Evaluation and mechanisms of host-plant resistance to the wireworm-Diabrotica-Systena complex in sweetpotatoes (Ipomoea batatas) and a commercial kairomone lure in Diabroticites

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Evaluation and mechanisms of host-plant resistance to the wireworm-Diabrotica-Systema complex in sweetpotatoes (Ipomoea batatas) and a commercial kairomone lure in Diabroticites

By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agricultural Life Sciences
in the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology

Mississippi State, Mississippi
August 2021
An evaluation of host-plant resistance to the wireworm-\textit{Diabrotica-Systena} (WDS) complex of root-feeding insects in sweetpotatoes was performed on a total of 15 cultivars and advanced lines over the course of 4 years (2017-2020). It was found that sweetpotatoes varieties can differ significantly in amount of damage incurred. Several mechanisms of resistance were proposed and tested: periderm toughness, dry weight percentage, and volatile organic chemical defenses. No significant difference was found amongst the varieties tested concerning the physical properties. Chemical analysis was limited in scope but did show some differences between a susceptible variety when compared to a resistant variety in the volatile chemical composition of the foliage. Finally, a commercial kairomone lure with sticky card for \textit{Diabroticites} was tested for efficacy when compared to sweep net sampling throughout the season. No correlation could be found amongst the methods in terms of number and species of insects caught by each.
ACKNOWLEDGEMENTS

I would like to thank USDA-ARS for funding for this research and providing slips for advanced lines from their breeding program as well as LSU AgCenter and NC State. I would also like to express my gratitude to my advisor, Dr. Fred Musser, for his guidance over the course of the last two and a half years. I would like to thank the other members of my committee: Dr. Blake Layton, Dr. Ashli Brown, and Dr. Stephen Meyers for their assistance in providing feedback on my research ideas and on this thesis. This research would not have been possible without the assistance from the other members of the lab who helped with planting and harvesting: Beverly Catchot, Lauren Sanders, Farrar Misso, Shannon Oswald, Coty Martin, Chance Anderson, and Blaye Brasher. I would like to thank Jessi Collier and other members of the Hand chemistry lab for their assistance with the gas chromatography and mass spectrometry analysis. Thank you to the faculty and staff of the Pontotoc Ridge-Flatwoods Branch Experiment Station for their hard work in replicating the research at their facility and providing slips each year from their plant beds. I am also grateful to Earp Farms, Topashaw Farms, and Ellison Farms for allowing me to put lures out in their fields. Last, but not least, I want to thank my family for their unwavering support during this whole process.
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CHAPTER I
INTRODUCTION

Introduction

Sweetpotatoes (*Ipomoea batatas*) are a burgeoning crop in both the national and international markets. Sweetpotatoes are the 7th most produced crop in the world following corn, wheat, rice, potatoes, cassava, and soybeans (Truong et al., 2018). They are (depending on the flesh color) high in β-carotene, anthocyanins, phenolics, dietary fiber, vitamins, and minerals. β-carotene, which is a precursor of vitamin A, could be a useful tool to combat vitamin A deficiency in the world (Low et al., 2007). Other benefits from the bioactive compounds in sweetpotatoes include anti-inflammatory, antioxidant, hepatoprotective activity, anticancer, and antidiabetic effects (Lim et al., 2013; Hu et al., 2016). Sweetpotatoes are produced in 114 countries with most production in the eastern hemisphere. In 2014, global production was 106.6 million metric tons (FAO, 2015). China accounts for 67% of global production, followed by Nigeria, Tanzania, Ethiopia, and Mozambique (Truong et al., 2016). Western hemisphere production accounts for 3.6% of global production (FAO, 2015). Because sweetpotatoes need a relatively warm climate (21-26°C), they are grown mostly in the tropics where they are the fifth most produced crop. However, they also do well in the warmer climes of the United States. They grow best in soils with a pH of 5.5-6.5 (Nedunchezhiyan et al., 2012). Sweetpotatoes are drought tolerant and have very low nutritional needs, but they are susceptible to both insect damage and disease which can lead to substantial loss if not managed (Mao et al., 2004).
Sweetpotatoes are a member of the morning-glory family (*Convulvulaceae*). Sweetpotatoes are usually a rotational crop often with cotton, corn, peanuts, tobacco, and soybeans in the southeastern U.S. (Reed et al., 2009); production is a very labor-intensive process. Most sweetpotatoes are produced from shoots, commonly called “slips”, that are grown from roots that have been stored from the previous year’s harvest. These slips are typically 25-35 cm in length and are cut between 24-48 hours prior to planting. Sweetpotatoes need 90-150 frost free days to reach a marketable size (Truong et al., 2018). Slips can be hand planted but are usually transplanted in commercial production using a vegetable transplanter in raised rows. Rows are approximately 1m apart and the slips are transplanted at 30cm intervals. In central Mississippi, the growing season extends from late May to mid-October. Sweetpotato yield and quality are optimized in a soil that is a mixture of sandy loam and clay. If the soil is too sandy, the roots tend to be long and narrow; if the soil has too much clay, the roots tend to be of good size but the number or roots per plant is diminished (Truong et al., 2018). Ideally, each plant will have 4-10 marketable roots (Swaider and Ware, 2002).

**Insect Pests of Sweetpotatoes**

Several insects can damage both the foliage and the roots of sweetpotatoes. Banded and spotted cucumber beetles (*Diabrotica balteata* LeConte and *D. undecimpunctata howardi* Barber, respectively), wireworms (*Conoderus* spp., *Melanotus* spp., and *Heteroderes* spp.), flea beetles (*Systena* spp.), sweetpotato flea beetles (*Chaetocnema* spp.), and white grubs (*Phyllophaga* spp.) all cause root damage (Schalk et al., 1993). Among the most significant in Mississippi and the Midsouth are the banded cucumber beetle, spotted cucumber beetle, flea beetle, and wireworm (Cuthbert and Reid, 1965). The damage symptoms caused by insects in this complex are not easily
distinguished between each other, so the causal agent is often referred to as the wireworm-
*Diabrotica-Systena* complex (WDS) (Schalk et al., 1991).

