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# Effects of Virus Infection and Volatiles on Aphid Virus Vector Behavior on Sweetpotato

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# EFFECTS OF VIRUS INFECTION AND VOLATILES ON APHID VIRUS VECTOR BEHAVIOR ON SWEETPOATO

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## Abstract

Sweetpotato is affected by a wide variety of viruses worldwide, which can cause yield losses of up to 90%. Many of these viruses are transmitted by aphids in a non-persistent manner. Non-persistent viruses are acquired and transmitted within minutes, and thus conventional control, such as insecticides, are ineffective. Altering aphid movement and feeding behavior may affect the rate of virus transmission. One potential method to alter aphid behavior is volatile organic compounds (VOC), including volatiles emitted by virus infected plants, plant hormones, and commercial control agents. Aphid movement near sweetpotato fields was monitored to determine trends throughout the growing season. Low vector numbers were recorded through the entire season, consistent with previous research. Volatiles for infected and uninfected sweetpotato were collected to determine what effect virus infection has on volatile emission. Infected plants emitted a greater diversity of volatiles than uninfected plants. The effect of virus infection, as well as volatile compounds methyl jasmonate (MEJA), methyl salicylate (MESA), stylet oil and neem oil, with the potential to alter aphid behavior, were tested. Y-tube and settling assays were performed with green peach aphid (GPA) and cotton aphid (CA). GPA was more attracted to virus infected than uninfected plants, as well as plants and MESA odor. GPA was less attracted to plants and MEJA or neem oil odor than plants alone. Orientation towards volatile sources did not always correspond to settling preference as GPA preferred to settle on uninfected plants and plants treated with neem oil as well as MESA treated plants. CA did not orient towards volatiles or show any settling preference in any treatment. The electrical penetration graph technique (EPG) was performed to determine the effect of headspace volatiles from the four compounds on aphid feeding behavior related to virus transmission on infected and uninfected plants. In all treatments tested, headspace volatiles altered behavior related to virus

transmission. However, headspace did not affect virus transmission rates in either aphid in virus transmission assays, suggesting that changes in aphid feeding behavior were not enough to alter transmission efficiency.

# Chapter 1. Introduction

## 1.1. Justification

Sweetpotato, *Ipomoea batatas* (L.), is a member of the morning glory family, Convolvulaceae, and is the seventh most important food crop worldwide (Thottappilly 2009). Sweetpotato has high nutritional value, with high levels of vitamin A and protein, and can be cultivated with low inputs, making it an ideal crop for developing countries (Jansson and Raman 1991). In developed countries, it is a high value specialty crop, which can be processed into a variety of foodstuffs and other commercial products including flour and animal feed (Thottappilly 2009).

Sweetpotato production is subject to a variety of weeds, pests, and pathogens, each with their own distinct management challenges. Some sweetpotato pests, such as the sweetpotato weevil and wireworms, are cryptic, feeding on the subterranean storage roots. Other pests such as aphids and whiteflies transmit viruses, such as the potyvirus *Sweetpotato feathery mottle virus* (SPFMV), the most common sweetpotato virus worldwide (Loebenstein et al. 2009). SPFMV, in combination with the whitefly transmitted crinivirus *Sweetpotato chlorotic stunt virus* (SPCSV), causes sweetpotato virus disease (SPVD) which can cause yield losses of over 50% (Loebenstein et al. 2009).

Due to the economic importance of sweetpotato viruses, growers implement a variety of management tactics to control them. These include the use of virus tested seed stock to reduce the level of primary inoculum in a field, host plant resistance to viruses and their vectors, and the removal of weedy hosts to reduce secondary inoculum (Loebenstein et al. 2009). Insect vectors must be controlled to reduce secondary infection (Moyer and Larsen 1991). However, aphids are difficult to manage due to their ephemeral nature: they require only a few minutes of contact

with their host to transmit nonpersistent viruses. Additionally, many aphid vectors are otherwise not economically pests of crops they transmit virus to, but rather are transient pests moving unpredictably through the landscape. For control of aphid vectored viruses, it is important to prevent viruliferous aphids from landing on a virus host. Aphids perform many host selection behaviors post landing (Döring 2014), including probing the plant. These probes are necessary for both host plant selection and nonpersistent virus transmission, as nonpersistent viruses are acquired and transmitted during cellular punctures performed during probes (Powell 2005).

Insecticides do not provide suitable control of aphid vectors, as insecticides can take hours to kill the aphids (Fereres 2000). Cultural control tactics may prove better at managing aphid vectors. Many of these tactics, such as barrier crops (Fereres 2000), horticultural oil sprays (Loebenstein and Raccah 1980), physical barriers, mulches, and host plant resistance (HPR) (Moyer and Larsen 1991) are deployed in other crops. Some of these tactics, such as physical barriers and reflective mulches, are designed to manipulate vector behavior, preventing them from landing in the crop. Other tactics, such as crop barriers, utilize aphid host finding behaviors and the short-term association between virus and vector to trap the virus in an immune crop.

Many control tactics for nonpersistent virus vector control involve manipulating host finding behavior. A similar strategy, the so called ‘push-pull’ or ‘stimulo-deterrent’ strategy, is successful at manipulating stem borer movement in East Africa. This strategy uses repellent (‘push’) and attractive (‘pull’) stimuli to manipulate the attractiveness of crop plants and distribution of pest insects in a crop system (Cook et al 2007). The prototypical example of the push-pull system intercrops maize with molasses grass (*Melinis minutiflora* P. Beauv.) and Napier grass (*Pennisetum purpureum* (Schumach)) to control stemborers (Khan et al. 2010).

Push-pull strategies can use a variety of stimuli, including olfactory cues, such as host and non-host volatile organic compounds (VOC) and an important step in determining host cues that may attract or repel virus vectors.

Plant volatile profiles are subject to change with plant phenotype. For example, different VOC are emitted after herbivore feeding (Pickett et al. 2012) or application of elicitors such as jasmonic acid (Rodriguez-Saona et al. 2001). These volatiles can alter the plant's relationship with other organisms, inducing a defensive response or attracting natural enemies (Arimura et al. 2009). Thus, by responding to herbivory, plants influence the organisms around them. Virus infection can also change a plant's volatile profile; these changes can make plants more visible or more attractive to vectors to enhance virus spread (Mauck et al. 2010; Jiménez-Martínez et al. 2004).

In sweetpotato, much of the volatile literature has focused on storage root volatiles and their effect on culinary attributes (Cui et al. 2010; Wang and Kays 2003). Several papers have focused on the influence of volatiles on host plant location by the economically important sweetpotato weevil (see Korada et al. 2013 and Wang and Kays 2002 for examples). As a research subject, sweetpotato offers the opportunity to examine the effects of multiple co-infecting viruses on a plant's volatile profile and how this affects virus vectors.

Beyond their use in the push-pull system, there is precedent for using VOC in pest management. Sex pheromones are used to enhance trap cropping in papaya (Shelton and Badeness-Perez 2006), and methyl salicylate, an herbivore induced plant volatile, is attractive to natural enemies of *Aphis glycines* in soybean (Zhu and Park 2005). Lures containing isolates or synthetic versions of virus-induced VOC could be used to enhance trap and border crops, that is, secondary crops planted in conjunction with the main crop to divert pests away from, or prevent

pests from entering, the main crop. Trap and border crops could be left untreated as a harbor for natural enemies (Shelton and Badeness-Perez 2006), or sprayed with an insecticide to kill vectors (Foster and Harris 1997). Alternatively, genetically engineering crops to express VOC would reduce costs associated with lures (Shelton and Badeness-Perez 2006). However, the area over which these cues are effective is unknown, as very few studies have examined the scale at which volatiles are active (Aartsma et al. 2017). Braasch and Kaplan (2012) found that phenylethyl alcohol manipulated arthropod natural enemy distribution out to 8 m in soybean fields. This suggests that volatile lures might be effective in manipulating herbivore populations as well. Aphids pose a problem in this regard, as their movement is often difficult to track.

Aphids are particularly poor fliers, and thus their initial migration is largely determined by wind currents (Loxdale et al. 1993). As migrating aphids are dispersed by wind currents, they often travel long distances. Aphids respond to the visual contrast between plant and ground in their initial landing, and may make subsequent trivial flights in the search for a suitable host (Döring 2014). While olfactory cues are not a factor in migratory movement, they may have a role in host finding after the initial flight. The role of olfactory stimuli in aphid host finding is poorly explored. Experiments in the laboratory suggest that olfaction has at least some role in aphid host finding. Research by Webster et al (2008) suggests that *Aphis fabae* orients towards host plant volatiles. *Myzus persicae* is attracted to and arrested by volatile blends more so than by single compounds (Ngumbi et al. 2007), which agrees with other research on herbivore host finding (Bruce and Pickett 2011). This suggests that elicitors that induce changes in VOC profile can alter host finding behavior.

Previous efforts at manipulating aphid host preference with chemical elicitors such as cis-jasmone and E-β-farnesene have met with mixed results. Cis-jasmone reduced damson-hop aphid

numbers in winter wheat (Birkett et al. 2000). In contrast, Bruce et al. (2015) genetically engineered wheat to produce the alarm pheromone E-β-farnesene, which was effective at repelling aphids in the lab and greenhouse, but not the field.

The goals of this research were four-fold, with the ultimate goal of better understanding how virus infection status and volatiles affect the spread of sweetpotato viruses. First, I propose to look at the type and number of aphids moving into sweetpotato fields to track potential virus vectors on which to focus our efforts. Second, I propose to determine the volatile profile of sweetpotato, particularly as it relates to virus infection status in order to test if aphids are attracted to VOCs released by virus infection. Third, I propose to examine how virus infection status and exogenous volatiles affect aphid host choice. Fourth, I propose to determine how volatile exposure affects aphid feeding behavior and virus transmission rate in order to begin to understand the mechanisms of VOCs on vector-plant-virus interactions.

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# Chapter 2. Literature Review

## 2.1. Sweetpotato

Sweetpotato, *Ipomoea batatas* (L.), is a member of the morning glory family, Convolvulaceae, originating from Central America (Thottapilly 2009). It is the seventh most important food crop worldwide and is particularly important in developing countries (Kays 2005). Sweetpotato was introduced to Europe from its native range in the 1500's and from there to Asia in the 1600s (Thottapilly 2009). Today, sweetpotato is primarily produced in Asia and Africa. In developing countries, it is a subsistence crop while in developed countries, it is considered a specialty crop.

Sweetpotato storage roots are the main part of the plant consumed and they are nutritionally rich. Sweetpotato roots are high in vitamins B and C as well as vitamin A precursors (van Jaarsveld et al. 2005). There are many varieties of sweetpotato with a great diversity in root flavor, texture, and color. Roots come in a variety of flesh colors, from purple to orange to white. These different flesh colored varieties vary in starch, protein, and secondary compound contents (Ji et al. 2015) and flavors (Thottappilly 2009). White flesh varieties tend to have lower moisture content and a firm, mealy texture when cooked (Thottappilly 2009). Orange flesh varieties are high in β-carotene, a vitamin A precursor and thus, these sweetpotato varieties are an important source of vitamin A in developing countries (Thottapilly 2009).

Sweetpotato is a low input crop and it is tolerant of drought and severe weather (Jansson and Raman 1991; Thottapilly 2009). Sweetpotato provides a consistent crop as it transfers nutrients to the storage roots throughout the growing season and is thus less susceptible to acute stress during production. However, it has higher initial production costs due to its vegetative propagation and greater storage requirements than many seed crops (Kays 2005). Sweetpotato

roots can be processed into a variety of foodstuffs such as chips, jams, and flour and they can also be used as livestock feed. Sweetpotato is also a potential source of bioethanol (Ziska et al. 2009). Other parts of the sweetpotato can be converted into commercial commodities: leaves and shoots can be consumed fresh or pickled or used as livestock feed (Padmaja 2009). Sweetpotato leaves are high in protein (Adewumi and Adebayo 2008) and higher in anthocyanins and phenolics than many commercial crops (Islam 2006).

### 2.1.1. Production

Sweetpotato production varies worldwide. China produces about 70% of all sweetpotato while the United States produces about 5% (FAO 2016). Sweetpotato is typically produced in poorer countries worldwide (Scott and Maldonado 1998), where it may be grown as a subsistence or cash crop. Sweetpotato grown for subsistence has lower quality demands, and production in these areas is often poor (Ebregt et al. 2004). For example, economic surveys of Kwara, Nigeria, suggest that much of the production in this area is limited to small (1 ha) farms run by family members and hired labor with an annual yield of 4-7 metric tons/ha (Adewumi and Adebayo 2008; Fawole 2007). This is far below the 23 metric tons/ha produced in the United States (FAO 2016). Small farm size limits production, as does lack of access to sufficient fertilizers and pesticides (Adewumi and Adebayo 2008). In fact, Adewumi and Adebayo (2008) found a negative correlation between fertilizer use and profit, suggesting inefficient use due to lack of technical knowledge. Lack of credit, as well as poor transportation and storage infrastructure also limit production in this area (Fawole 2007). Storage time can be increased by leaving roots in the ground however this increases exposure to pests and pathogens (Ebregt et al. 2004). Other factors that affect sweetpotato production include poor marketing and uncertain

pricing (Scott and Maldonado 1998), while Kays (2005) suggests that sweetpotato's strong flavor limits its marketability compared to other staple crops such as rice.

In the United States, sweetpotatoes are primarily produced in California, North Carolina, Mississippi, and Louisiana (Smith et al. 2009). The orange-fleshed varieties Covington and Beauregard are most commonly planted (Carpena 2009). Sweetpotato production begins in February or March, with hotbed production. Seed potatoes, purchased from virus tested stock or saved from the previous year's production, are placed in seed beds and covered with plastic mulch, which induces the production of vines or 'slips.' These slips are cut from the seed potatoes between April and June and transplanted into production fields (Smith et al. 2009). Sweetpotatoes are harvested (mechanically or by hand) 110-150 days after planting, as late as November, and graded (Smith et al. 2009). Sweetpotato roots are cured through storage at high temperature and humidity for several days to several weeks after harvest. This technique toughens the skin and aids in wound healing, reducing loss to postharvest disease (Smith et al. 2009). Additionally, curing can affect the chemical composition of the roots, such as altering the concentration of boehmerol and boehmeryl acetate, compounds associated with resistance to sweetpotato weevil (Son et al. 1991). Cured sweetpotatoes may be stored several months to up to a year after harvest in proper storage conditions (Smith et al. 2009).

**2.2. Sweetpotato Pests**

In the United States, sweetpotato is attacked by a variety of insects in the field, including foliar herbivores such as *Diabrotica* and a lepidopteran complex (Sorenson 2009), and root herbivores including white grubs (Scarabaeidae), wireworms (Elateridae), *Diabrotica*, flea beetles (*Systena*) (The WDS complex) and the sweetpotato weevil (SPW), *Cylas formicarius* (Fabricus) (Chafalt et al. 1990). Aphids and whiteflies also feed on sweetpotato and are the most

common vectors of sweetpotato viruses. Foliar pests are not particularly important, and usually do not require treatment (Sorenson 2009). Sweetpotato can withstand high levels of defoliation, as root formation occurs during the entire season. Defoliation may cause temporary delay in root growth, which can affect harvest time and may expose the crop to further pest damage (Chalfant et al. 1990).

Root feeders are the most economically important herbivores in sweetpotato, as they directly injure the roots, resulting in loss of harvestable tissue and aesthetic damage that reduces marketability. Damage by larval SPW causes roots to produce unpalatable terpenes that make roots unfit for sale (Uritani et al. 1975). Current control of these pests includes chemical and cultural control techniques. Liquid chemicals or soil fumigation can be effective against soil dwelling root feeders, however, many of these pests are cryptic, residing in the roots, and these treatments may therefore not be effective (Chalfant et al. 1990). Transgenic sweetpotato expressing Bt proteins may be a control option against SPW, however, current transgenic plants are not yet effective, providing poor control against SPW (Moran et al. 1998; Rukarwa et al. 2013).

As even minor SPW infestations may cause heavy yield losses and result in unmarketable product, SPW control focuses on keeping the insect out of the field. Quarantines are used to keep SPW confined to its current range (Chalfant et al. 1990). Males are attracted to a sex pheromone, which may be used to track movement and potentially disrupt mating (Sorenson 2009). Additional tactics to prevent weevil infestation include use of clean planting material, clearing of crop debris and weedy hosts, intercropping, and crop rotation, all of which work to disrupt the weevil life cycle; (Chalfant et al. 1990). Planting in suitable soil that does not crack or modifying

soil (via banking or irrigation) to reduce cracking impedes SPW access to roots, and thus reduces root infestation (Chalfant et al. 1990).

Several natural enemies of SPW exist, including parasitoid wasps, nematodes, and fungi. Nematodes in the families Heterorhabditidae and Steinernematida attack SPW, as does the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium brunneum*. Biological control varies in effectiveness against SPW (Chalfant et al. 1990; Schalk et al. 1993). Entomopathogenic fungi are effective against SPW, however, they require contact with enough conidia, which does not often happen in the field (Yasuda 1999). Host plant resistance (HPR) is also used against SPW. HPR results in reduced preference (antixenosis) for and reduced oviposition on sweetpotato roots (Barlow and Rolston 1981; Chen 2017; Hue and Low 2015). One factor of HPR against SPW is the chemical composition of the sweetpotato root periderm. Boehmerol and boehmeryl acetate are oviposition stimulants and low levels of boehmeryl acetate are associated with SPW resistance (Son et al. 1991) while other compounds, such as caffeic acid are associated with decreased larval survivorship (Stevenson et al. 2009).

The WDS complex is another major problem for United States growers. The complex is usually managed with insecticides such as bifenfrin Insecticides are incorporated into the soil before planting or as a layby treatment, despite its component insects varied life histories (Chalfant et al.1990). Wireworms that attack sweetpotato often have multi-year life cycles, and thus can be managed to a degree with monitoring via trapping, removal of weedy hosts, and crop rotation (Sorenson 2009). Insecticides used for WDS may be incorporated into the soil during slip transplantation or applied as liquid or granules during production. Proper irrigation aids the uptake of insecticides into the soil (Chalfant et al. 1990).

Several sweetpotato insect pests also feed on roots post-harvest. These include invasive pests such as SPW, *Euscepes postfasciatus*, and the sweetpotato vine borer, *Omphisa anastomosalis* (Follett 2006). SPW is a particularly harmful post-harvest pest in developing countries that do not have adequate storage facilities (Ray and Ravi 2005). These pests also threaten quarantines. They and other minor storage pests may be treated with insecticides such as phosmet post-harvest (Smith and Beuzelin 2015), or with gamma irradiation (Follett 2006). Curing of sweetpotato may also alter root characteristics to deter pests (Son et al. 1991).

### 2.2.1. Sweetpotato Pathogens

Sweetpotato is subject to a variety of pathogens in the field, including fungus, bacteria, and viruses. Root borne diseases, those caused by infected seed roots, include scurf (caused by *Monilochaetes infuscans*), black rot (caused by *Ceratocystis fimbriata*) and foot rot (caused by *Plenodomus destruens*). They may be controlled by crop rotation and the use of disease free propagation material (Clark et al. 2012). Soil borne diseases, those present in the soil before planting, include sclerotium rot, *Streptomyces* rot, and fusarium wilt (Clark et al. 2012). These diseases may persist in the soil for many years and are controlled via crop rotation and the use of resistant cultivars (Clark et al. 2012). Foliar diseases, such as stem and leaf scab and *Alternaria* blight may affect yields in some growing areas. They are controlled with fungicides and resistant cultivars (Clark et al. 2012). Bacterial root and stem rot and *Rhizopus* soft rot affect sweetpotato post-harvest and can quickly destroy stored roots (Clark et al. 2012). The prevention of wounds in harvested sweetpotato, curing, the use of resistant cultivars, and the application of pesticides can prevent post-harvest losses.

## 2.3. Sweetpotato Viruses

Sweetpotato is subject to 30 different viruses from 9 families (Clark et al. 2012). Sweetpotato viruses are diverse in composition, including ssDNA, dsDNA, and plus and minus strand ssRNA viruses of varying size (Clark et al. 2012). Sweetpotato viruses are transmitted in a variety of ways, including mechanical transmission, persistent, semipersistent, and nonpersistent transmission by aphids and persistent and semipersistent transmission by whiteflies (Loebenstein et al. 2009). Multiple virus infection can cause yield losses of 20-40% in the United States, and 80-90% in East Africa (Clark et al. 2012).

Many sweetpotato viruses have symptomless infections, making identification difficult without access to molecular techniques. Furthermore, many viruses are only present at low titer, and may not be detected, such as SPFMV when not coinfecting with SPCSV (Loebenstein et al. 2009) or may only be detected by grafting onto an indicator plant (such as *I. setosa*) which displays symptoms (Green et al. 1988). Grafting is laborious and time consuming compared to molecular techniques. Furthermore, sweetpotato viruses may also have complex interactions within the plant, affecting virility, and thus affecting detection.

The most notable example of sweetpotato virus interactions is SPVD caused by the interaction between SPCSV and SPFMV (Loebenstein et al. 2009). Individually, SPFMV causes little to no yield loss, and SPCSV causes moderate (30%) loss (Clark et al. 2012). However, combined, SPVD causes yield losses of upwards of 50% to 90% in Africa (Loebenstein et al. 2009). In plants with SPVD, it appears that SPCSV synergizes SPFMV, resulting in up to a 600-fold titer increase (Clark et al. 2012). This is probably caused by its ability to affect host RNAi (Clark et al. 2012). SPVD is common in Africa, South America, and parts of Europe, however it is not present in other growing regions, notably North America. It is unknown why this disease is

geographically isolated despite the presence of both components in nearly all sweetpotato growing locations (Clark et al. 2012).

SPFMV also commonly occurs in mixed infections with other potyviruses such as *Sweetpotato virus G*, *Sweetpotato virus C*, and *Sweetpotato virus 2*. Infections of multiple potyviruses increase yield loss, probably due to the viruses' shared molecular machinery (Syller, 2011). However, naturally infected sweetpotato had significantly higher yield losses than those artificially infected with mixed potyviruses (Clark and Hoy 2006). This is probably due to the presence of unknown viruses in the field, such as the poorly characterized, symptomless, whitefly transmitted begomoviruses (Clark et al. 2012). These so called 'sweepoviruses' can reach high titers despite their lack of symptoms (Clark et al. 2012).

### 2.3.1. Virus control

Currently, the most effective way to control viruses is by using uninfected propagation material. This reduces the amount of primary inoculum in the field (Lobenstein et al. 2009). This can be accomplished to a degree by selecting symptomless vine cuttings, however, meristem tip culture is much more effective. Meristem tip culture uses the apical tip of the meristem to propagate new plant material. This tissue is not connected to the plant vascular system and therefore likely to be virus free (Wang and Charles 1991). Currently the United States has virus tested (VT) seed stock programs which provide tissue free of known viruses to farmers. These programs have expanded into other countries but are not usually employed by subsistence farmers (Clark et al. 2012). The disadvantage of these programs is that farmers must purchase this stock every year, as VT sweetpotato will inevitably acquire viruses over the course of the season, resulting in reduced yield in subsequent seasons. Sweetpotato grown from virus infected material may lose some viruses via a process known as viral reversion. Viral reversion appears to

be an RNAi mediated gene silencing process in which low titer viruses are eliminated from the plant (Clark et al. 2012).

RNAi is also one of the focuses of transgenic virus resistant sweetpotato to prevent SPVD by creating dsRNA of SPFMV RNA polymerase. However, it does not yet appear to be effective in the field (Kreuze et al. 2008). Other transgenic approaches include coat protein expression and rice cystein proteinase inhibitor (ORI) expression. Because viral coat proteins are recognized and induce a defensive response in plants, transgenic plants expressing coat proteins are resistant to that and related viruses (Beachy et al. 1990). ORI expression appears to inhibit the potyvirus cystein proteinase that modifies the potyvirus viral poly protein (Gutierrez-Campos et al. 1999). It may also be effective against SPCSV, which has a similar protein (Clark et al. 2012). Transgenic resistance is attractive in sweetpotato because breeding this crop is difficult: sweetpotato is a hexaploid with 90 chromosomes (Valverde et al. 2007).

Traditional breeding has produced some varieties with HPR effective against some viral diseases. Resistant varieties may be effective against sweepoviruses. Several varieties, such as NASPOT1 and NASPOT11 are resistant to SPVD (Clark et al. 2012). Resistant varieties appear to acquire viruses less frequently, and may have fewer symptoms, lower virus titers and higher yields (Mpembe et al. 2011). They may also undergo viral reversion at a higher rate. (Clark et al. 2012) Resistance to vectors must also be considered, as it is effective against persistently transmitted viruses.

