BIOLOGY AND MANAGEMENT OF LEAFHOPPERS AND ASTER YELLOWS PHYTOPLASMA IN MICHIGAN CELERY AND CARROT AGROECOSYSTEMS

By

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ABSTRACT

BIOLOGY AND MANAGEMENT OF ASTER LEAFHOPPERS AND ASTER YELLOWS PHYTOPLASMA IN MICHIGAN CELERY AND CARROT AGROECOSYSTEMS

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Managing aster yellows phytoplasma (*Candidatus* Phytoplasma sp.) and its leafhopper vectors is complex and requires better control methods than those currently used and a greater understanding of the phytoplasma-insect relationship. In this thesis, I determine the effectiveness of a decision support tool focused on managing phytoplasma infected leafhoppers and determine whether leafhoppers in celery and carrot field edges contribute to phytoplasma transmission within the crop fields.

We informed farmers about phytoplasma infectivity on their farms via a web-based text messaging system to shift county-level management of *Macrosteles quadrilineatus* from using leafhopper abundance to infectivity. We found that infected *M. quadrilineatus* abundance decreased after farmers were informed about their numbers, suggesting that our decision support tool allowed growers to successfully manage infected *M. quadrilineatus*. We also identified temporal differences in infected and uninfected leafhopper peak abundance in celery but not in carrot cropping systems, suggesting that farmers should account for these phenological shifts across crops and over time.

In the field edge surveys, leafhoppers were collected from celery and carrot fields and field edges. I identified leafhoppers through DNA barcoding and conducted real-time PCR to determine phytoplasma infection status. The most abundant species were *M. quadrilineatus* (57%) and *Empoasca fabae* (23%). Our results confirmed that *M. quadrilineatus* was the primary vector in celery and carrots, although there is evidence that *E. fabae* may also vector this pathogen.

This thesis is dedicated to my husband Christopher for his constant love and support

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1 Economic impact

Celery (*Apium graveolens* L.; Apiales: Apiaceae) and carrot (*Daucus carota* subsp. *Sativus* (Hoffm.) Schübl. & G. Martens; Apiales: Apiaceae) are both economically important crops. In 2018, the USA produced over $1 billion worth of carrots and celery on 110,000 acres,[1] while Michigan produced 4,000 acres of carrots worth approximately $14.5 million and 1,900 acres of celery worth approximately $19.5 million.[1]

Aster yellows phytoplasma (*Candidatus* Phytoplasma sp.), a bacterial plant pathogen, causes considerable damage to vegetables, field crops, and ornamentals in North America and Europe.[2] This disease can infect more than 350 plant species, including important vegetable crops such as carrots, celery, onions, lettuce, and potatoes, which become unmarketable when infected.[3] Aster yellows phytoplasma is vectored by 24 leafhopper species in North America.[4] The primary vector in North America is the aster leafhopper (*Macrosteles quadrilineatus* Forbes, formerly considered a part of *M. fascifrons* Stål; Hemiptera: Cicadellidae) due to its abundance in sensitive crops.[5,6]

The primary strategy to control phytoplasmas in commercial agriculture is through controlling the vector populations with insecticides.[7,8] Traditionally, insecticides have been applied prophylactically on a calendar-basis to control leafhopper populations, but due to numerous negative effects of this practice, growers, in collaboration with researchers, have developed better approaches that involve the use of an aster yellows phytoplasma infectivity index to guide insecticide applications.[9] Current methods for identifying phytoplasma infected aster

leafhoppers have a relatively quick turnaround of about 24 hours,[10] but it is unknown whether other leafhopper species contribute to the spread of the pathogen or maintain it in the vegetation around fields, and it is important to identify those that can may help minimize the spread of the disease.

2 Aster leafhoppers

2.1 Biology

Adult aster leafhoppers (Fig 1.1A) are light yellow-green, with gray-green wings. Sizes range from 3–4 mm with the females generally larger than males. The most distinctive features are the markings on the head with 4–6 dark brown-black lines and 2 spots.[11] Aster leafhoppers are polyphagous and feed on over 300 species of plants,[12] although adults prefer cereal crops over carrots when presented with a choice.[13] Aster leafhoppers feed on plants using a piercing/sucking type mouth which they insert into leaves and feed on sap within the phloem. While they feed, they secrete saliva to protect and guide their mouth parts.[11]

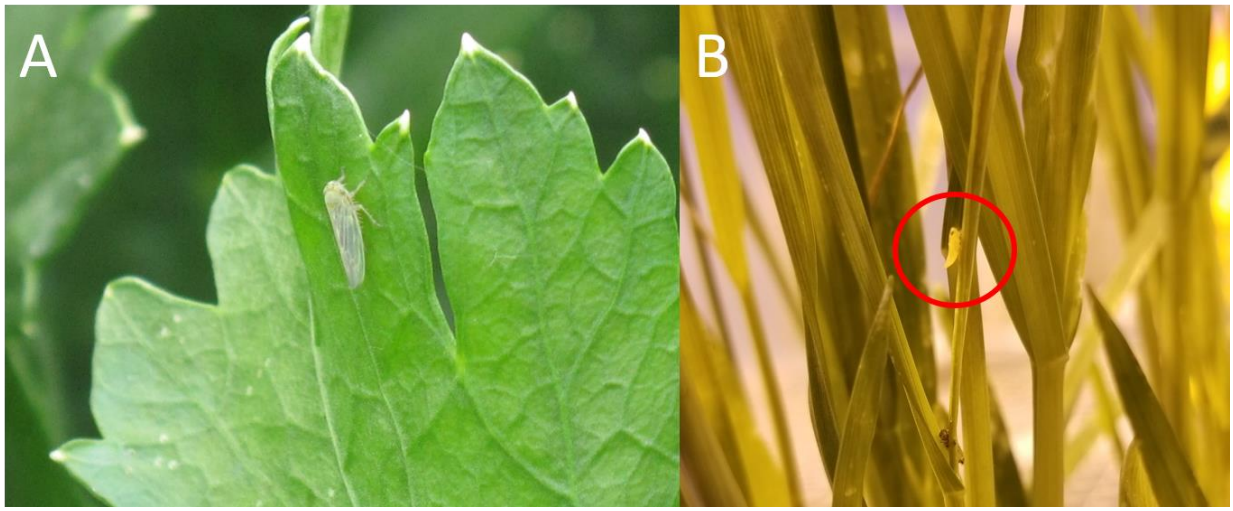

Figure 1.1. (A) Adult aster leafhopper on celery. (B) Aster leafhopper nymph on oats.

2.2 Life history

Aster leafhoppers reproduce sexually and are hemimetabolous with egg, nymph, and adult stages.[11] Adult females typically remain on the plants where they molted to adult, while males move among plants attempting to find unmated females.[14] Once mated, females will move to younger plants to lay eggs.[14] A generation is about 27–34 days (average 30 days to get from egg to reproductive adult) .[11] The adult lifespan is about 18–20 days (Beanland et al, 2000). An adult aster leafhopper's life span decreases from 20 to 7 days when temperatures are above 30°C, although all life stages can survive between 0–35°C.[15]

Eggs are laid on leaf veins, close to the petiole, and take about 7–8 days to hatch. Aster leafhoppers will lay eggs on cereals and wild plants, such as clovers, grasses, and weeds, at the end of the growing season. These eggs will overwinter until spring when the nymphs will move to available host plants (Fig 1.1B).[11] Typically, females will lay one to five eggs a day,[11] and approximately 30 eggs are laid in a lifetime.[16] Females can lay eggs between 5–35°C.[15]

Newly hatched nymphs will feed on the plant from which they emerged and continue to grow and molt, completing 5 nymphal instars before the final molt to an adult.[11] When eggs are laid on less suitable host plants, the nymphs have a lower survival rate and longer developmental time compared to nymphs laid on preferred host plants.[17] For example, lettuce is one of the few vegetable host plants that is suitable for egg laying.[11]

2.3 Migration

In the southern USA, aster leafhoppers have a continuous life cycle and do not undergo diapause as it does not get cold enough to require a quiescent state. [18] During the spring, the winds move in a northerly direction from states like Texas, to the Great Plains states, then into the Midwest (including Michigan), and Canada; thus, some of these southern populations will migrate

to the northern U.S. using wind currents. [18] The wind speed and direction may change every year, which contributes to variability in the numbers of leafhoppers that arrive in Michigan each year (Fig 1.2).[18]

Figure 1.2. Generalized migration map of aster leafhopper movement from Texas and Mexico, to the Midwest. Migration begins in mid-May and lasts through early-June. During this time, the leafhoppers move using seasonal wind currents. This map is based on those provided by Hoy at al.[18] for 1988–1990.