The holes in sweetpotatoes from the WDS complex vary from less than 1mm to 8mm in
diameter, depending on the pest. The youngest larvae of the *Systena* flea beetle can cause pinholes
of 1mm or less (Thomas, 1927). The older larvae can make holes that more closely resemble that
of cucumber beetles (Schalk et al., 1991). Cucumber beetles can make holes that range from 1-3mm in diameter. These holes are sometimes found in clusters of 3-5 holes. Wireworm holes are
normally deeper than flea beetle or cucumber beetle holes and can range from 2-8mm in diameter.
These are normally found in random locations throughout the potato (Chalfant and Seal, 1991).
Because of the amount of overlap in the diameters of the holes created by each of these pests, it
can be difficult to determine exactly which pest caused the damage. For example, a 2mm hole
could have been created by a large flea beetle larva, a cucumber beetle, or a young wireworm. The
sweetpotato flea beetle causes narrow (1-2 mm wide) channels across the periderm (Tysowsky,
1971). White grubs create gouges that are often broad and rough; these gouges are almost always
contiguous (Hammond et al., 2001).

The adult banded cucumber beetle is characterized by distinct alternating green and yellow
bands across the elytra. The spotted cucumber beetle is yellow green with 12 distinct spots on the
elytra (Alston and Worwood, 2008). While there are some differences in the minutiae of
development, the following description is true for both species. Copulation occurs between 4-8
days after emergence. On average, the female will oviposit 15-16 days after emergence. Eggs are
laid in groups of 20-100 over the course of 24 hours. Depending on temperature, the eggs will
hatch 5-8 days after oviposition. Increased temperatures up to 27°C decrease the incubation
period. Depending on the diet, the larvae will be white to yellowish. Larvae feeding on storage roots of potatoes will be more yellow than white (Pitre and Kantak, 1962). *Diabrotica* larvae are differentiated from other larvae by a dark spot on both the anterior end and the last abdominal segment. There are three larval instars: in tests performed by Drs. Pitre and Kantak at LSU it was determined that each of the first two instars fed for approximately 7 days; the last instar averaged 10 days for a total mean larval development of 23.3 days at 21 degrees celsius (this period decreased with increasing temperatures with mean larval development of 17 days at 27°C). The pupae are exarate and will emerge in approximately 7-9 days. In the southern United States, there can be 7-8 generations per season (Pitre and Kantack, 1962).

Wireworms (*Conoderus* spp., *Melanotus* spp., and *Heteroderes* spp.) (Schalk, 1984) vary greatly in number of generations and length of larval development, but many are almost morphologically identical to one another in their larval forms. The largest difference amongst many wireworms found as agricultural pests is the amount of time they spend in their larval form. Some can stay in that form for several years, moving deeper into the soil as the weather gets colder. In a study conducted in 1962 by Robert L. Rabb, the tobacco wireworm (*Conoderus vespertinus*), which is the most common species found in fields in Mississippi (Cuthbert and Reid, 1965), had a larval development period in a laboratory environment from 287-347 days. Rabb does, however, speculate that these larvae may not be indicative of field colonies because of the multiple inspections they underwent. These lab colonies, in general, required longer to complete their development than larvae in field cages. The eggs of wireworms are white, spherical, and approximately 0.5mm in diameter on average. The incubation period lasts between 9-16 days, over which the egg would darken. This period seemed to increase with increasing temperatures (Rabb, 1962). One feature that characterizes larvae is that they are scleroterized in all except for
the first instars. The number of instars varies with species. The adults are brown to black in color and share a common general form that includes a long, narrow body with a large prothorax. Flight, feeding, mating and ovipositing almost exclusively occurs at night. During the day, the adults display thigmokinesis, a cessation of movement due to a stimulus; in this case, light (Fraenkel and Gunn, 1940). During the day, the adults are tightly wedged into crevices, such as flower bracts of cotton or between leaf sheaths of corn. Wireworms overwinter as larvae. Emergence of adults occurs, according to temperatures, around May with complete emergence, in *C. vespertinus*, occurring by late July (Rabb, 1962).

The flea beetle, specifically *Systena frontalis*, displays complete metamorphosis. The eggs are oval, white, and about 1mm in length. There are three larval instars where the larvae can range from 5mm-10mm in length. These larvae are white with a brown spot on the anterior end (Cloyd, R.A. & Herrick, N., 2018). The larvae feed on plant roots. The adults are approximately 5mm long with enlarged hind femora and a red head. The adults will emerge in the Midsouth beginning in May and will have many overlapping generations until early October (Shimat, J & Hudson W., 2020).

The sweetpotato flea beetle overwinters as an adult in leaf litter and other detritus. They begin to feed when sweetpotatoes are planted and by the end of June they migrate to bindweed where they will oviposit and die. The larvae will hatch after three weeks (Jolivet, 2008). The larvae are small, white, and scarabaeiform (“C” shaped larval form). They feed for 4-8 weeks in the mid-south. The larvae will develop into pupae and in 7-8 days will emerge as adults (Tsatsia & Graham, 2017).
White grubs are the immature forms of many species of scarab beetles. The grubs can be 6mm-25mm in length. Due to the diversity of species, they have life cycles that can differ dramatically. Some species lay their eggs in grasses, soybeans, corn, and many other substrates. There are species that have a one year life cycle, the masked chafer and Japanese beetle for example, and some like the May/June beetles have a life cycle that can take two to three years to complete (https://extension.entm.purdue.edu/fieldcropsipm/insects/corn-whitegrubs.php).