Cultural control tactics may be used to control viruses. Removal of weedy hosts (*Ipomoea sp*.) prevents secondary inoculum from entering the field (Loebenstein et al. 2009). Rogueing, the removal of symptomatic plants may be effective against whitefly transmitted viruses, as this vector rarely spreads far in the crop (Clark et al. 2012). However, rogueing can

increase the spread of nonpersistent aphid transmitted viruses due to the vector's visual attraction to the gap (Davis et al. 2009). Several other cultural control tactics are used to control the spread of vectors. Stylet oils can also prevent virus transmission, through an unknown mechanism possibly by inhibiting viron adhesion to the stylet (Powell 1992; Simons and Zitter 1980), and can be mixed with insecticides to provide better vector control (Fereres 2000). Barrier crops have also been effective at reducing virus transmission in peppers (Fereres 2000).

### 2.3.2. Vector Control

Control of insect vectored viruses depends on the type of virus transmission. Persistent (such as *Sweetpotato leaf speckling virus*) and semipersistent (such as SPCSV) transmitted viruses require extended contact with the host plant to acquire and transmit while nonpersistent transmitted viruses (such as SPFMV) may be acquired and transmitted in minutes (Whitfield et al. 2015). These viruses differ not only in acquisition and transmission speed, but also in retention by vectors. Nonpersistent viruses are exclusively transmitted by aphids and bind to receptors in the stylet. They are acquired and transmitted in seconds to minutes and lost during feeding activity and upon molt (Whitfield et al. 2015). Semipersistent viruses bind to the stylet or foregut and are acquired and transmitted in minutes to hours. They are retained upon feeding, but lost during molt (Ng and Falk 2006; Whitfield et al. 2015). Persistent viruses are phloem bound in the plant, require hours of feeding for acquisition and transmission, and are circulative in the host body. They require a latent period before transmission and are retained upon feeding and molting (Ng and Perry 2004).

The transmission type of the virus affects management strategies. The use of virus tested propagation material reduces the level of primary inoculum in a field, however, insect vectors must be controlled to reduce secondary infection (Moyer and Larsen 1991). Insecticides provide

mixed control of virus vectors. Thackray et al. (2000) showed that pyrethroids were effective in controlling colonizing aphid vectors of *Cucumber mosaic virus* (CMV) in lupin, however, they did not provide consistent enough control to be used as a management tactic. Furthermore, pyrethroids were not effective in controlling the non-colonizing vector green peach aphid (GPA), which showed high levels of insecticide resistance. Perring et al. (1999) found that most of cases in which insecticides were effective at controlling viruses were semipersistent and persistent transmitted viruses. In nonpersistent transmitted viruses, the vector does not interact with the plant long enough to receive a lethal dose of insecticide. Furthermore, some insecticides agitate vectors, causing them to move to new plants and further spread disease (Fereres and Raccah 2015). Despite this lack of efficacy, insecticides are often used for virus prevention: virus epidemics are difficult to predict, and therefore, insecticides are used as a prophylactic, as cost of application is much lower than the potential cost of a virus infection (Perring et al. 1999). Another factor promoting insecticide use to control vectors may be limited knowledge of, or limited ability, to implement other techniques.

Alternate methods to control vectors in sweetpotato have not been adequately explored. HPR may reduce incidence of some viruses either by reducing feeding or repelling vectors, however, resistance to aphids and whiteflies is poorly explored in sweetpotato (Valverde et al. 2007). A variety of cultural control tactics are employed to reduce virus transmission in other crops, however, they have not been evaluated in sweetpotato (Clark et al. 2012). Currently employed cultural control tactics in other crops include barrier crops (Fereres 2000), oil sprays (Loebenstein and Raccah 1980), removal of secondary inoculum sources, physical barriers, and mulches.

Barrier or border crops are crops planted around the main crop. Barrier crops are effective at reducing incidence of the nonpersistent *Potato virus Y* in potato, which has similar cultivation to sweetpotato (Difonzo et al. 1996). They work through two main mechanisms to prevent virus transmission. Alate aphids are attracted to the contrast between soil and plants when searching for landing sites. (Damicone et al. 2007; Davis et al. 2015) Thus, aphids tend to land at field borders, and barrier crops act as a physical barrier, preventing vectors from entering the main crop (Hooks and Fereres 2006). Borders also act as a 'sink' for viruses: vectors landing on and probing the border crop lose their ability to transmit nonpersistent viruses to the main crop (Fereres 2000).

Barrier crops can also mask the odors of the main crop, preventing vectors from finding the crop; thus, they can be used as a trap crop (Hooks and Fereres 2006). Border crops typically reduce virus incidence in the outer rows but do little to reduce it in the center rows (Difonzo et al. 1996). This could be mitigated by planting border crops that are more attractive to aphids than the main crop, causing aphids to preferentially land on the border crop (Schröder et al. 2015).

Intercropping, the planting of a secondary crop either as a cover crop between rows or in alternate rows (Damicone et al. 2007), can be more effective at preventing virus spread than border crops. Intercrops can also act to repel vectors, as in the push-pull technique discussed later (Cook et al. 2007). Intercropping is more difficult for growers to implement than traditional monoculture due to differences in crop requirements such as nitrogen use (Baumann et al. 2001) and the potential for interspecific competition (Mushagalusa et al. 2008). Interactions between the crops may also negatively impact yield. Damicone et al. (2007) found that while intercrops of peanut (*Arachis hypogaea*), soybean (*Glycine max*), and sorghum (*Sorghum bicolor*) reduced disease incidence in pumpkin (*Cucurbita pepo*), this did not translate into yield gains, and in the

case of sorghum, resulted in yield loss of up to 50% due to competition. In addition to competition, the logistical issues and costs of managing two crops in the same field may make this tactic impractical (Hooks and Fereres 2006).

Physical barriers and mulches also work to prevent vectors from accessing the crop. Physical barriers include row covers and other manmade barriers that physically prevent vectors from reaching the host (Orozco-Santos et al. 1995). Mulches, particularly reflective mulches, work to obscure the visual contrast between plant and soil. Kaolin clay and latex whitewash sprays also increase the reflectivity of plants reducing contrast with the soil; these techniques are compatible with other cultural control techniques (Lowrey et al. 1990).

Oil sprays are low (>1-4%) concentrations of mineral oil sprayed on the plant. They are effective against aphid and whitefly vectors (Singh et al. 1973). They interfere with virus transmission through an as yet unknown mechanism that probably interferes with adherence of virus particles to the stylet (Simons and Zitter 1980), and may be repellent or toxic (Davis et al. 2009). Oil sprays can also be combined with insecticides to reduce vector populations (Lowrey et al. 1990), and are compatible with other tactics, but are phytotoxic at high concentrations (Simons and Zitter 1980). Oil sprays are typically more effective in high density crops. As sweetpotato production fields are typically planted at low density, it may be a less effective tactic in this crop.

Removal of secondary inoculum, in the form of weedy hosts or other crops is important for reducing virus spread. In sweetpotato, this is important because weedy hosts often have high virus titers (Wosula et al. 2012). The clearing of weedy hosts, volunteer plants, and other debris from field edges also reduces infestation by other insect pests (Loebenstein et al. 2009). Planting

sweetpotato away from potential sources of inoculum (such as other susceptible crops) may also reduce the incidence of viral diseases (Davis et al. 2009b; Nault et al. 2004), as may altering planting and harvesting dates (Davis et al. 2009b).

Monitoring and forecast of aphid flight activity may be used in concert with other tactics to reduce virus transmission. Monitoring via pan and suction traps can give an area wide estimate of aphid number and species (Davis et al. 2009b). Other data, such as meteorological data, may be analyzed to predict aphid movement based on temperature and jet stream duration (Davis et al. 2009b). Monitoring insect movement is an important aspect of cultural control, as it can be used to inform the timing of other tactics. For example, timing oil sprays for periods of high aphid density (Clark et al. 2012).

Aphid monitoring is accomplished via trapping and identification. Suction traps record aphids moving throughout the landscape, while pan traps with yellow or green tiles record aphids attracted to landing cues (Radcliff and Ragsdale 2002). Aphids are poor fliers, and long-distance aphid dispersal is generally dictated by atmospheric currents (Irwin et al. 2007). Aphids can travel long distances via jet stream currents colonizing areas in which they cannot overwinter (Zhu et al. 2006). However, aphids are weak fliers, and this dispersal is mostly out of the insect's control. (Loxdale et al. 1993). Aphid landing is mediated by physiological (depletion of energy stores) and physical cues (light wavelength, wind speed) (Irwin et al. 2007). Landing aphids are attracted to contrast and are thus most likely to land at field edges or on widely spaced plants; increased row spacing increases virus infection in potato (Davis et al. 2015). Landing aphids are also attracted to green or yellow-green wavelengths, dependent on species (Irwin et al. 2007).

Long distance flights are followed by subsequent short distance flights to find a suitable host. These flights are influenced by plant cues, including visual and olfactory cues such as plant volatiles.

**2.4. Plant Volatiles**

Plants emit a variety of volatile organic compounds (VOC). The simplest of these is the plant hormone ethylene ($C_2H_4$), which mediates growth and senescence (Sisler and Yang 1984). Other notable volatiles include the six-carbon aldehydes, alcohols, and acetates known collectively as 'green leaf volatiles' (GLV), terpenes ($nC_5H_8$) and terpenoids, and benzenoids. VOC act as both intra- and interplant signals. As a signal, VOC are not constrained by the plant vascular system, and thus can transmit information to other parts of the plant more quickly than a vascular signal as well as reach parts of the plant that are not connected by the vascular system (Heil and Bueno 2007). However, this also means that VOC are dispersed into the environment, and can be used as a signal by any organism that can receive and interpret them. Other plants can use these signals for their own purposes (Kobayashi, and Yamamura 2007).

VOC are synthesized through different pathways. Ethylene, for example, is synthesized from methionine metabolism (Sisler and Yang 1984). Terpenes are synthesized by the methylerythritol phosphate pathway (from pyruvate and glyeraldehyde-3-phosphate) and mevalonate pathway (from acetyl COA) (Dudareva et al. 2004). GLV are synthesized via the hydroperoxide lyase pathway of oxylipin metabolism. Volatiles are typically synthesized in epidermal tissues and may be secreted directly through the cell membrane or stored as liquid in specialized tissues (resin ducts, trichomes, extrafloral nectaries, flowers) (Fahn 1988; Pichersky and Gershenzon 2002). VOC are emitted constitutively but are also emitted due to stress or physical damage to cells (Holopainen and Gershenzon 2010). For example, both GLV and

jasmonic acid are released rapidly after mechanical damage. These VOC are synthesized from the free fatty acids and are produced when a cell membrane is ruptured (Maffei 2010).

VOC perform a variety of functions within and without the plant. VOC produced by flowers function as pollinator attractants (Pichersky and Gershenzon 2002). Isoprene and monoterpenes reduce oxidative stress within and outside plant leaves by reacting with oxygen radicals (Holopainen 2004). Volatiles also reduce abiotic stress including heat, drought and UV light (Maffei 2010). Volatiles are released in response to biotic stress and serve several functions in this regard. They induce defensive responses to herbivores and pathogens in the plant (Arimura et al. 2009) or prime the plant for defensive response against future attack. This can be specific to the attacking organism, or a broader defensive reaction (Stout et al. 1998). The response is modified by how plants perceive injury. VOC are released in response to both mechanical damage and herbivore specific cues, such as the volicitin, a compound in caterpillar saliva that induces volatile production (Alborn et al. 1997). These cues affect the plant's response.

Plant volatiles may be exploited by other organisms. At the lowest trophic level, other plants may receive volatile signals and prime their own defenses (Heil and Karban 2009). This probably evolved to increase fitness among conspecifics. As volatile cues typically only work over a short distance, plants of the same species in this area are likely related, and thus priming by volatiles increases group fitness (Kobayashi and Yamamura 2007). Plants of other species may be primed by wounded plants (Baldwin et al. 2006). The prototypical example of this can be found with wild tobacco priming sage brush defenses, in which wild tobacco downwind of clipped sagebrush increased defensive enzymes which reduced herbivory (Karban et al. 2000).

VOC affect herbivore behavior. Herbivores are typically attracted to the host plant's volatiles blend (Bruce and Pickett 2011). Alterations to this blend (both the number and intensity of components) may affect the insect's ability to find hosts or otherwise affect herbivore behavior (Webster et al. 2010). A stark example of this is that exposure to day or night time volatiles affects *Mythimna separata* behavior on corn, regardless of actual light conditions (Shiojiri et al. 2006). Another example of this is plants that emit the aphid alarm pheromone, E-β-farnesene as defense against aphids. Aphids counter this by reducing their response to the alarm pheromone in the presence of other plant produced terpenes, including β-caryophyllene, another herbivore induced compound (Dawson et al. 1984).

Induced VOC may act as attractants or repellents. This relationship varies with plant and herbivore species. As induced plants often emit more VOC than their un-induced neighbors, they are more visible to herbivores (Dicke et al. 2000). Spider mites, for example, are attracted to volatiles released after feeding by conspecifics, but only up to a certain density before they become repellent (Dicke et al. 2000). GLV may act as a feeding stimulant for *Manduca sexta* on tobacco (Halitschke et al. 2004) and are attractive to the aphid *Toxoptera aurrantii* (Han et al. 2012). GPA perform significantly better on potato plants lacking hydroperoxide lyases, required to synthesize GLV, suggesting these volatiles are used in aphid resistance (Vancannyet et al. 2001). Cis-jasmone deters *Nasonovia ribis-nigri* from lettuce, and reduced aphid numbers in winter wheat, as well as induced other plant volatiles (Birkett et al. 2000). The GLV (Z)-hexenyl acetate acts to deter *Heliothis virescens* oviposition on tobacco (Pichersky and Gershenzon 2002). Oviposition deterrents may be specific to species: *Manduca* caterpillars can distinguish between GLV isomers emitted by plants attacked by conspecifics versus those attacked by other

insects (Allmann et al. 2013). These examples suggest that VOC convey information that allows insects assess their suitability as hosts.

VOC also affect other trophic levels. Floral VOC are used to attract pollinators (Pichersky and Gershenzon 2002) and there may be trade of between constitutive floral VOC and herbivore induced VOC as feeding by herbivores can increase or reduce floral volatile production (Pareja et al. 2012). Induced VOC may be attractive to natural enemies; this has been likened to plants "crying for help" (Dicke 2009) but is the result of natural enemies adapting to use plant volatile cues to locate their food source. Methyl salicylate and other stinkbug induced volatiles attract parasitic wasps in soybean (Michereff et al. 2013). Methyl salicylate is also induced by aphid feeding and is attractive to lacewings and coccinellids (de Vos and Jander 2010; Zhu and Park 2005). Cotton and cowpea GLV attract the parasitoid wasps *Microplitis croceipes* and *Netelia heroica* (Whitman and Eller 1990). Thus, VOC mediate interactions between multiple trophic levels, and herbivory can influence natural enemies and pollinators.

VOC are also induced by pathogens. Volatile oils have antibacterial properties (Dorman and Dean 1999), as do GLV (Scala et al. 2013). GLV have antifungal properties (Kubo et al. 2003), and *Arabadopsis* genetically engineered to produce higher levels of GLV were more resistant to *Botrytis cinereal* (Shiojiri et al. 2006). As GLV are emitted in response to wounding, it is likely this evolved to prevent infection (Scala et al. 2013). Methyl salicylate, the methyl ester of salicylic acid, the plant hormone which mediates pathogen resistance, is released by tobacco plants infected with *tobacco mosaic virus*, suggesting this volatile induces a defensive response to the virus (Shulaev et al. 1997).

Pathogens may also alter plant volatiles to their own gain. Methyl salicylate released by *Candidatus Liberibacter asiaticus* infection in citrus attracts the psyllid vector of this bacterium,

which then acquires the pathogen and moves to more suitable uninfected hosts, spreading the disease (Mann et al. 2012). Virus infection also changes a plant's volatile profile; several studies have shown that virus infection can induce plants to release VOC attractive to virus vectors. *Rhopalosiphum padi* were more attracted to headspace volatiles from *Barley yellow dwarf virus* infected wheat than volatiles from noninfected wheat (Jiménez-Martínez et al. 2004). Both GPA and *Aphis gosypii* were more attracted to CMV infected cucumber than uninfected cucumber, despite infected cucumber being a poorer host (Mauck et al. 2010). The attraction found in both studies may be due to an overall increase in the amount of volatiles released by virus infected plants, which makes them more apparent compared to uninfected plants (Mauck et al. 2010).

Changes to volatile profiles may be due to viruses inducing defense responses. In maize infected with *Maize chlorotic mottle virus*, release of (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), methyl salicylate and (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), commonly defensive volatiles, attracts the thrips vector (Mwando et al. 2018). A similar effect is seen with GLV induced by *Black raspberry necrosis virus* and *Raspberry leafmottle virus* attracting their aphid vector (McMenemy et al. 2012). However, VOC may also be triggered by the virus itself. The CMV 2b gene, in addition to increasing host susceptibility to GPA in tobacco, appears to increase the release of volatiles, although this did not increase aphid attraction in the lab (Tungadi et al. 2017). The CMV 2b protein also interacts with and inhibits jasmonic acid signaling, increasing host attractiveness to vectors (Wu et al. 2017)

**2.5. Integration of VOC into Pest Management**

VOC can influence pest and beneficial insects, which can be exploited for pest management. Essential oils, a product of steam distillation of plant foliage, include a volatile component, and may be used as repellents or contact insecticides against a wide variety of pests

(Isman 2000). These properties appear to come from high concentrations of monoterpenes including eugenol and carvacrol, which may act on the octopamine pathway (Isman 2000). Azadirachtin, the terpenoid main component of neem oil, also has antifeedant and insecticidal activity (Isman et al. 1990). Essential oils have found significant use as organic insecticides for home and garden use (Koul et al. 2008). Essential oils are also effective as fumigants against stored crop pests (Shaaya et al. 1990).

Intercropping works partially via volatile cues. The secondary crop may be more attractive to the pest, acting as a trap crop. An example of this is intercropping of peppers with maize, which acts as a trap crop for *A. gossypii* (Hussein and Samad 1993). Secondary crops may also mask host plant odors. α-pinene from rosemary oil rendered onion volatiles unattractive to *Neotoxoptera formosana* (Hori 1998), and VOC from savory and thyme mask host odors to *Aphis fabae* (Nottingham et al. 1991). Secondary crops may repel pests; planting onion and garlic with mustard reduced numbers of *Lipaphis erysimi* (Sarker et al. 2009). Secondary crop VOC may induce defenses in the main crop, making them less attractive or a less suitable host (Ben-Issa et al. 2017). Finally, secondary crops may attract natural enemies, though this is often not due solely to VOC, but is also due to secondary crops providing additional prey or favorable microclimates.

Plants may be genetically engineered to alter their volatile profile. The most dramatic example of this is 'whiffy' wheat, genetically engineered to produce E-β-farnesene to repel aphids (Bruce et al. 2015). Another proposed idea is increasing the emission of terpenes to attract natural enemies (Degenhardt et al. 2003). Whiffy wheat was promising in the lab and greenhouse, however, failed in the field. VOC are only active over limited distances, limiting their effectiveness in attraction natural enemies, (Braasch and Kaplan 2012). Additionally, there

is the potential for altering the spatial abundance of natural enemies. Braasch and Kaplan found movement of parasitoid braconids towards volatile lures resulted in depletion from the surrounding area. Another issue is habituation. Natural enemies may become habituated to volatile cues, as is the case with *Podius maculiventris* habituated to exogenous methyl salicylate (Vidal-Gomez et al. 2018). Pest insects, too, may become habituated to VOC. Colorado potato beetle became habituated to a synthetic attractant (Martel et al. 2005), and aphids become habituated to their own alarm pheromone (De Vos and Jander 2010).

### 2.5.1. Push Pull System

'Push-pull' or 'stimulo-deterrent' describes pest management strategies that use repellent ('push') and attractive ('pull') stimuli such as plant VOC to manipulate the distribution of pest insects in a crop system (Cook et al. 2007). The push-pull strategy was first developed for use against stemborers in maize in East Africa (Khan et al. 2010). Intercropping maize with the repellent *M. minutiflora* or *Desmodium sp.*, removes stemborers from the crop, while *Desmodium* also controls parasitic *Striga* plants. Adding *P. purpureum*, which is attractive to stemborers, but a poor larval host, provides a sink for the insect population (Khan et al. 2010). This push pull strategy uses a combination of intercrop and trap plant volatiles to control pests, but other stimuli, including visual cues, synthetic volatiles, or host plant VOC can potentially be used for this strategy (Cook et al. 2007).

## 2.6. Objectives

This research has four objectives. First exploring aphid movement in and around sweetpotato fields determine any pattern in aphid abundance, which can affect vector management, and informs when potential volatile based control tactics should be deployed. Second, to identify volatile compounds released by infected and uninfected sweetpotato to

determine how virus infection influences the production of compounds related virus vectors. Third, to examine aphid host choice in regard to both virus infection status and exogenous volatiles with potential for vector management. Fourth, to determine how exposure to volatiles during feeding affects aphid behavior related to virus transmission, and if this in turn affects virus transmission rate to better understand the effects of VOCs on vector-plant-virus interactions.

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# Chapter 3. Monitoring of Aphid Migration and Movement Near Sweetpotato in Louisiana

## 3.1. Introduction

Aphids are major crop pests worldwide, both due to the direct injury they cause and the viruses they transmit. Approximately half of all plant viruses are transmitted by aphids (Ng and Perry 2005). Aphids are poor fliers and their movement is affected by different conditions and stimuli at different levels (Irwin et al. 2007). Aphid movement may be appetitive (towards a food source) or migratory (Irwin et al. 2007). Aphids require still air and a high enough temperature in order to initiate flight and thus usually fly during the day (Loxdale et al. 1993). Migratory aphids are attracted exclusively to ultraviolet light (Irwin et al. 2007), and migratory flights are mainly governed by external forces as once the aphid attains altitude, it is at the mercy of atmospheric conditions. High altitude currents, such as jet streams, govern the aphid's movement and eventual deposition (Loxdale et al. 1993; Zhu et al. 2006). These atmospheric conditions can be important predictors of future aphid infestation and virus incidence (Zhu et al. 2006). Landing aphids typically use visual cues to find plants. Aphids are attracted to the contrast between the plant and soil (Fereres et al. 1999). This typically attracts aphids to the edge of fields (A'Brook 1968). Aphids are also attracted to yellow green and saturated yellows (Kennedy et al. 1961; Robert 1987). Olfactory cues, such as host and non-host odors, could also have a role in determining landing sites (Nottingham and Hardie 1993).

In the field, migratory aphids can be categorized into four different types, after Irwin et al. 2007. These four types are transient non-vectors, transient vectors, colonizing non-vectors, and colonizing vectors. Transient non-vectors are aphids that do not regard the plant as a host and pass through while searching for a suitable host. These aphids are not considered pests. Transient vectors do not colonize the plant; however, they may vector viruses, introducing

secondary inoculum (infectious material not initially present at the beginning of the growing season) into the field. Colonizing non-vectors feed on the plant but do not transmit viruses. However, they may be economically important pests in their own right due to the injury they cause to plants (Östman et al. 2003). Colonizing vectors both feed on the plant and transmit viruses. Due to their extended contact with their host, they often transmit persistently transmitted viruses.

In sweetpotato, viruses are often transmitted by transient vectors (Wosula et al. 2013). Predicting the arrival of these vectors may allow for the implementation of better management tactics (Irwin et al. 2007). Previous research on sweetpotato in Louisiana examining aphid landing rates and virus spread found high variability in species composition and landing rates, with peaks in the early and late summer (Wosula et al. 2013). This research examines aphid landing rates as well as local aphid movement via trapping to determine if there is any change in aphid and vector abundance in sweetpotato fields, as identifying patterns in vector abundance allows for the better implementation of management strategies.

## 3.2. Methods

Aphid populations in the field were monitored by sticky cards, pan traps, and by suction trap. Pan traps and sticky cards were used in 2015 and 2016 in a single field at the the Burden Central Research Station (Baton Rouge, Louisiana, 30.41°N, 91.11°W), and 2015 in a single field at the Sweetpotato Research Station in Chase, Louisiana. The suction trap was operated from 2016 to 2018 in Chase, Louisiana (32.10°N 91.70°W).

