Figure 2). Buds were removed weekly by hand as previously described from 30 March 2015 to 28 April 2015 again corresponding to *A. signatus* activity at grower locations throughout North Carolina, and the number of buds removed per plot during each week was recorded. The center 10 plants of each plot were marked and harvested for yield assessment. All plots were harvested once or twice a week, depending on ripe fruit availability, and total yield was recorded. To examine potential compensation mechanism, single berry weights were recorded for a random sample of ten berries per treatment per cultivar from the southern two rows. Harvest started on 15 April 2015 concluded on 15 June 2015.

Data analysis. All data were analyzed via Proc Mixed (SAS v. 9.4, Cary NC) and assessed for normality via the Shapiro-Wilkes test and homogeneity of variance by Levene's test. The number of buds removed within treatments was analyzed via mixed model analysis of variance (ANOVA). Buds removed was the dependent variable, treatment and cultivar were independent variables, and replicate was considered a random effect. Yield was analyzed via mixed model ANOVA with season-long total yield as the dependent variable. Independent, fixed variables were cultivar, and treatment. Replicate was considered random variables. Per plant weekly yield totals were also calculated within each year and compared using a repeated measure mixed model ANOVA with means separated by least significant difference with a Tukey-Kramer adjustment. Treatment, and cultivar were independent, replicate was a random effect, and week was repeated. An unstructured covariance (UN) for repeated effects was the best fit for the model. Weekly average single berry weights were also calculated; weeks where a treatment had less than 20 fruit were dropped from the analysis. Average berry weight data were compared across cultivar and treatment (fixed effects), with replicate as a random effect and week as a repeated measure. Variance components (VC) covariance structure for repeated effects was the best fit for the model.

Results

The number of buds removed from simulated clipping varied between treatments across all cultivars, but did not differ between years for Camarosa ($F=0.04$, $df=1, 21$, $p=0.8386$; Table 1). Regardless of how many buds were removed or the timing of removal, total yield did not

differ significantly between treatments for Camarosa in 2014 (F=0.04, *df*= 1, 21, *p*=0.8386; Table 2), and while total yield differed between varieties in 2015 (F=21.73, *df*=4, 48, *p*<0.001; Figure 3), it did not differ between treatments within any variety (2015:F= 1.57, *df*= 12, 48, *p*=0.1344; Table 2). In 2015, Benicia and Camarosa had higher total yields than Albion and Chandler, and total yield for Sweet Charlie and Chandler were significantly higher from Albion (Figure 3). When weekly harvest totals were compared within cultivar, bud removal significantly impacted yield for all cultivars tested but Albion (Table 3).

Although total yield was not impacted by bud removal, yield timing did differ significantly for Camarosa in both years (2014 (Camarosa): F= 4.58, *df*= 21,93, *p* < 0.0001 ; 2015 (all cultivars): F = 1.42, *df* = 75,453, *p* = 0.0170). During 2014, Camrosa yield was reduced relative to the untreated control in the second week of harvest (23-27 April 2014) for primary bud removal plots when compared to the untreated control. Yield was reduced relative to the untreated control in secondary bud removal plots during the fourth and five weeks of harvest (11-18 May 2014) (Table 3). During 2015, Camarosa had reduced yield in the third and fourth weeks of harvest (27 April – 4 May 2015) in the primary bud removal treatment compared to the control. Tertiary bud removal plots had increased yield compared to the control in the fourth week of harvest (4-8 May 2015) (Table 3).

In addition to Camarosa, bud removal for all other cultivars except Albion also significantly impacted yield timing (Table 3). Benicia experienced a significant reduction in yield in plots with primary buds removed compared to the untreated control during the second and third weeks of harvest (22-27 April 2015). During the fourth and fifth weeks of harvest (4-11 May 2015), a significant increase of available fruit was observed in plots with

tertiary bud removal compared to the untreated control. In the sixth week of harvest (14-18 May 2015) there was a significant reduction in yield of plots with secondary bud removal when compared to the untreated control (Table 3).

Sweet Charlie, an early fruiting cultivar, experienced a significant decrease in weekly yield across all treatments during at least one week of harvest when compared to the untreated control. During the second week of harvest (22-24 April 2015) Sweet Charlie had a significant decrease of fruit in plots that had primary buds removed. During the fourth and fifth weeks of harvest (4-11 May 2015) secondary bud removal decreased weekly yield totals, and the removal of the tertiary buds reduced fruit yield during the fifth week of harvest (7-11May 2015; Table 3).

Despite differences in yield overtime, weekly average berry weights did not significantly for any of the treatments within a cultivar when compared to the untreated control in 2015 ($F = 1.01$, $df = 84, 136$, $p = 0.4891$; Table 4).

Discussion

Our results suggest that no significant total yield loss occurs at damage levels up to 18 buds per plant, dependent upon variety. Despite this potential elevated injury level, damage exceeding this amount were observed at more than 30% of commercial farms monitored during 2014 and 2015 in North Carolina, with the greatest loss of buds occurred during the period of secondary bud emergence (McPhie 2016). Although there was no

difference in total yield, weekly yields did vary relative to untreated controls between treatments and across cultivars. Timing of availability of the fruit is of great importance to pick-your-own farms, where specific dates are considered to have higher levels of customer traffic. However, a number of factors, including weather, may also influence yield and customer presence on a weekly basis. Understanding the relationship between bud removal and the timing of fruit availability may allow for better timing of applications of treatments, when determined to be necessary, to avoid economic loss. The average time for a strawberry to develop from a bud to a fruit is approximately 30 days. If damage begins to be observed more than 30 days prior to target picking dates, it may be advisable to apply treatments against *A. signatus* in order to reduce possible loss.