3 Phytoplasma

3.1 Biology

Phytoplasmas (*Candidatus* Phytoplasma spp., Acholeplasmatales: Acholeplasmataceae) are a genus of globally distributed pathogens that infect 98 plant families, consisting of several hundred plant species, including peanuts, fruit trees, lettuce, and canola.[2-3] These small, wall-less, obligate parasitic nano-microbes require plant hosts or insect vectors for survival.[19] Their genomes are the smallest of any self-replicating organism ranging from 530–1,350 kb.[19] Officially, there are no named phytoplasma species as they cannot be grown in axenic culture, therefore the group

has been granted the taxonomic classification of *Candidatus*.[19] One study has demonstrated that it may be possible to grow phytoplasmas in axenic culture, but this work is still in the early stages.[20] Currently, there are 32 sub-groups that are distinguished by their 16S rDNA sequences, the species of plants they affect, and their disease symptoms.[19] Common disease symptoms include yellow or purple leaves, virescence, phyllody, proliferation of shoots, witches' broom, stunting, decline in health, and premature death.[19]

3.2 Identification

Infected plants vary in the severity of their symptoms, ranging from asymptomatic to yellowing and rapid decline. In both cases, these plants could be tested to reveal infections of the same magnitude.[19] To determine which phytoplasma subgroup caused an infection in both plants or insects, 16S rDNA universal primers are used in conventional PCR.[21] To identify phytoplasma subgroups, PCR products can be digested with restriction enzymes, which cleave the PCR product into phytoplasma subgroup specific segments, and the banding patterns allow sorting into subgroups.[19] This method is useful in identifying the specific phytoplasma subgroup that may be causing the infection, but it cannot determine phytoplasma titer in plants. Phytoplasmas are unevenly distributed in plants, thus it may be most effective to test samples using tissue taken from multiple parts of the plant.[19]

3.3 Transmission

Phytoplasmas are vectored by herbivorous hemipterans, which secrete phytoplasma in their saliva as they feed on the phloem. The main vectors are leafhoppers, treehoppers (Cicadelloidea), psyllids (Psylloidea), and planthoppers (Fulgoroidea).[19] Transmission can also occur in laboratory settings by grafting infected plant tissue onto healthy plants from a related species or by attaching dodder (*Cuscuta* spp., Solanales: Convolvulaceae) to both the infected and healthy plants. These

methods will connect the phloem of the two specimens allowing transmission of phytoplasma from the infected plant to the healthy plant.[19]

In agriculture, leafhoppers are the most common source of phytoplasma infection. Leafhoppers acquire phytoplasmas by feeding on infected plants. The phytoplasma replicates within the leafhopper during the latency period where it will migrate to the salivary glands. Once the infection has reached a sufficiently high titer in the salivary glands, the leafhopper is infectious. The leafhopper can inoculate uninfected plants when feeding for the remainder of its life.[19] This method is similar for all Hemipteran vectors of phytoplasmas (Fig 1.3), however, the rate of infectivity varies depending on the specific vector and the phytoplasma acquired. For efficient vectors, transmission can be near 100%,[22] but for poor vectors, rates can be as low as 40%.[23]

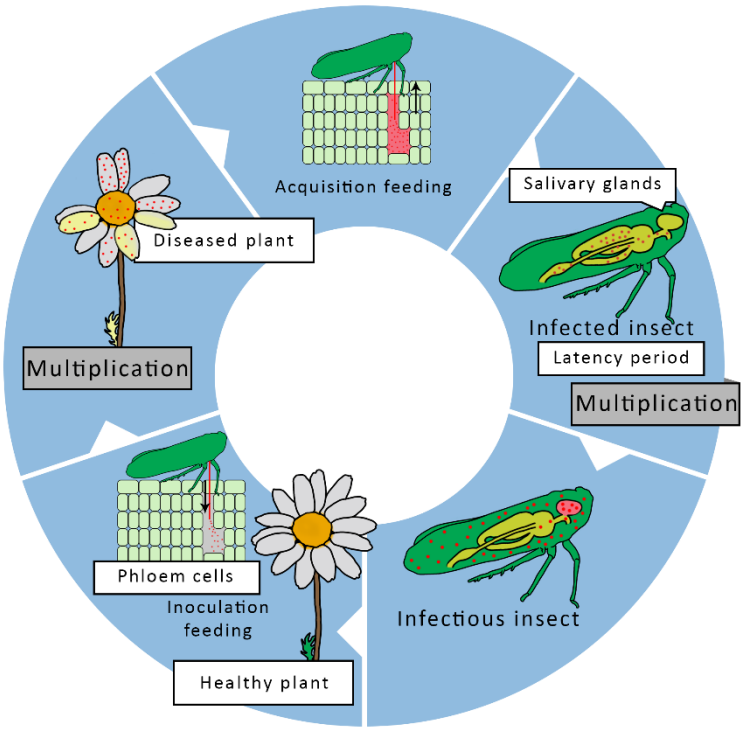

Figure 1.3. Pathway for phytoplasma acquisition and transmission by hemipteran vectors.

4 Aster leafhoppers as vectors of aster yellows phytoplasma

Aster leafhoppers are known to vector at least four different phytoplasmas, including clover phyllody, European aster yellows, North American aster yellows, and stolbur.[4] The most prevalent of the four is North American aster yellows which aster leafhoppers can transmit to at least 191 plant species.[19] The major factor limiting the spread of the phytoplasmas is the vector's host plant preferences.

Male aster leafhoppers are twice as likely to be infected by aster yellows phytoplasma than females, although females have a higher rate of success at inoculating plants.[24] This is likely due to the different behaviors of the sexes: males jump around from plant to plant looking for mates, while females are less mobile.[24]

When aster leafhoppers are infected with aster yellows phytoplasma, the infection benefits females by extending their lifespan (~ 10 days) and doubling the number of eggs they can lay compared to uninfected aster leafhoppers.[16]

4.1 Management

Aster leafhoppers often form large populations in carrot and celery fields, but their feeding does little damage to crops. Thus, the primary reason for keeping aster leafhopper abundance low is not to prevent direct crop damage but rather to limit leafhopper transmission of aster yellows phytoplasma. Once infected, aster leafhoppers can transmit phytoplasma for the remainder of their lives.[24]

A cultural management method for aster leafhopper control is to avoid planting host plants in adjacent fields, such as celery and alfalfa. When one crop is harvested, the aster leafhoppers will move over to the adjacent field in search of a new food source, (personal observation, Z. Szendrei). Another cultural management method is controlling the host plant reservoirs in the field edge as

these may provide shelter for aster leafhoppers when the field is being treated with insecticides or when harvested.[25]

Foliar insecticides are the most common chemical control for aster leafhoppers. Plants may need to be sprayed frequently, especially when repeated rain events wash the insecticides off the plants.[25] In celery, since aster leafhoppers prefer younger plants, frequent scouting and management is critical (personal observation, Z. Szendrei). In carrots, it is unknown if aster leafhoppers prefer younger plants.

New means of control are being investigated. For example, several carrot cultivars have been bred to be resistant/tolerant to aster yellows phytoplasma.[25] Selectively breeding crops is currently the only means of preventing aster yellows phytoplasma from infecting a crop, but there are many susceptible cultivars currently is use.

4.2 Monitoring

Aster leafhoppers can be monitored using yellow sticky traps, inverted cage trapping, or sweeping.[14] Sweeping has been identified as the best way to catch an even ratio of males and females, unlike the other two methods.[14] Males are more likely to be caught with yellow sticky traps as they are actively flying between plants looking for unmated females, while females are more likely to be caught by inverted cage trapping as they are more sedentary.[14]

Sweeping can be combined with laboratory techniques to identify the infectivity levels of aster leafhoppers in the field. Currently in Michigan, a qPCR protocol[10] is used to determine the infectivity level of field caught aster leafhoppers, then an action threshold is calculated, and sent to growers in a text message (Fig 1.4).