### 3.2.1. Sticky Cards

Yellow sticky cards were used to obtain total aphid population counts. Yellow sticky traps (7.35 by 12.25 cm) (Whitemire Micro-Gen Research Laboratories Inc., St. Louis) were

attached to stakes with binder clips (Staples Inc., Framingham, MA) approximately 1 m above the ground and a single trap was placed at each of the four corners of each field. The traps were exposed for weekly intervals (16 June to 18 August (Chase, 2015), 3 June to 19 August (Burden, 2015), and 5 April to 6 August (Burden, 2016) before collection and replacement. Sticky cards were wrapped in plastic film to prevent them from sticking to each other, returned to the lab, and stored in a freezer until total aphid counts were performed under a dissecting scope.

### 3.2.2. Pan Traps

Yellow and green pan traps were used to monitor aphid species composition. Pan traps were constructed of rectangular 1.4 liter plastic containers (Servin Saver; Rubbermaid, Wooster, OH) with a yellow or green 7.35 by 7.35 cm ceramic tile (Imola, Cooperativa Ceramica D' Imola S.C., Vittorio Veneto, Italy) in the center. The trap was filled with 50 mL of a 50:50 (v:v) solution of water and propylene glycol (Chemistrystore.com, Cayce, SC). One pan trap with a yellow tile and one pan trap with a green tile were placed at the same location as the sticky cards in tomato wire cages, just above the sweetpotato canopy for weekly intervals (16 June to 18 August (Chase, 2015), 3 June to 19 August (Burden, 2015), and 5 April to 6 August (Burden, 2016). In the lab, aphids were removed from the water/glycol mixture with tweezers and put into scintillation vials filled with 95% ethyl alcohol and then identified under a compound scope after slide mounting. Aphids were cleared overnight in a 10% sodium hydroxide solution (Mallinckrodt Chemicals, Staines-Upon-Thames, UK) at 4.4°C. The body cavity was pierced with a #0 black enameled insect pin (Entomoravia, Slavkov u Brna, Czech Republic), and body contents removed. Aphids were transferred to distilled water, then mounted on a slide (Fisher

Scientific, Pittsburgh, PA) in CMC-10 mounting media (Master's Company, INC, Bensenville, IL). Aphids were identified to lowest possible taxonomic level (genus or species) with aid of taxonomic keys by Pike et al (2003) and Smith et al (1992).

#### 3.2.3. Suction Trap

The suction trap was operated after Bahlai et al. 2014 and Lagos-Kutz et al. 2018. Briefly, the trap samples air 6.7 m above ground at a rate of approximately 570 $m^3$/h. The trap was operated daily from 7:00AM to 8:PM April to December in 2016, May to October in 2017, and May to September in 2018. Aphids were collected weekly by J. Ronsonet and shipped to D. Lagos-Kutz for identification.

### 3.3. Results

#### 3.3.1. Sticky Cards

Average aphid counts per week are recorded in Figure 3.1. Counts vary by site and year but suggest a late season spike in aphid collection.

#### 3.3.2. Pan Traps

Aphid species and number from both years are recorded in Table 3.1. More aphids were collected the second year, representing a more diverse group of species.

#### 3.3.3. Suction Trap

16 pest aphid species were identified from suction trap collection. Table 3.1 records the aphid species collected and the total number of each species collected each year; Table 3.2 shows the percentage of total aphids captured each species represents as well as the average percentage of the total each aphid species comprised over the three year study period. The sugarcane aphid, *Melanaphis sacchari*, comprised an average of 70.6% of all aphids collected over the three years sampled. Known sweetpotato virus vectors (see Wosula et al. 2013) were an average of 8.9% of

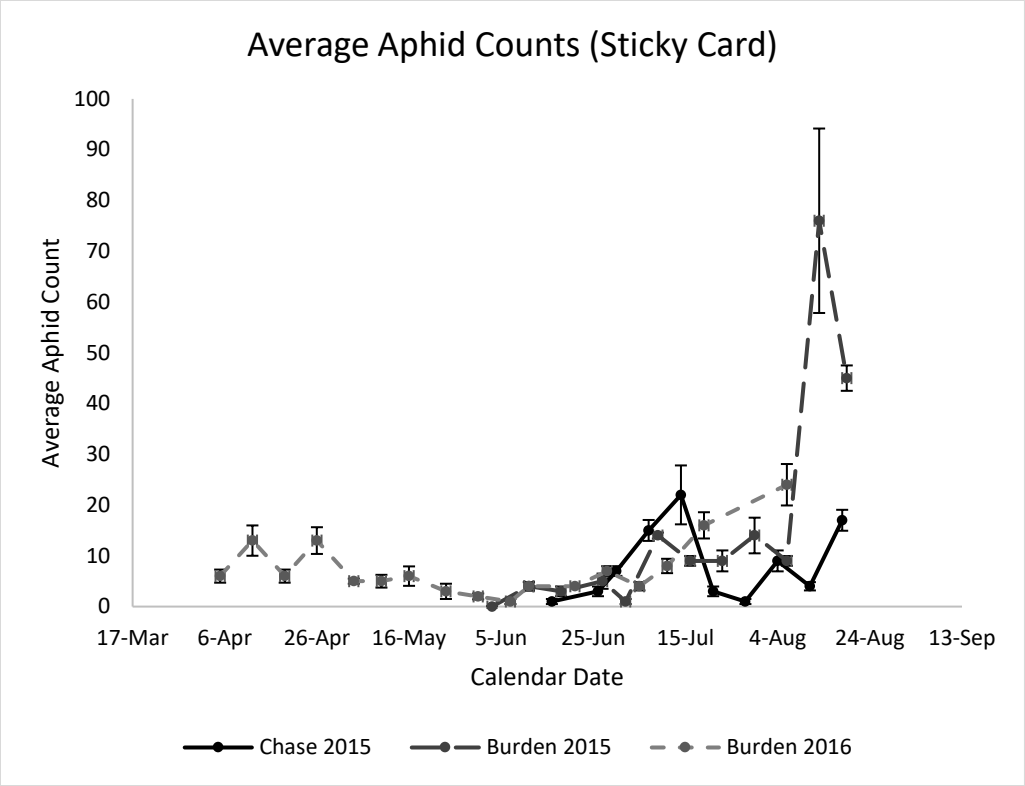


Figure 3.1: Number of aphids collected with sticky cards on each date by site and year. Sticky cards were collected from 16 June to 18 August (Chase, 2015), 3 June to 19 August (Burden, 2015), and 5 April to 6 August (Burden, 2016). Data points indicate average number of aphids collected on the four sticky cards per field, ± s.e.

Table 3.1: Total number and species of aphids identified from all pan traps by year. 2015 count includes aphids from two separate sites, 2016 includes only one site. Pan traps were collected from 16 June to 18 August (Chase, 2015), 3 June to 19 August (Burden, 2015), and 5 April to 6 August (Burden, 2016)

| **Species** | **2015** | **2016** |
|---|---|---|
| *Aphis armoraciae* | 0 | 6 |
| *Aphis craccivora* | 2 | 5 |
| *Aphis gossypii* | 1 | 3 |
| *Aphis nasturtii* | 0 | 2 |
| *Cavariella sp.* | 0 | 1 |
| *Eriosoma sp.* | 0 | 1 |
| *Hyperomyzus pallidus* | 0 | 1 |
| *Melanaphis sacchari* | 1 | 3 |
| *Rhopalosiphum insertum* | 0 | 1 |

the aphids collected over three years. The total number of aphids collected by date for each of the three years is recorded in Figure 3.2. Total numbers of the 6 known virus vectors collected by date each year is recorded in Figure 3.3.

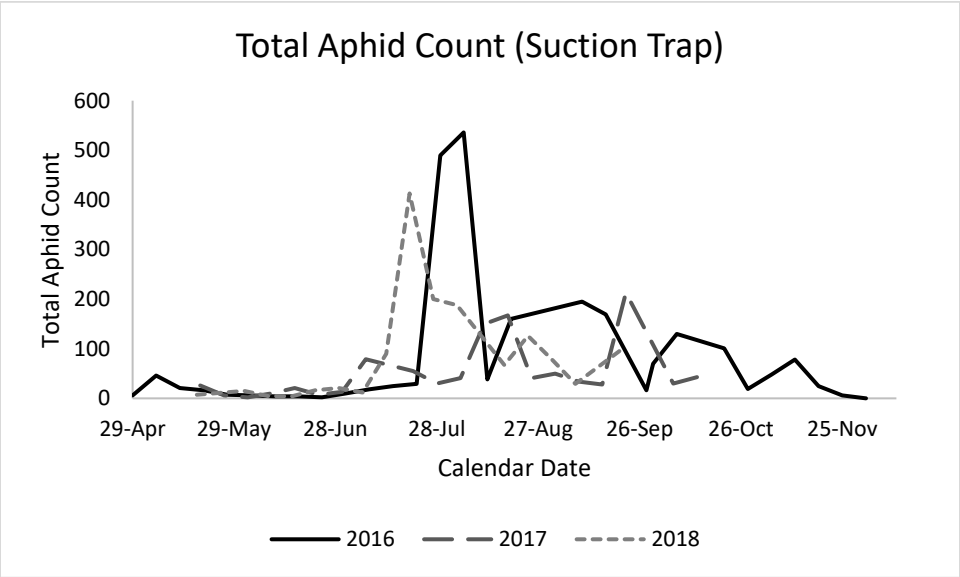


Figure 3.2: Total number of aphids collected by the suction trap, by collection date. The trap was operated daily from April to December in 2016, May to October in 2017, and May to September in 2018. Aphids were collected weekly.

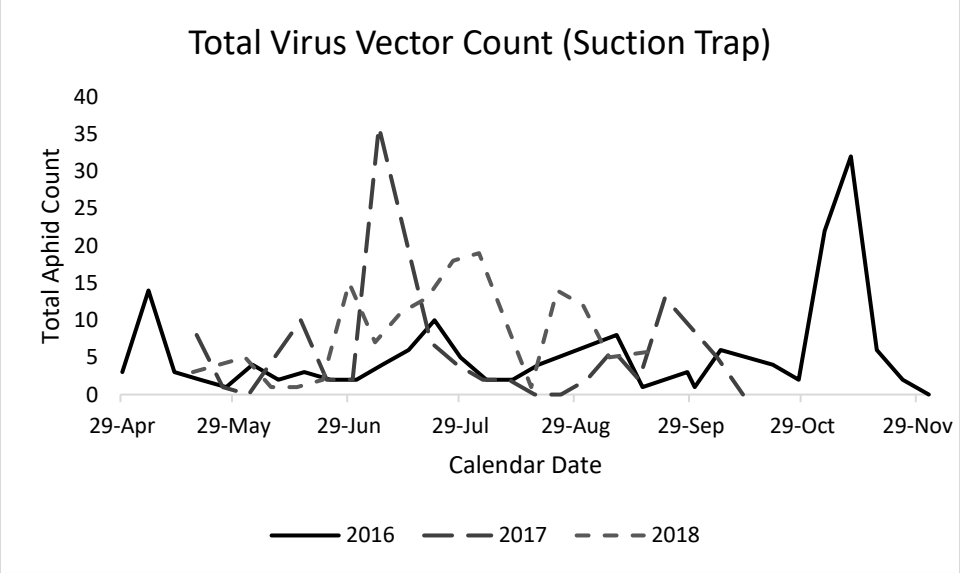


Figure 3.3: Total number of the 6 known vector species collected by suction trap, by collection date. The trap was operated daily from April to December in 2016, May to October in 2017, and May to September in 2018. Aphids were collected weekly.

Table 3.2: Total number of aphids of each aphid species collected by the suction trap, by year. Aphids are listed alphabetically and species names in bold are known virus vectors of sweetpotato viruses.

| Species | Count (2016) | Count (2017) | Count (2018) |
|---|---|---|---|
| *Acyrthosiphon pisum* | 7 | 2 | 0 |
| ***Aphis craccivora*** | 16 | 5 | 21 |
| *Aphis glycines* | 1 | 2 | 0 |
| ***Aphis gossypii*** | 76 | 54 | 85 |
| *Aphis spiraecola* | 29 | 6 | 2 |
| ***Lipaphis pseudobrassicae*** | 1 | 29 | 1 |
| *Macrosiphum euphorbiae* | 1 | 0 | 0 |
| *Melanaphis sacchari* | 1674 | 582 | 1143 |
| ***Myzus persicae*** | 1 | 0 | 0 |
| *Protaphis middletonii* | 36 | 15 | 27 |
| ***Rhopalosiphum maidis*** | 1 | 2 | 19 |
| ***Rhopalosiphum padi*** | 59 | 17 | 11 |
| ***Rhopalosiphum*** *rufiabdominale* | 308 | 313 | 71 |
| *Schizaphis graminum* | 5 | 6 | 1 |
| *Sitobion avenae* | 4 | 5 | 7 |
| *Therioaphis trifolii* | 40 | 8 | 0 |

Table 3.3: Aphid species listed as the percentage of the total number of aphids collected each species represents per year, with the average percentage over all three years and standard error. Species names in bold are known virus vectors of sweetpotato viruses.

| Species | Percentage (2016) | Percentage (2017) | Percentage (2018) | Percentage (Three Year Average) |
|---|---|---|---|---|
| *Acyrthosiphon pisum* | 0.30 | 0.19 | 0 | 0.16±0.09 |
| ***Aphis craccivora*** | 0.70 | 0.47 | 1.51 | 0.89±0.31 |
| *Aphis glycines* | 0.04 | 0.19 | 0 | 0.07±0.05 |
| ***Aphis gossypii*** | 3.36 | 5.16 | 6.12 | 4.88±0.80 |
| *Aphis spiraecola* | 1.28 | 0.57 | 0.14 | 0.66±0.33 |
| ***Lipaphis pseudobrassicae*** | 0.04 | 2.77 | 0.07 | 0.96±0.90 |
| *Macrosiphum euphorbiae* | 0.04 | 0 | 0 | 0.01±0.01 |
| *Melanaphis sacchari* | 74.10 | 55.64 | 82.34 | 70.69±7.89 |
| ***Myzus persicae*** | 0.04 | 0 | 0 | 0.01±0.01 |
| *Protaphis middletonii* | 1.59 | 1.43 | 1.94 | 1.65±0.15 |
| ***Rhopalosiphum maidis*** | 0.04 | 0.19 | 1.36 | 0.53±0.41 |
| ***Rhopalosiphum padi*** | 2.61 | 1.62 | 0.79 | 1.67±0.52 |
| ***Rhopalosiphum*** *rufiabdominale* | 13.63 | 29.92 | 5.11 | 16.22±7.27 |
| *Schizaphis graminum* | 0.22 | 0.57 | 0.07 | 0.28±0.14 |
| *Sitobion avenae* | 0.17 | 0.47 | 0.50 | 0.38±0.10 |
| *Therioaphis trifolii* | 1.77 | 0.76 | 0 | 0.84±0.51 |

### 3.4. Discussion

Aphids were hand sorted from pan traps, which may explain low numbers compared to previous research in which aphids were suctioned through a Büchner funnel. The composition of species identified did not differ substantially from that of previous research (Wosula et al. 2013).

Data collected from the suction trap represents a single location over a limited period of time, so it is important not to draw too many conclusions from this data. Previous studies using similar suction traps found that aphid counts were correlated with infestation in nearby fields, suggesting that suction traps mainly record local movement (within 50 km of the trap) (Bahlai et al. 2015). Thus, collections from this trap indicate aphid movement in a limited area. These results suggest that in the area and years collected, known vectors of sweetpotato viruses comprised a small percentage (less than 9%) of aphids collected (Table 3.2). The vast majority (70%) of aphids collected were *Melanaphis sacchari*, a poor vector of nonpersistent viruses (Paudel 2019), but a major pest of sugarcane and sorghum. As *M. sacchari* dominated the sample, it is likely that movement of this species were responsible for the trends in aphid abundance over time (Figure 3.2). Aphid counts from sticky cards (Figure 3.1) show a similar trend in aphid numbers over time, with a peak in July or August, however, when only looking at known sweetpotato virus vectors, the trends over time do not match up, with a similar peak only in 2018 (Figure 3.3).

Management of vector borne viruses requires an understanding of vector ecology. How and when potential vectors move is necessary to refine management tactics so that they are deployed in a temporally accurate manner, ensuring control agents, including insecticides, volatile lures and oil sprays are not being deployed at a time when the crop is at low threat of infection. (Park et al. 2005). Sweetpotato is most often infected after transplantation from seed

beds to production fields, despite the presence of potential vectors throughout the growing season (Wosula et al. 2013). The suction trap did not find any distinct multi-year trends in vector movement over three years, however, this it is a single trap and thus cannot show any trends that may occur at a larger (parish, state) scale. Weekly collections of vectors were consistently low (0-36 aphids per) throughout the collection period. This suggests that tactics to control vectors might be most effective during early season, regardless of vector numbers.

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# Chapter 4. Identification of Volatile Compounds Released by Virus Infected and Uninfected Sweetpotato Plants

## 4.1. Introduction

All plants emit volatile organic compounds (VOC), constitutively or induced by stimuli (Maffei 2010). These small molecules with low vapor pressure perform a variety of roles including stress mediation and con- and hetero-specific signaling (Picherskey and Gershenzon 2002). Compounds such as isoprene and monoterpenes function to reduce heat stress and oxidative stress; isoprene reduces heat stress on photosynthetic membranes (Sharkey et al. 2001) and isoprene and monoterpenes scavenge for oxygen radicals within the plant (Loreto and Velikova 2001; Vickers et al. 2009). Volatiles also mediate biological stress by signaling to activate defensive responses. Exposure to green leaf volatiles (GLV), which are often emitted during herbivory, increases jasmonic acid levels in undamaged plants (Scala et al. 2013).

Volatile signals have the advantage of circumventing the vascular system, reaching the rest of the plant much faster (Heil and Beuno 2007). For instance, ethylene, a gaseous plant hormone, has a role regulating growth and senescence (Sisler and Yang 1984). As these are airborne signals, they are detectable to other organisms. Monoterpenes emitted by vegetative tissue and absorbed in the soil can inhibit seed germination (Zunino and Zygadlo 2005) while root volatiles from neighbors stimulate root growth, a method of securing resources from neighbors (Grøndahl and Ehlers 2008). Other plants may respond to VOC to activate their defenses against herbivores, upregulating defenses (Dicke and van Loon 2000). This reaction is not limited to conspecific plants as heterospecific plants may react to damaged plant volatiles, upregulating their own defenses. For example, wild tobacco (*Nicotinia attenuata*) near induced sagebrush (*Artimisia tridentata*) plants showed increased levels of jasmonic acid, a defensive hormone (Karban et al. 2003).

Other trophic levels use plant VOC. Pollinators use floral VOC to find flowers (Picherskey and Gershenzon 2002). Herbivores use plant volatiles among other cues to find their hosts, orienting towards host plant odors (Webster et al. 2008). Natural enemies also use plant volatiles to find their hosts, often searching for cues such as GLV or methyl salicylate emitted in response to herbivory. For example, several species of parasitoid wasps oriented towards GLV in wind tunnel experiments (Whitman and Eller 1990) and *Telenomus podisi* oriented towards α-farnesene and methyl salicylate emitted by soybean after feeding by their stink bug hosts (Michereff et al. 2013).

Induction of plant VOC by biotic factors can alter plant volatile profiles, affecting trophic interactions. VOC induced by herbivores can attract natural enemies or alter host attractiveness. Induced VOC may indicate that the plant is being utilized by competitors, and thus herbivores may be repelled by VOC induced by other herbivores, as is the case with several Lepidopterans (de Moraes et al. 2001; Zakir et al. 2013). Alternatively, induced VOC may indicate that the plant is a more desirable resource and herbivores may be attracted to induced VOC. Synthetic release of GLV attracted several herbivores in maize causing increased herbivory over control plants (von Mérey et al. 2011). Two-spotted spider mites (*Tetranychu urticae*) were significantly attracted to VOC induced by conspecifics in laboratory experiments (Pallini et al. 1997). Similarly, the Asian citrus psyllid (*Diaphorina citri*) is attracted to VOC induced by conspecifics on citrus trees (Martini et al. 2014).

Plant pathogens may also alter volatile profiles. VOC can act as direct defenses against pathogens: common beans (*Phaseolous vulagaris*) infected with *Pseudomonas syringae* emitted bactericidal GLV (Croft et al. 1993). VOC are an important signaling component in inducing a defensive response (Yi et al. 2009). Methyl salicylate, the methyl ester of the plant hormone

salicylic acid, is used as an airborne signal to prime plant immune response (Shulaev et al. 1997) and is emitted along with methyl jasmonate and sesquiterpenes in response to avirulent *P. syringae* infection by tobacco (Huang et al. 2003).

On the other hand, induced VOC can also benefit pathogens. VOC induced by pathogens can be attractive to insect vectors, thus promoting spread of the disease. This is observed in psyllids on citrus, where induced methyl salicylate is attractive to the psyllid vector (Mann et al. 2012) and on the psyllid *Cacopsylla picta* which transmits *Candidatus phytoplasma mali*, to apple (*Malus pumila*), for which β-ocimene is the attractive signal (Mayer et al. 2008). Viruses are particularly adept at manipulating plant VOC to attract vectors. Viruses more than any other pathogens have direct access to cellular machinery. For example cucumber mosaic virus (CMV) increases the total amount of volatiles produced by infected plants, increasing plant visibility to vectors (Mauck et al. 2010). The CMV 2b protein is responsible for this, altering plant defensive signaling to make hosts more attractive to vectors (Sharifi et al. 2017). Other viruses with different hosts alter plant volatile profiles as well. Volatiles from potatoes (*Solanum tuberosum*) infected with *Potato leafroll virus* were more attractive to the vector green peach aphid (*Myzus persicae*) than volatiles from uninfected plants due to differences in the volatile profile (Eigenbrode et al. 2002). *Nicotiana benthamiana* transformed to express the *Tomato yellow leaf curl* China virus βC1 protein emitted more linalool (Salvaudon et al. 2013). This suggests a general trend in viruses altering plant VOC emission.

Sweetpotato (*Ipomoea batatas*) is an interesting study system for plant-virus-vector interactions due to virus symptomatology and synergistic effects, and the challenges associated with virus control in a vegatatively propagated crop. Sweetpotato is affected by over 30 viruses from 9 different virus families (Clark et al. 2012). These viruses are often symptomless and

occur in mixed infections capable of causing yield loss of over 40% (Clark and Hoy 2006). Many of these viruses are vectored by insects, including *Sweetpotato feathery mottled virus*, the most common sweetpotato virus worldwide.

Current research on sweetpotato VOC has focused on two aspects. First, root volatiles and volatiles related to culinary quality (Cui et al. 2010; Tui et al. 1985). Understanding the volatile aspect of sweetpotato flavor is an important aspect in breeding new sweetpotato varieties for flavor (Wang and Kays 2000). The other aspect of sweetpotato volatile chemistry researched is the effect of sweetpotato VOC on sweetpotato weevil host finding (Korada et al. 2013; Wang and Kays 2002). Sweetpotato weevil is an economically important pest of sweetpotato, and understanding how it finds hosts can lead to better management strategies. While previous research in other crop systems suggests that viruses induce VOC production, there is currently no research on this in sweetpotato. Identifying how plant viruses affect the volatile cues released by their hosts is the first step in using these cues to create better management strategies for their vectors. To this end, sweetpotato volatiles were captured through headspace collection and identified and quantified via GC/MS to determine the effect of virus infection on VOC emission in sweetpotato.

## 4.2. Methods

### 4.2.1. Plants and Viruses

Sweetpotato plants (cv. Beauregard (B-14)) were derived from virus-tested mericlones maintained by nodal propagation in tissue culture at the LSU AgCenter Department of Plant Pathology and Crop Physiology to ensure that they were virus free. Cultivar Beauregard was chosen because it is one of the most common sweetpotato varieties grown in the United States (Firon et al. 2009). Plants were maintained under greenhouse conditions which entailed large

changes in temperature and humidity in 13 cm plastic pots using Miracle Gro (Miracle Gro, Marysville, OH) potting soil and Osmocote fertilizer (Miracle Gro, Marysville, OH) (NPK 13:13:13). Singly and mixed virus infected cuttings were provided by the Louisiana State University Department of Plant Pathology (Wosula et al. 2012). Mixed infected cuttings were infected with the potyviruses *Sweetpotato virus* G (SPVG), *Sweetpotato virus* 2 (SPV2), and *Sweetpotato feathery mottle virus* (SPFMV). Singly infected cuttings were infected with only SPFMV.