Timing of flower bud emergence could be an additional method of protection from yield loss due to *A. signatus* in strawberries. *A. signatus* are active for a short period of time, between four to six weeks, which coincides with bud emergence and flowering of commonly grown June-bearing cultivars. It has previously been observed that primary buds are rarely injured by *A. signatus* in June-bearing cultivars (English-Loeb et al. 1999). This is likely because primary buds open prior to emergence of *A. signatus* from overwintering sites making them unsuitable hosts for oviposition.

Albion is a day neutral cultivar and has relative few buds available during the period of *A. signatus* activity, resulting in primary, secondary, and tertiary bud removal counts of 4.33, 2.63, and less than 1 per plant on average respectively. Noticeable decrease in yield would not be expected at the levels of injury. The timing of bud development of day neutral

cultivars may be out of sync with active time of *A. signatus.* Albion has been used to extend the growing season in the southeast United States, due to its late blooming period.

Chandler, the most commonly grown strawberry cultivar in North Carolina, showed increased availability of fruit over three weeks despite bud removal. Chandler was also the cultivar that demonstrated no significant yield loss, even with up to 18 buds per plant removed, the highest number removed per plant of any cultivar we observed. It is very likely that the number buds we removed are conservative estimates of the level of injury that some cultivars could compensate for.

We observed no differences in single berry weights in any cultivar, suggesting that they compensate for bud loss through the production of more berries and not the reallocation of fruit mass to remaining fruit. However, we did not distinguish what bud hierarchy produced the berries harvested.

It is important to continue testing new cultivars as they are introduced into the southeastern plasticulture system for the ability to compensate for damage caused by *A. signatus* because it is clear that cultivars do not respond to damage in a uniform fashion. It would also be beneficial to the growers to have a clearer picture of the limits of the compensational ability of cultivars; this would require more testing with higher numbers of buds removed to truly test the limit of the compensational abilities.

Tables and Figures

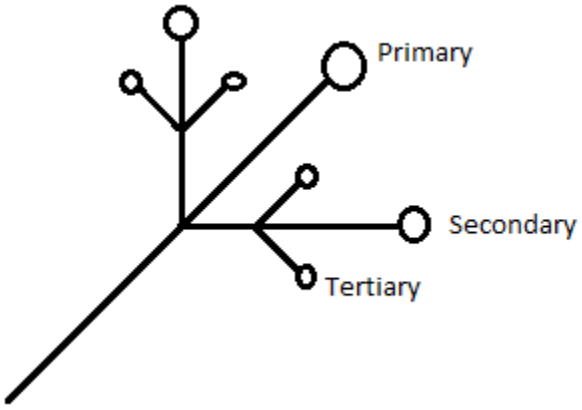


Figure 2.1. Schematic of the bud emergence pattern of a “typical” strawberry inflorescence illustrating primary, secondary and tertiary bud placement.

Figure 2.2. Plot layout of test plots from Central Crops Research Station, Clayton NC for the 2015 season.