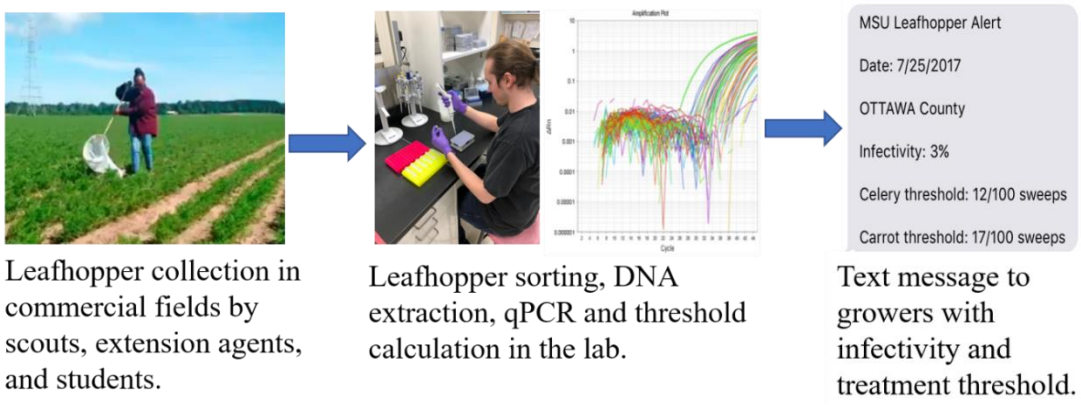

Figure 1.4. Summary of the current diagnostics workflow to inform growers about leafhopper infectivity in their fields. Aster leafhoppers are collected from celery or carrot fields, DNA is extracted from the leafhoppers, qPCR is performed to determine if the leafhoppers are infected with aster yellows, and then the results are provided to farmers via a text message.

5 Leafhoppers of Michigan

5.1 Vectors of aster yellows

In Michigan, there are several leafhoppers known to vector aster yellows, in addition to the aster leafhopper, including: *Aphrodes bicinctus* (Schrank), *Athysanus argentarius* (Metcalf), *Endria inimica* (Say), *Fieberiella florii* (Stål), and *Scaphytopius acutus acutus* (Say).[4,26] All of these species' host plants include grasses, clovers, and cereals, except *F. florii* which predominantly feeds on ornamental shrubs and fruit trees.[23,26–30] Grasses, clovers, and cereals are all common plants along celery and carrot field edges, and aster leafhoppers can feed on them. If an aster leafhopper infects grasses or cereals in the field edge, other leafhoppers that normally do not interact with infected crops, can acquire aster yellows. This can lead to an overwintering source of aster yellows in the resident leafhopper populations as well as in the plants near crop fields.[31]

Besides feeding on plants in the field edge, *A. bicinctus* can vector aster yellows to celery.[28] Thus other leafhopper species are likely present in Michigan that can infect celery with aster yellows, although their populations are likely smaller than those of aster leafhoppers.

5.2 Non-vector leafhoppers

Besides the known vectors, potato leafhoppers (*Empoasca fabae* Harris) are abundant in celery and carrot fields and are the second most prominent leafhopper species collected in sweep nets (personal observation, P. Stillson). Currently, it is unknown if potato leafhoppers vector aster yellows, but due to their abundance in numerous crops, understanding how they interact with this pathogen is important.

6 Knowledge gaps

There are many gaps in our knowledge of phytoplasma transmission. For example, we need to better understand the factors that contribute to phytoplasma acquisition and infection in both the insect vector as well as in the host plant (Fig 1.5). Does the manner in which the leafhopper feeds (phloem vs. xylem feeders) affect the probability of acquiring phytoplasma from infected plants? Different leafhopper species feed in different ways and on different plant tissues, but in some polyphagous leafhoppers, like the potato leafhopper, this changes based on the plant they are feeding on.[32] After leafhoppers acquire the phytoplasma, the pathogen must migrate to the salivary glands by moving through the midgut and salivary glands. These barriers are likely what prevents ingested phytoplasma from making leafhoppers into vectors, but what exactly is preventing the migration is unknown. After becoming a vector, some leafhoppers develop detrimental side effects due to the phytoplasma infection, such as shortened lifespans.[33] It is unknown why this may occur, but it may potentially be due to an immune response in the leafhoppers.

In addition to the knowledge gaps associated with phytoplasma acquisition and transmission, there are some gaps associated with the disease ecology. It is unknown how many total plants an infectious leafhopper can infect during its life as this can be affected by their sex,[14]

age when they acquire the phytoplasma,[28] how long it takes to transmit the phytoplasma, and how long latency periods are for different leafhoppers (e.g. for chrysanthemum yellows phytoplasma latency period lasts 18 days for *Macrosteles quadripunctulatus* and 30 days for *Euscelidius variegatus*).[22,34] Additionally, there is not much research on which leafhoppers may contribute to outbreaks in different susceptible cropping systems as most work focuses on a single species and potential vectors are ignored.

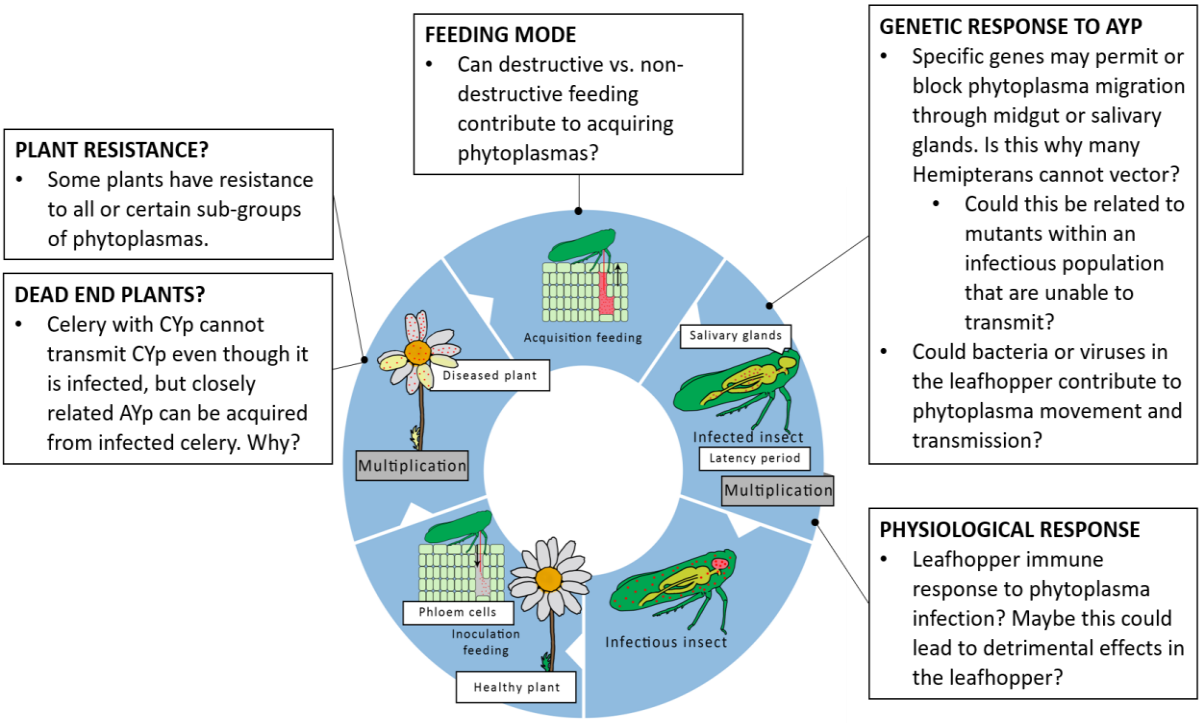

Figure 1.5. Current knowledge gaps in our understanding of phytoplasma acquisition and transmission. These relate to how the vectors feed on the plants, molecular and physiological responses to the phytoplasma, and how the plants interact with the pathogen. Aster yellows phytoplasma = AYp, chrysanthemum yellows phytoplasma = CYp.