**Control of the WDS Complex**

Insecticides are the main tool used to combat root-feeding pests in commercial production fields in the U.S. (Chalfant et al., 1991). Insecticides are usually incorporated into the soil prior to transplanting the slips in a process known as pre-plant incorporation, or PPI. Once the plants begin to vine, an insecticide (sometimes the same insecticide used PPI) can be applied and incorporated into the soil prior to full canopy coverage. This “lay-by” insecticide application is used to control mid- to late-season damage by root-feeding insects. Chlorinated hydrocarbons were used effectively to combat root-feeding insects prior to de-registration by the Environmental Protection Agency (Schalk et al., 1993). Currently used insecticides generally are in the organophosphate and pyrethroid classes. Foliar sprays are used to control the adults of these species. Sampling is a tool used to monitor adult populations. One of the more common methods of sampling is using a sweep net. The Mississippi Insect Control Guide for 2021 sets thresholds for these adult pests. These thresholds are the number of insects per 100 sweeps. Thresholds recommended are as follows: 2 or more flea beetles, 2 or more white grub beetles, 2 or more cucumber beetles, and 4 or more click beetles. One insecticide that can treat these adults is Bifenthrin which is in the pyrethroid class of insecticides (Mississippi Insect Control Guide, 2021). While damage to foliage is not a large economic factor, controlling populations of adults can help curtail subsequent larval damage.
With the risk of organophosphorus insecticides like Lorsban (used by a large percentage of commercial growers in Mississippi) being deregistered soon, alternative methods of control must be considered. One such method is host-plant resistance. Many of the commercially produced varieties grown in the United States have shown minimal resistance to the WDS complex (Collins et al., 1991). However, the diversity of insect resistance found among sweetpotato breeding lines provides potential for breeding cultivars with a greater resistance to insect feeding (Gichuki et al., 2003). Host-plant resistance to insects has been found in several traditional and heirloom varieties (Jackson and Harrison, 2013). Unfortunately, increased resistance is often inversely correlated with yield. To get growers to adopt insect-resistant varieties for commercial production, lines that show both resistance and high yield need to be found. The attributes that contribute to insect resistance in sweetpotato varieties have yet to be fully established.
References


CHAPTER II
RESISTANCE TO THE WIREWORM-DIABROTICA-SYSTENA COMPLEX IN SWEETPOTATO (IPOMOEA BATATAS) CULTIVARS AND ADVANCED LINES

Abstract

Commercial cultivars and advanced sweetpotato lines were tested in Mississippi from 2017-2020 for resistance to the wireworm-\textit{Diabrotica-Systena} (WDS) insect complex. Numerous lines were more resistant to WDS than the commercial standard Beauregard cultivar. However, yield on the more resistant lines tended to be lower. The one exception was the cultivar Bayou Belle, which showed both good yield and some resistance to WDS when compared to Beauregard and other high yielding varieties. While highly resistant lines are not commercially acceptable at this time, the range in resistance observed indicates that WDS resistance should be a reachable target for plant breeders.

Introduction

The wireworm-\textit{Diabrotica-Systena} (WDS) complex of root-feeding insects can cause significant reductions to marketable yield in sweetpotato production. In the mid-south United States, the most significant species are spotted cucumber beetles (\textit{Diabrotica undecimpunctata howardii} Barber), banded cucumber beetles (\textit{Diabrotica balteata} LeConte), and \textit{Conoderus} spp. of wireworms (Cuthbert & Reid, 1965). This complex mainly causes direct damage to roots during their larval stages but can also cause minor defoliation during the adult stage. The
damage to the roots are holes of 1-8 mm in diameter that are bored into the sweetpotato by the larvae (Reed et al., 2010). Commercial varieties have shown little resistance to this insect complex which can lead to considerable economic losses if not effectively controlled. The deregistration of persistent chlorinated hydrocarbon insecticides, which provided good control of this complex, led to a shift in reliance on non-persistent insecticides, but these are not reliable (Reed et al., 2009). Therefore, alternative strategies such as biological control and selective breeding for resistance to the WDS complex are needed (Schalk et al., 1993). These strategies could prove to be a valuable resource in mitigating the potential damage of this complex.

Sweetpotatoes have 90 chromosomes and are hexaploidal (Truong et al., 2018). Sweetpotatoes have been cultivated over a wide range of areas for many years which creates a large genotypic variability in extant cultivars and lines, especially when considered from region to region (Truong et al., 2018). As a polyploid that is mostly an obligate out-crosser with high levels of heterozygosity and many incompatible genotypes, it can be especially difficult to breed (Jones, 1986; Collins et al., 1999; Truong et al., 2018), but resistance to soil damaging insects in certain lines of sweetpotatoes, including WDS, has been known for at least 50 years (Cuthbert & Reid, 1970). Many breeding programs utilized the large germplasm bank in an attempt to produce new advanced lines (Koehler & Kays, 1991; Arancibia et al., 2018; Nwosisi et al., 2017; Laurie et al., 2020), but few of them focused on insect resistance (Cuthbert & Davis, 1970; Schalk et al., 1993; Collins et al., 1999; Jackson & Bohac, 2006; Jackson et al., 2012). The purpose of this study was to evaluate cultivars and new advanced lines for resistance to WDS damage with the hope that improved understanding of resistance levels available will encourage more breeding for insect resistance and higher grower adoption of more resistant lines.
Materials and Methods

A total of eight site-years of cultivar and advanced line testing was conducted from 2017 to 2020 at the R.R. Foil Plant Science Research Center in Starkville, MS and the Pontotoc Ridge-Flatwoods Branch Experiment Station in Pontotoc, MS. At all site-years, plots were 4 rows wide (97 cm spacing) by 7 m long and arranged in a randomized complete block design. The treatments included at each site-year are listed in Table 2.1. For all trials, slips were either cut from plant beds at the Pontotoc location or they were obtained from plant breeders with the USDA-ARS Vegetable Laboratory, North Carolina State University, or the Louisiana State University AgCenter. Slips were transplanted at 30 to 40 cm spacing. Fields were treated with recommended herbicides (Mississippi Extension Service, 2021), but no insecticides were applied. Sweetpotatoes were grown for 90-120 days and hand harvested. Planting dates that were recorded for each site-year are listed in Table 2.2. Each root of marketable size was washed, sized, weighed, and assessed for insect damage. Damage caused by larvae of the WDS complex are holes in the roots that are approximately 1-8mm in diameter. Individual roots were given a size rating of No. 1, Canner, or Jumbo according to their diameter and length (Benedict & Smith, 2009). Damage type was assessed using the descriptions and diagrams of Reed et al. (2010).