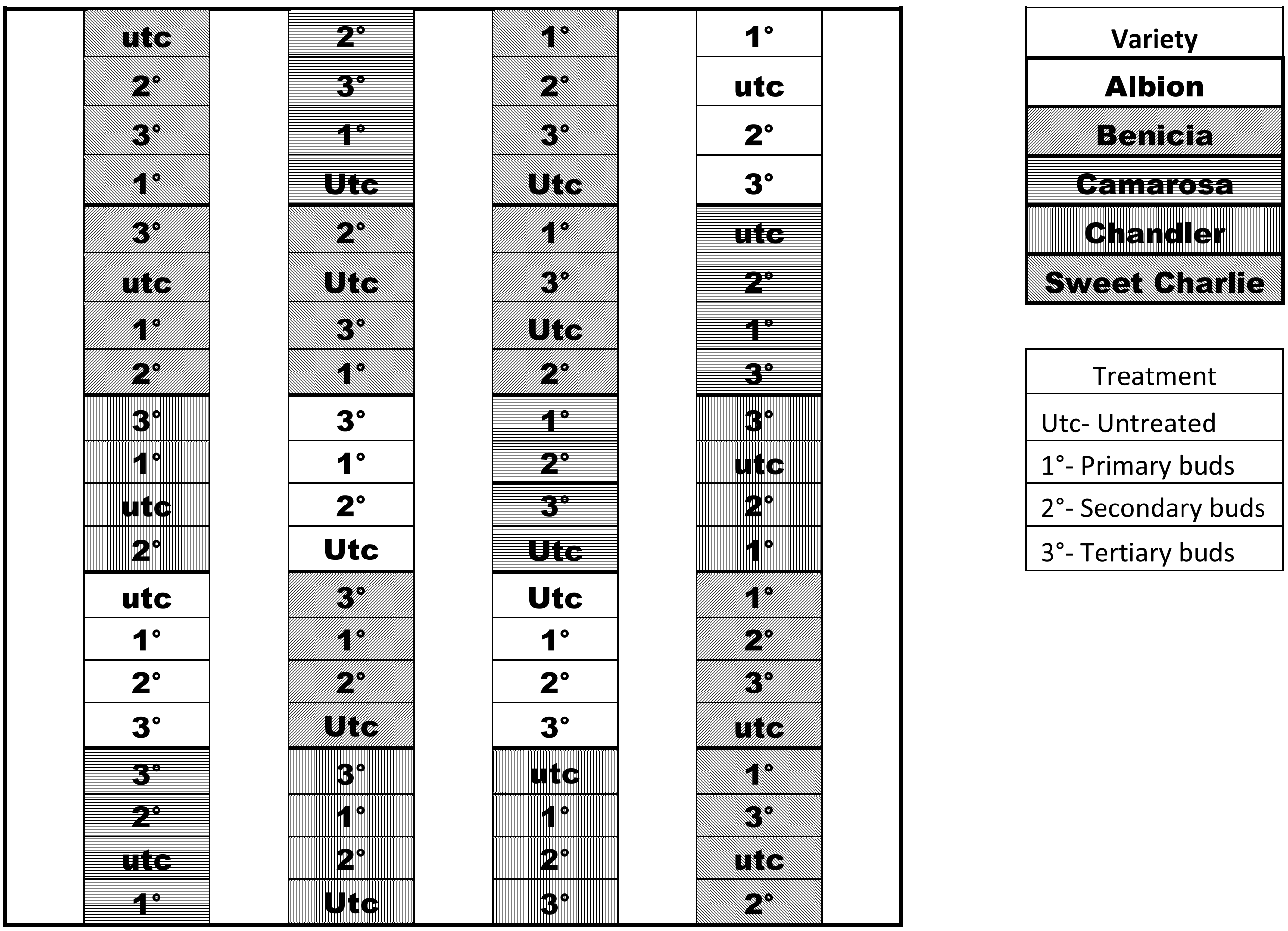


Table 2.1. Mean ± SE buds removed per plant by treatment. No significant difference was found between the two years of Camarosa data for mean clipped buds per treatment, so data for both years were combined. Values within a cultivar across treatments (across rows) followed by the same letter (outside parentheses), and values within a treatment across varieties (down columns, within parentheses) are not significantly different via mean comparison using the Tukey-Kramer adjustment ($\alpha = 0.05$).

Cultivar	UTC	Primary	Secondary	Tertiary
Albion	0.0±0.0 a (a)	4.33±0.61 b (a)	2.63±0.30 ab (a)	0.7±0.19 ab (a)
Benicia	0.0±0.0 a (a)	11.68±1.31 c (b)	8.65±0.90 bc (b)	3.83±0.53 ab (b)
Camarosa	0.0±0.0 a (a)	7.29±0.36 b (ab)	8.45±0.42 b (b)	2.76±0.43 a (ab)
Chandler	0.0±0.0 a (a)	9.53±1.37 b (b)	16.95±1.38 c (c)	9.83±1.14 b (c)
Sweet Charlie	0.0±0.0 a (a)	10.25±1.20 c (b)	13.78±1.27 c (c)	4.33±0.61 b (b)

Table 2.2. Mean yield per plant ± SE. No significant difference occurred within any cultivar across treatments. No significant difference was observed between treatments within a cultivar, via mean comparison using the Tukey-Kramer adjustment, $\alpha = 0.05$.

		Yield (kg)			
Cultivar	**Year**	**UTC**	**Primary**	**Secondary**	**Tertiary**
Albion	2015	0.29±0.03	0.21±0.03	0.24±0.01	0.28±0.05
Benicia	2015	0.48±0.04	0.41±0.04	0.43±0.02	0.57±0.05
Camarosa	2014	1.66±0.09	1.58±0.03	1.47±0.10	1.71±0.01
Camarosa	2015	0.53±0.04	0.42±0.06	0.50±0.04	0.58±0.05
Chandler	2015	0.32±0.06	0.43±0.02	0.42±0.06	0.37±0.08
Sweet Charlie	2015	0.53±0.02	0.44±0.01	0.45±0.03	0.42±0.05

Figure 2.3. Mean per plant yield of cultivars in kg ± SE during the 2015 season. Bars indicated by the same letter are not significantly different (via Tukey-Kramer adjustment, $\alpha = 0.05$). ($F = 21.73$, $df = 4, 48$, $p < 0.001$)