#### 4.2.2. Dynamic Headspace Volatile Collection

Sweetpotato slips used for volatile collection were collected from greenhouse maintained stock. Vines of approximately 60cm in length were cut and allowed to root for one week under greenhouse conditions. A single planted slip was placed in a teflon guillotine (Analytical Research Systems, Gainsville, FL), which was then covered with aluminum foil to prevent soil volatiles from contaminating the sample. This was covered with a glass collection vessel (Analytical Research Systems, Gainsville, FL). Airgas Ultra Zero Grade air (Airgas, Radnor PA) was pushed into the collection vessel at 1 L per minute by a controlled air delivery system 4Push4Pull system (Sigma Scientific, Micanopy, FL) and pulled out of the collection vessel at 0.5 L per minute into a HaySep Q trap (Volatile Assay Systems, Rensselaer, NY) via vacuum generated by the 4Push4Pull system. Concurrently, headspace collection was performed on an empty vessel set up in the same manner. Headspace collection was performed for three hours. Collection traps were eluted with 100 µL dichloromethane (Fisher Scientific, Hampton, NH) and analyzed by GC/MS the same day (See below). This was repeated five times with both virus tested and mixed infected slips.

### 4.2.3. GC/MS

Volatile samples were quantitatively analyzed by gas chromatography and mass spectrometry. GC/MS samples were analyzed on a Varian CP-3800 gas chromatograph connected to a Saturn 2200 ion trap mass spectrometer (Varian, Inc., Walnut Creek, CA). GC/MS was run with a Zebron ZB-semivolatile GC column (Phenomenex, Torrance, CA). The injector temperature was 250°C and Helium was the carrier gas with a flow rate of 1 mL/min. The column was held at 40°C for 4 minutes, heated to 180°C at 5°C/min and held for 2 minutes, heated to 280°C at 20°C/min and held for 5 minutes, and then heated to 300°C at 20°C/min and held for five minutes. Compounds were identified via National Institute of Stands and Technology mass spectra library matches.

## 4.3. Results

Identified volatile compounds are listed in tables 4.1 and 4.2. Thirteen compounds were identified in the headspace of virus tested slips and 21 compounds were identified in the headspace of mixed infected slips. Chromatograms of collected headspace volatiles are presented in the appendix.

Table 4.1: Volatile compounds detected in headspace of virus tested sweetpotato slips listed by retention time. TIC=total ion count. Value displayed is the percentage of TIC the compound occupied (±s.e.), averaged over the collections it was identified in. Compounds that were also identified in mixed infected plants are marked with an asterisk.

| Compound | Retention Time | TIC |
|---|---|---|
| 2-Ethyl-1-hexanol | 10.282 | 1.96±0.125 |
| Anisole | 11.3 | 4.749±0.303 |
| 3,3-dimethyl-hexane | 11.543 | 0.824±0 |
| Naphthalene * | 12.989 | 2.495±0.214 |
| Isothiocyanato-cyclohexane * | 13.697 | 1.475±0.614 |
| Dodecane | 14.907 | 1.309±0.083 |
| Eicosane | 15.157 | 0.786±0 |
| Nerylacetone | 16.662 | 1.944±0.082 |

(Table cont'd.)

| Compound | Retention Time | TIC |
|---|---|---|
| Methyl tetradecanoate | 19.915 | 0.831±0.038 |
| Methyl palmitate | 21.766 | 2.012±1.136 |
| Palmitic acid, methyl ester | 22.002 | 2.020±0.492 |
| Oleic acid, methyl ester | 23.698 | 1.8005±0.076 |
| Squalene * | 29.444 | 40.305±10.631 |

Table 4.2: volatile compounds detected in infected sweetpotato slips listed by retention time. TIC=total ion count. Value displayed is the percentage of TIC the compound occupied (±s.e.), averaged over the collections it was identified in. Compounds that were also identified in virus tested plants are marked with an asterisk.

| Compound | Retention Time | TIC |
|---|---|---|
| (E)-2-Hexanal | 7.2 | 0.526±0.18 |
| (E)-3-Hexen-1-ol | 7.269 | 7.691±0 |
| Heptanal | 8.852 | 0.394±0 |
| Sulcatone | 11.716 | 0.830±0.53 |
| Octanal | 12.346 | 0.340±0 |
| (Z) 3-Hexen-1-ol acetate | 12.421 | 9.139±0 |
| 2-ethyl-1-hexanol | 13.195 | 0.591±0.165 |
| B-ocimene | 13.790 | 2.762±1.541 |
| Linalool | 15.511 | 0.341±0 |
| Nonanal | 15.661 | 3.885±0.729 |
| E]4,8-dimethylnona-1,3,7-triene | 15.938 | 6.365±5.115 |
| Naphthalene * | 18.029 | 4.275±3.317 |
| Methyl salicylate | 18.289 | 0.413±0 |
| 4z-Hexenyl angelate | 19.461 | 0.663±0 |
| Isothiocyanato-cyclohexane * | 19.467 | 0.604±0.003 |
| Indole | 21.147 | 1.138±0 |
| β-elemene | 23.839 | 1.365±0.0185 |
| Caryophyllene | 24.636 | 14.604±3.972 |
| Humulene | 25.542 | 1.102±0.577 |
| Germacrene D | 26.178 | 10.00±2.617 |
| Squalene * | 28.395 | 2.662±0 |

## 4.4. Discussion

Both infected and virus tested sweetpotato emitted a variety of VOC. Virus tested plants emitted predominately unbranched alkanes as well as carboxylic acids and their derivatives. Long chain hydrocarbons make up a portion of epicuticular waxes (Barthlott et al. 1998). Several compounds emitted by virus tested sweetpotato, including anisole (methoxybenzene), nerylacetone (6,10-dimethylundeca-5,9-dien-2-one), methyl tetradecanoate, methyl palmitate (methyl 9-hexadecenoate) are recognized as distinct odors and flavors by humans (Goff and Klee 2006; Schwab et al. 2008). Their function in sweetpotato is unknown. Also present were the methyl esters of oleic acid (9-octodecenoic acid), and palmitic acid (hexadecanoic acid). These are metabolites of linoleic acid, and may be metabolic byproducts, or components of the cuticular wax. All of these compounds are previously recorded in floral volatiles of other species (Knudsen et al. 1993). There is a great diversity of plant volatile compounds, and while the biological and ecological role of many has been discovered, it can be difficult to ascribe specific purpose to each compound in a plant blend.

Mixed infected plants emitted mostly green leaf volatiles (GLV), terpenes, and aldehydes. Mixed infected plants emitted methyl salicylate, suggesting that the plant is mounting a defensive response to the virus infection. GLV and terpenes have important ecological functions, mediating trophic interactions. GLV are typically produced constitutively at low levels but can be rapidly induced by stress (Holopainen and Gershenzon 2010). GLV function as an airborne signal to induce defenses against both pathogens (Kishimoto et al. 2001) and herbivores (Shiojiri et al. 2006). Terpenes are a very diverse class of compounds, with a variety of functions in the plant. They mediate plant defense at different levels. B-ocimene induces direct defense against aphids in Chinese cabbage (*Brassica rapa*), reducing performance of green peach aphid

(Kang et al. 2018). Linalool, a compound emitted by sweetpotato in this study, applied directly to leaf surfaces had a repellent effect on green peach aphid (Gabrys et al. 2005), and both compounds were attractive to parasitoid wasps (Du et al. 1998).

Both infected and virus tested plants emitted naphthalene, isothiocyanato-cyclohexane, and squalene. Naphthalene is a constitutive component of several plant volatiles (Koedam 1986), however, it was also an environmental contaminant in the area where volatile collection took place. Similarly, isothiocyanates are important constitutive plant defenses in some species (Agrawal and Kurashige 2003; Wittstock and Gershenzon 2002). However, isothiocyanato-cyclohexane is a common environmental pollutant (Gallego et al. 2007), suggesting that these compounds might be contaminants. Squalene is a triterpene, and a precursor to plant sterols (Goad and Goodwin 1966), but also functions as a volatile signal in flowers (Ecroyd et al. 1995) and mediates tri-trophic interactions in a leafminer-parasitoid system in apple (Dutton et al 2002). Its function in sweetpotato is unknown.

The volatile profiles of virus tested and potyvirus infected sweetpotatoes are vastly different, sharing only three compounds in common. An ANOVA of average TIC percentage of these compounds indicated no difference in the emission of these compounds (Naphthalene: $F$ = 0.2867, df = 1, $P$ = 0.6207 isothiocyanato-cyclohexane: $F$ = 1.2075, df = 1, $P$ = 0.3521 squalene: $F$ = 3.1314, df = 1, $P$ = 0.2187). However, as the amount of eluant was not standardized, this does not preclude differences. The difference in type and number of compounds suggests that virus infection induces changes in sweetpotato volatile emission. The lack of compounds from virus tested plants in mixed infected volatiles could be due to the emission of these volatiles beings suppressed. Both herbivores and pathogens have the ability to suppress the production of specific volatiles (Ponzio et al. 2013). It is also possible that virus infected sweetpotatoes

produce a higher volume of induced volatiles, causing these volatiles to get lost in the GC/MS baseline recording. Infection with cucumber mosaic virus both causes changes in the volatile blend of and induces greater volatile emission in tobacco (*Nicotiana tabacum*) (Tungadi et al. 2017). *Potato leafroll virus* induces production of volatiles similar to the baseline blend (Eigenbrode et al. 2002), most likely to make the plant more apparent to vectors.

Although long distance movement in aphids is not affected by volatiles, wind tunnel experiments suggest that host volatiles can affect aphid orientation during flight. Previous research on sweetpotato volatiles (Chapter 2) consistently report sweetpotato producing a variety of sesquiterpenes, some of which were present in the headspace of mixed infected slips but were absent in headspace of virus tested sweetpotato in this study. Previous research did not attempt to control for virus infection status. Sweetpotato in the field is often infected with a mixture of unknown viruses (Clark and Hoy 2006), suggesting that these compounds are the result of biotic stress. While this research looked at the effect of potyvirus infection on sweetpotato volatiles, future research could focus on the sweetpotato-virus-herbivore system. Sweetpotato is affected by many viruses with aphid and whitely vectors and different modes of transmission. Additionally, sweetpotato is attacked by both root and foliar herbivores, and the effect of these herbivores on virus transmission is unknown.

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# Chapter 5. The Effect of Virus Infection and Commercial Volatiles on Aphid Attraction to Sweetpotato

## 5.1. Introduction

Insects use a variety of cues to find their hosts, including visual, olfactory, and temperature cues, as well as constitutive and induced plant volatiles from long distances (Webster and Carde 2017). At shorter distances, host specific cues (such as host plant volatiles) are used to locate suitable host plants and by altering host plant cues, we can affect the insects' ability to find their host. For example, reflective mulches reduce aphid landings by obscuring the distinction between plant and soil that aphids use to orient (Brown et al. 1993). Alteration of volatile cues can have the same effect, as often seen in intercropping. Intercropping wild tomato (*Lycopersicon hirsutum*) or cabbage (*Brassica oleracea*) with potato (*Solanum tuberosum*) reduced Colorado potato beetle's (*Leptinotarsa decemlinata*) orientation response towards potato (Thiery and Visser 1986).

Altering insect behavior with volatile cues requires knowledge of how the specific insect utilizes these cues. Often, the specific volatile blend of a host is important for insect host location (Bruce and Pickett 2011). Insects that orient towards a host's volatile blend may or may not orient towards individual compounds from that blend. For example, female coddling moths, *Cydia pomonella*, prefer the odor of the apple volatile butyl hexanoate over that of combined headspace volatiles from apples (*Malus pumila*) (Hern and Dorn 2003). However, for *Aphis fabae*, host odors presented individually are repellent, only becoming attractive when presented in a blend (Webster et al. 2010). Additions to host volatile blends can also affect behavior, for example, wheat (*Triticum aestivum*) genetically engineered to constitutively emit E-β-farnesene repelled aphids in the lab and greenhouse, although this did not translate into repellency in the field (Bruce et al. 2015).

In aphids, long distance movement seems to be directed by visual and environmental cues (See Chapter 3), while olfaction affects aphid host choice at short distances. For example, methyl salicylate released by *Prunus padus* is repellent to spring migrant *Rhopalosiphum padi* (Glinwood and Petterson 1999) and host and nonhost odors affected *Aphis fabae* orientation in wind tunnels (Nottingham et al. 1993). However, the role of volatile cues in aphid host location is not fully understood, particularly as it relates to the aphid's role as a virus vector. As aphids are economically important vectors (Ng and Perry 2005), understanding how they react to volatile cues may lead to better management strategies.

Some compounds already used in pest management have a volatile component that may affect aphid orientation. Stylet oil, a mineral or paraffin oil sprayed on crops, is effective at reducing non-persistent virus transmission by aphids (Olubayo et al. 2010; Simons and Zitter 1980), probably by inhibiting the binding of virions to aphid stylets (Boquel et al. 2013). As these oils are composed of short chain hydrocarbons, they volatilize, and may have an effect on aphid behavior. Neem oil, extracted from the seeds of the *Azadirachta indica* tree, is a natural insecticide that exhibits antifeedant properties (Isman et al. 1990). It also may exhibit repellent effects towards aphids (Hunter and Ullman 1992), that, coupled with its antifeedant properties, could reduce virus transmission. Methyl salicylate (MESA), the volatile form of the plant hormone salicylic acid, is used commercially to lure aphid natural enemies under the name 'Predalure' (Rodriguez-Saona et al. 2011). MESA is also released from virus infected sweetpotato (see Chapter 4). Jasmonates, including methyl jasmonate (MEJA) function in the plant response to aphid feeding (Smith and Boyko 2006).

This research was performed to determine the effect of virus infection status on aphid orientation and settling behavior in sweetpotato. The effects of the plant hormones MESA and MEJA, stylet (horticultural) oil, and neem oil on aphid orientation and settling were also examined.

## 5.2. Methods

### 5.2.1. Plants and Viruses

Beauregard (B-14) sweetpotato plants were derived from virus-tested mericlones maintained by nodal propagation in tissue culture at the LSU AgCenter Department of Plant Pathology and Crop Physiology to ensure that they were virus free. Plants were maintained under greenhouse conditions which entailed large shifts in temperature and humidity throughout the year. Plants were grown in 13 cm plastic pots using Miracle Gro (Miracle Gro, Marysville, OH) potting soil and Osmocote fertilizer (Miracle Gro, Marysville, OH) (NPK 13:13:13). Singly and mixed virus infected cuttings were provided by the Louisiana State University Department of Plant Pathology (Wosula et al. 2012). Mixed infected cuttings were infected with the potyviruses *Sweetpotato virus* G (SPVG), *Sweetpotato virus* 2 (SPV2), and *Sweetpotato feathery mottle virus* (SPFMV). Singly infected cuttings were infected with only SPFMV.

### 5.2.2. Aphids

Green peach aphids (*Myzus persicae* (Sulzer)) (GPA) and cotton aphids (*Aphis gossypii* Glover) were from colonies that were established from single apterae and maintained under laboratory conditions in screened cages at room temperature (20 to 22°C) and a photoperiod of 14:10 (L:D). *M. persicae* was collected from eggplant, *Solanum melongena* L., and developed from a single aptera in 2009. *A. gossypii* was collected from cotton at the LSU AgCenter Macon Ridge Research Station in Winnsboro, LA in 2006. *M. persicae* was reared on ‘Tendergreen’

mustard (Seed Savers, Decorah, IA) (*Brassica cretica* L.); *A. gossypii* was reared on DP174RF (DeltaPine, Monsanto Company, St. Louis, MO, USA).

### 5.2.3. Volatiles

Volatile solutions were mixed as follows: 0.02 mM methyl jasmonate and 0.02 mM methyl salicylate solutions were created by mixing 4.3 µL methyl jasmonate (Sigma Aldrich, St. Louis, MO) and 2.5 µL methyl salicylate respectively with 1 mL 95% ethanol, then diluting into 100 mL DI water. Stylet oil solution was created by mixing 750 µL JMS Stylet Oil (JMS Flower Farms, Vero Beach, FL) with 100 mL DI water. Neem oil solution was created by mixing 781 µL pure neem oil (Dyna Gro, Richmond, CA) and 781 µL Top Surf nonionic 80/20 surfactant (Agriliance, St Paul, MN) with 100 mL DI water

### 5.2.4. Y-Tube Assays

A Y-tube apparatus was constructed with a Sigma Scientific CADS 4Push4Pull system (Sigma Scientific, Micanopy, FL) and glass collection vessels (Analytical Research Systems, Gainsville FL). Airgas Ultra Zero Grade (Airgas, Radnor PA) air was pushed over test plants in two collection vessels at 1 L per minute by a Sigma Scientific CADS 4Push4Pull system (Sigma Scientific, Micanopy, FL) into the short ends of the Y-tube. A single adult apterous aphid (visually identified by size) was introduced to the long end of the 'Y' with a # 000 paint brush. The back of the Y tube was covered with aluminum foil to induce a phototaxis response. The aphid could travel along the tube until it reached a mark halfway down one of the short ends of the 'Y,' or 15 minutes passed, whichever came first. This was conducted 50 times with a single pair of plants, then replicated three times for a total of 150 aphids per treatment. Each aphid was used only once and the odor sources were switched to the opposite arm every five assays to

avoid any bias. For each volatile treatment, 9 cm filter paper (410 Qualitative, VWR International, Sugar Land, TX) was impregnated with 2 mL of volatile solution via a micropipette. Fresh impregnated filter paper was used every five assays.

### 5.2.5. Arena Assays

An arena was constructed out of a Gladware 739 mL resealable medium square entrée container (The Glad Products Company, Oakland, CA) and an Aligent wash vial (Chrom Tech, Apple Valley, MN). The lid of the vial was glued to a hole in the center of the Gladware container, and two holes were cut in the container for the insertion of sweetpotato leaves (Figure 5.1). Twenty-five apterous adult aphids were starved for one hour in scintillation vials. Leaves were treated immediately before insertion into the arena with a volatile solution or a control solution of DI water with a Preval aerosol sprayer (CA Acquisition, Coal City, IL). The number of aphids on each leaf was counted at 1, 2, 3, and 24 hours.

Figure 5.1 Arenas for arena assays were constructed from a plastic container and GC wash vial. Holes cut in the side allow for the admission of single leaves of otherwise intact plants, and were sealed with parafilm to prevent aphid escape.

### 5.2.6. Data Analysis

Data were tested for normality with the Shapiro-Wilk test. Y-tube data were analyzed for differences between treatments with analysis of variance (ANOVA) in JMP Pro 14 (SAS 2018). Arena assay data followed a Poisson distribution. It was analyzed with the LSMEANS statement in PROC GLIMMIX (SAS 2013) with a model accounting for observation time.

## 5.3. Results

### 5.3.1. Y-tube Assays

Only GPA oriented towards a volatile source in the Y-tube assays. When placed in the Y-tube, CA did not move towards any odor source. Significantly more GPA oriented towards mixed infected plants than towards virus tested plants ($F$ = 50.0, df = 1, $P$ = 0.0021) (Figure 5.2). There was no difference in orientation towards the odors of virus tested plants and those of SPFMV infected plants ($F$ = 0.2, df = 1, $P$ = 0.7000). Aphids were significantly more attracted to the plant plus MESA treatment ($F$ = 10.7, df = 1, $P$ = 0.0309) than control plants, and significantly less attracted to the MEJA ($F$ = 38.7, df = 1, $P$ = 0.0034) and neem oil ($F$ = 73.5, df = 1, $P$ = 0.001) treatments than control plant odors (Figure 5.2).

### 5.3.2. Arena assays

Only GPA showed significant differences in settling. Significantly more GPA settled on virus tested sweetpotato in the second hour than mixed infected sweetpotato ($t$ = 2.2, df=10, $P$ = 0.0458) (Figure 5.3). Significantly more GPA settled on neem treated sweetpotato in the first hour than control plants ($t$ = -2.3, df = 10, $P$ = 0.0464) (Figure 5.11). Significantly more GPA settled on MESA treated sweetpotato in the first ($t$ = -2.2, df = 10, $P$ = 0.0453) and second ($t$ = -

3.2, df = 10, *P* = 0.0083) hour than control plants (Figure 5.5). There was no significant difference in settling at any time points in any other treatments and there was no significance effect of time on settling in any treatment (Figures 5.4, 5.6-5.10, 5.12).

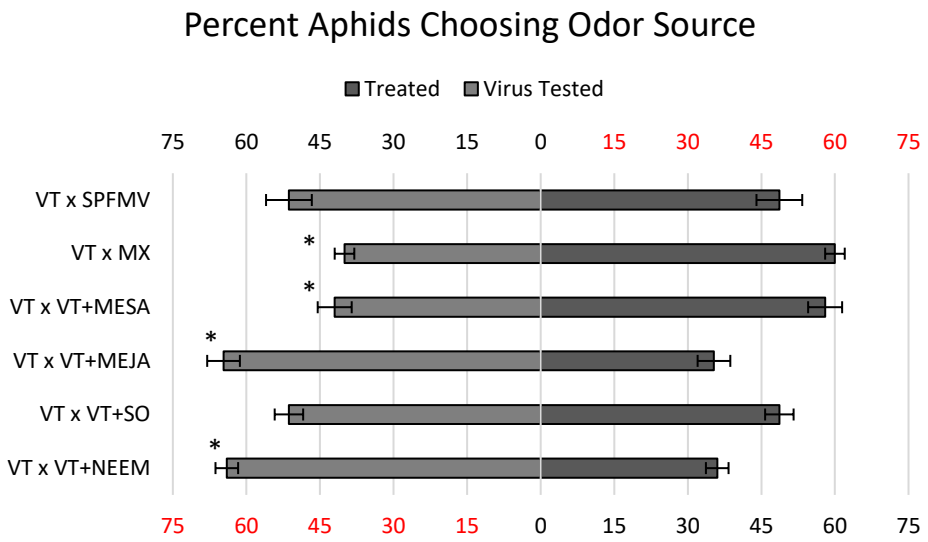


Figure 5.2 Percentage of green peach aphid choosing an odor source in Y-tube assays. SPFMV= *Sweetpotato feathery mottle virus* infected plant. MX= mixed infected plant. Asterisk indicates that aphid response to treatments are significantly different.

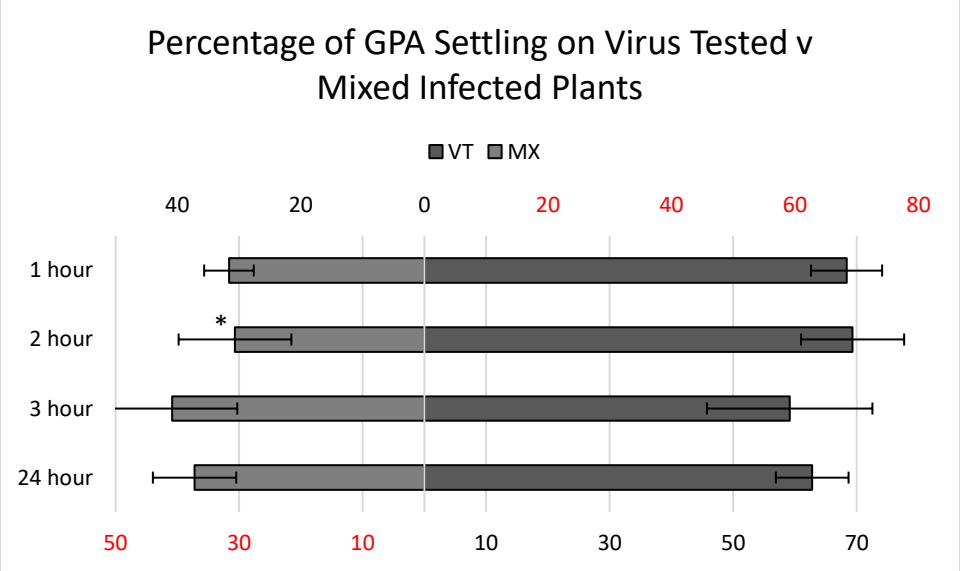


Figure 5.3 Percentage of green peach aphid (GPA) settling on virus tested versus mixed infected sweetpotato at each time point in the arena assay. Asterisks indicate significant differences in settling between treatments.