Insect damage was measured as number of WDS holes per sweetpotato storage root. Yield is presented as an expression of the percentage marketable yield of Beauregard (the industry standard variety commercially produced in the Mid-South) because it is a consistently high yielding variety that was used in every year of the study. Data were analyzed using the GLIMMIX procedure (SAS 9.4, SAS Institute, Cary, NC). Different cultivars and advanced line were compared using Fisher’s Protected Least Significant Difference method for $\alpha = 0.05$. 
Results

Overall site years breeding line, or cultivar, was significant with regard to WDS damage (F = 8.97; df = 15, 172; P < 0.0001) as well as yield (F = 6.93; df =14, 171; P < 0.0001). Some white grub damage was seen in 2019 and 2020, but it was very limited, so no conclusions regarding susceptibility of lines could be made for white grubs (data not shown). Large standard errors, like those found in Stokes in Fig 2.2, are partially due to only being tested for one site year. When comparing yield and resistance, highly resistant lines (Murasaki, NC-04531, NC-140589, and USDA- 04136) yielded less than 60% than that of Beauregard. Murasaki showed good resistance amongst the lines tested for multiple years, but yield was only 44% ± 2.8% of Beauregard when grown for the same number of days. When regarding both yield and resistance of lines tested over multiple years, Bayou Belle had a yield of 103 ± 8% when compared to Beauregard. When the insect damage of the two lines is compared, Bayou Belle (0.55 ± 0.08 holes per potato) had almost half the damage of Beauregard (1.09 ± 0.17 holes per potato). Even though it was only tested for one year, USDA-09130 showed promise as well with a yield that was 115.3% ± 9.4% than that of Beauregard and a damage rating of 0.56 ± 0.15 WDS holes per potato.

Discussion

For most lines, the highest yielding varieties showed poor resistance to the WDS complex. Murasaki showed good resistance but small yield. If Murasaki was planted early so it could grow for a longer amount of time, it could be possible to see a significant increase in yield. The roots of Murasaki were much smaller than those of the susceptible varieties. This decrease in size could account for the decreased damage. Canners of all varieties tested had much less
damage than the larger roots. It would be valuable to compare damage amongst the lines when
the roots are of similar size to account for this difference. The yield results and
susceptibility/resistance of many of the commercial varieties tested are consistent with similar
studies in the southern United States (Jackson et al., 2012; Nwosisi et al., 2017; Arancibia et al.,
2018). In summary, two lines (Bayou Belle and USDA-09130) showed promise based on yield
and WDS damage. If a line could be bred that showed yield consistent with that of Beauregard
and resistance on par with the more resistant lines, that could provide increased value to
producers. Of course, there are many more factors to consider for commercial producers such as
storage quality, consumer preference, and resistance to other biotic and abiotic factors. Given
the wealth of genetic diversity amongst sweetpotato lines, resistance breeding, coupled with
other IPM practices such as chemical and biological control measures, could be a valuable tool in
the control of damage due to the WDS complex.
Table 2.1 Cultivars and advanced lines included at each trial site-year

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Averre</th>
<th>Bayou Belle</th>
<th>Beauregard</th>
<th>Bellvue</th>
<th>Burgundy</th>
<th>Covington</th>
<th>L14-41</th>
<th>Murasaki</th>
<th>NC 04531</th>
<th>NC 140589</th>
<th>Orleans</th>
<th>USDA 04-136</th>
<th>USDA 04-671</th>
<th>USDA 09-130</th>
<th>Vardaman</th>
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<tr>
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<td>Starkville</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>Starkville</td>
<td>2019</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Starkville</td>
<td>2020</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Pontotoc</td>
<td>2020</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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Table 2.2  Site-year agronomic details including replications, planting date, and information pertinent to that site-year

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Replications</th>
<th>Planting Date</th>
<th>Harvest</th>
<th>Comments?</th>
</tr>
</thead>
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<tr>
<td>Pontotoc-E</td>
<td>2017</td>
<td>8</td>
<td>6/15</td>
<td>10/11</td>
<td></td>
</tr>
<tr>
<td>Pontotoc-L</td>
<td>2017</td>
<td>4</td>
<td>7/24</td>
<td>11/1</td>
<td></td>
</tr>
<tr>
<td>Starkville</td>
<td>2017</td>
<td>4</td>
<td>7/17</td>
<td>10/31</td>
<td></td>
</tr>
<tr>
<td>Pontotoc</td>
<td>2018</td>
<td>4</td>
<td>6/5</td>
<td>10/11</td>
<td></td>
</tr>
<tr>
<td>Starkville</td>
<td>2018</td>
<td>4</td>
<td>7/5</td>
<td>10/31</td>
<td>Only 50 potatoes per plot harvested due to field conditions</td>
</tr>
<tr>
<td>Pontotoc</td>
<td>2019</td>
<td>4</td>
<td>6/24</td>
<td>11/11</td>
<td>3 weeks of heavy rains prior to harvest</td>
</tr>
<tr>
<td>Starkville</td>
<td>2019</td>
<td>4</td>
<td>7/11</td>
<td>11/13</td>
<td>3 weeks of heavy rains prior to harvest</td>
</tr>
<tr>
<td>Pontotoc</td>
<td>2020</td>
<td>4</td>
<td>6/11</td>
<td>10/21</td>
<td>No advanced lines due to pandemic</td>
</tr>
<tr>
<td>Starkville</td>
<td>2020</td>
<td>4</td>
<td>6/17</td>
<td>10/26</td>
<td>No advanced lines due to pandemic; 3 reps of L14-41; 2 reps of Bellvue</td>
</tr>
</tbody>
</table>
Figure 2.1  Mean percent marketable yield (±SEM) of each line when compared to Beauregard; 2017-2020. Any values less than 100 yielded less than Beauregard, any values higher than 100 yielded more than Beauregard (SAS 9.4).
Figure 2.2  Mean WDS holes (±SEM) per potato by line; 2017-2020. Higher values indicate an increased susceptibility to WDS damage. Large standard error bars are a product of having only one site-year for Stokes.
References


Reed, J.T., Shankle, M.W., Williams, M.R., and Burdine, W. 2010. Results of southern sweetpotato IPM project in Mississippi. Bulletin 1181 of the Mississippi Agricultural and Forestry Experiment Station


CHAPTER III

PHYSICAL AND CHEMICAL MECHANISMS OF RESISTANCE TO THE WIREWORM-
DIABROTICA-SYSTENA COMPLEX IN SWEETPOTATO

Abstract

Sweetpotato [Ipomoea batatas (L.) Lam.] lines were tested for damage from the wireworm-
damage data determined there was a significant difference in host-pant resistance between lines.
Periderm toughness and dry weight were measured on lines during 2020 to determine if there was
a correlation between these attributes and insect resistance. There were no differences in periderm
toughness, but dry weight percentage varied among lines. Two moderately resistant lines, Averre
and Bayou Belle had the lowest dry weight percentages. Volatile organic compounds (VOCs) were
measured from foliage and root flesh of three lines and differences in the presence of a few known
plant-defensive compounds were detected between resistant and susceptible lines. While firm
conclusions cannot be made concerning the mechanisms of WDS resistance in sweetpotatoes,
these data provide potential targets for further research.