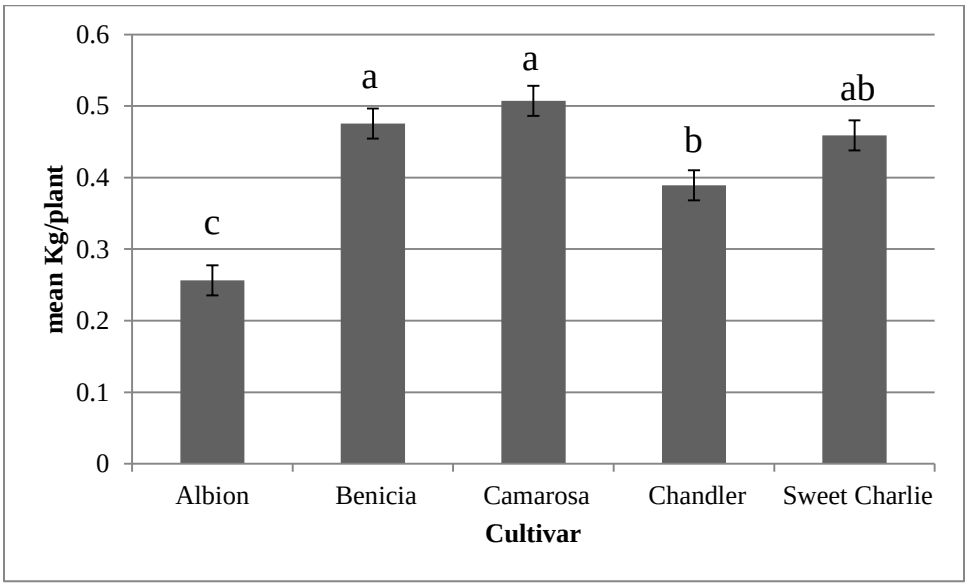


Table 2.3. Mean per plant yield (kg) ±SE by week for treatments with different letter indicates significant differences compared to the untreated control across treatments within the cultivar and week. Values for a given variety within an individual week followed by the same letter are not significantly different via Tukey-Kramer adjustment ($\alpha = 0.05$).

		Week of year							
		17	18	19	20	21	22	23	24
Albion	UTC	0.00±0.00	0.024±0.0033	0.048±0.0151	0.042±0.0007	0.032±0.0075	0.029±0.0118	0.045±0.0076	0.035±0.0079
	Primary	0.00±0.00	0.003±0.0027	0.026±0.0094	0.037±0.0108	0.030±0.0048	0.033±0.0083	0.048±0.0053	0.020±0.0024
	Secondary	0.00±0.00	0.024±0.0039	0.036±0.0071	0.029±0.0033	0.028±0.0035	0.016±0.0021	0.050±0.0078	0.032±0.0023
	Tertiary	0.00±0.00	0.027±0.0073	0.047±0.0118	0.051±0.0201	0.034±0.0115	0.019±0.0058	0.046±0.0045	0.035±0.0071
Benicia	UTC	0.038±0.0134 a	0.081±0.0140a	0.101±0.0027 a	0.129±0.0180 b	0.091±0.0176 a	0.027±0.0085 a	0.011±0.0041	0.003±0.0027 a
	Primary	0.001±0.0006 a	0.018±0.0053 b	0.078±0.0141 b	0.156±0.0299 b	0.104±0.0097 a	0.040±0.0082 a	0.014±0.0061	0.003±0.0021 a
	Secondary	0.027±0.0069 a	0.100±0.0094 a	0.113±0.0178 a	0.113±0.0156 b	0.046±0.0054 b	0.014±0.0075 a	0.017±0.0066	0.006±0.0022 a
	Tertiary	0.031±0.0075 a	0.099±0.0083 a	0.135±0.0133 a	0.179±0.0163 a	0.103±0.0205 a	0.017±0.0052 a	0.005±0.0028	0.002±0.0009 a
Chandler	UTC	0.00±0.00	0.029±0.0084 a	0.091±0.0135 a	0.093±0.0174 b	0.049±0.0153 b	0.043±0.0135 b	0.017±0.0034	0.003±0.0016 a
	Primary	0.00±0.00	0.010±0.0040 a	0.066±0.0091 a	0.133±0.0058 a	0.103±0.0141 a	0.093±0.0120 a	0.025±0.0050	0.005±0.0017 a
	Secondary	0.00±0.00	0.048±0.0049 a	0.120±0.0153 a	0.087±0.0174 b	0.052±0.0014 b	0.040±0.0042 b	0.022±0.0035	0.005±0.0019 a
	Tertiary	0.00±0.00	0.085±0.0088 a	0.226±0.0183 a	0.264±0.0243 a	0.061±0.0189 b	0.035±0.0145 b	0.029±0.0043	0.008±0.0020 a
Sweet Charlie	UTC	0.084±0.0216 a	0.039±0.0033 a	0.099±0.0116 a	0.092±0.010 a	0.037±0.0034 a	0.065±0.0060 a	0.069±0.0057	0.006±0.0020 a
	Primary	0.037±0.0046 b	0.018±0.0028 a	0.086±0.0153 a	0.073±0.0076 ab	0.047±0.0060 a	0.084±0.0071 a	0.067±0.006 a	0.008±0.0015 a
	Secondary	0.108±0.0182 a	0.028±0.0053 a	0.053±0.0071 b	0.043±0.0085 c	0.022±0.0025 a	0.065±0.0080 a	0.075±0.0040	0.013±0.0048 a
	Tertiary	0.082±0.0181 a	0.031±0.0068 a	0.073±0.0111 a	0.060±0.0137 bc	0.033±0.0078 a	0.052±0.0095 a	0.057±0.0048	0.011±0.0040 a
Camarosa (2014)	Utc	0.000±0.0052 a	0.265±0.0159 a	0.341±0.0315 a	0.311±0.0537 a	0.180±0.0491 a	0.303±0.0138 a	0.164±0.0309	0.070±0.0281 a
	Primary	0.000±0.0000 a	0.199±0.0270 b	0.306±0.0230 a	0.311±0.0177 a	0.158±0.0367 a	0.332±0.0171 a	0.190±0.0296	0.078±0.0221 a
	secondary	0.001±0.0063 a	0.292±0.0116 a	0.334±0.0198 a	0.193±0.0563 b	0.081±0.0310 b	0.280±0.0276 a	0.191±0.0363	0.087±0.0373 a
	Tertiary	0.002±0.0126 a	0.304±0.0024 a	0.348±0.0119 a	0.302±0.0316 a	0.179±0.0282 a	0.287±0.0213 a	0.182±0.0487	0.085±0.0243 a
Camarosa (2015)	Utc	0.00±0.00	0.056±0.0089 a	0.130±0.0143 b	0.137±0.0131 a	0.092±0.0074 a	0.055±0.0056 a	0.025±0.0016	0.010±0.0030 a
	Primary	0.00±0.00	0.011±0.0038 b	0.072±0.0199 c	0.110±0.0165 a	0.096±0.0075 a	0.064±0.0148 a	0.040±0.0067	0.018±0.0044 a
	secondary	0.00±0.00	0.055±0.0054 a	0.128±0.0198 b	0.132±0.0187 a	0.082±0.0155 a	0.041±0.0061 a	0.019±0.0035	0.019±0.0059 a
	Tertiary	0.00±0.00	0.068±0.0147 a	0.162±0.0281 a	0.143±0.0161 a	0.104±0.0089 a	0.050±0.0033 a	0.027±0.0046	0.012±0.0017 a