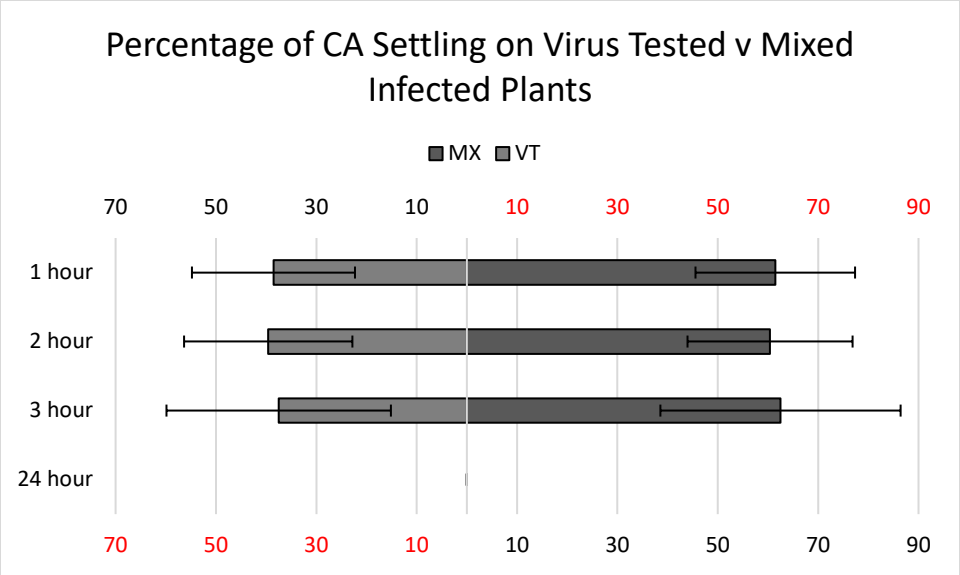


Figure 5.4 Percentage of cotton aphid (CA) settling on virus tested versus mixed infected sweetpotato. Asterisks indicate significant differences in settling between treatments. No aphids were observed settling on either treatment at the 24 hour observation.

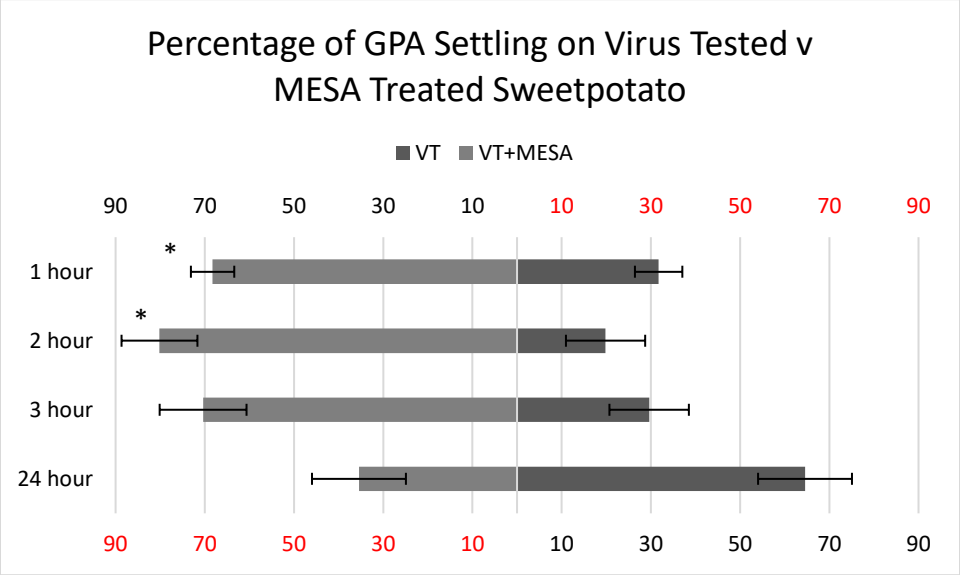


Figure 5.5 Percentage of green peach aphid (GPA) settling on virus tested versus sweetpotato treated with MESA. Asterisks indicate significant differences in settling between treatments.

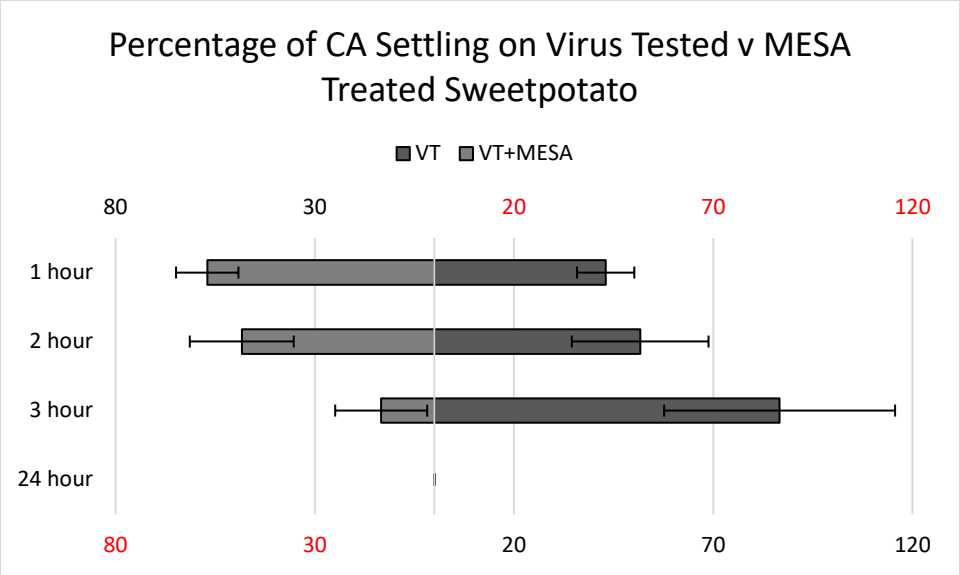


Figure 5.6 Percentage of cotton aphid (CA) settling on virus tested versus sweetpotato treated with MESA. Asterisks indicate significant differences in settling between treatments. No aphids were observed settling on either treatment at the 24 hour observation.

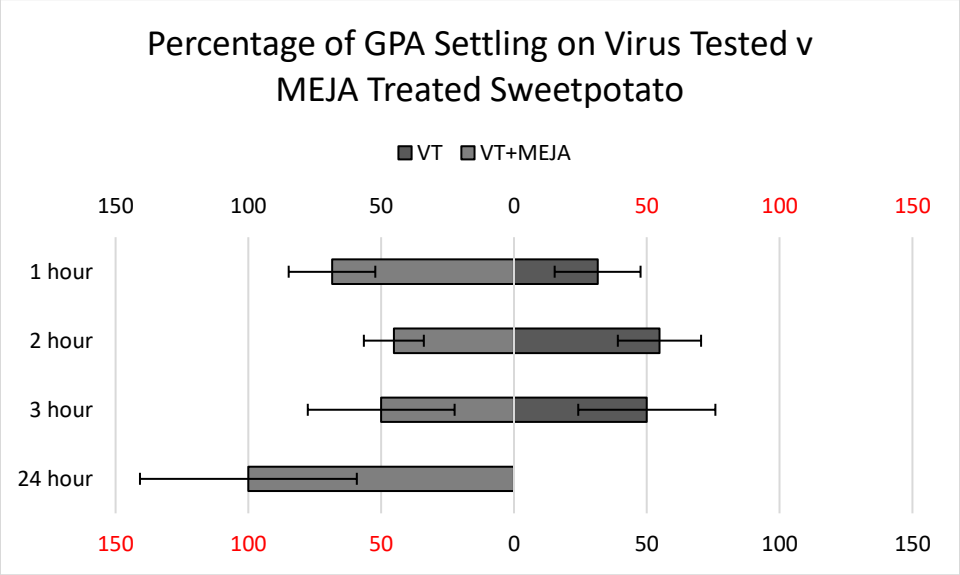


Figure 5.7 Percentage of green peach aphid (GPA) settling on virus tested versus sweetpotato treated with MEJA. Asterisks indicate significant differences in settling between treatments.

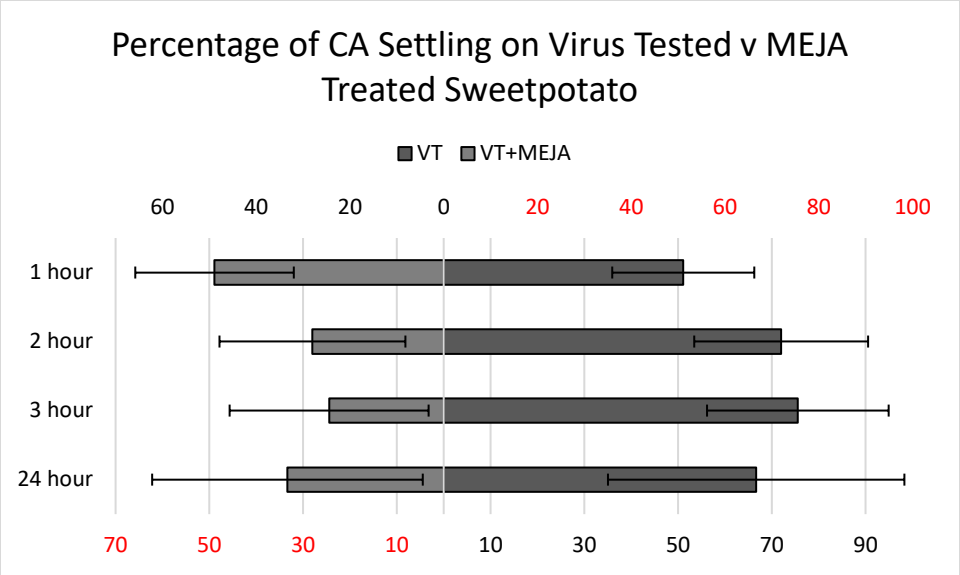


Figure 5.8 Percentage of cotton aphid (CA) settling on virus tested versus sweetpotato treated with MEJA. Asterisks indicate significant differences in settling between treatments.

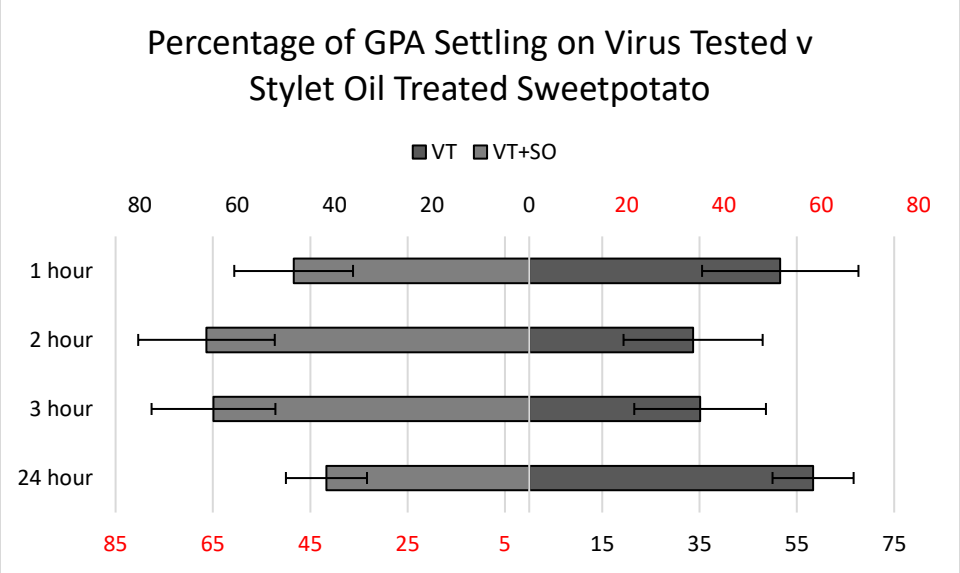


Figure 5.9 Percentage of green peach aphid (GPA) settling on virus tested versus sweetpotato treated with stylet oil. Asterisks indicate significant differences in settling between treatments.

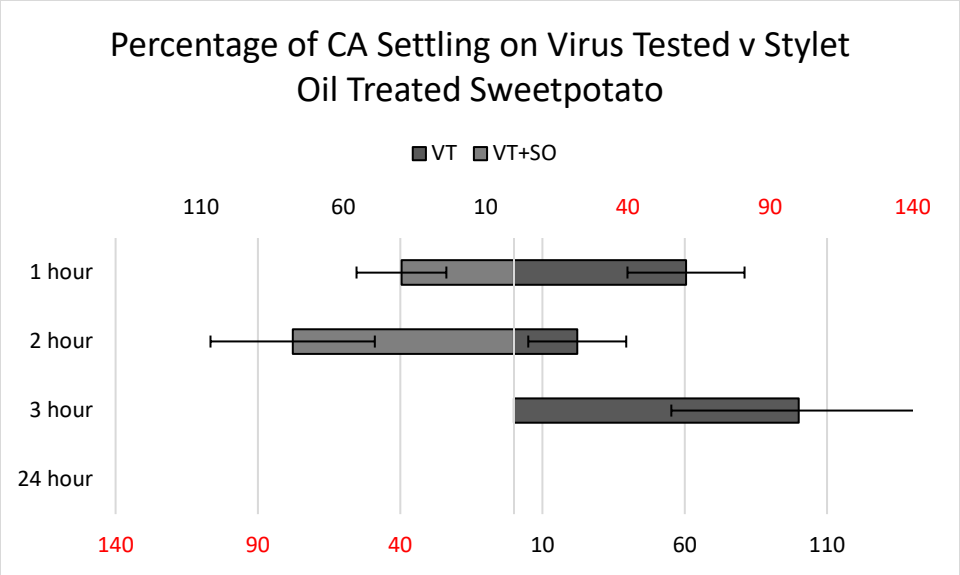


Figure 5.10 Percentage of cotton aphid (CA) settling on virus tested versus sweetpotato treated with stylet oil. Asterisks indicate significant differences in settling between treatments. No aphids were observed settling on either treatment at the 24 hour observation.

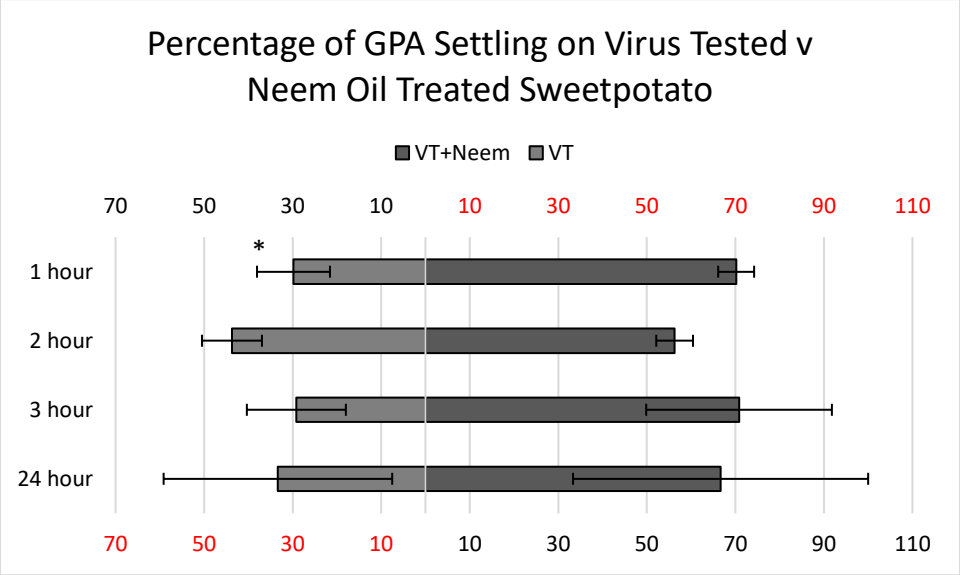


Figure 5.11 Percentage of green peach aphid (GPA) settling on virus tested versus sweetpotato treated with neem oil. Asterisks indicate significant differences in settling between treatments.

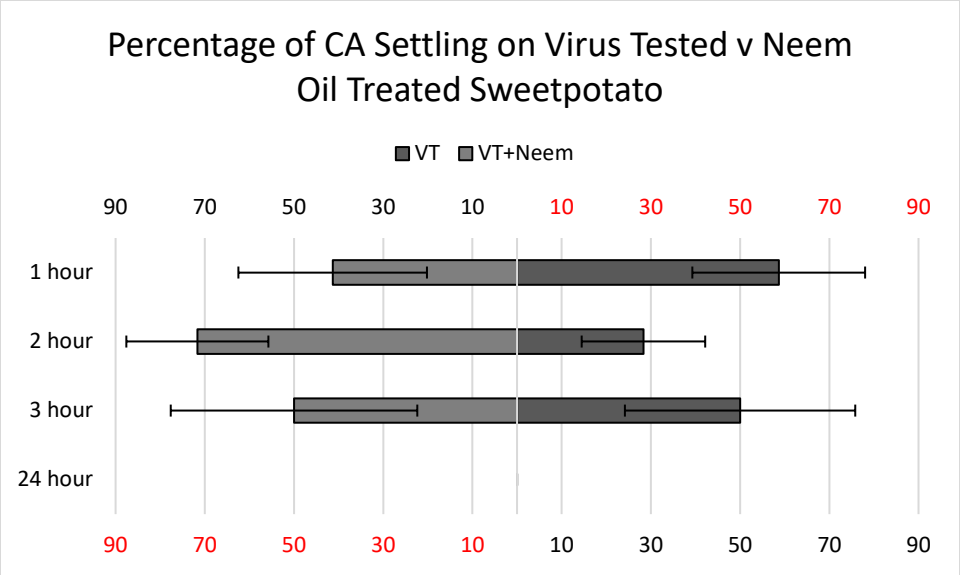

Figure 5.12 Percentage of cotton aphid (CA) settling on virus tested versus sweetpotato treated with neem oil. Asterisks indicate significant differences in settling between treatments. No aphids were observed settling on either treatment at the 24 hour observation.

**5.4. Discussion**

These results suggest several things about the aphids tested, their affinity for sweetpotato, and the effect of virus infection status and exogenous volatiles on that affinity. Only GPA exhibited an orientation response in the Y tube assays. GPA is a polyphagous aphid, feeding on over 400 different plant species. GPA may orient towards and colonize sweetpotato, however, it may not be a preferred host. CA has a wide host range; however, this may be due to high genetic diversity within the species (Vanlerberghe-Masutti and Chavigny 1998). In no-choice survivorship studies, GPA was capable of colonizing sweetpotato, but CA was not (Wosula et al 2013). Previous research indicates that both species are important and competent vectors of sweetpotato viruses (Wosula et al. 2012), however, they may only be transient vectors in sweetpotato. Byamukara et al. (2004) found few aphids within the sweetpotato canopy, despite their abundance in traps. A poor affinity for sweetpotato may account for CA not orienting towards odors in Y-tube assays.

GPA were more attracted to mixed infected sweetpotato than virus tested sweetpotato in Y-tube olfactometer tests. Previous research in other systems indicates that virus infected plants are often more attractive to vectors than uninfected plants (Mauck et al. 2010). However, when given the choice between odors virus tested and SPFMV infected plants, there was no significant difference in GPA orientation. This is may be due to the low virus titer level in singly infected Beauregard sweetpotatoes, as found in other studies (Wosula et al. 2012). Because sweetpotato potyviruses share much of their molecular machinery, infections with multiple potyviruses has a synergistic effect, increasing virus titer and yield loss (Clark et al. 2012). This possibly alters the number and quantity of volatiles the plant emits. In wheat infected with *Barley yellow dwarf virus* (BYDV), increase in virus titer was associated with increased volatile emissions (Jiménez Marténez et al. 2004) and this correlated to increased attractiveness to *R. padi*. However, Medina-Ortega et al. (2009) did not find similar results in the same system, suggesting that this relationship should be explored further.

Despite orientation towards mixed infected sweetpotato odor in arena assays (Figure 5.2), GPA preferred to settle on virus tested sweetpotato (Figure 5.3). This may be due to the time frame of the experiment. Aphids were starved for one hour before the experiment to encourage settling, and this can influence feeding behavior (Collar and Fereres 1998). It is possible that aphids may have sampled the mixed infected plants first, then rejected them due to low quality. However, in no-choice life table analyses, GPA performed better on mixed infected sweetpotato than virus tested sweetpotato (Wosula et al. 2013), suggesting that this is not the case. This suggests that there is another cue, perhaps visual, responsible for aphid settling. As the headspace in the arena was static, volatile host cues may have a reduced role in host settling. The quality of the host plant may have an effect on aphid choice. The GPA colony used in these

experiments was raised on mustard and there is evidence that previous host behavior can influence host selection behavior in aphids (Gorur et al. 2007).

GPA were more attracted to sweetpotato plus MESA odor, and less attracted to sweetpotato plus MEJA in olfactometry tests (Figure 5.2). As MESA is emitted by virus infected sweetpotato, it is not surprising that this odor is attractive to GPA, as it could indicate a more palatable host plant. MESA is repellent to several species of aphids (Hardie et al. 1994). As MESA is emitted by plants after aphid attack and attractive to aphid predators (Zhu and Park 2005), it may indicate that a plant is an unsuitable host. However, there is no research indicating this is the case with GPA. MEJA could be repellent for similar reasons. MEJA is emitted by plants in response to herbivore attack and jasmonates were repellent to damson-hop aphids (*Phorodon humuli*) in the field (Birkett et al. 2000). However, while MESA promoted settling in arena assays (Figure 5.5), MEJA did not (Figure 5.7). As leaves were directly sprayed with the volatile solution, these assays do not reflect only the effect of volatiles on aphid settling but also the effect of the volatile on the plant. Assays were conducted immediately after treatment, however, due to the length of the experiment, the treatments may have induced a response in the plant. As these two hormone derivatives activate plant defenses (Thaler et al. 1999), this may affect aphid settling even after the volatile component dispersed throughout the arena headspace, perhaps by altering the plant in ways that make it more palatable to GPA.

Neem oil odor was repellent to GPA in Y-tube assays (Figure 5.2) but more GPA settled on neem treated leaves at the first hour that on the control plants (Figure 5.11). Neem extracts show antifeedant (Lowery and Isman 1993) and aphidicidal properties (Aziz et al. 2014; Das et

al. 2008), making GPA preferentially settling on treated leaves curious. This suggests that neem oil alters sweetpotato perhaps by altering plant defenses in a way that makes the plant more attractive to GPA.

Stylet oil odor had no effect on GPA attraction or settling (Figures 5.2, 5.9). While stylet oil is effective at reducing non-persistent virus transmission by aphids, this suggests that it elicits no attractive or repellent response in aphids. Previous research suggests that other horticultural oils are repellent to the whitefly *Bemisa argenfolii* and the spider mite *Tetranychus urticae* (Liu and Stansly 1995; Walsh and Grove 2005). However, it appears there is no previous research on the effect of stylet oil on aphid settling behavior. Both Liu and Stansly (1995) and Walsh and Grove (2005) found that other brands of oil were repellent to pests, however, Walsh and Grove found that JMS stylet oil was 'repulsive' (reduced movement from untreated to treated plant tissue), but did not repel settled insects. This suggests that other oils may have different effects on aphid host choice. Two important things must be noted about the aphids used in these experiments. First, apterous aphids were used in these experiments, and thus may not be indicative of the behavior of colonizing alate aphids. Apterous aphids lack the secondary rhinaria alate aphids possess which may result in different host preference between the two. However, apterous aphids do move among plants in the field, including by walking across soil in response to biotic (Gish and Inbar 2005) and abiotic stimuli (Mann et al. 2005). This movement may further spread plant viruses throughout the field as demonstrated by displaced apterous *R. padi* spreading BYDV further throughout oat (*Avena sativa*) fields (Bailey et al. 1995) and GPA spread *Potato leafroll virus* in the field to plants that did not touch, suggesting movement across the soil (Barker and Woodford 1992). The aphids used in these experiments were also not viruliferous. *R. padi* vectoring BYDV prefer uninfected wheat plants (Ingwell et al. 2012),

however, BYDV is a persistently transmitted virus that has a more sustained interaction with its vector than do nonpersistently transmitted viruses. It cannot be ruled out that this altered behavior may affect host preference.

Overall, these results suggest that aphids respond to both plant and exogenous volatile cues, however, the effect of olfactory cues on aphid settling behavior is unclear. Further research would be required to determine how these volatiles affect alate aphids, and what effect they have on aphid orientation and settling in the field.

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# Chapter 6. Effects of Common Commercial Volatiles on Aphid Feeding Behavior Associated with Virus Transmission and Vector Efficiency

## 6.1. Introduction

Plant viruses cause economically important losses in food crops; in sweetpotato, mixed infections of viruses cause an average of 33 to 41% yield loss in Louisiana compared to virus tested plants (Clark and Hoy 2006). As vectors of half of all arthropod borne plant viruses, aphids are economically important pests of many crops. Aphids transmit plant viruses in several ways which can be categorized into non-persistent, semi-persistent, and persistent based on their association with their aphid vectors (Ng and Perry 2004). Persistent viruses require sustained phloem feeding by aphids for transmission while non- and semi-persistent viruses require much shorter interactions with the plant. Non-persistent viruses, in particular, can be acquired and transmitted within minutes (Ng and Falk 2006). This poses a particular problem for the management of these vectors. Insecticides are ineffective as the virus may be acquired and transmitted before an insecticide can kill the insect (Perring et al. 1999). Other tactics are needed to reduce virus transmission. Altering vector feeding behavior may reduce virus transmission.