Introduction

Several insect species damage the roots of sweetpotatoes. In the mid-southern United
States, the most prevalent of these species are banded and spotted cucumber beetles (Diabrotica
balteata LeConte and Diabrotica undecimpunctata howardi Barber, respectively), wireworms
(Conoderus, Melanotus, and Heteroderes spp.), flea beetles (Chaetocnema and Systena spp.), and
white grubs (*Phyllophaga* spp.) (Schalk et al., 1993). Wireworms, cucumber beetles, and flea beetles are often considered a pest complex of root-feeding insects (Cuthbert & Reid, 1965) because the diameter of the holes in the roots caused by this complex range from 1 to 8 mm and cannot be used to clearly identify the pest that created them. Therefore, the causal agent is often referred to as the wireworm-*Diabrotica-Systena* or WDS complex (Schalk et al., 1991).

Prior to deregistration of persistent chlorinated hydrocarbons, chemical insecticides were an effective means of control of these sweetpotato pests (Schalk et al., 1993). Currently, insecticides such as organophosphates and pyrethroids are used in chemical control (Crow et al., 2021). However, with the risk of some organophosphorus insecticides being deregistered soon (Brown, 2021), other means of insect control should be developed. One such method is the use of selective breeding techniques for host-plant resistance to these pests. Most commercially grown sweetpotato varieties have shown little resistance to the WDS complex (Collins et al., 1991). The large diversity among sweetpotato breeding lines as well as land race varieties could allow for an increase in host-plant resistance to insect feeding (Gichuki et al., 2003). There have been some research projects that have studied the mechanisms of this resistance. Cuthbert and Davis (1971) tested the time and depth of root enlargement, sugar content, carotene, dry matter, latex flow, and flesh pH, but found that none of these factors were associated with insect resistance. They also observed that when the periderm of the sweetpotato was stripped off, cucumber beetle larvae had decreased mortality. When the periderm and cortex were tested on lab colonies of spotted and banded cucumber beetles, both species had higher mortality after feeding on resistant lines than after feeding on susceptible lines (Jackson & Bohac, 2007), supporting the earlier observation that the periderm is important in insect resistance.
Volatile organic compounds (VOCs) are another potential mechanism of resistance as they have been shown to play a role in anti-herbivory in numerous insect/plant relationships (Brilli et al., 2019; Zhou et al., 2013; Smith et al., 2012; Naznin et al., 2014). VOC’s are semio-chemicals that are produced by plants, either constitutively or after being induced by damage to the plant or to a nearby plant (Hiltpold & Hibbard, 2018; Walling, 2000). Induced chemical response to herbivory has been documented in over 100 plant species (Thaler & Karban, 1997). These chemicals can attract insect natural enemies such as parasitic wasps, entomopathogenic nematodes, and even beneficial bacteria (Hiltpold & Hibbard, 2018; Liu & Brettell, 2019). This study is an attempt to ascertain what mechanisms are involved in host-plant resistance in sweetpotatoes by examining differences in plants that are susceptible and resistant to the WDS complex.

**Materials and Methods**

Four sweetpotato cultivars and one advanced breeding line were planted and managed at Starkville, MS at the R.R. Foil Plant Science Center in 4-row plots (7.5 m long) with four replications during June 17, 2020 using recommended practices (Nedunchezhiyan et al., 2012) except that no insecticides were applied. Based on previous insect resistance trials (Chapter 2), Beauregard was selected as a representative insect-susceptible line and Murasaki and Averre were selected as insect-resistant lines. Slips of all lines were obtained from the Pontotoc Ridge-Flatwoods Branch Experiment Station in Pontotoc, MS. The middle two rows were hand harvested on 29 October 2020. Harvested roots were hand washed, air dried, and stored in plastic crates in a storage facility until analyses could be conducted.

To test skin toughness, the force needed to push a 7.9 mm diameter tip through the periderm was measured with a penetrometer (Fruit Hardness Tester, Agriculture Solutions model FHP-803; Kingfield, ME) on each sweetpotato storage root that was ≥ 6 cm in diameter. A minimum of 10
and an average of 27 roots were tested from each plot during December 2020. To estimate the dry weight, five sweet potatoes from each line were sampled by excising approximately 10g of flesh (excluding the periderm) from a 2.5 mm cross section of the largest portion of each potato. Each sample was weighed before drying, labelled, placed in a Cusimax aluminum cupcake liner (7 cm x 5 cm x 3 cm) and placed in an oven for 24 h at 38° C. After drying, the dry weight was recorded, and a dry weight percentage was calculated using the formula:

\[
\text{Dry wt \%} = 100 \times \frac{b}{a}
\]

where \(a\) = wet weight in grams and \(b\) = dry weight in grams. Skin toughness and dry weight data were analyzed using the GLIMMIX procedure (SAS 9.4, SAS Institute, Cary, NC). Means were separated using the Fishers Protected LSD test with a \(P\)-value of 0.05. A correlation analysis (Proc Corr, SAS 9.4) was conducted to determine if there was a relationship between dry weight or skin toughness with respect to insect damage.