7 Thesis objectives

I conducted my research at commercial celery and carrot farms to address knowledge gaps associated with vector identification and management within susceptible cropping systems. These crops are both economically important in Michigan and susceptible to aster yellows phytoplasma.

My first goal was to determine whether the Vegetable Entomology Laboratory's text messaging-based decision support tool was useful to celery and carrot farmers in controlling the populations of aster yellows infected leafhoppers. The objectives of this work were 1) to determine whether our decision support tool informed farmer management and directed insecticide applications at the infected population rather than the overall leafhopper populations, 2) to determine whether there were similarities between leafhopper abundances and infectivity between celery and carrot systems during the growing season and if these similarities could be used to improve aster yellows management across cropping systems. My second goal was to identify the different leafhopper species associated with commercial celery and carrot farms and how they might interact with aster yellows phytoplasma. The objectives of this work were 1) to identify the species of leafhoppers that reside within crop fields and the field edges, 2) to determine whether there are differences in leafhopper distributions between the field and the field edge and if any species were found among both cropping systems, 3) to determine whether any of the collected leafhoppers are known vectors of aster yellows or new potential vectors for the phytoplasma.

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CHAPTER 2: A novel plant pathogen management tool for vector management

1 Introduction

Decision support systems have existed for decades to manage insect pests across many cropping systems.[1–4] These tools often provide management thresholds based on scouting data and promote control of pest insects that damage crops while preventing unnecessary insecticide applications.[4,5] While these decision support systems are increasingly adopted in agriculture, few are available specifically for insect vector management.[5] Moreover, the implementation of these decision support systems may be complicated by behavioral and biological differences between populations of pests infected and uninfected with plant disease.

When data needed for decisions support tools are lacking, a calendar-based spray schedule is sometimes followed where insecticide applications are performed without knowledge of pest abundance.[6,7] However, calendar-based management approaches are not ideal given the use of insecticides is cost prohibitive, environmentally damaging, and increases insecticide resistance.[8,9] When decision support based on abundance thresholds does exist, these tools are again inadequate because the abundance of infected vectors is frequently a better predictor of pathogen prevalence in crops than vector abundance alone.[7,10,11] Therefore, contemporary pest management is shifting to the use of diagnostics to identify and verify the presence of insect vectored pathogens which can then inform pest management.[12,13] However, decision support systems linking the results of diagnostic laboratories to farmers remain rare, indicating that improving the delivery of diagnostics to farmers could enhance insect vectored plant pathogen management and reduce the use of calendar sprays (Fig 2.1).

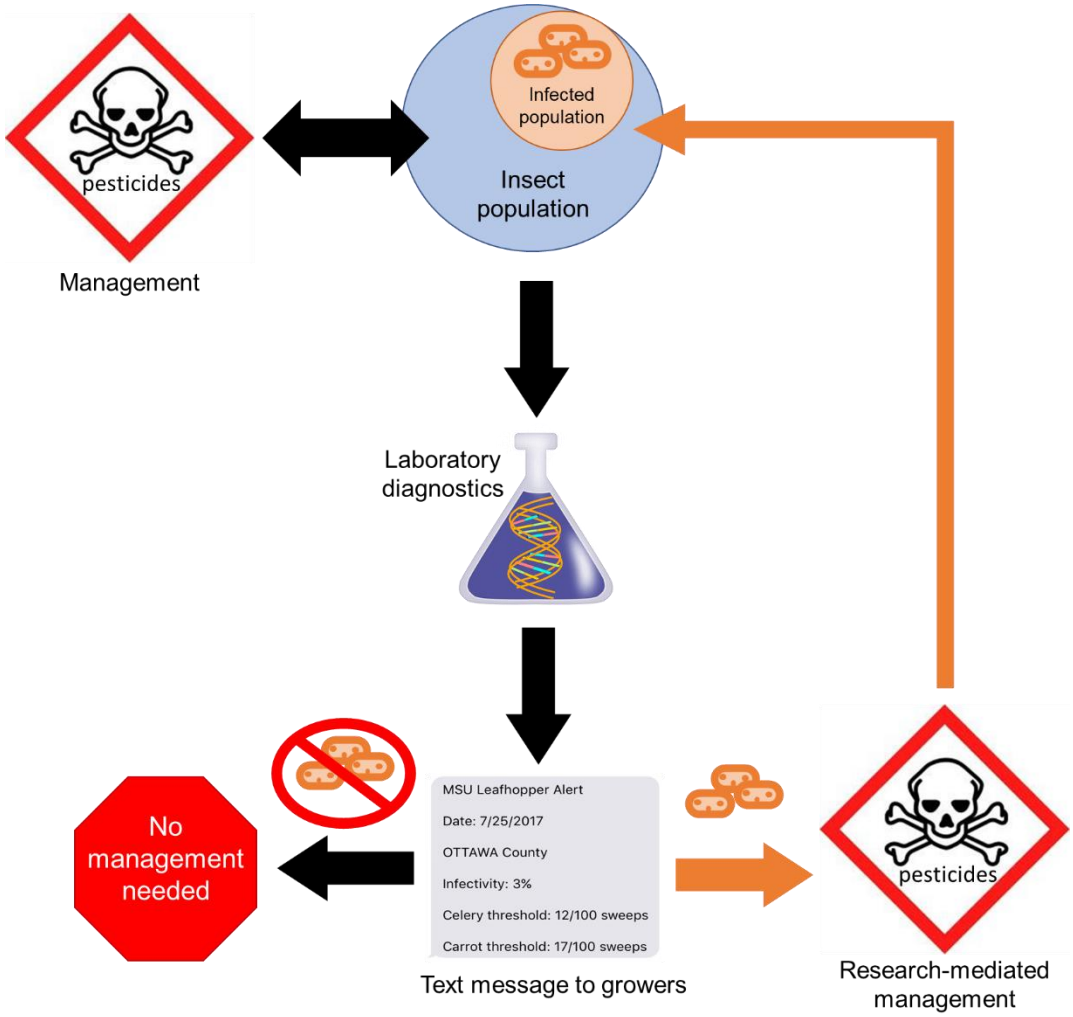

Figure 2.1. Aster yellows phytoplasma is transmitted by aster leafhoppers to carrots and celery. The economic damage is caused by the phytoplasma; therefore, it is important to assess the proportion of the leafhopper population that is infected. Leafhopper management that is based on detecting the pathogen in the leafhopper could reduce yield losses. In our system, the results of disease diagnostics are sent to farmers via a group text messages indicating rates of leafhopper infectivity and the action threshold for carrots and celery. If the text message indicates that infected leafhoppers have been detected, then farmers will respond by applying insecticides to their fields (research-mediated management). If the text message indicates that leafhoppers are not infected with the phytoplasma, then management action is not needed and we recommend growers not use insecticide management. Leafhoppers are collected regularly during the growing season and text messages are sent out approximately 24 h after collecting leafhoppers from the field, providing an opportunity for quick response, if necessary.

Time delays between the insect vector acquiring the pathogen and transmitting it (latency) may contribute to differences between the population abundances of infected and uninfected individuals.[14,15,16] Infections may occur at various spatial scales, both within a cropping system (as a patchwork of infected and uninfected plants) and across cropping systems due to differences in host suitability and management. This heterogeneity means that some insects are infected and some are not, reflecting differences in the infectivity status of their host plants.[17–19] Identifying patterns between the abundance of infected and uninfected individuals is challenging, but it is necessary to shift pest management from abundance to infectivity-based models[17,20] and reduce uncertainty in the appropriate timing of insecticide applications.

We used an infectivity-based decision-support system to provide celery (*Apium graveolens*, L., Apiaceae) and carrot (*Daucus carota* subsp. *Sativus*, Hoffm., Schübl. & G. Martens, Apiaceae) farmers with rapid diagnostics information to manage an economically damaging insect-vectored plant pathogen, aster yellows phytoplasma (*Candidatus* Phytoplasma spp.). This pathogen is transmitted by its primary vector, the aster leafhopper (*Macrosteles quadrilineatus*, Forbes).[21] The main objective of this support tool was to support farmers in changing their management strategy from one of abundance-based insecticide applications to a directed spray program focusing on the infected population. We identified leafhopper population patterns before and after farmers received diagnostics results, and to understand the relationship between infected and uninfected vector populations, we examined temporal and spatial patterns. Our results help identify the spatial scale at which decisions support tools can inform management and indicate that temporal shifts in management based on infectivity thresholds may help to reduce the prevalence of an economically important plant pathogen in two high value vegetable cropping systems.