Specific behaviors are required for non-persistent virus acquisition and transmission. First, the aphid vector must probe the plant with its stylet mouthparts. After initial landing, the decision to probe is based on volatile, tactile, and chemical cues such as cuticular waxes (Fereres and Moreno 2009). Once aphids probe the plant, they sample cell contents to determine host suitability. This behavior is also required to acquire and transmit non-persistent viruses (Powell et al. 1995; Powell et al. 2005).

Distinct behaviors in cellular punctures are associated with non-persistent virus transmission and acquisition. Cellular punctures are divided in to three phases, the second of which is required for non-persistent virus transmission and is further divided into three sub-

phases (II-1, II-2, and II-3). In phase II-1, aphids salivate into the cell, transmitting virions (Powell et al. 1995). In phase II-3, aphids ingest cell contents and acquire virions. Archlets, which occur in phase II-3, are also associated with acquisition of virions (Collar et al. 1997). Other behaviors, such as the length of probing are associated with virus transmission (Collar et al. 1997).

Because aphid feeding occurs within the plant and cannot be observed without destructive sampling, the electrical penetration graph technique (EPG) is used to analyze aphid feeding behavior. In EPG, a circuit is created between plant and aphid. When the aphid mouthparts connect with the plant, this closes the circuit (Walker 2000). Cellular punctures are essential for the transmission of plant viruses (Collar et al. 1997; Powell et al. 1995). By analyzing the resistance created by moving fluids and voltage differences between different plant tissues, a picture of aphid feeding behavior is created. Thus, for example, cellular punctures are recorded as distinct potential drops, as plant cells have a lower voltage than the intercellular space (Walker 2000).

Altering probing behavior may reduce virus transmission. Some insecticides alter behavior. For example, cypermethrin reduced probing by green peach aphid (GPA) on pepper plants infected with *Potato virus Y*, however, this only reduced transmission in experiments where aphids were exposed long enough to be paralyzed (Collar et al. 1997). Other insecticides have also proven infective at controlling plant viruses. Aphids probe regardless of potential deterrents (Fereres and Moreno 2009), and the repellent effects of insecticides such as pyrethroids may encourage further virus spread (Lowrey and Boiteau 1988).

Newer insecticide chemistries may show promise in reducing virus transmission. Ryanodine receptor agonists affect muscle tissue and have shown promise in reducing the

transmission of persistently transmitted viruses in thrips and whiteflies (Jacobson and Kennedy 2013). Kir channel inhibitors alter aphid feeding behavior (Li et al. 2019), and thus may reduce virus transmission rates. In the case of Kir channel inhibitors, several compounds inhibited the aphid's ability to reach the phloem, likely reducing the transmission of persistent viruses. It is yet to be determined how these insecticides affect non-persistent virus transmission.

Resistant crop varieties also alter aphid probing behavior. GPA feeding on resistant lettuce (*Lactuca sativa*) probed longer than on susceptible plants. However, it performed fewer cellular punctures, suggesting that resistance reduces virus transmission (Montllor and Tjallingii 1989). Induced resistance, resistance exhibited after stimuli, also affects aphid feeding behavior. *Sitobion avenae* (Fab.) feeding on wheat (*Triticum aestivum*) treated with methyl jasmonate (MEJA) had more, but shorter probes while wheat treated with methyl salicylate (MESA) exhibited shorter phloem feeding periods (Cao et al. 2013). GPA feeding on soybean (*Glycine max*) treated with jasmonic acid salicylic acid to induce resistance exhibited an increase in probing behavior associated with virus transmission (Dryburgh 2015).

Virus infection status may also affect aphid probing behavior. In a previous study, GPA feeding on the sweetpotato cultivars 'Beauregard' and 'Evangeline' performed more behaviors associated with virus transmission on infected plants than non-infected plants. Aphids feeding on virus infected plants had a shorter time to first intracellular puncture, more and longer potential drops, and a longer duration of potential drop subphase II-3 (Wosula et al. 2014).

A poorly explored aspect of virus transmission is the effect of volatiles on vector feeding behavior. Plant volatiles serve a wide variety of ecological roles, from within plant signaling (Heil and Bueno 2007), to mediating tritrophic interactions and herbivore host location (Arimura et al. 2009; Webster 2008) While the effect of plant volatiles on host location is well

documented, there is no research on the effect of volatiles on vector feeding behavior. Plant volatiles serve an important ecological role in mediating plant herbivore interactions (Arimura et al. 2009) and may have a role on aphid host location (Nottingham and Hardie 1993). However, while the effect of exogenous volatile leaf treatments on aphid feeding behavior has been studied (Dancewicz et al. 2016), the role of headspace volatiles once the aphid has landed on the plant has not been studied, and it is unknown what effect host plant volatiles, such as MEJA and MESA, or exogenous volatiles, such as essential oils, may have on feeding behavior. In order to determine the effect of these volatiles on virus transmission, EPG technique was used to examine the effect of exposure to volatiles on the feeding behavior of two common vectors of sweetpotato potyviruses related to virus transmission and acquisition on virus tested and virus infected plants. Further experiments were performed to examine the effect of these volatiles on vector efficiency.

## 6.2. Methods

### 6.2.1. Plants and viruses

Beauregard (B-14) sweetpotato plants were derived from virus-tested mericlones maintained by nodal propagation in tissue culture at the LSU AgCenter Department of Plant Pathology and Crop Physiology to ensure that they were virus free. Plants were maintained under greenhouse conditions, which varied widely in temperature and humidity, in 13 cm plastic pots using Miracle Gro (Miracle Gro, Marysville, OH) potting soil and Osmocote fertilizer (Miracle Gro, Marysville, OH) (NPK 13:13:13). Mixed virus infected cuttings infected with the potyviruses *Sweetpotato virus* G (SPVG), *Sweetpotato virus* 2 (SPV2), and *Sweetpotato feathery mottle virus* (SPFMV) were provided by the Louisiana State University Department of Plant Pathology and Crop Physiology Sweetpotato Pathology Laboratory under the direction of Dr. C. Clark (Wosula et al. 2013). *Ipomoea nil* cv ‘Scarlet O’Hara’ was used as an indicator plant

(Wosula et al. 2013). *I. nil* plants were grown from seed, five to six plants per pot, under the same conditions as the sweetpotato plants. Plants were grown to the cotyledon stage, four to five days after planting before use in experiments.

### 6.2.2. Aphids

Green peach aphids (*Myzus persicae* (Sulzer)) (GPA) and cotton aphids (*Aphis gossypii* Glover) are from colonies that were established from single apterae and maintained after Wosula et al (2013) (GPA) and Li et al. (2018) (CA). Colonies were maintained under laboratory conditions in screened cages at room temperature (20 to 22°C) and a photoperiod of 14:10 (L:D). *M. persicae* was collected from eggplant, *Solanum melongena* L., and developed from a single aptera in 2009. *A. gossypii* was collected from cotton at the LSU AgCenter Macon Ridge Research Station in Winnsboro, LA in 2006. *M. persicae* is reared on 'Tendergreen' mustard (Seed Savers, Decorah, IA) (*Brassica cretica* L.); *A. gossypii* is reared on DP174RF (DeltaPine, Monsanto Company, St. Louis, MO, USA).

### 6.2.3. Treatments

Solutions were mixed as follows: 0.02 mM methyl jasmonate and 0.02 mM methyl salicylate solutions were created by mixing 4.3 μL methyl jasmonate (Sigma Aldrich, St. Louis, MO) or 2.5 μL methyl salicylate with 1 mL 95% ethanol and then diluting into 100 mL DI water. Stylet oil solution was created by mixing 750 μL JMS Stylet Oil (JMS Flower Farms, Vero Beach, FL) with 100 mL DI water. Neem oil solution was created by mixing 781 μL pure neem oil (Dyna Gro, Richmond, CA) and 781 μL Top Surf non-ionic 80/20 surfactant (Agriliance, St Paul, MN) with 100 mL DI water.

### 6.2.4. EPG

To quantify aphid probing behavior during exposure to volatiles on infected and virus tested sweetpotato plants, EPG experiments were performed in a Faraday cage using a Giga8 DC amplifier (Wageningen Agricultural University, The Netherlands) with 1 gigaohm input resistance and an AD conversion rate of 100 Hz running only the first four channels. A DI-710 (DATAQ Instruments, Inc., Akron OH) acquisition card converted the analog signals to digital signals, which were recorded using WinDaq Serial Acquisition software (DATAQ Instruments, Inc., Akron OH). 18-µm gold wire (Semiconductor Packaging Material, Armonk, NY) was attached to the dorsal tergum of an apterous adult aphid with silver paint (Pelco Colloidal Silver Liquid no. 16034, Ted Pella, INC., Redding, CA). Aphids were placed on either an infected or virus tested sweetpotato plant and exposed to volatiles via 9 cm filter paper (410 Qualitative, VWR International, Sugar Land, TX) impregnated with 2 mL of volatile solution suspended approximately 60 cm above the test plant. A 1% ethanol solution was used as a control. Aphids feeding behavior was recorded for 20 min. Four aphids were tested at a time, this was repeated sixteen times for each treatment, for a total of 64 aphids per treatment. Ten different behaviors were analyzed for each aphid per each 20 min recording: time to the aphid's first probe, time to the aphid's first potential drop (cellular puncture), total number of probes, number of potential drops per probe, duration (sec) of the aphid's first probe, duration of all of the aphid's probes (sec), number of archlets, duration of cellular puncture phase II-1 (sec), and the duration of cellular puncture phase II-3(sec). These ten behaviors were analyzed because they correlate with virus transmission. The time to first probe indicates acceptance of the plant based on external

plant cues, including volatiles (Fereres and Moreno 2009). Probe duration indicates likelihood of virus transmission and acquisition; the longer an aphid is probing the higher the chance of transmission.

### 6.2.5. Virus transmission assay

Aphids were starved for 1 hour. A single mixed infected Beauregard leaf was placed on moist filter paper under a dissecting scope. A single aphid was placed on the leaf and monitored until it assumed resting position (antennae laid back over the abdomen), an indicator that the aphid has begun to probe the plant. The aphid the length of the acquisition access period was five minutes or until the aphid left resting position. The aphid was then gently removed with a #000 brush and transferred to an *I. nil* 'Scarlet O'Hara' plant at cotyledon stage. A piece of 9 cm filter paper (410 Qualitative, VWR International, Sugar Land, TX) soaked with 2 mL of volatile solution (ethanol solution for control) was suspended above the plant. The aphid given an inoculation access period of 10 minutes before being removed. Plants were then transferred to a greenhouse for symptom monitoring. Plants were monitored for fourteen days for virus symptoms. Ten aphids were used per replicate, with two replicates per treatment.

### 6.2.6. Data Analysis

Data was tested for normality with The Shapiro Wilk test. EPG data was nonparametric. Each of the tested variables were analyzed with the Wilcoxon Each Pair test in JMP Pro 14 (SAS 2018). Virus transmission assays were analyzed by analysis of variance (ANOVA) in JMP Pro 14 (SAS 2018).

## 6.3. Results

### 6.3.1 EPG.

**6.3.1.1. Green Peach Aphid on VT sweetpotato** Exposure volatiles altered green peach feeding behavior. GPA feeding on virus tested plants exposed to MEJA had a significantly longer time to initiate first probe that those feeding while exposed to stylet oil ($Z$ = -2.5, df = 1, $P$ = 0.0102) or MESA ($Z$ = -2.2, df = 1, $P$ = 0.0225) (Figure 6.1). GPA feeding while exposed to MEJA had a significantly longer duration of their first probe ($Z$ = 2.7, df = 1, $P$ = 0.0065) than GPA feeding on control plants, as did those feeding on plants exposed to stylet oil ($Z$ = 2.8, df = 1, $P$ = 0.0052) and neem oil ($Z$ = 2.2, df = 1, $P$ = 0.0258). GPA exposed to MEJA had a significantly longer time to first potential drop than aphids exposed to stylet oil ($Z$ = -2.2, df = 1, $P$ = 0.0229) or MESA ($Z$ = -2.2, df = 1, $P$ = 0.0260). GPA exposed to MESA had a significantly longer potential drop duration compared to control ($Z$ = 2.4, df = 1, $P$ = 0.00129), as did GPA exposed to MEJA ($Z$ = 4.4, df = 1, $P$ < 0.0001) and neem oil ($Z$ = 3.7, df = 1, $P$ = 0.0001). Additionally, potential drop duration was significantly long for aphids exposed to MEJA than those exposed to MESA ($Z$ = -2.0, df = 1, $P$ = 0.0377) or stylet oil ($Z$ = -2.7, df = 1, $P$ = 0.0061). GPA exposed to all volatile treatments had a higher number of archlets than those exposed to control (MESA: $Z$ = 3.0, df = 1, $P$ = 0.0025; MEJA: $Z$ = 6.0, df = 1, $P$ < 0.0001; stylet oil: $Z$ = 4.5, df = 1, $P$ < 0.0001; neem oil: $Z$ = 4.1, df = 1, $P$ < 0.0001). GPA exposed to MEJA had significantly more archlets than those exposed to neem ($Z$ = -2.4, df = 1, $P$ = 0.00158) or MESA ($Z$ = -3.0, df = 1, $P$ = 0.0020). GPA exposed to MESA ($Z$ = 3.7, df = 1, $P$ = 0.0002) and stylet oil ($Z$ = -2.3, df = 1, $P$ = 0.0212) had a longer duration of phase II-1 than aphids exposed to control, while aphids exposed to MEJA had significantly shorter phase II-1 duration ($Z$ = -4.5, df = 1, $P$ < 0.0001). Duration of phase II-3 was significantly longer in GPA exposed to MEJA

Table 6.1: Comparison of ten feeding behaviors of green peach aphid while exposed to volatile treatments on virus tested sweetpotato (mean±se). All times are in seconds. Letters indicate that treatments are significant different from each other.

| | Ethanol Control | Methyl Salicylate | Methyl Jasmonate | JMS Stylet Oil | Neem Oil |
|---|---|---|---|---|---|
| Time to 1st probe (s) | 326.609±58.941 ac | 249.547±43.489 a | 362.923±53.053 bc | 237.109±40.664 a | 320.157±54.904 ac |
| Time to 1st pd (s) | 391.78±62.865 a | 335.198±55.37 a | 519.485±76.29 a | 313.832±50.559 a | 422.783±59.792 a |
| Duration of 1st probe (s) | 194.077±59.482 a | 237.645±52.442 ab | 240.693±55.491 bc | 264.123±57.294 bd | 255.001±54.873 be |
| Total probe duration (s) | 544.346±57.455 a | 681.451±56.299 a | 561.37±65.361 a | 666.083±60.054 a | 669.112±59.792 a |
| Mean pd duration (s) | 4.58±0.059 a | 4.88±0.083 b | 5.2±0.127 c | 4.96±0.095 ab | 5±0.07 bc |
| # of archlets per pd | 0.218±0.064 a | 0.621±0.118 b | 1.162±0.204 c | 0.959±0.133 bc | 0.811±0.116 b |
| Duration of II-1 (s) | 1.335±0.016 a | 1.417±0.018 b | 1.213±0.025 c | 1.312±0.014 d | 1.393±0.026 a |
| Duration of II-3 (s) | 1.512±0.053 a | 1.662±0.071 a | 1.892±0.093 b | 1.942±0.08 bc | 1.723±0.006 ac |
| # of probes | 3.321±0.385 a | 2.911±0.302 a | 2.333±0.245 a | 2.735±0.25 a | 2.484±0.242 a |
| # pds per probe | 9.5±1.073 a | 6.55±0.895 b | 5.37±1.03 b | 8.79±1.284 ab | 9.454±1.079 a |

($Z$ = 3.8, df = 1, $P$ = 0.0001) and stylet oil ($Z$ = 3.1, df = 1, $P$ = 0.0017) than those exposed to control on VT plants. In addition, duration of phase II-3 was significantly long in GPA exposed to stylet oil than those exposed to MESA ($Z$ = 2.1, df = 1, $P$ = 0.0282). Phase II-3 was significantly shorter in aphids exposed to MEJA than those exposed to MESA ($Z$ = -2.7, df = 1, $P$ = 0.0062) or neem oil ($Z$ = -2.3, df = 1, $P$ = 0.0181). GPA feeding on VT plants exposed to MESA ($Z$ = -2.2, df = 1, $P$ = 0.0249) and MEJA ($Z$ = -2.7, df = 1, $P$ = 0.0056) had significantly fewer potential drops than aphids feeding on control plants. They also had significantly fewer potential drops than GPA expose to neem oil (MESA: $Z$ = 2.1, df = 1, $P$ = 0.0329; MEJA: $Z$ = 2.6, df = 1, $P$ = 0.0077).

**6.3.1.2. Green Peach Aphid on mixed infected sweetpotato** GPA feeding on mixed infected sweetpotato while exposed to neem oil volatiles had a significantly shorter time to first probe than aphids exposed to MEJA ($Z$ = -2.7, df = 1, $P$ = 0.0052) or the control treatment ($Z$ = -3.0, df = 1, $P$ = 0.0020) (Table 6.2). GPA exposed to neem also had a significantly shorter time to first potential drop than aphids exposed to control ($Z$ = -3.4, df = 1, $P$ = 0.0006), MESA ($Z$ = -3.1, df = 1, $P$ = 0.0015), or MEJA ($Z$ = -2.6, df = 1, $P$ = 0.0073). GPA exposed to MESA while feeding on mixed infected plants had significantly longer first probe duration than any other treatment (Control: $Z$ = 2.5, df = 1, $P$ = 0.0108; MEJA: $Z$ = 4.4, df = 1, $P$ < 0.0001; Stylet oil: $Z$ = -4.0, df = 1, $P$ < 0.0001; neem oil: $Z$ = -3.6, df = 1, $P$ = 0.0002). Additionally, GPA exposed stylet oil had a significantly shorter first probe duration than aphids exposed to the control treatment ($Z$ = -2.0, df = 1, $P$ = 0.0378). GPA probing on mixed infected sweetpotato while exposed to neem oil had longer total probe duration than those exposed to MESA ($Z$ = 3.6, df = 1, $P$ = 0.0003) or MEJA ($Z$ = 2.0, df = 1, $P$ = 0.0051); those exposed to style oil also had longer total probe durations than those exposed to MESA ($Z$ = 2.0, df = 1, $P$ = 0.0453). GPA probing on

Table 6.2: Comparison of ten feeding behaviors of green peach aphid while exposed to volatile treatments on mixed infected sweetpotato. (mean±se) All times are in seconds. Letters indicate that treatments are significant different from each other.

| | Ethanol Control | Methyl Salicylate | Methyl Jasmonate | JMS Stylet Oil | Neem Oil |
|---|---|---|---|---|---|
| Time to 1st probe (s) | 418.526±54.989 a | 354.645±56.51 ab | 420.453±60.824 a | 359.499±63.755 ab | 205.604±43.483 b |
| Time to 1st pd (s) | 485.368±56.15 a | 453.442±54.948 a | 424.798±55.797 a | 378.162±62.132 ab | 234.511±44.42 b |
| Duration of 1st probe (s) | 470.14±70.335 a | 469.144±63.891 b | 327.542±55.328 ac | 307.833±62.237 c | 337.561±71.015 ac |
| Total probe duration (s) | 644.371±60.919 ab | 729.635±50.985 a | 570.719±51.988 ac | 646.446±63.314 bc | 812.907±48.172 b |
| Mean pd duration (s) | 5.141±0.111 a | 5.369±0.107 bc | 5.381±0.096 bc | 3.96±0.083 d | 4.302±0.061 e |
| # of archlets per pd | 0.296±0.114 a | 0.723±0.137 b | 0.669±0.126 b | 0.424±0.0757 ab | 0.555±0.093 b |
| Duration of II-1 (s) | 1.475±0.018 a | 1.402±0.015 bc | 1.401±0.021 bc | 1.355±0.015 b | 1.445±0.021 bc |
| Duration of II-3 (s) | 1.646±0.074 a | 2.036±0.083 bc | 2.044±0.083 bc | 1.165±0.04 de | 1.318±0.048 de |
| # of probes | 1.703±0.218 a | 1.857±0.238 ac | 1.935±0.274 ac | 2.437±0.32 bc | 2.692±0.287 b |
| # pds per probe | 7.111±1.01a | 7.607±1.115 a | 7.322±1.09 a | 9.454±1.344 ab | 12.23±1.272 b |

plants exposed to MEJA had longer potential drops than those probing on plants exposed to the control ($Z$ = 2.4, df = 1, $P$ = 0.0145), stylet oil, ($Z$ = -13.9, df = 1, $P$ < 0.0001), and neem oil ($Z$ = -10.3, df = 1, $P$ < 0.0001). GPA feeding on plants exposed to MESA had longer potential drops than those probing on plants exposed to the control ($Z$ = 2.2, df = 1, $P$ = 0.0278), stylet oil, ($Z$ = -13.7, df = 1, $P$ < 0.0001), and neem oil ($Z$ = -10.2, df = 1, $P$ < 0.0001). Potential drops of aphids exposed to stylet oil were significantly shorter than those exposed to the control ($Z$ = -11.9, df = 1, $P$ < 0.0001), as were those exposed to neem oil ($Z$ = -8.2, df = 1, $P$ < 0.0001). furthermore, there was a significant difference in the duration of potential drops of GPA exposed to neem and stylet oil ($Z$ = -3.9, df = 1, $P$ < 0.0001). Aphids exposed to MESA during feeding had more archlets than those exposed to the control ($Z$ = 2.6, df = 1, $P$ = 0.0073), as did those feeding while exposed to MEJA ($Z$ = 3.0, df = 1, $P$ = 0.0024), and neem oil ($Z$ = 2.6, df = 1, $P$ = 0.0077). GPA exposed to all treatments had shorter phase II-1 durations than the control (MESA: $Z$ = -3.0, df = 1, $P$ = 0.0025; MEJA: $Z$ = -2.0, df = 1, $P$ = 0.0020; stylet oil: $Z$ = -5.0, df = 1, $P$ < 0.0001; neem oil: $Z$ = -2.0, df = 1, $P$ = 0.0367). Additionally, duration of phase II-1 was significantly different for aphids exposed to stylet oil and neem oil ($Z$ = -3.8, df = 1, $P$ < 0.0001). Aphids exposed to MESA while feeding had significantly longer phase II-3 than those feeding on those exposed to control ($Z$ = 4.0, df = 1, $P$ < 0.0001), stylet oil ($Z$ = -11.7, df = 1, $P$ < 0.0001), or neem oil ($Z$ = -9.3, df = 1, $P$ < 0.0001). Aphids exposed to MEJA while feeding had significantly longer phase II-3 than those feeding on those exposed to control ($Z$ = 3.9, df = 1, $P$ < 0.0001), stylet oil ($Z$ = -11.5, df = 1, $P$ < 0.0001), or neem oil ($Z$ = -9.1, df = 1, $P$ < 0.0001). GPA exposed to stylet oil had significantly shorter phase II-3 durations compared to those exposed to control ($Z$ = -8.1, df = 1, $P$ < 0.0001) as did aphids exposed to neem oil ($Z$ = -5.7, df = 1, $P$ < 0.0001). Aphids exposed to neem oil probed significantly more compared to those

exposed to the control ($Z$ = 2.6, df = 1, $P$ = 0.0085), MESA ($Z$ = 2.2, df = 1, $P$ = 0.0236), or MEJA ($Z$ = 2.3, df = 1, $P$ = 0.0192). GPA exposed to stylet oil also probe significantly more than those exposed to the control ($Z$ = 2.0, df = 1, $P$ = 0.0382). GPA exposed to neem oil performed more potential drops than those exposed to the control ($Z$ = 2.8, df = 1, $P$ = 0.0046), MESA ($Z$ = 2.6, df = 1, $P$ = 0.0070), or MEJA ($Z$ = 2.7, df = 1, $P$ = 0.0052).

**6.3.1.3. Cotton Aphid on VT sweetpotato** No feeding was observed by CA exposed to stylet or neem oil. CA exposed to both MESA ($Z$ = -2.8, df = 1, $P$ = 0.0050) and MEJA ($Z$ = -3.5, df = 1, $P$ = 0.0005) had shorter potential drops than those exposed to the control (Figure 6.3). CA exposed to MESA had significantly longer phase II-1 durations than aphids exposed to control ($Z$ = 2.7, df = 1, $P$ = 0.0060) but significantly shorter phase II-3 ($Z$ = -2.0, df = 1, $P$ = 0.0371). CA exposed to MEJA had significantly shorter phase II-3 durations than aphids exposed to control ($Z$ = -4.1, df = 1, $P < 0.0001$).