Leaves or roots were collected from these lines grown in a sweetpotato field in Starkville, MS during the week of harvest (November 13, 2019 and October 26, 2020). Flesh of Beauregard and Murasaki in 2019, and foliage of Beauregard and Averre lines in 2020, were tested for volatile organic compounds by performing mass spectrometry and gas chromatography on the headspace of homogenized samples of one sweet potato root or 3 to 4 leaves. A solid-phase microextraction (SPME) fiber (85 µm Carboxen/PDMS StableFlex, Supelco, Bellefonte, PA) was used to absorb the volatiles given off by each sample at room temperature and, again, in a 40°C water bath for 24 h. The higher temperature was used in an attempt to elicit a greater absorbtion of VOCs. After extraction, the SPME fibers were analyzed using gas chromatography (GC) (Agilent 7890B, Agilent Technologies, Santa Clara, CA), column length 30m with a diameter of 0.25mm, and mass spectrometry (MS) (Agilent 5977A, Agilent Technologies, Santa Clara, CA). The gas
chromatography oven temperature was programmed to go from 60°C (held for 2 min) to 105°C (held for 0 min) at 15°C/min rate, then to 165°C (held for 0 min) at 10°C/min rate, and lastly to 290°C (held for 4 min) at 5°C/min rate. Agilent MassHunter software (Agilent Technologies, Santa Clara, CA) was used to perform a library search through the National Institute of Standards and Technology chemical library to find matches of 50% or greater certainty with our GC-MS data. Independently, a list of VOCs that have been found to affect insect resistance was compiled from the literature (Heil & Bueno, 2007; Holopainen & Gershenzon, 2010; Hiltpold & Hibbard, 2018). The list of VOCs identified from our samples was then compared to the list of known insect resistance VOCs.

**Results and Discussion**

Dry weight percentage varied among the lines (F= 3.08; df = 5,24; P = 0.027). Bayou Belle had the lowest dry weight percentage while L14-41 and Orleans had the highest dry weight percentages (Fig. 3.1). There were no differences among the lines in skin toughness (F=2.23; df = 4,9; P = 0.15) (Fig. 3.2).

As reported in Chapter 2, Bayou Belle had the least insect damage among the high yielding varieties tested. Bayou Belle also had the lowest skin “toughness” and percent dry weight, suggesting that these factors may contribute to insect resistance. However, correlation analyses over all tested lines (Proc Corr, SAS 9.4) between WDS damage in holes per sweetpotato and dry weight percentage (r = 0.05 ± 0.136; F = 0.14; df = 1,3; P = 0.74) and WDS damage and skin “toughness” at harvest (r = 0.014 ± 0.026; F = 0.29; df = 1,13; P = 0.60) showed that neither of these physical properties were correlated to WDS damage data. Schalk et al. (1986) suggested that periderm thickness may play an important role in protection of the root from herbivory early in the season, especially for cucumber beetles which are considered to cause more damage early.
in the growing season (~60 days after plant). Assuming that a thicker periderm will be more difficult to penetrate, penetrometer tests at various points in the growing seasons would be useful to see how much variability exists among resistant and susceptible lines. The VOCs from the lines were similar between the two flesh samples, but different between the two foliage samples (Table 3.1). Beta-carophyllene, a VOC found in the resistant foliage but not in the susceptible, has been shown to increase resistance in maize to fall armyworms (Smith et al., 2012). The other 5 compounds on the list have been shown to play a role in defense in lima beans (Phaseolus lunatus [L.] Fabricius), lettuce (Lactuca sativa L.), French marigold (Tagetes patula L.), and many other plant species (Karban & Baldwin, 1997, Heil & Bueno, 2007, Song et al., 2017, Wonglom et al, 2020)

It is likely that several factors play a role in host-plant defense in sweetpotatoes. Although we found no significant correlation between insect resistance and the physical properties tested, and few VOC differences in the lines evaluated, more research could be done to further study these factors. It is possible that mechanically damaging the tissues prior to headspace analysis could have skewed the results (Karban & Adler, 1996; Karban & Baldwin, 1997; Thaler & Karban, 1997; Rasmann et al., 2012). A broader range of lines used in these tests as well as looking at the constitutive chemicals would help to create a more comprehensive study of what factors influence resistance. Also, as mentioned, it might be useful to study the toughness of the sweetpotato throughout the growing season to see if more variability between lines could be established.
Table 3.1  A list of Volatile Organic Compounds (VOCs) that could play a role in insect resistance identified in the sweetpotatoes tissues tested using Gas Chromatography and Mass Spectrometry

<table>
<thead>
<tr>
<th>VOC</th>
<th>Beauregard flesh</th>
<th>Murasaki flesh</th>
<th>Beauregard foliage</th>
<th>Averre foliage</th>
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<tr>
<td>2-Ethylhexanol</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-Carophyllene</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Cis-3-Hexen-1-ol-acetate</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Decanal</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonanol</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1  Mean dry weight percentage (± SEM) of flesh samples in sweetpotato lines grown in Starkville, MS during 2020. Bars containing the same letter are not significantly different (Fishers Protected LSD, P=0.05).
Figure 3.2  Mean periderm resistance (± SEM) in sweetpotato lines grown in Starkville, MS during 2020 using a penetrometer with a 7.9 mm diameter tip. Lines were not significantly different (F=2.23; df = 4,9; P = 0.15).
References


Song, B., Liang, Y., Liu, S., Zhang, L., Tang, G., Ma, T., and Yao, Y. 2017. Behavioral responses of Aphis citricola (Hemiptera:Aphididae) and its natural enemy Harmonia axyridis (Coleoptera: Coccinellidae) to non-host plant volatiles. Florida Entomologist 100(2): 411-421


CHAPTER IV

EFFICACY OF KAIROMONE TRAPS FOR CUCUMBER BEETLES IN SWEETPOTATOES

Abstract

Commercially available kairomone lures in conjunction with sticky traps were tested for their effectiveness compared to sweep nets in sampling for banded and spotted cucumber beetles in sweetpotatoes over five locations in north/northeast Mississippi. The lures were not as effective as sweep nets. These data along with previous research suggest that these kairomones are not as effective in trapping when compared with traditional sweep net sampling. Pheromones could be a possible alternative when used in conjunction with sticky cards.