2 Materials and methods

2.1 System description

2.1.1 Pathogen-vector system

Aster yellows phytoplasma is a cell wall-less bacteria that is transmitted by phloem feeding insects; it is one of the largest and most diverse group of phytoplasmas.[22] This pathogen can infect over 300 plant species, including crops (e.g., carrots, celery, lettuce) and ornamentals.[22,23] Plants infected with aster yellows phytoplasma are unmarketable due to chlorotic, deformed, and stunted growth[23,24] and farmers have reported yield losses of up to 10% due to aster yellows phytoplasma.[25] Aster leafhoppers are the main vector of aster yellows phytoplasma;[21] while aster leafhoppers cause minimal damage to most crops, leaving small marks where they fed, once infected they transmit the phytoplasma in a persistent manner for the remainder of their lives.[26] Aster leafhoppers acquire phytoplasmas from the environment while feeding on infected plants[27] and remain latent for two to three weeks before becoming infectious; once infectious they remain so for the rest of their lives.[26,28] Aster leafhoppers annually migrate north from the southern USA in early May, acquiring aster yellows along the way.[21] Little is known about overwintering aster leafhopper populations and sources of aster yellows in the Midwestern USA.[26] However, once in the Midwest, aster leafhoppers move short distances between adjacent crops, fields, and field edges to feed on grasses and weeds[29] which are known disease reservoirs.[30] Currently, insecticides are applied when leafhopper abundance is high, but this practice is unnecessary, as uninfected leafhoppers rarely cause direct damage to plants and the relationship between population abundance and infectivity is unknown.[27]

2.1.2 Cropping systems

We studied leafhopper populations and the incidence of aster leafhoppers infected with aster yellows phytoplasma in two cropping systems, carrots and celery. While these crops are taxonomically similar, their production methods differ. Celery is grown in greenhouses for eight weeks before transplanting into fields, and farmers continue to transplant weekly for approximately two months providing a mixture of plant age classes throughout the growing season.[31] Carrots are direct seeded over a shorter period of time and are more similar in age across fields.[32] All farms in the study were large-scale commercial operations (field sizes from 1.2–36.2 ha) and used synesthetic pesticides for pest management. Fungicides were applied weekly in both celery and carrots; however, insecticide application frequency varied based on scouting reports. Overall, aster leafhoppers were collected from 10 celery and 12 carrot farms, totaling 40 and 20 different fields respectively, between 2014 and 2019 (Fig 2.2; Tables S2.1 – S2.2).

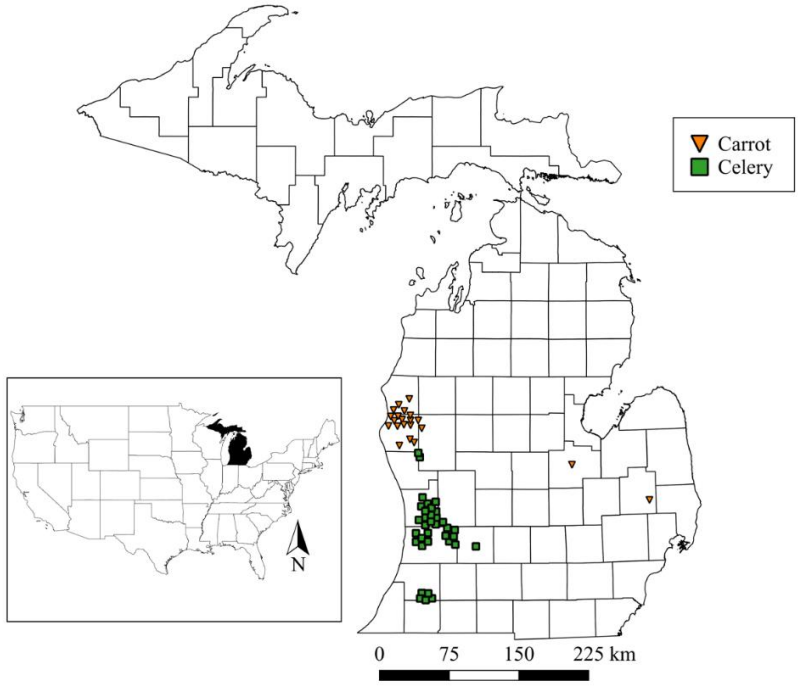

Figure 2.2. Map of Michigan, USA, symbols indicate the locations of commercial carrot and celery fields where aster leafhoppers were collected from 2014-2019. Leafhoppers were collected using sweep nets and were transported to the laboratory to determine aster yellows infectivity.

2.2 Aster leafhopper diagnostics

2.2.1 Leafhopper collection

Leafhoppers were collected weekly from mid-May through early August, 2014–2019 (*n* = 365 samples). Crop consultants performed sampling using a sweep net (38 cm diameter aerial net), with a minimum of 100 sweeps per field. Fourteen celery and five carrot farms were scouted, on average, each year, with weekly scouting consisting of at least one field per farm sampled; in larger farms samples were taken from multiple fields (Tables S2.1 – S2.2). The numbers of collected leafhoppers varied depending on leafhopper presence and abundance in fields at a given time. Consultants reported the density of aster leafhoppers found within the field to each farmer for the respective survey as the abundance of leafhoppers collected per 100 sweeps. After collection, leafhoppers were transferred to plastic bags, placed in a cooler, transported to our laboratory at Michigan State University, East Lansing, MI, USA, and stored at -20°C overnight. Since aster leafhoppers are the only leafhopper of economic concern in celery and carrot,[21] scouts sorted leafhoppers morphologically into aster leafhoppers and all other leafhoppers. Leafhoppers not identified as aster leafhoppers in were excluded from subsequent analyses.

2.2.2 Laboratory processing

We performed DNA extractions to determine the number of aster leafhoppers infected with aster yellows phytoplasma. One to three adult aster leafhoppers (three leafhoppers were used when more than 50 leafhoppers were collected from one field) were placed in a 2 ml homogenization tube (Sarstedt, Nümbrecht, Germany), along with high salt extraction buffer[33] (70 µl) and three homogenization beads (2.3 mm diameter, zirconia/silica; BioSpec Products, Inc., Bartlesville, OK). Aster leafhoppers were homogenized for 60 s at 4.0 m/s (FastPrep-24, MP Biomedicals, Santa Ana, CA). Dneasy Blood & Tissue DNA isolation kit (Qiagen, Valencia, CA) was used to

extract DNA, following the manufacturer's insect DNA extraction protocol. We modified the protocol to include incubating samples in the proteinase K/Buffer ATL solution for 1 h. DNA was suspended in elution buffer (100 µl for samples with 1–2 leafhoppers and 200 µl for samples with 3 leafhoppers). Varying elution buffer volumes were used to standardize the DNA concentration across samples. Final DNA concentrations ranged from 0.50–350 ng/µl. The presence of aster yellows phytoplasma was detected using a TaqMan qPCR assay[34] with universal phytoplasma primers and probe[35] (Thermo Fisher Scientific, Waltham, MA). Leafhopper samples with a cycle threshold < 32 were recorded as positive for aster yellows phytoplasma.[34]

2.3 Disseminating information

2.3.1 Infectivity threshold calculations

Action thresholds in pest control are designed to decrease pest populations before disease transmission can cause economic damage.[36] When working with vectored pathogens, action thresholds must take into account both pest abundance and the proportion of the infected population, providing a better predictor of disease incidence.[11,37]

We used the following equations in determining an action threshold:[38]

$$\text{Percent of infected leafhoppers} = (\text{infected leafhoppers} / \text{total leafhoppers}) \times 100, \quad (1)$$

$$\text{Aster yellows index} = \text{percent of infected leafhoppers} \times \text{leafhoppers per 100 sweeps}, \quad (2)$$

$$\text{Celery threshold} = (35 / \text{aster yellows index}) \times 100, \quad (3)$$

$$\text{Carrot threshold} = (50 / \text{aster yellows index}) \times 100, \quad (4)$$

where the values of 35 and 50 in eqns. 3–4 represent constants based on resistance to aster yellows phytoplasma in celery and carrot respectively.[38] When the number of infected leafhoppers increase, values found with eqns. 3–4 decrease indicating that insecticide applications should take place when leafhoppers are found at or above these threshold values. Conversely, if no infected

leafhoppers are detected, then the equation gives an illegal fraction, suggesting that an infinite number of leafhoppers can be caught and an action remains unnecessary.