Table 6.3: Comparison of ten feeding behaviors of cotton aphid while exposed to volatile treatments on virus tested sweetpotato. (mean±se) All times are in seconds. Letters indicate that treatments are significant different from each other.

| | Ethanol Control | Methyl Salicylate | Methyl Jasmonate |
|---|---|---|---|
| Time to 1st probe (s) | 371.048±88.956 a | 489.505±183.534 a | 359.445±54.379 a |
| Time to 1st pd (s) | 482.913±109.02 a | 511.±242.463 a | 519.485±76.29 a |
| Duration of 1st probe (s) | 349.601±83.09 a | 234.053±124.655 a | 574.878±140.41 a |
| Total probe duration (s) | 628.243±86.653 a | 632.558±56.299 a | 781.821±119.956 a |
| Mean pd duration (s) | 5.183 ±0.209 a | 3.928±0.334 b | 4.146±0.194 b |
| # of archlets per pd | 0.481±0.146 a | 0.388±0.204 a | 0.441±0.22 a |
| Duration of II-1 (s) | 1.219±0.024 a | 1.307±0.0485 b | 1.281±0.048 a |
| Duration of II-3 (s) | 2.152±0.188 a | 1.492±0.153 b | 1.309±0.156 b |
| # of probes | 1.866±0.255 a | 1.8±0.583 a | 1.888±0.200 a |
| # pds per probe | 5.4±1.182 a | 7.2±4.641 a | 4.888±1.549 a |

**6.3.1.4. Cotton Aphid on mixed infected sweetpotato** CA exposed to stylet oil had a significantly shorter time to first probe than aphids exposed to MESA ($Z$ = 2.8, df = 1, $P$ = 0.0050) or MEJA ($Z$ = -2.1, df = 1, $P$ = 0.0345) (Figure 6.4). CA exposed to stylet oil had a significantly longer total probe duration than aphids exposed the control ($Z$ = 1.9, df = 1, $P$ =

0.0480), MESA ($Z$ = -3.1, df = 1, $P$ = 0.0018), or neem oil ($Z$ = -2.1, df = 1, $P$ = 0.0307). CA exposed to MEJA had significantly shorter potential drops than those exposed to the control ($Z$ = -2.0, df = 1, $P$ = 0.0396) or MESA ($Z$ = 2.1, df = 1, $P$ = 0.0333). CA exposed to stylet oil had significantly shorter potential drops than those exposed to the control ($Z$ = -3.6 df = 1, $P$ = 0.0003) or MESA ($Z$ = 3.2, df = 1, $P$ = 0.0012). CA exposed to neem oil had significantly shorter potential drops than those exposed to the control ($Z$ = -2.1, df = 1, $P$ = 0.0330) or MESA ($Z$ = -2.0, df = 1, $P$ = 0.0378), or MEJA ($Z$ = -1.9, df = 1, $P$ = 0.0466). CA exposed to MESA performed significantly more archlets than those exposed to the control ($Z$ = 2.0, df = 1, $P$ = 0.0374), MEJA ($Z$ = 3.3, df = 1, $P$ = 0.0008), or stylet oil ($Z$ = 4.5, df = 1, $P$ < 0.0001). CA exposed to MEJA ($Z$ = -2.2, df = 1, $P$ = 0.0275) and stylet oil ($Z$ = -2.8, df = 1, $P$ = 0.0042) performed significantly more archlets than those exposed to the control. CA exposed to stylet oil had shorter phase II-1 durations than those exposed to the control ($Z$ = -4.5, df = 1, $P$ < 0.0001), MESA ($Z$ = 2.7, df = 1, $P$ = 0.0056), or MEJA ($Z$ = 3.4, df = 1, $P$ = 0.0006). CA exposed to MESA had significantly longer phase II-3 durations than those exposed to the MEJA ($Z$ = 3.3, df = 1, $P$ = 0.0008), or stylet oil ($Z$ = 3.5, df = 1, $P$ = 0.0004). CA exposed to MESA had significantly shorter phase II-3 durations than those exposed to the control ($Z$ = -2.1, df = 1, $P$ = 0.0304). CA exposed to stylet oil performed significantly more potential drops than those exposed to the control ($Z$ = 2.9, df = 1, $P$ = 0.0027), MESA ($Z$ = -4.1, df = 1, $P$ < 0.0001), or neem oil ($Z$ = -2.0, df = 1, $P$ = 0.0431). CA exposed to MEJA performed more potential drops than those exposed to MESA ($Z$ = -2.2, df = 1, $P$ = 0.0273).

Table 6.4: Comparison of ten feeding behaviors of cotton while exposed to volatile treatments on mixed infected sweetpotato. (mean±se) All times are in seconds. Letters indicate that treatments are significant different from each other.

| | Ethanol Control | Methyl Salicylate | Methyl Jasmonate | JMS Stylet Oil | Neem Oil |
|---|---|---|---|---|---|
| time to 1st probe (s) | 335.257±65.71 ab | 512.2±85.464 a | 483.17±41.881 a | 190.635±41.881 b | 725.862±280.462 a |
| time to 1st pd (s) | 408.462±73.571 a | 515.74±91.37 a | 499.825±70.555 a | 353.788±70.555 a | 1042.692±0 a |
| duration of 1st probe (s) | 325.295±70.548 a | 299.644±72.975 a | 375.656±90.805 a | 505.669±90.805 a | 107.625±86.61 a |
| total probe duration (s) | 601.834±67.529 a | 437.256±68.642 a | 804.101±65.36 ab | 804.101±65.36 b | 186.437±7.23 a |
| Mean pd duration (s) | 5.73±0.223 a | 6±0.331 a | 4.582±0.197 b | 4.77±0.248 bc | 3.674±0.113 c |
| # of archlets per pd | 1.411±0.231 a | 2.444±0.427 b | 0.533±0.249 b | 0.62±0.136 b | 0±0 ab |
| duration of II-1 (s) | 1.257±0.0289 a | 1.186±0.027 a | 1.256±0.065 a | 1.177±0.069 b | 1.242±0.193 ab |
| duration of II-3 (s) | 2.568±0.197 ac | 3.146±0.305 a | 1.619±0.182 b | 1.831±0.108 ac | 1.106±0.196 abc |
| # of probes | 1.875±0.173 a | 1.684±0.23 a | 2±0.436 a | 1.933±0.153 a | 1±0 a |
| # pds per probe | 5.625±0.861 ab | 3.263±0.517 a | 6.428±1.306 bc | 10±1.15 c | 2±2 ab |

### 6.3.2. Virus Transmission Assays

There were no significant differences in the percentage of plants showing symptoms in any of the treatments compared to the control plants in transmission assays with either aphid (Figures 6.1 and 6.2).

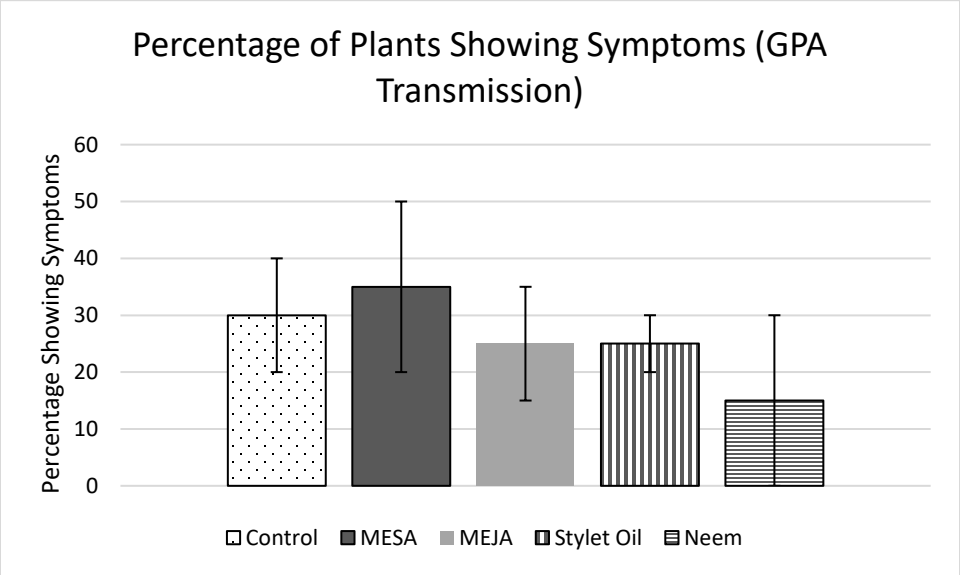


Figure 6.1: Percentage of plants exposed to GPA showing virus symptoms per treatment.

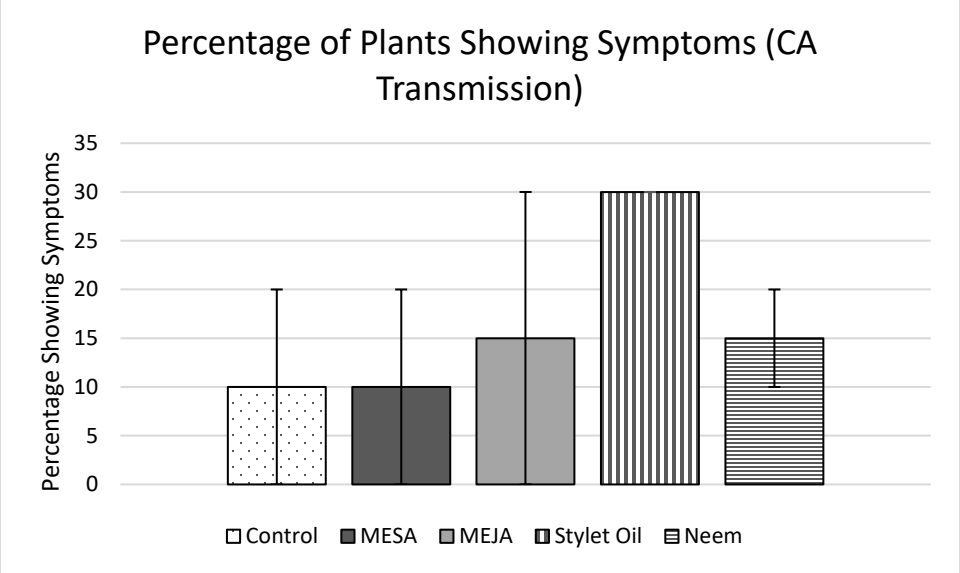


Figure 6.2: Percentage of plants exposed to CA showing virus symptoms per treatment.

## 6.4. Discussion

Exposure to volatiles affected behaviors related to virus transmission and acquisition in every treatment compared to the ethanol control, with the exception of CA exposed to neem oil on mixed infected plants and CA exposed to stylet oil or neem oil on virus tested plants. These results suggest that headspace volatiles alter aphid behavior during probing. This experiment was designed to fill the headspace with volatiles, however, there are some parts of the experimental design that may affect results. First, the release rate and amount of volatiles released from the filter paper during the experiment is unknown and likely does not resemble natural conditions. In order to gauge release rates and the effects of different volatile concentrations on aphid feeding behavior, a capillary tube or other system in which the evaporation of volatiles could be quantified would be more accurate. As the goal of these experiments was not to mimic nature conditions, but to test the hypothesis that headspace volatiles would affect feeding aphid behavior, this was not attempted. Secondly, aphids were placed on the plant and tethered during the experiment, so these experiments do not account for the effect of volatiles on alighting behavior. Thirdly, this experiment does not account for the effect of headspace volatiles on the sweetpotato plant. Both MESA and MEJA induce defensive responses to aphids (Smith and Boyko 2006). Thus, any of the volatile chemicals could have induced a defensive response to the aphids, affecting feeding behavior. Induced resistance tends express after a delay of hours to days (Underwood 1998), for example, tomato exposed to 100 nL MEJA only showed an increase in proteinase inhibitor levels five hours after exposure (Farmer and Ryan 1990). Thus, 20 minutes of exposure would not be enough to induce resistance at any meaningful level. However, volatiles physically interact with the plant surface, and may be adsorbed and rereleased (Himanen et al. 2010). Thus, the changes in aphid behavior could be affected by volatiles on the

plant surface, not just an olfactory response. Finally, the aphids in these experiments were not viruliferous, which may affect aphid feeding behavior.

In previous research, GPA feeding on soybean treated with jasmonic acid and salicylic acid exhibited longer probing time and increase in the duration of phases II-1 and II-3 (Dryburgh 2015). In this study, volatiles from the methylated forms of these compounds had a mixed effect on these behaviors. GPA feeding on virus tested sweetpotato exposed to these compounds showed an increased potential drop duration, and more archlets, with a mixed effect on the duration of potential drop subphases. MEJA exposed GPA had a longer first probe, but both treatments had fewer potential drops. GPA feeding on mixed infected plants while exposed to these volatiles had increased potential drop and phase II-3 durations as well as an increased number of archlets, however, they had shorter phase II-1 durations. CA feeding on virus tested plants while exposed to MESA had shorter potential drops and phases II-1 and II-3, while those exposed to MEJA had increased potential drop durations but shorter phase II-3. CA feeding on mixed infected plants exposed to MESA had more archlets, while those exposed to MEJA had fewer archlets, and shorter potential drops and phase II-3. These results suggest a mixed effect on virus transmission. Increases in the duration of potential drops and their subphases indicated a higher likely hood of virus transmission. They also may indicate that the plant is a better host for the aphid, as it is not encountering any unpalatable chemicals within the epidermal cells (Fereres and Moreno 2009). The increased first probe duration by GPA on virus tested sweetpotato exposed to MEJA suggests a higher likelihood of virus transmission, while the reduced number of potential drops in these treatments suggests the opposite.

While stylet oil did not affect aphid orientation or settling behavior (Chapter 5), it did affect feeding behavior. This suggests that foreign headspace volatiles affect aphid probing

behavior. Like MESA and MEJA, it had a mixed effect on probing behavior, increasing the number of archlets and duration of the first probe and phase II-3 in GPA on virus tested plants, while decreasing the duration of phase II-1. In GPA feeding on mixed infected plants, it decreased the number of archlets and the duration of the first probe, potential drop and phases II-1 and II-3. However, exposure increased the average number of probes. CA exposed while feeding on mixed infected plants exhibited an increased total probe duration and number of potential drops, but a decrease number of archlets and potential drop and phase II-1 duration. As stylet oil applied to the plant reduces virus transmission by interfering with the adhesion of virons with the aphid stylet (Powell 1992), it is unclear what affect this would have in the field.

Neem oil similarly displayed inconsistent effects on aphid feeding behavior, In GPA feeding on virus tested plants, exposure increased the time to first potential drop, potential drop duration, and number of archlets. In GPA feeding on mixed infected plants, exposure decreased the time to first probe and potential drop, while increasing the number of probes and potential drops. Exposure also reduced the duration of the potential drops and subphases but increased the number of archlets. Neem oil, like stylet oil, reduces virus transmission by physically inhibiting virion uptake (Lowrey et al. 1997) in addition to its antifeedant and potential repellent properties. As neem oil, like stylet oil, is effective at preventing virus transmission in the field; it is unclear what effect the volatile component of neem oil has on virus transmission in the field.

Overall, these results suggest that headspace volatiles have an effect on aphid probing behavior, suggesting that aphids use these cues in addition to internal plant cues in order to make a decision about the acceptability of plants as a host. Additionally, it appears that aphid species and virus infection status have an effect on how aphids interpret headspace volatiles while feeding.

Given the differences in aphid feeding behavior due to volatile exposure during feeding, virus transmission assays were performed to determine if this had an effect on virus transmission rates. There was no difference in the number of plants showing symptoms between any of the treatments in transmission assays by either. This suggests that the differences in aphid feeding behaviors between treatments are not significant enough to change virus transmission rates. Duration of phase II-1 by pea aphids that successfully transmitted *Pea enation mosaic virus* were longer than those that did not successfully transmit the virus (Powell 2005). Starved aphids have longer phase II-3, leading to greater virus acquisition (Collar et al. 1998; Powell et al. 1995). However, aphids reflexively probe surfaces, and will do so even in the presence of strong antifeedants such as dodecanoid acid (Fereres and Moreno 2009). It is likely that the time it takes to initiate a probe is the key factor in transmission of plant viruses, along with possibly the number of potential drops, as each new potential drop affords the opportunity to transmit the virus. In all treatments in the EPG experiments, the average time to first potential drop was under the ten minutes, the amount of time aphids were allowed for transmission. In an agricultural setting, reducing the number of vectors entering the field is most likely a more feasible method of reducing virus transmission. However, how factors such as host plant resistance and volatile cues affect virus transmission remains an interesting ecological question.

**6.5. References**

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# Chapter 7. Summary and Conclusions

Aphid transmitted plant viruses are a major economic problem in crops worldwide. Non-persistently transmitted viruses pose management problems as they are quickly acquired and transmitted by their vectors. In order to better control these viruses, the ecology of their vectors, including movement, host finding, and feeding behavior must be better understood. In many insects, host plant volatiles are an important part of host finding and plant acceptance. This research was performed to examine the effect of plant derived and exogenous volatiles on aphid behavior.

First, field movement of aphids was examined to identify trends in vector abundance throughout the sweetpotato growing season. Aphid abundance at the sites sampled appears to reflect the population dynamics of the most commonly collected species, the non-vector *Melanaphis sacchari*. Sweetpotato virus vectors numbers were consistently low throughout the years sampled, and there were no discernable trends in vector abundance, which suggest that aphid control tactics should be deployed early in the season when sweetpotato is most vulnerable to virus transmission.

Virus infection affects volatile emission in many different plant species which can affect insects' ability to find hosts. Thus, headspace volatiles of sweetpotato were collected to determine if virus infection status had an effect on the sweetpotato volatile profile. Thirteen compounds were produced by virus tested sweetpotato and 21 compounds were produced by infected sweetpotato. Compounds induced by virus infection were mainly green leaf volatiles and terpenes, many of which affect insect behavior. Additionally, this may be the first recorded effort to identify volatiles from sweetpotato free of known diseases.

In addition to volatiles induced by virus infection, other volatiles may have an effect on aphid behavior. To explore this, the effect of four compounds that emit volatiles on aphid behavior, in addition to the effect of virus infection status, was tested on two common sweetpotato virus vectors, green peach aphid (GPA, *Myzus persicae*) and cotton aphid (CA, *Aphis gossypii*). Methyl jasmonate (MEJA) and methyl salicylate (MESA) were chosen due to their importance in plant defense against insects. JMS stylet oil was chosen due to its effects on virus transmission, and the lack of research into the effect of its odor on insect behavior. Neem oil was chosen for previous research on repellency and its antifeedant behavior, and its potential use for vector management. In Y-tube assays, only GPA oriented towards odors. GPA preferred the odor of infected plants over virus tested plants, and the odor of virus tested plants and MESA over the odor of virus tested plants alone. GPA preferred the odor of virus tested plants alone over that of plant and MEJA or plant and neem oil. Settling assays were performed to determine if these preferences had any effect on aphid settling behavior. While GPA preferred plants treated with MESA, contrary to the result of the Y-tube assays, aphids preferred to settle on virus infected plants and neem oil treated plants over virus tested plants. This suggests that orientation towards odors does not necessarily indicate settling preference in GPA.

Finally, the effect of headspace volatiles on aphid feeding on infected and virus tested sweetpotato was examined in order to determine if volatile treatments that do not directly affect the plant affect aphid feeding behavior. Both GPA and CA exhibited changes in behaviors related to virus transmission in the presence of all volatile treatments compared to controls when feeding on both virus tested and virus infected plants, suggesting that exposure to headspace volatiles while feeding affects aphid feeding behavior. However, in virus transmission assays,

exposure to these volatiles during feeding did not affect the number of plants showing virus symptoms, suggesting that the changes in behavior are too subtle to affect transmission rates.

Sweetpotato remains an interesting system in which to study plant-virus-vector interactions. Future research may examine the effect of individual volatiles emitted by virus infected sweetpotato on vectors. It could focus on the effects of infection with different viruses, including different potyvirus mixes, on the volatiles sweetpotato emits. Another interesting avenue of research may be how these volatiles affect the economically important sweetpotato weevil, and whether the sweetpotato weevil in turns affects virus transmission.

# Appendix. GC/MS Chromatograms

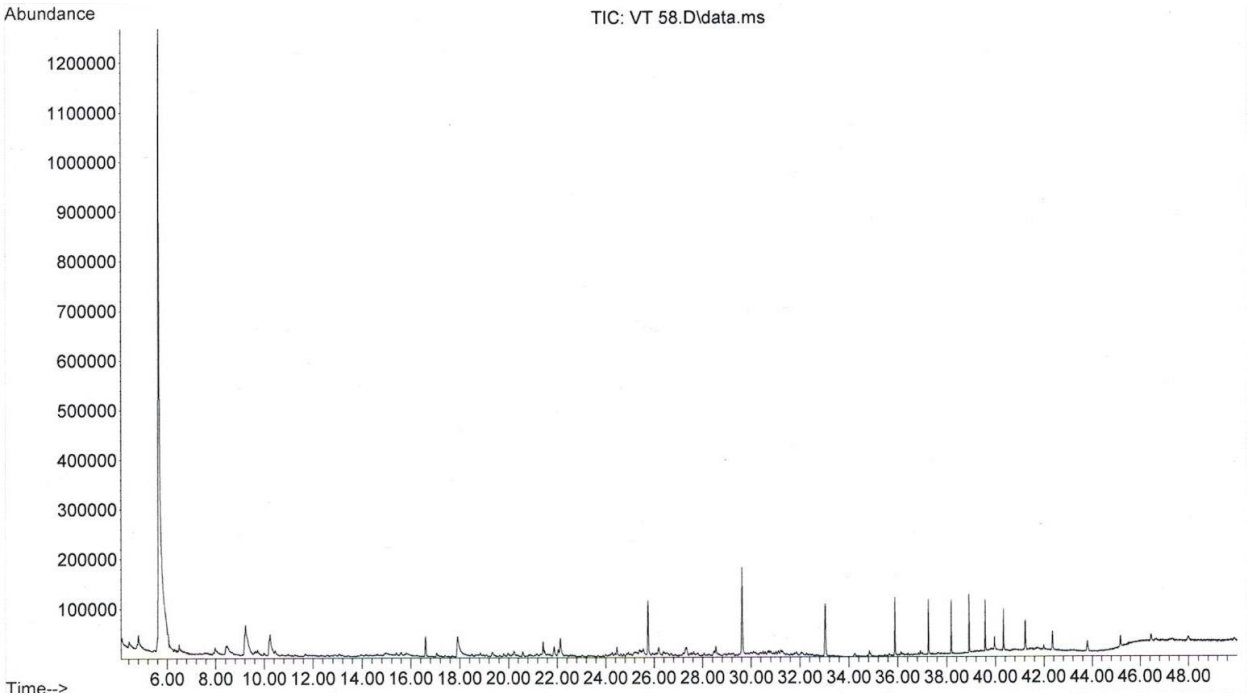


Figure A.1: Chromatogram of volatiles collected from the headspace of a virus tested sweetpotato slip on 5/8/2019

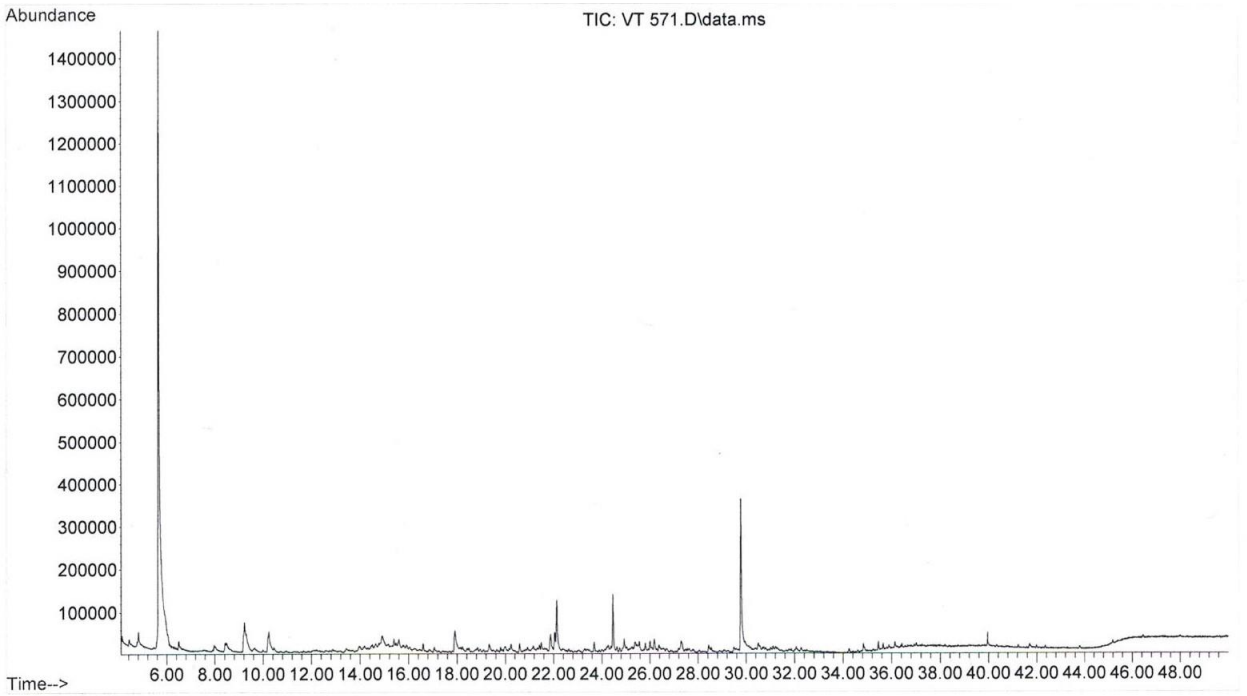


Figure A.2: Chromatogram of volatiles collected from the headspace of a virus tested sweetpotato slip on 5/7/2019