Table 2.4. Mean single berry weight (g) ± SE for all cultivars by treatment for all weeks in 2015. No significant difference (via Tukey-Kramer adjustment, $\alpha = 0.05$) in any week for any treatment within a cultivar when compared to its untreated control (UTC).

		Week of year						
		18	19	20	21	22	23	24
Albion	UTC	25.59±9.58	22.49±1.81	22.93±0.72	23.90±3.40	18.56±0.69	18.56±2.23	18.62±1.97
	Primary	19.54±6.91	18.76±6.27	19.52±1.96	16.20±1.08	23.40±6.20	22.17±1.12	21.28±0.99
	Secondary	26.51±1.96	21.95±2.00	27.67±0.09	20.62±1.53	28.25±0.60	17.11±2.36	17.74±1.35
	Tertiary	26.60±4.39	22.00±0.87	29.83±1.61	25.36±3.18	19.89±0.25	10.40±2.71	19.71±2.90
Benicia	UTC	32.85±4.26	26.69±0.81	30.55±1.65	24.65±0.93	18.84±3.44	14.06±1.06	14.32±1.01
	Primary	27.36±3.77	28.00±2.24	30.15±5.11	20.75±3.08	17.17±2.28	12.75±3.89	16.24±0.59
	Secondary	34.35±1.48	28.86±1.27	28.90±1.22	20.99±1.20	15.80±1.35	13.82±1.64	11.94±3.34
	Tertiary	30.82±0.63	30.97±0.05	29.63±2.03	19.68±2.44	15.97±3.84	12.57±1.53	7.62±1.38
Camarosa	UTC	30.11±4.50	22.32±4.61	25.52±0.60	23.62±1.11	21.75±1.90	17.14±0.54	16.60±4.76
	Primary	34.00±1.83	25.75±0.08	29.07±0.85	24.64±2.09	27.27±1.97	22.07±4.29	20.89±2.52
	Secondary	32.94±6.62	21.69±2.09	30.08±0.44	21.48±0.99	22.64±2.60	17.02±0.28	18.66±0.47
	Tertiary	28.59±2.36	26.85±4.85	27.57±2.38	19.18±1.45	17.04±3.16	20.18±1.13	16.92±1.02
Chandler	UTC	24.55±2.30	17.17±1.67	15.32±3.16	10.27±3.31	12.26±3.37	11.06±0.57	10.12±4.53
	Primary	22.31±0.78	18.06±3.45	16.44±1.82	13.25±1.86	16.02±1.58	14.09±3.06	12.81±3.62
	Secondary	24.89±3.48	20.17±4.87	19.90±0.29	14.29±1.77	17.20±3.37	12.23±2.12	10.48±2.16
	Tertiary	18.73±4.12	17.88±1.15	14.37±0.43	16.20±1.06	14.26±1.86	11.87±4.75	9.59±4.09
Sweet Charlie	UTC	18.27±1.07	12.07±0.49	13.50±0.53	12.74±2.09	11.10±2.13	16.19±1.67	15.62±0.26
	Primary	13.57±0.79	15.35±1.52	15.88±0.70	12.43±0.88	13.04±3.18	17.70±1.95	15.69±2.00
	Secondary	21.44±1.36	11.12±0.28	12.16±3.45	12.65±2.75	10.01±0.91	14.27±0.75	16.23±2.60
	Tertiary	17.00±1.93	11.69±2.88	15.31±2.97	12.06±0.07	9.89±0.01	14.38±1.84	14.72±3.67

Effects of Microbial, Organically Acceptable, and Reduced Risk Insecticides on *Anthonomus signatus* (Curculionidae: Coleoptera) in Strawberries (*Fragaria* × *ananassa*)

Abstract

Anthonomus signatus, the strawberry bud weevil, is active in early spring coinciding with the bloom period of host plants and when managed and unmanaged pollinators are active. Female *A. signatus* cause injury to the host plant during egg laying. Female *A. signatus* deposit a single egg inside an unopened flower bud and then girdle or "clip" the buds at the pedicel. Each female is capable of laying up to 75 eggs, so damage levels may increase rapidly over a short period of time. Past efforts to control *A. signatus* populations have relied on the use of broad spectrum insecticides. Locations where damage varies from year to year are typically treated at the first signs of damage. In fields with a history of damage, precautionary treatments are often applied prophylactically without confirmation of damage. Because *A. signatus* damage occurs during bloom, there is concern about the potential harm to pollinators caused by these treatments. In order to identify materials more compatible for use during bloom, the efficacy of reduced risk materials against *A. signatus* were tested in a semi-field bioassay study over two years. None of the reduced risk materials compared during the first year caused significant *A. signatus* mortality. Two materials tested in the second year, acetamiprid and spinosad, had higher *A. signatus* mortality when compared to an untreated control, and exposure to spinosad resulted in similar mortality to bifenthrin, an industry standard material.