Introduction

Cucumber beetles are a major pest of sweetpotatoes in the Mid-South. They belong to a root-feeding complex that has the potential to cause considerable yield loss. In Mississippi, there are two species commonly found in sweetpotatoes: the banded cucumber beetle (Diabrotica balteata LeConte) and the spotted cucumber beetle (Diabrotica undecimpunctata howardii Barber). The banded cucumber beetle is characterized by alternating green and yellow bands on the elytra of the adult. The spotted cucumber beetle is characterized by a yellow elytra marked with twelve black spots (Alston and Worwood, 2008). The adults of both species cause indirect damage by defoliation and the larvae cause direct damage by making holes that can range from 1-3 mm in diameter in the root of the sweetpotato plant (Reed et al., 2010).
The economic threshold in Mississippi for cucumber beetle adults in sweetpotatoes is two per 100 sweeps with a sweep net (Crow et al., 2021). An economic threshold is an estimate of the pest density at which control measures should be initiated to prevent economic loss (Weinzierl et al., 1987). In the case of cucumber beetles, the threshold is based on minimizing oviposition since the more damaging larval stage lives in the soil where it is protected from foliar insecticide applications. Sweep nets are cloth or mesh nets that are attached to a 38 cm diameter wire frame. Sweep net samples are collected by swinging the net through the foliage with a pendulum motion, making sure to get the sweep net below the top of the foliage as you move down the rows with each sweep (Seiter et al., 2015). While a sweep net is an efficient sampling tool in crops that are 25-100 cm tall such as soybeans and alfalfa, it is challenging in sweetpotatoes since the foliage is never very high above the ground, so other sampling methods should be explored. One possible sampling method is trapping using a semiochemical lure.

Kairomones are volatile semiochemicals that benefit the receiver. In the case of cucumber beetles and sweetpotatoes, natural kairomones are emitted by the sweetpotato and detected by the herbivorous cucumber beetle, attracting the beetle to the plant. The chemical components of various plant kairomones have been identified and are commercially available in slow-release packets that can be used to monitor the density of insects in a field. In spotted and striped cucumber beetles (*Acalymma vittatum* F.), the use of different mixtures of these kairomones has been well documented, especially those containing some mixture of 1,2,4-trimethoxybenzene, indole, (E)-cinnamaldehyde (collectively known as TIC), 4-methoxycinnamaldehyde, chavicol, phenylacetaldehyde, benzyl acetone, phenethyl alcohol, phenyl acetate, veratrole, methyl eugenol, methyl isoeugenol, and isoeugenol (Lampman and Metcalf, 1987; Metcalf and Lampman, 1989;
Metcalf et al., 1998; Lewis et al., 1990; Jackson et al., 2005; Piñero, 2018). It was found that the mixture of these chemicals, even slightly changed, could have a significant impact on how attractive these lures are and to which species (Jackson et al., 2005). Many Diabroticite spp. have been shown to be attracted to cucurbit flowers (squash, watermelon, gourds, cucumbers, etc.) (Metcalf and Lampman, 1991). The synergistic effects on Diabroticite spp. from Cucurbita flowers were studied and it was found that indole was the primary chemical synergist in many kairomonal mixtures for Western corn rootworm (D. vergifera vergifera), Northern corn rootworm (D. barberi), and striped cucumber beetles (A. vittatum) (Anderson and Metcalf, 1986; Metcalf, et al., 1995). Banded cucumber beetles, however, did not respond well to any of the mixtures that were studied. Banded cucumber beetles in a laboratory setting have shown phagostimulation from various doses of a cucurbit kairomone mixture (exact ingredients not specified) (Peterson and Schalk, 1985), but none of the literature reviewed showed a preference for a kairomonal mixture over any other phagostimulant in the banded cucumber beetle.

The objective of this study is to determine if commercial kairomone lures can be effective tools for sampling cucumber beetles compared to a sweep net under field conditions. If so, this could be a useful tool to monitor these species before they can oviposit and to track population densities throughout the growing season for evaluating control strategies.

**Materials and Methods**

Three commercially available lures from Alpha Scents, Inc (Canby, OR) were used in this experiment: a banded cucumber beetle lure (Banded) containing eugenol and 4-methoxycinnamaldehyde (ASIl, 2021), a spotted/striped cucumber beetle lure (Spotted) containing indole, trans-cinnamaldehyde and 1, 2, 4-trimethoxybenzene (ASIlb, 2021) and an ethyl
alcohol (Ethanol) lure. Each of the lures were placed on 25 cm X 25 cm double-sided yellow sticky cards that were attached to metal stakes approximately 60 cm above the ground (Fig. 4.1) in five fields of Beauregard sweetpotatoes in northeast Mississippi. The fields were located near the communities of Starkville, Woodland, West Houlka, Atlanta, and Pontotoc, MS. The traps were checked weekly from July 16 to mid-October (apart from inclement weather on two occasions). Each location included one replication of each trap. Traps were placed at 7.6 m intervals along the border of each field. The sticky cards were replaced each week while the lures were replaced every two weeks. Twenty-five sweeps using a 38 cm sweep net were performed at each site every time the sticky cards were checked. Larval Baits were made using two methods: corn/wheat and rolled oats/honey. For the corn and oat bait, equal parts untreated corn and wheat were soaked for 24 hours and then placed in a mesh stocking deposited in a hole in the soil 20 to 25 cm deep. The wheat and honey mixture consisted of 1 kg of rolled oats, 200 mL of raw clover honey, and 1 quart of water. These ingredients were hand mixed and rolled into balls approximately 6 cm in diameter and placed in a mesh stocking and deposited into the soil in the same manner as described for the corn and wheat bait.

Results and Discussion

The larval bait traps yielded zero larvae for cucumber beetles or wireworms in the two years tested. A total of 168 cucumber beetles were captured by all sampling methods over the course of the season, 149 of which were banded and 19 of which were spotted. A total of 13 banded cucumber beetles were captured on banded lure traps, while 113 banded cucumber beetles were captured using a sweep net (Table 4.1). Cucumber beetles of both species were captured by lures each month tested except for July, when only 1 spotted cucumber beetle and no banded cucumber beetles were trapped. No sweep net data were collected in the beginning of the season.
as the plants were too small for sweep net sampling. Sweep net sampling was initiated approximately 30 days after transplanting. Fig. 4.2 shows that on some dates, the sweep net collections for banded cucumber beetles were more than 12 times the economic threshold, but the kairomone traps caught very few banded cucumber beetles. Correlation analyses (Proc Corr, SAS 9.4, SAS Institute, Cary, NC) showed a positive correlation between sweep net sampling and banded lure traps for *D. balteata* (R = 0.37; P = 0.008) and between sweep nets and spotted lure traps for *D. undecimpunctata* (R= 0.15; P = 0.28). Although the correlation was significant for banded cucumber beetles, no economic threshold for these lures could be determined. The banded lure seemed to be much less efficacious when compared to the sweep net samples after September 10th. We see a steady incline in population densities from sweeps from that point until almost the end of harvest, but the banded lure did not follow the same trend. There were too few non-zero trap catches for the ethanol lures to enable statistical analysis for any species. Catches of *D. balteata* varied by sampling method (F=46.64; df = 3,230; P <0.0001). Catches of *D. undecimpunctata* did not significantly vary by sample method (F = 2.29; df = 3,230; P = 0.08). These data suggest that the commercial lures were significantly less effective than sweeps for sampling banded cucumber beetles, which was suggested by previous research. The *D. undecimpunctata* lure captured more banded cucumber beetles over the course of the season than the *D. balteata* lure. The density of spotted cucumber beetles was too low to provide a meaningful test of sampling efficiency with the lures. There is still a need to find an alternative to the sweep net for monitoring cucumber beetles in sweetpotatoes. Based on our findings, further research for spotted cucumber beetles should test various concentrations and mixtures of kairomones, while banded cucumber beetle monitoring could be more successful using sex pheromones as the attractant rather than kairomones (McLaughlin et al., 1991).
### Table 4.1  Summary of Trap Catches by Sticky Cards\Kairomone Lure and Sweeps