2.3.2 Text messages

Beginning in 2016, we contacted celery and carrot farmers and encouraged them to enroll to receive group text messages providing the percent of infected aster leafhoppers and management thresholds determined by each leafhopper survey. The text message (Eztexting.com) was sent to those signed up for the group messaging system the day after leafhoppers were collected, with a standard turnaround time of 24 h from collection. Text messages were sent out from 2016 to 2019 in May, June, July, and August (30 in 2016, 31 in 2017, 43 in 2018, and 25 in 2019). Text messages were sent 1 to 8 times per week based on the number of collections performed by crop consultants. Over the course of our study, the number of people receiving our text messages increased approximately 16% from 36 in 2016 to 42 in 2019. Each text message was based on information from leafhoppers collected in a single field but in order to keep the precise location confidential, we identified the county as the sample origin in the message. The messages also included the date, percent of aster leafhoppers testing positive for aster yellows phytoplasma, and the threshold adjusted for level of infectivity of aster leafhoppers per 100 sweeps for carrots and celery (Fig 2.1).

2.4 Statistical analysis

2.4.1 Text messaging and infectivity

To determine whether the abundance of infected aster leafhoppers in the fields decreased after farmers received text messages indicating that infectivity was greater than 0%, we calculated the total abundance of infected leafhoppers at three time points: ‘one-week before text message’, ‘week of text message’, and ‘one-week after text message’. A Kruskal-Wallis rank sum test (function = ‘kruskal.test’)[39] was used to determine differences in the number of infected

leafhoppers across the three time points and Dunn's test (function = 'dunn.test', package = 'dunn.test')[40] was used to identify pairwise differences between weeks.

2.4.2 Leafhopper populations across and within cropping systems

Insect abundance is well known to change as host plant suitability varies.[19,38] However, whether differences in the abundance of infected and uninfected leafhoppers varies across cropping systems is relatively unknown and likely driven by both host plant suitability and pesticide management practices.[6] To examine these population patterns, we used a Kruskal-Wallis rank sum test (function = 'kruskal.test')[39] to compare the mean abundance of infected and mean density of leafhoppers across the two crops (carrot and celery).

These analyses, however, do not account for variation across the cropping systems between infected and uninfected leafhopper populations.[10,19,41] Insect populations can temporally vary in abundance across plant resources[19] suggesting that differences in plant management across our study systems may drive temporal differences in leafhoppers over the production season. To examine when populations of leafhoppers in carrot and celery were most similar over time, we performed a cross-correlation analysis (function = 'ccf').[39] We evaluated the correlation of weekly population patterns at four time lags (two positive and negative) centered on zero, with a correlation at zero indicating that no temporal lag existed across the cropping systems, a negative lag indicating that populations in carrot were temporally delayed when compared to celery, and a positive lag indicating the opposite, where populations in celery were temporally delayed when compared to carrot. To prepare our data, leafhopper densities (abundance of leafhoppers collected per 100 sweeps) and infected leafhopper abundances were summed by week across years (n = 365 collections; Tables S2.3 – S2.4) (2014–2019) and by crop (celery and carrot) yielding one time point for each week of the season. There were 15 and 13 time points (weeks of sampling) in celery

and carrot respectively. For the purpose of analyses comparing carrot and celery, the first two time points were removed from the celery data to align the sampling weeks between the two crops, but when comparing timepoints within the celery system, all 15 time points are used (Tables S2.3). Prior to evaluating our data with the cross-correlation function, we confirmed that our data met the assumptions of the analysis using the Kwiatkowski-Phillips-Schmidt-Shin test for stationarity (function = 'kpss.test', package = 'tseries').[42] We then evaluated the relationship between the populations of infected and uninfected individuals across the cropping systems (13 sampling weeks are used for this analysis) by finding the sample cross-correlation function, r_k^{xy}, for the aforementioned lags *k*:[43]

$$g_k^{xy} = \frac{1}{n}\sum_{t=1}^{n-k}(y_t - \bar{y})(x_{t+k} - \bar{x}), \quad (5)$$

$$r_k^{xy} = \frac{g_k^{xy}}{\sqrt{SD_x \times SD_y}}, \quad (6)$$

where g_k^{xy} is the sample cross-covariance function and numerator of our desired statistic, r_k^{xy}. In eqns. 5–6, *n* is the number of weeks in the sampling season (13 sampling weeks), x_t and y_t are the total density of leafhoppers per week for celery and carrot, $\bar{x}$ and $\bar{y}$ are the mean density of leafhoppers across all weeks for celery and carrot, and SD_x and SD_y are the standard deviation of leafhopper densities across all weeks for celery and carrot, respectively. We also determined the 95% confidence interval for the cross-correlation function:[39]

$$-\frac{1}{n} \pm \frac{2}{\sqrt{n}}, \quad (7)$$

Where *n* is the number of time points (13 weeks) used in eqns. 5–6. Cross correlation values found at each lag (4 total) and at no lag using eqn. 6 were compared to the 95% confidence interval. We also used the vector of cross correlation values for each lag to find two-sided p-values which were computed using the *pnorm* function in R, with a mean of 0 and standard deviation of $1/\sqrt{n}$.[39] Values

found with eqn. 6 that were above the 95% confidence interval (eqn. 7) and with p-values below an α-level of 0.05 indicated a correlation between population patterns of infected and uninfected leafhoppers across the cropping systems.

We also suspected that temporal differences existed between infected and uninfected leafhopper populations within each cropping system.[15] Therefore, we carried out an additional cross-correlation analysis that addressed differences in the population patterns between uninfected and infected individuals within each cropping system. Several mechanisms exist that could explain the temporal differences between the infected and uninfected populations. We speculated that the latency period would give rise to a lagged correlation between the uninfected and infected populations, whereby the population of infected individuals would be most similar to the population of uninfected individuals when delayed by up to two weeks which would allow for the mechanisms of disease acquisition and transmission to take place.[44] We also suspected that diseased plants could promote greater leafhopper abundance.[45] While we could not test this directly, we assumed that patterns of infected individuals could be a proxy of plant infectivity in the field. Therefore, we also investigated whether populations of uninfected leafhoppers were most similar to the infected population when delayed for up to two weeks temporally. Therefore, this approach accounts for two lags in the positive and negative direction (four lags total) and no lag, where the density of uninfected individuals were treated as the predictor "x" and the abundance of infected individuals was treated as the response "y" in eqns. 5–6. The value for "n" varied by cropping system with 15 and 13 sampling weeks for celery and carrot respectively (Tables S2.4).

2.4.3 Spatial variation in leafhopper populations

To determine whether leafhopper samples of similar density and infectivity clustered together, we calculated spatial autocorrelation as a function of distance bands using Moran's I with

the *moran.mc* function (n = 2000 simulations) in R,[46] split by cropping system (carrot and celery). P-values below an α-level of 0.05 indicated a correlation between populations of infected and uninfected leafhoppers across collection points within each distance band. Scouts did not collect spatial data for all samples, therefore we subset our data to those where the collection point was known. In sum, there were 18 and 7 unique collection points (fields) for celery (n = 191 samples) and carrot (n = 65 samples), respectively. Distance bands were defined based on *a priori* knowledge of sites and allowed to vary across cropping systems. For example, in celery sites less than 2.5 km apart were known to be fields within a farm, and these coordinates were placed within one distance band (Fig S2.1). Using *a priori* knowledge to create distant bands has important practical implications, as strong positive correlations within farms would suggest that sampling need not occur in multiple fields to inform leafhopper management farm-wide.