Keywords: *Anthonomus signatus,* Strawberry, reduced-risk, microbial insecticide

Introduction

Anthonomus signatus Say (Curculionidae: Coleoptera), the strawberry bud weevil, is considered a key pest in eastern strawberry production in North America (Clarke and Howitt 1975, Schaefers 1978). In early spring adults emerge from overwintering sites when temperatures are consistently over 16°C and move to host crops with early season bud development (Schaefers 1978). *A. signatus* have been reported on a wide taxonomic range of hosts including caneberries (*Rubus spp.)*, strawberries (*Fragaria* × *ananassa*), and red bud (*Cercis spp.)* (Kovach et al. 1999). Damage occurs when a female beetle deposits an egg in an unopened flower bud and then girdles the pedicel just below the sepal, causing the bud to desiccate and drop off the plant (Headlee 1916). The egg hatches, the larva feeds on immature pollen in order to develop through three instars, and pupates protected inside the dried unopened bud (Clarke and Howitt 1975, Mailloux and Bostanian 1993). The assumption that the loss of one bud is equal to the loss of an average sized fruit has led to the development of very low economic threshold of two clipped buds per meter (Schaefers 1978, Kovach et al. 1999). Research in perennial matted row strawberry plantings suggests that, dependent on individual cultivar ability to tolerate damage, the economic threshold could be as high as 20 clipped buds per meter. Even if a higher threshold is in use, one study (Handley et al. 2002) found that two thirds of matted row fields still had damage levels higher than 20 clipped bud per meter, and significant yield loses were observed. Most prior observations of *A. signatus* have been made in perennial strawberry production (Mailloux and Bostanian 1993, Pritts et al. 1999, Handley et al. 2002). The majority of strawberries grown in the eastern United States are grown as annual plants in plastic mulch. While comparatively little

is known about *A. signatus* significance and biology in these environments, thirty percent of the annual plasticulture strawberry fields monitored over two years in North Carolina also exceeded the upper limit of any currently available economic threshold recommendations (McPhie 2015).

There are no known biological control agents for *A. signatus* and host plant resistance among cultivars grown in annual plasticulture is unclear. Therefore, insecticides are the only management tools available. Current insecticides recommended to control *A. signatus* are broad spectrum in the carbamate, organophosphate, and pyrethroid families, and some can result in later season two spotted spider mite flare ups caused by non-target mortality of beneficial predatory mites (Louws et al. 2015). These insecticides are also listed as highly toxic to honey bees (*Apis mellifera*) and other pollinators (Michaud and Grant 2003, Dai et al. 2010). Strawberries benefit from bee pollination, and managed honey bees are an important part of the pollinator community (Chagnon et al. 1993). Pesticide application during bloom, such as those targeted at *A. signatus*, have the potential to negatively impact pollinators and reduce pollination services. Organic production systems, in which fewer total pesticide applications are made of materials which persist in the environment for relatively short periods, appear to facilitate greater strawberry pollination than conventional systems (Andersson et al. 2012).

Several potential alternatives to broad spectrum insecticides exist for *A. signatus* and are already registered for use in strawberries. Efforts to control *Anthonomus rubi* (strawberry blossom weevil), a related pest found throughout Europe, have identified reduced risk materials with various levels of efficacy, most notably acetamiprid (Labanowska 2002,

Fitzgerald 2004). Additionally observations in Canada have demonstrated that some strains of the entomopathic fungi *Beauveria bassiana* show efficacy in lowering populations of *A. signatus* in matted row strawberry plantings (Sabbahi et al. 2009). Cyantraniliprole, a material in the anthranilic diamide class, has been shown to cause morality in the congeneric species, *Anthonomus eugenii* (pepper weevil), when applied to peppers in bioassay experiments (Caballero et al. 2015), and novaluron an insect growth regulator has been shown to perform significantly better than an untreated control against *A.eugenii*, for both adult weevils and infested fruit in treated plots (Seal 2007). Pyrethrins, an OMRI listed material, has previously been recommend for treatment for control of *A. signatus* in organically grown strawberry systems(Hazelrigg and Kingsley-Richards 2007). Shown to be effective in control of sucking insect pests, flonicamid has a novel mode of action that disrupts feeding causing mortality (Fitzgerald and Jay 2011, Golmohammadi and Mohammadipour 2015).

The objective of our study was to determine if these materials were acceptable alternatives to broad spectrum insecticides for the control of *A. signatus* populations in annual plasticulture strawberry growing system.