<table>
<thead>
<tr>
<th>Sampling Method</th>
<th># samples</th>
<th>Total <em>Diabrotica</em> spp. Captured</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>D. balteata</em></td>
</tr>
<tr>
<td>Banded lure</td>
<td>50</td>
<td>13B</td>
</tr>
<tr>
<td>Spotted lure</td>
<td>50</td>
<td>16B</td>
</tr>
<tr>
<td>Ethanol lure</td>
<td>50</td>
<td>7B</td>
</tr>
<tr>
<td>Sweep Net</td>
<td>50</td>
<td>113A</td>
</tr>
</tbody>
</table>
Figure 4.1  25 cm X 25cm yellow sticky trap and lure affixed to metal stake (pictured left to right Topashaw Farms – June 17, July 17)
Figure 4.2  Mean (±SEM) number of *D. balteata* captured by trap type by date in Mississippi during 2020. A sample for banded was one 25 x 25 cm sticky card in a week. A sample for sweeps was 25 sweeps with a 38 cm diameter sweep net in sweetpotato foliage. The threshold for *D. balteata* using a sweep net is 0.5/25 sweeps.
References

Alston D.G., and Worwood D.R. 2008. Western striped cucumber beetle, Western spotted cucumber beetle (*Acalymma trivitatum* and *Diabrotica undecipunctata undecipunctata*). Utah Pests Fact Sheets. ENT-118-08. Utah State University Extension and Utah Plant Pest Diagnostic Laboratory.


Reed, J.T., Shankle, M.W., Williams, M.R., and Burdine, W. 2010. Results of southern sweetpotato IPM project in Mississippi. Mississippi Agricultural and Forestry Experiment Station Bulletin 1181


APPENDIX A

SUPPLEMENTARY DATA
Table A.1  List of VOC’s Identified in Plant Defense

<table>
<thead>
<tr>
<th>Compound</th>
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<tbody>
<tr>
<td>Cis-Hexenylacetate</td>
</tr>
<tr>
<td>2-Ethylhexanol</td>
</tr>
<tr>
<td>Cis-beta-Ocimene</td>
</tr>
<tr>
<td>Trans-beta-Ocimene</td>
</tr>
<tr>
<td>Linalool</td>
</tr>
<tr>
<td>Nonanal</td>
</tr>
<tr>
<td>2-Ethenyl-cyclohexan</td>
</tr>
<tr>
<td>C_{11} Homoterpene</td>
</tr>
<tr>
<td>Cis-3-Hexen-1-yl-butyrate</td>
</tr>
<tr>
<td>Methyl Salicylic Acid</td>
</tr>
<tr>
<td>Decanal</td>
</tr>
<tr>
<td>Cis-3-Hexenylhexoate</td>
</tr>
<tr>
<td>Cis-Jasmone</td>
</tr>
<tr>
<td>Beta-Carophyllene</td>
</tr>
<tr>
<td>Trans-Geranylacetone</td>
</tr>
<tr>
<td>Cis-3-Hexen-1-ol-benzoate</td>
</tr>
<tr>
<td>4,8,12-Tetramethyldeca</td>
</tr>
<tr>
<td>Methyl Jasmonic Acid</td>
</tr>
<tr>
<td>Stearyl Acetate</td>
</tr>
<tr>
<td>Palmitinic Acid-Isopropylester</td>
</tr>
<tr>
<td>1,3,7,11-tetraene</td>
</tr>
</tbody>
</table>
Figure A.1 2017 Pontotoc mean (±SEM) yield in kg/25 row feet, separated by size. Cultivars with the same letters are not significantly different from each other. Analyzed using the GLIMMIX procedure (SAS, 9.4)
Figure A.2 2017 Mean (±SEM) percent WDS damage and holes per 50 Potatoes. Cultivars with the same letter are not significantly different. Analyzed using the GLIMMIX procedure (SAS, 9.4)
Figure A.3 2018 Mean (±SEM) of percent insect damage in Starkville and Pontotoc. Analyzed using the GLIMMIX procedure (SAS, 9.4)
Figure A.4  2018 Mean (±SEM) WDS holes per 50 roots in Starkville and Pontotoc. Analyzed using the GLIMMIX procedure (SAS, 9.4)
Figure A.5  2018 Yield as a percentage of Beauregard in Starkville and Pontotoc. Percentages higher than 100% yielded more sweetpotatoes than Beauregard. Analyzed using the GLIMMIX procedure (SAS, 9.4)
Figure A.6  2019 Mean (±SEM) marketable yield in Starkville. Data Analyzed using the GLIMMIX procedure (SAS, 9.4).
Figure A.7  2019 Mean (±SEM) WDS damage in holes per potato in Starkville. Data analyzed using the GLIMMIX procedure (SAS, 9.4).
Figure A.8  2020 Mean (±SEM) WDS damage in Starkville. Damage is in holes per potato. Analyzed using the GLIMMIX procedure (SAS, 9.4)
Figure A.9  2020 Mean (±SEM) marketable yield in Kg. Data analyzed using the GLIMMIX procedure (SAS, 9.4)