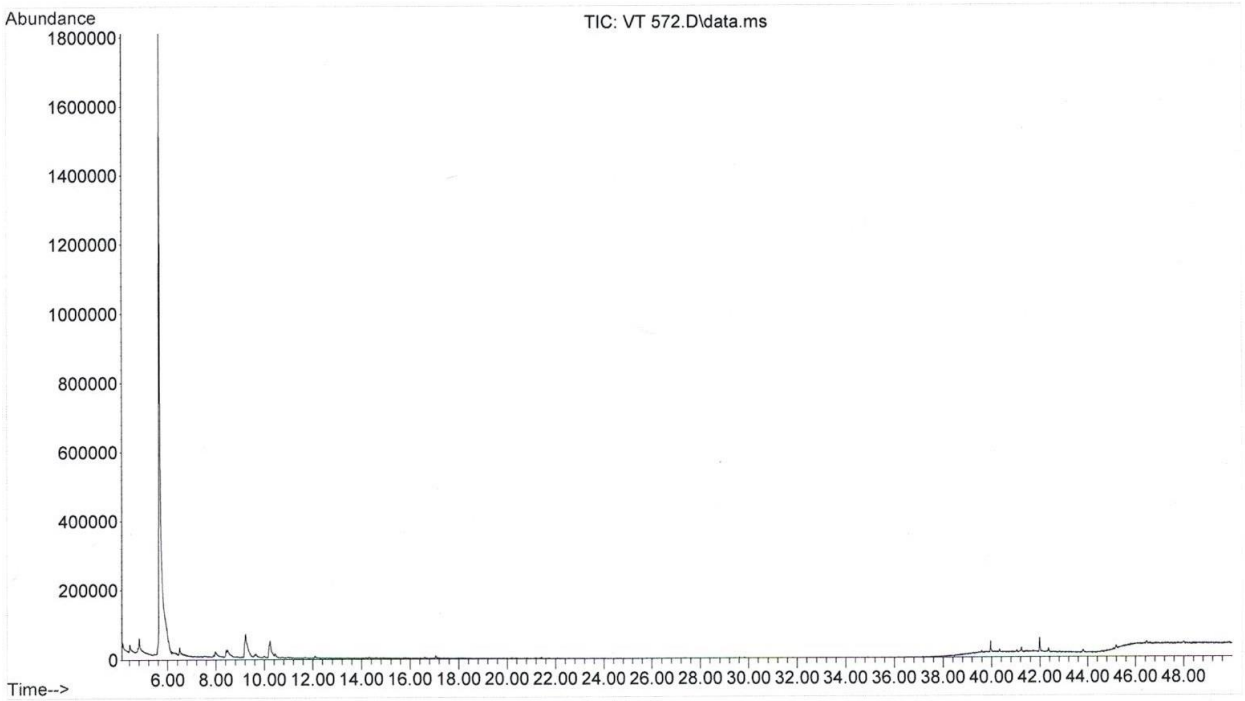


Figure A.3: Chromatogram of volatiles collected from the headspace of a virus tested sweetpotato slip on 5/7/2019

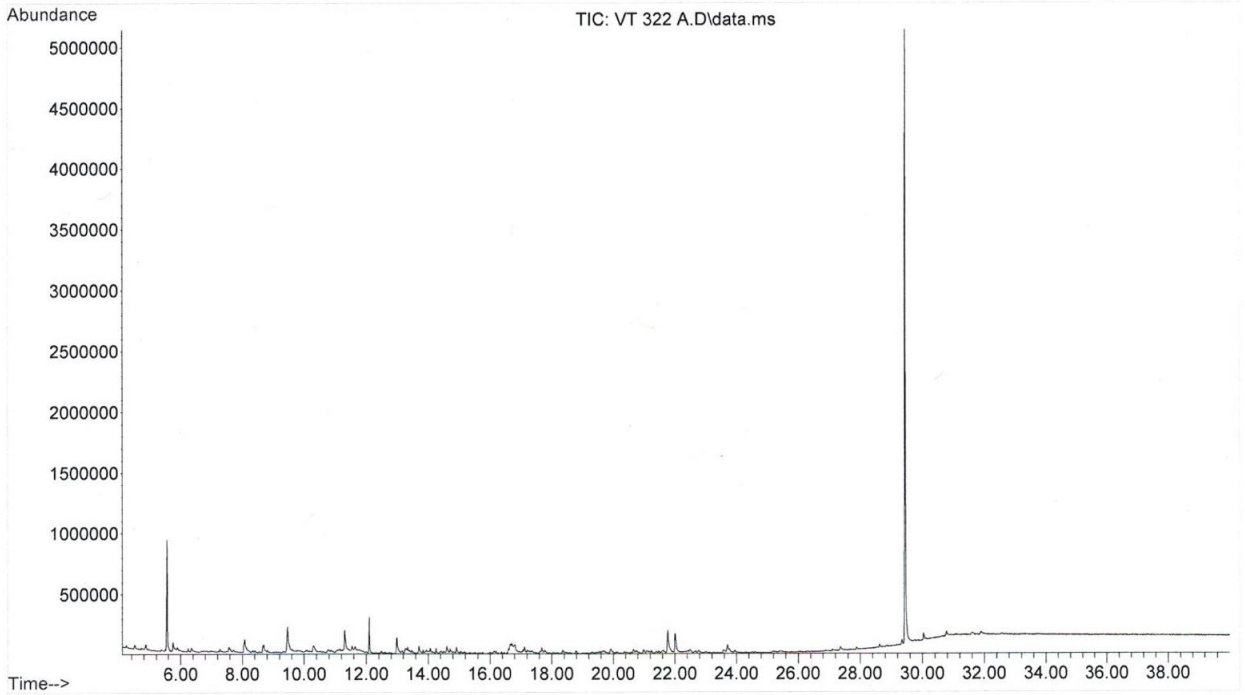


Figure A.4: Chromatogram of volatiles collected from the headspace of a virus tested sweetpotato slip on 3/22/2019

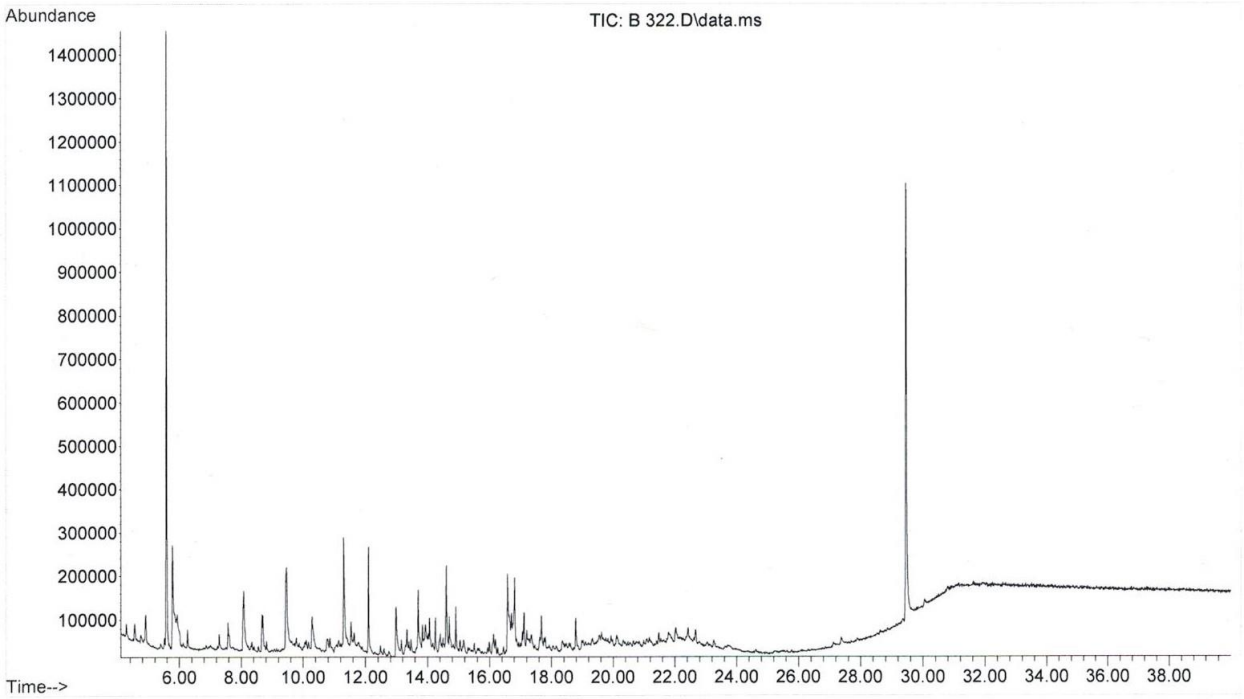


Figure A.5: Chromatogram of volatiles collected from the headspace of a virus tested sweetpotato slip on 3/22/2019

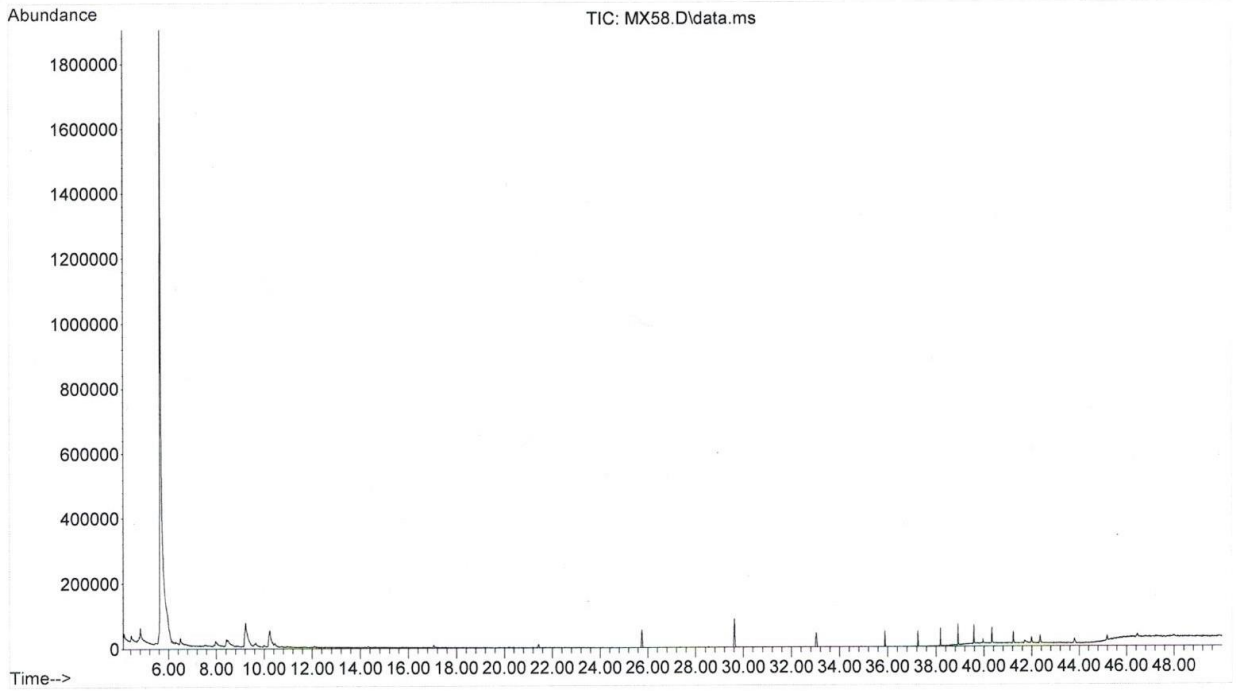


Figure A.6: Chromatogram of volatiles collected from the headspace of a mixed infected sweetpotato slip on 5/8/2019

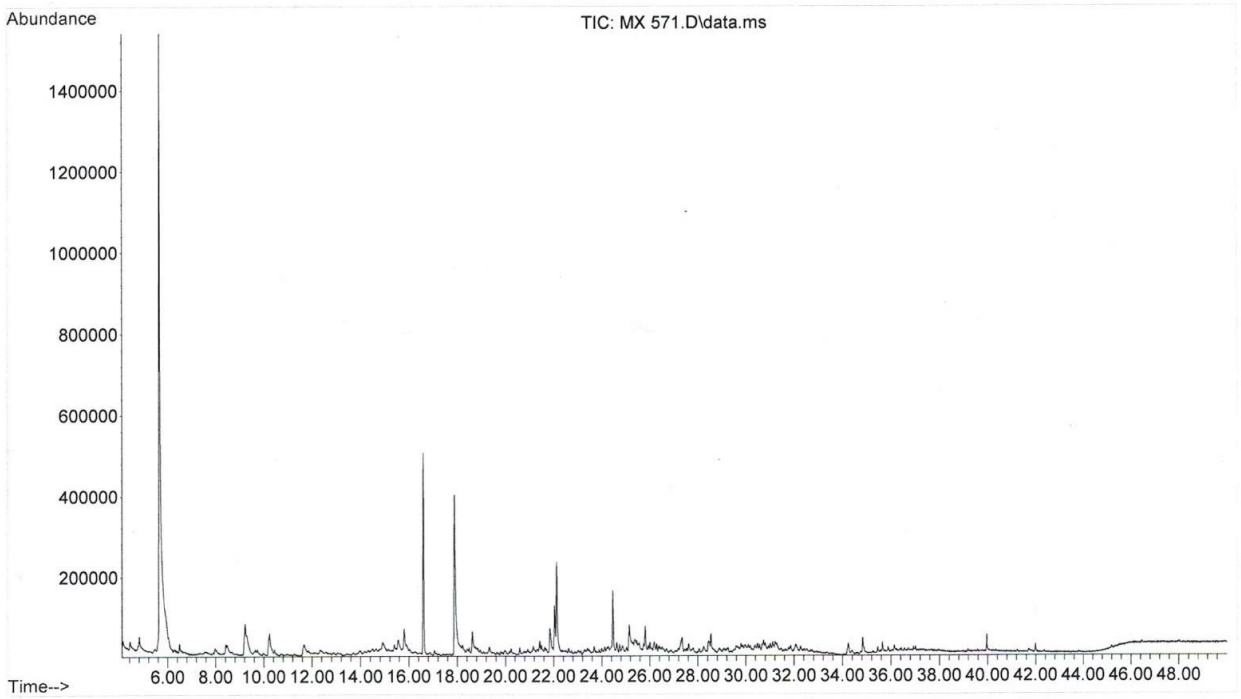


Figure A.7: Chromatogram of volatiles collected from the headspace of a mixed infected sweetpotato slip on 5/7/2019

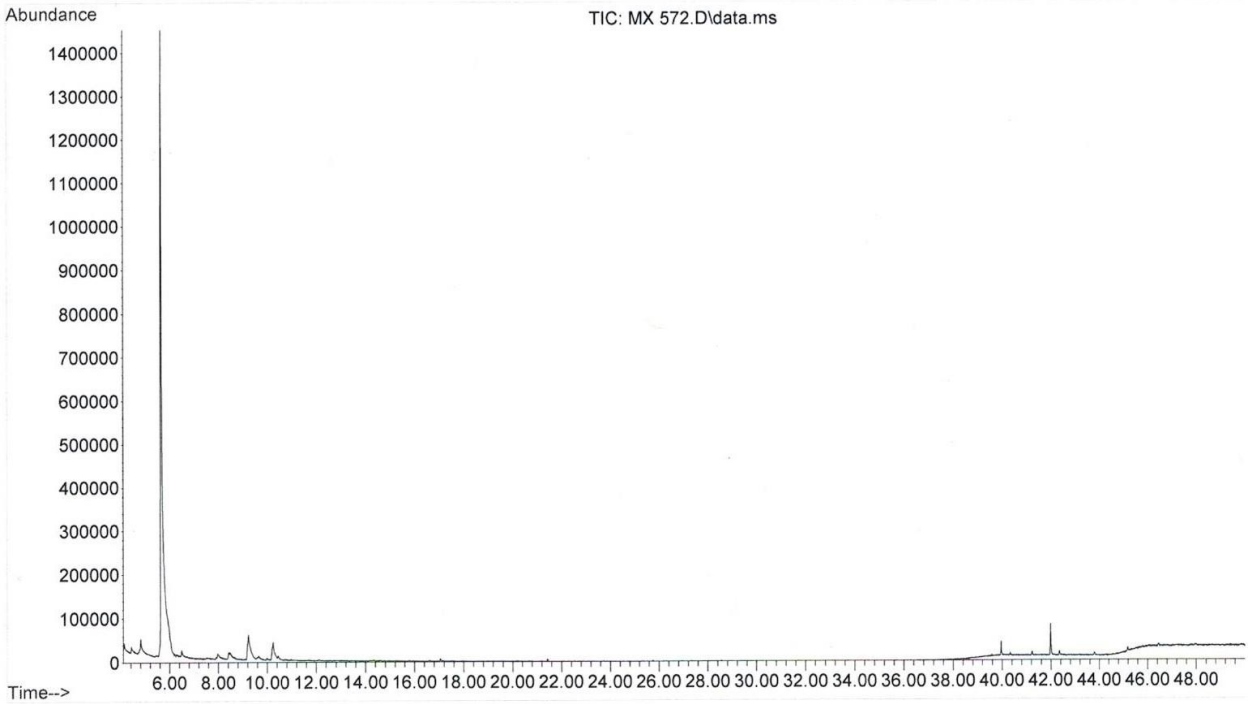


Figure A.8: Chromatogram of volatiles collected from the headspace of a mixed infected sweetpotato slip on 5/7/2019

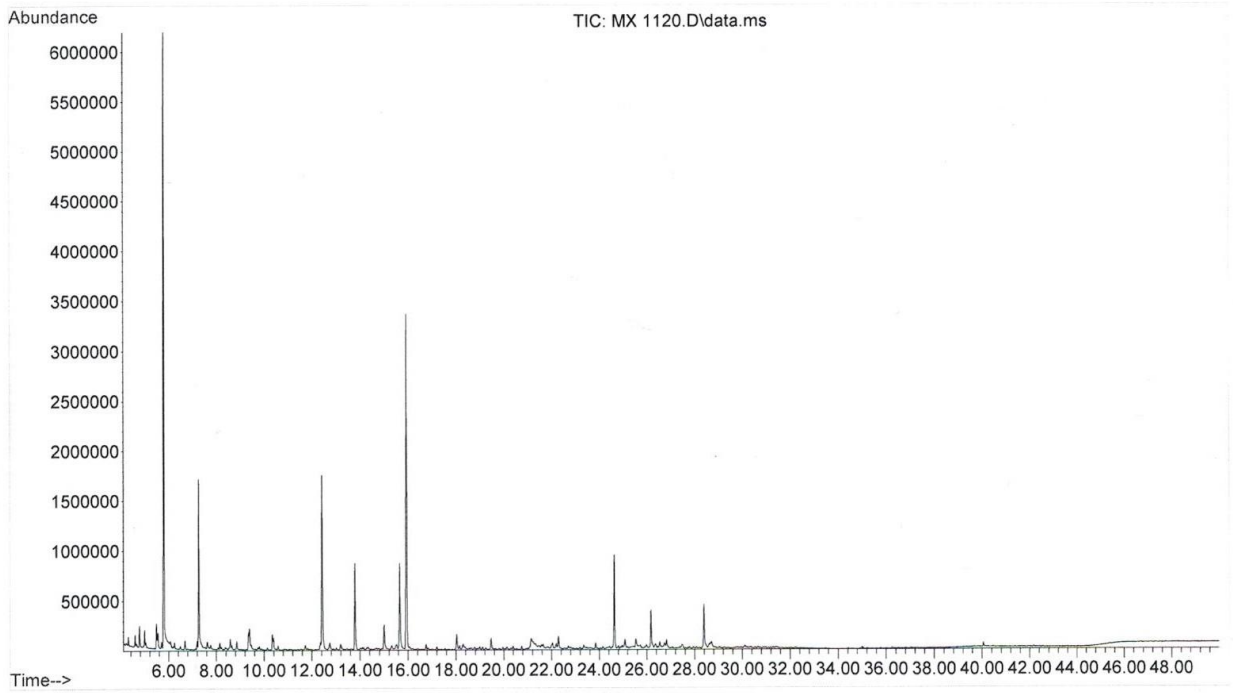


Figure A.9: Chromatogram of volatiles collected from the headspace of a mixed infected sweetpotato slip on 11/20/2018

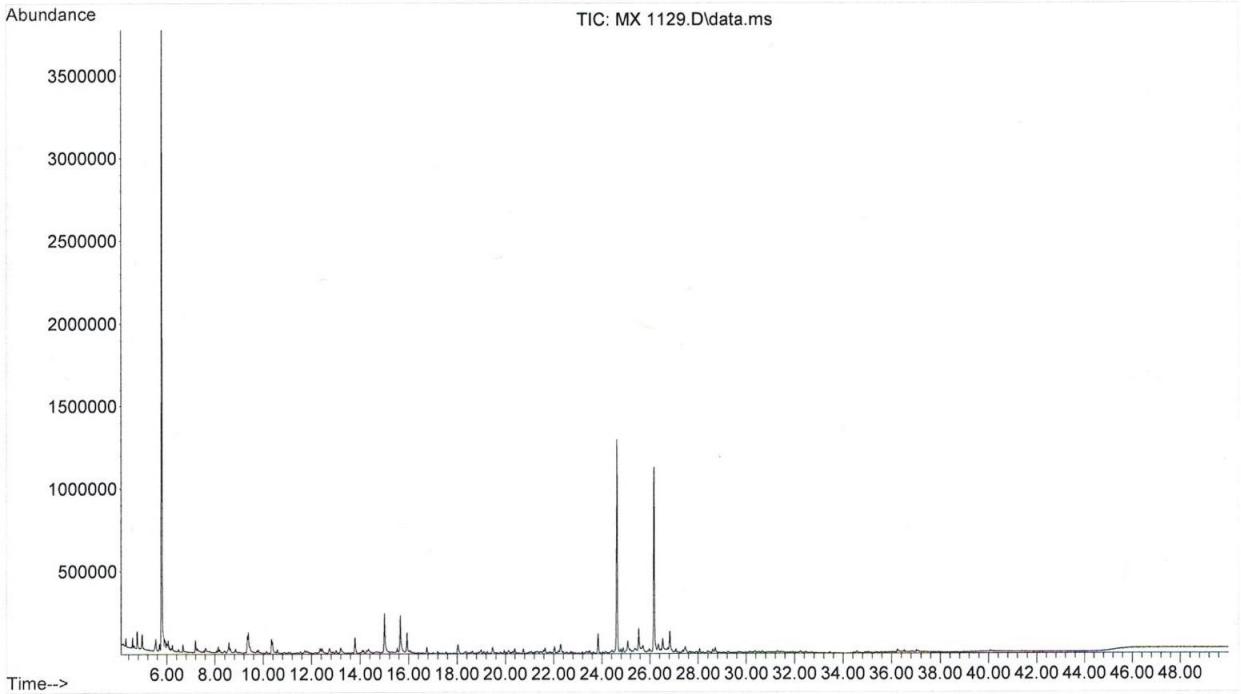


Figure A.10: Chromatogram of volatiles collected from the headspace of a mixed infected sweetpotato slip on 11/29/2018

# Vita

John Dryburgh is from King of Prussia, Pennsylvania. He graduated from Susquehanna University in 2012 with a Bachelor's degree in Biology. He completed his MS in Entomology (Thesis title: Herbivore response to soybean under differing induction methods) under the guidance of Dr. Jeffrey A. Davis in 2015.