Materials and methods

Because *A. signatus* populations are variable under field conditions, semi-field bioassays were conducted to assess toxicity of candidate insecticides. Strawberry plants were

grown at the Central Crops Research Station, Clayton, North Carolina (N 35.6686, W 78.5060) in 2013-2014 and 2014-2015. In North Carolina, strawberries are typically planted in September through October, harvested from April through June, and plants are removed in July or August. In the first year of our study, strawberries (*Fragaria* x *ananassa,* cultivar Camarosa) were planted October 15, 2013, and in the second year planting occurred on October 7, 2014. Plants were planted in four double-row beds on 1.53 meter center in black plastic mulch with a plant spacing of 0.36 meters. The field was fumigated with Pic Clor 60 at a rate of 134.43kg per ha prior to bed formation and mulch application. Plots consisted of 20 plants each in 2014 (0.00036 ha), and 17 plants each in 2015 plots (0.00056 ha). In 2014, bud emergence and bloom began on 25 March, and harvest began on 23 April. For 2015, bud emergence and bloom began on 30 March, and harvest began on 15 April. Plants were fertilized and treated with fungicides following standard recommendation (Louws et al. 2015), but received no insecticide applications except for those made in our treatments.

Materials to be compared were selected due to prior reporting of the active ingredient showing some effect on *A. signatus,* related species, or other insects with similar feeding or egg laying behavior. All materials selected are considered to have reduced environmental risk relative to materials that are currently used to control *A. signatus* or to have relatively low pollinator toxicity (Table 1). Treatments were applied at a distance of two feet above the top of the plants via a CO_2 pressurized back pack sprayer fitted with a three-nozzle boom using two Tee-Jet™ TG1 nozzles on either side and one TG2 nozzle in the center (TeeJet Technologies Carol Springs, IL). In 2014 the experiment was repeated twice. On 8 May

2014, the materials were applied at a spray volume equivalent 467.68 L/ha, and on 20 May, 2014 materials were applied to previously untreated plots at a spray volume equivalent of 935.41 L/ha. During 2015 a single application of all materials was made on 20 April, 2015 at a volume equivalent of 935.41L/ha. All materials were applied at the maximum rate according to the manufactures label (Table 3.1). Treatments were allowed to air dry, then one medium sized trifoliate leaf and 3 to 4 unopened flower buds were placed in a bioassay container. Bioassay containers consisted of a 946 ml (DeliPRO brand, Tri-pack Industrial USA, White Plains, NY) fitted with a water pick through a hole in the bottom to prevent wilting of the plant material during the duration of the assay and closed with a lid with a circular screened opening, approximately 24cm² wide (Figure 3.1). Gloves were changed between treatments to minimize risk of cross contamination. Bioassay containers were transported to the laboratory, and approximately five *A. signatus* were added. Bioassay arenas were monitored for 120 hours with mortality recorded every 24 hours. Each treatment was replicated four times per experiment.

A. signatus used in 2015 experiments were collected from overwintered populations at commercial farms in Granville, Orange, and Durham Counties in early April 2015. All were held for a minimum of 72 hours at approximately 21°C and 10:14 L:D to insure no mortality resulted from injury cause during collection.

Data analysis. The proportion of dead *A. signatus* within a bioassay arena was calculated for each time point. Data were analyzed via SAS 9.4 (SAS, Cary NC) using a mixed model analysis of variance (ANOVA) with mean separation by least significant difference and

adjusted for multiple comparisons using a Tukey-Kramer adjustment. Insecticide treatments were considered fixed variables while replication was considered a random variable, and proportion of mortality observed at 24 and 120 hours are the dependent variables. Data were not normally distributed due to large numbers of zeros in the response of proportion of mortality. Log transformation plus a constant of 0.5 normalized the data as confirmed via Shaprio-Wilkes test (W=0.90, p=0.12). Satterthwaite's approximation for degrees of freedoms was applied to account for differences in variance.

Results and Discussion

In 2014, no material caused significantly more mortality as compared to the untreated control when applied at 467.68 L/ha except for the grower standard, bifenthrin after 24 hours (F=17.16, df=5,15, p<0.001) and 120 hours (F=17.78, df=5,18, p<0.001) (Table 3). Spray volume was increased for the second trial in 2014 from 467.68 L/ha to 935.41 L/ha to improve coverage. However, the increased spray volume did not lead to a significant increase of mortality at 24 hours (F=16.09, df=5,18 , p<0.001) or 120 hours (F= 21.52, df= 5, 15, p< 0.001) post-exposure. Despite promising reported results using multiple strains of *B. bassiana* (Sabbahi et al. 2009), commercially available formulations had no effect on adult *A. signatus*.

In 2015 all materials were applied at the higher spray volume of 935.41 L/ha. Of the materials compared during 2015, only bifenthrin differed significantly from the untreated control 24 hours after treatment (F=28.05, df=4, 15, p<0.001). At 120 hours after treatment flonicamid still did not differ from the untreated control. However, acetamiprid exposed

beetles had higher mortality than the untreated control and flonicamid after 120 hours of exposure but lower mortality rates than spinosad or bifenthrin. Mortality in spinosad exposed beetles was the same as bifenthrin ($F= 77.55$, $df= 4, 15$, $P < 0.001$) (Table 3.3). The use of field collected *A. signatus* that had overwintered in the second year of the experiment more closely represents the insects which would be subject to field control tactics.

The use of broad spectrum insecticides during bloom has become a topic of concern relative to pollinator health. Most strawberry growers utilize managed populations of pollinators which have increasing regulation and requirements for their protection. Concerns about dramatic decline in the populations of some economically important pollinators (Potts et al. 2010) and non-target mortality of biological controls have increased the need to identify effective lower risk materials. Some materials used in this study are involved in relabeling and stricter use guidelines in the interest of managed pollinator population protection. The bee advisory box has been added to materials to promote awareness and proper pesticide use of materials when pollinators are active. Two of the reduced risk materials tested have been marked with the bee advisory box, spinosad and the neonicotinoid acetamiprid. However, applying the materials when pollinators are not active and allowing time to dry greatly reduces the risk. In a recent study comparing 40 common pesticides for honey bee toxicity acetamiprid was determined to cause less than 1% mortality (Zhu et al. 2015).