3 Results

3.1 Leafhopper collections

From 2014–2019, a total of 8,343 aster leafhoppers were collected, and 99 infected leafhoppers were detected (Tables S2.3–S2.4). In carrot and celery, there were 1,870 and 6,473 leafhoppers and 39 (2.09%) and 60 (0.93%) infected individuals respectively, which was similar to others studies that detected 0.09%–6.25% infectivity.[15] During the growing season, the number of infected leafhoppers peaked at week 26 in celery and week 28 in carrots, while the total number of leafhoppers peaked during week 24 in celery and 31 in carrots.

3.2 Text messaging and infectivity

There was a 29.17% decrease in the mean number of infected leafhoppers between the week before a text message was sent compared to the week of sending a text message (χ^2 = 6.63, df = 2, p-value = 0.06; Fig 2.3). There was also a 73.33% decrease in the mean number of infected leafhoppers between the week a text message was sent and the following week (χ^2 = 6.63, df = 2, p-value = 0.39), and an 81.11% reduction between the week before a text message was sent and the week after the text message was sent (χ^2 = 6.63, df = 2, p-value = 0.02) (See supplementary Information for follow-up discussion).

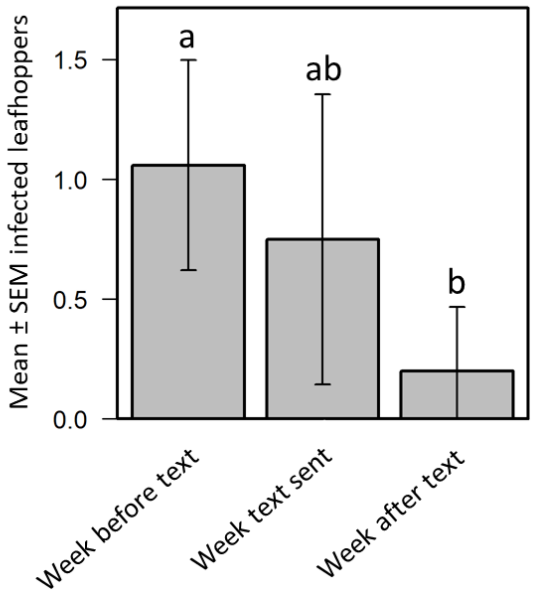

Figure 2.3. Abundance (mean ± SEM) of aster yellows phytoplasma infected leafhoppers during the 2016–2019 growing season by the number of weeks since farmers received a text message indicating leafhopper infectivity. Text messages were sent to inform stakeholders of the percent of aster yellows phytoplasma infected aster leafhoppers in the population and the action threshold for carrot and celery. Different letters above bars denote significant differences in abundance of infected leafhoppers across weeks.

3.3 Leafhopper populations across cropping systems

While we collected more leafhoppers in celery, the mean density of uninfected leafhoppers in carrots was 1.84 times higher than the mean density in celery (χ^2 = 5.75, df = 1, p-value = 0.02; Fig 2.4A). However, no difference was found between the mean abundance of infected leafhoppers when comparisons were made between the two crops (χ^2 = 0.26, df = 2, p-value = 0.61; Fig 2.4B).

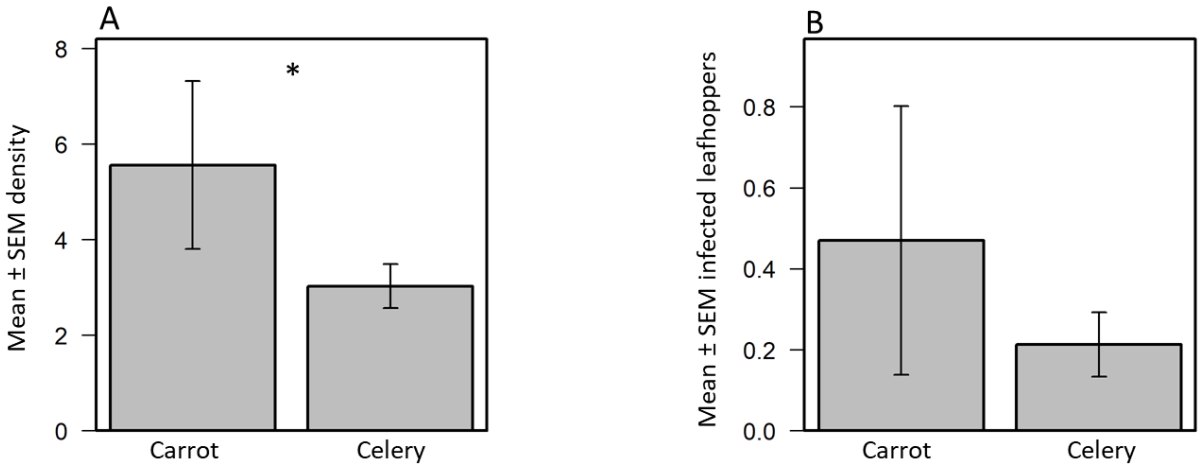

Figure 2.4. (A) Mean ± SEM aster leafhopper density (leafhoppers per 100 sweeps) and (B) mean ± SEM abundance of aster leafhoppers infected with aster yellows phytoplasma found in carrots and celery. Leafhoppers were collected with sweep nets from commercial celery and carrot farms in Michigan from 2014 to 2019 and tested for phytoplasma using a qPCR based diagnostic method. Asterisk indicates significant differences between carrot and celery.

When we compared the weekly population patterns of infected and uninfected leafhoppers between the two cropping systems, we found no temporal relationship when comparing the density of uninfected leafhoppers in celery to that in carrot (Fig S2.2A, B). However, the population of infected leafhoppers in carrot lagged that in celery by two weeks ($r = 0.79$, p-value = 0.004; Fig 2.5A) indicating that the temporal pattern of infected individuals across weeks 24–34 in carrot was similar to the population pattern in celery across weeks 22–32 (Fig 2.5B).

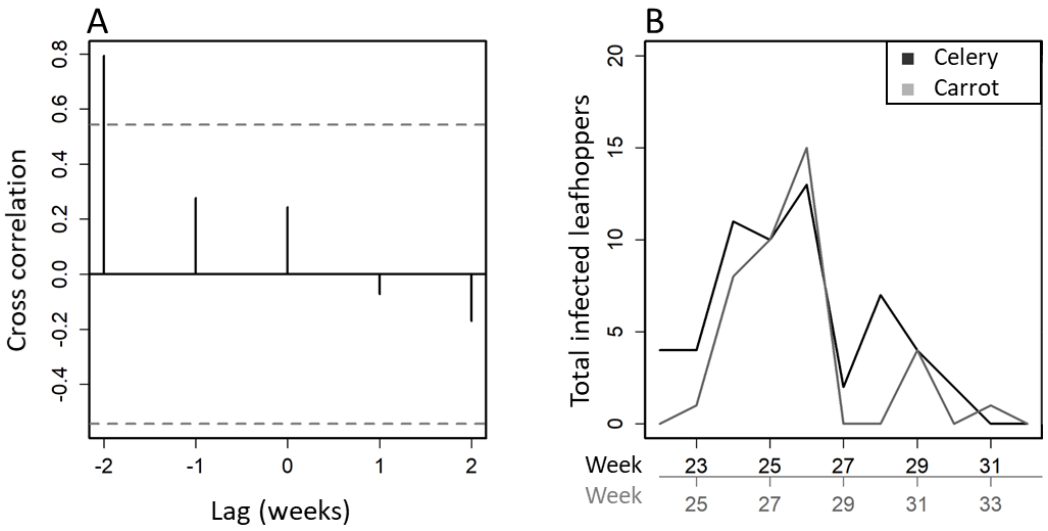

Figure 2.5. Cross correlation analysis of the abundance of aster yellows phytoplasma infected aster leafhoppers in celery and carrot fields in Michigan from 2014 to 2019. Dotted lines indicate a 95% confidence interval and each lag represents a week. (A) The cross-correlation value at a lag of -2 indicates that the pattern of infected leafhopper abundance in carrot may be delayed by two weeks when compared to the weekly population pattern observed in celery. (B) Total number of aster yellows phytoplasma infected aster leafhoppers plotted by week across the season in Michigan celery and carrot fields. The weeks on the x-axis correspond with the weeks of the calendar year. The line for carrots is shifted by two weeks to illustrate the two-week lag that was identified in the cross correlation.