The OMRI listed spinosad had similar levels of control compared to the grower standard bifenthrin, and acetamiprid had some efficacy in reducing populations of *A. signatus*.

Acetamiprid is locally systemic within plants, and may have further effects on egg, larval, and pupal stages which were not assessed in our experiment.

It is necessary to continue to investigate reduced risk and organic materials for efficacy against *A. signatus.* Elevated levels of regulation on materials considered acceptable for use during the bloom period while pollinators are active will increase the need for effective reduced risk materials. Further investigation is needed into the possible systemic effects of acetamiprid and any possible roll it could play in disruption of the development of the summer generation and life cycle of *A. signatus* populations.

Tables and figures

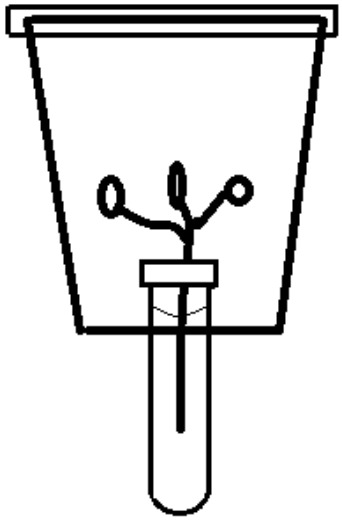


Figure 3.1. Bioassay arena consisting of 946ml clear plastic cup and a lid with a circular screened opening approximately 24cm² (DeliPRO brand, Tri-pack Industrial USA, White Plains, NY) with a water pick attached through a hole in the bottom.

Table 3.1. Rates of materials compared against *A. signatus*, with honey bee contact LD_{50}, and year of comparison.

Insecticide /(trade name)	**Maximum application rate**	**Honey bee contact LD_{50}**	**Year(s) compared**
Acetamiprid (Assial 30SG, United Phosphorus Inc.)	6.9oz/Acre	7.1 µg/bee	2015
Beauveria bassiana Strain GHA (Mycotrol-O, BioWorks Inc.)	32oz / Acre	Not available	2014
Bifenthrin (Bridgade WSB, FMC Corp.)	32oz / Acre	0.01462 µg/bee	2014, 2015
Cyantraniliprole (Exirel, DuPont)	20.5oz /Acre	2.78 µg/bee	2014
Flonicamid (Beleaf, FMC Corp.)	4.2oz /Acre	100 µg/bee	2015
Novaluron (Rimon0.83 EC, Makhteshim Agan)	12oz / Acre	>100 µg/bee	2014
Pyrethrins (Pyganic EC 5.0 , MGK)	17oz / Acre	5.52 µg/bee	2014
Spinosad (Entrust, DOW Agroscience)	2.5oz / Acre	0.050 µg/bee	2015

Table 3.2. Mean±SE. proportion dead *A. signatus* in 2014 experiments. Values followed by different letter within a column indicates significant differences, α=0.05. Data are presented as untransformed mean proportions.

Material	Rate	24hr	120hr
Bifenthrin	467.68 L/ha	0.66±0.160**a**	0.90±0.10**a**
Untreated Control	467.68 L/ha	0.0±0.0**b**	0.10±0.058**b**
Cyantraniliprole	467.68 L/ha	0.0±0.0**b**	0.163±0.055**b**
Beauveria bassiana Strain GHA	467.68 L/ha	0.0±0.0**b**	0.25±0.15**b**
Pyrethrins	467.68 L/ha	0.0±0.0**b**	0.0±0.0**b**
	Df	5,15	5,18
	F	17.6	17.78
	P	<0.001	<0.001
Bifenthrin	935.41 L/ha	0.7±0.129**a**	0.95±0.050**a**
Untreated Control	935.41 L/ha	0.0±0.0**b**	0.063±0.063**b**
Cyantraniliprole	935.41 L/ha	0.050±0.050**b**	0.063±0.064**b**
Beauveria bassiana Strain GHA	935.41 L/ha	0.15±0.096**b**	0.063±0.065**b**
Pyrethrins	935.41 L/ha	0.0±0.0**b**	0.063±0.066**b**
	Df	5, 18	5, 15
	F	16.09	21.52
	P	<0.001	<0.001

Table 3.3. Mean±SE. proportion dead *A. signatus* in 2015 experiments. Values followed by different letter within a column indicates significant differences, α=0.05. Data are presented as untransformed means proportions.

Material	Rate	24hr	120hr
Acetamiprid	935.41 L/ha	0.275±0.123**b**	0.325±0.043**b**
Flonicamid	935.41 L/ha	0.050±0.050**b**	0.050±0.050**c**
Bifenthrin	935.41 L/ha	0.90±0.058**a**	1.00±0.0**a**
Untreated Control	935.41 L/ha	0.0±0.0**b**	0.0±0.0**c**
Spinosad	935.41 L/ha	0.10±0.058**b**	0.850±0.096**a**
	Df	4, 15	4, 15
	F	28.05	77.58
	P	<0.001	<0.001

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