3.4 Leafhopper populations within cropping systems

When we compared the weekly population pattern between uninfected and infected leafhoppers within cropping systems, we found support for a temporal relationship between infected and uninfected individuals in celery (r = 0.61, p-value = 0.02; Fig 2.6A). Patterns of infected leafhopper abundance across weeks 22–34 were similar to the pattern of uninfected leafhoppers across weeks 20–32 (Fig 2.6B), indicating that the population pattern of infected individuals was similar to that of uninfected individuals but at a two-week delay. No temporal relationship was found between the density of uninfected and infected leafhoppers in carrot (Fig S2.3A, B).

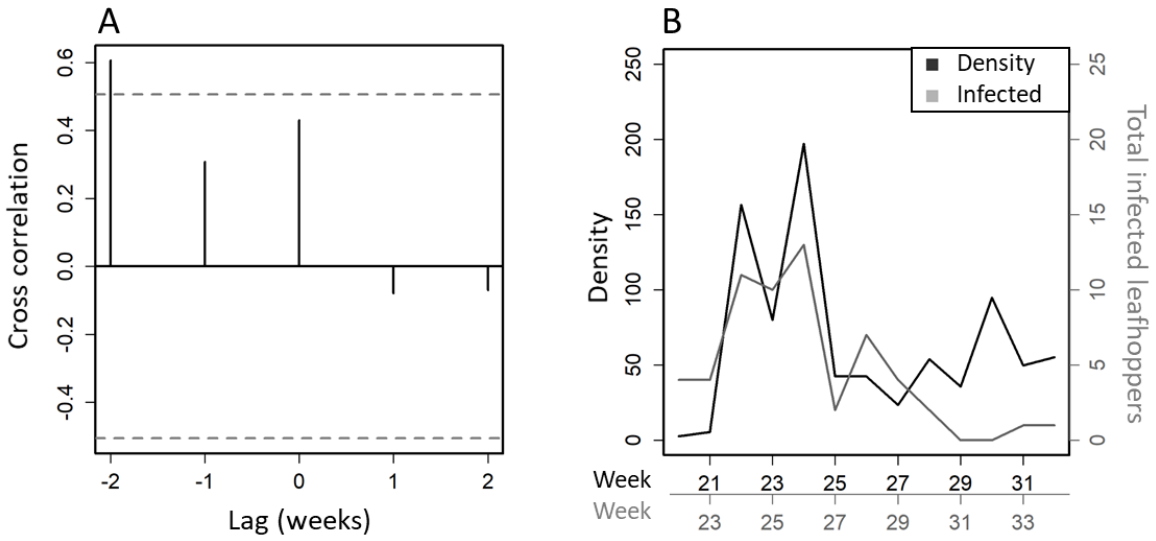

Figure 2.6. Cross correlation analysis of the density of aster leafhoppers (leafhoppers per 100 sweeps) and the abundance of aster yellows phytoplasma infected aster leafhoppers in celery fields in Michigan from 2014 to 2019. Dotted lines indicate 95% confidence interval and each lag represents a week. (A) The cross-correlation value at a lag of -2 indicates that the weekly patterns of infected leafhopper abundance may be delayed by two weeks when compared to the weekly pattern of uninfected individuals. (B) Total density of aster leafhoppers and the total abundance of aster yellows phytoplasma infected aster leafhoppers in Michigan celery fields, plotted by week. The line for infected leafhoppers was shifted two weeks to illustrate the two-week lag that was identified in the cross-correlation.

3.5 Leafhopper populations across sampling points

When we compared the abundance of infected and uninfected leafhoppers across sites by cropping system, celery fields within 2.5 km were similar in leafhopper density (*Moran's I* = 0.56, p-value = 0.03) and infectivity (*Moran's I* = 0.70, p-value ≤ .001). Celery fields > 2.5 km apart, however, did not correlate strongly in leafhopper density or infectivity (Fig 2.7A, S2.1A). When evaluated, carrot fields within 6 km where highly dissimilar in leafhopper density (*Moran's I* = -0.65, p-value = 0.99), while fields in distance bands > 6 km suggested no positive or negative correlation in leafhopper density across fields (Fig 2.7B, S2.1B). No correlation was observed in the infected leafhopper population between carrot fields at any distance (Fig 2.7B, S2.1B).

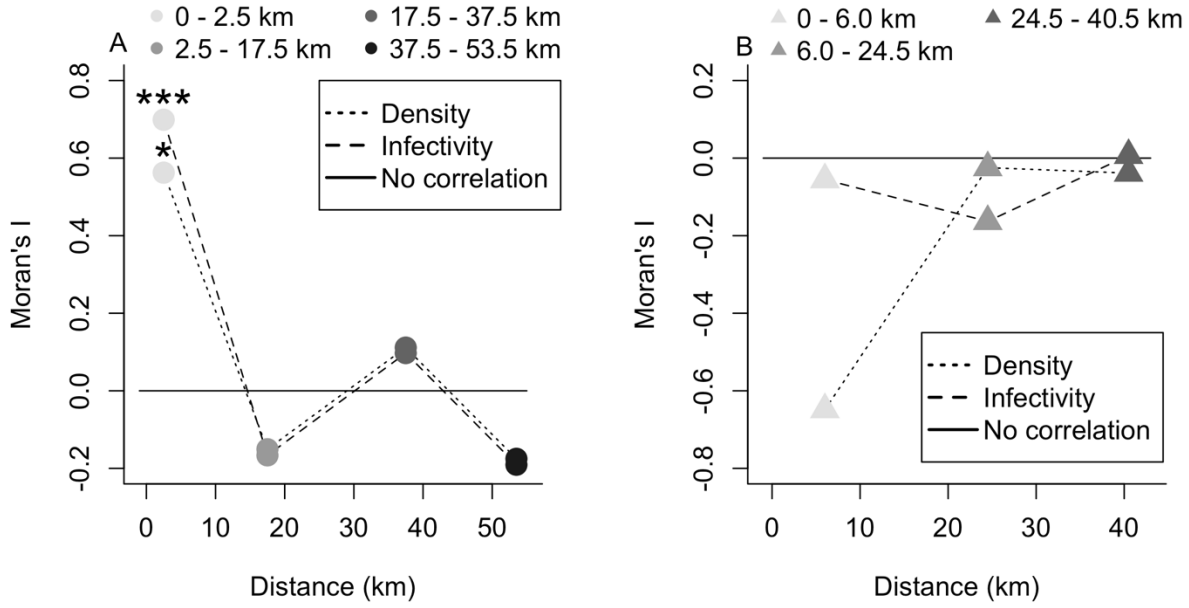

Figure 2.7. Correlation of leafhopper density and infectivity between sampling sites as a function of distance bands split by (A) celery and (B) carrot. (A) Celery sampling sites within 2.5 km were similar in leafhopper density and infectivity. However, no correlation was found between sites beyond 2.5 km apart for either density or infectivity. (B) Carrot sites within 6 km where dissimilar in leafhopper density. No strong correlation was found for leafhopper density in carrot for sites in distance bands beyond 6 km, nor was any correlation observed in the infected leafhopper population between carrot sites at any distance.

4 Discussion

Few decision support tools exist for insect vector management, and those that do, focus mainly on insect abundance rather than pathogen vector prevalence.[5] We addressed this gap by developing a decision support tool which informed farmers of vector infectivity in two cropping systems, carrot and celery. Using our tool, we found when we sent out text messages reporting infected leafhoppers were present, there was a decrease in infectivity, but this downward trend could have been associated with several factors including a natural decline in leafhoppers over the season as well as changes in weather. Additionally, we were unable to explore this trend further as there were no controls to compare to, as not sending farmers the text messages would be ethically questionable. Despite these issues, farmers found the tool useful and with the diagnostics data we gathered, we made some important temporal and spatial discoveries in this pathosystem.

We identified that temporal differences and spatial correlations exist between uninfected and infected leafhopper populations and that these depend on the crop context. Specifically, in celery our results indicated a temporal difference between populations of infected and uninfected leafhoppers with a 2-week delay between leafhopper populations which were uninfected compared to those infected with phytoplasma. In practice, this suggests that aster leafhopper management should be delayed to focus control on the disease carrying vectors, rather than the inconsequential damage caused by leafhopper feeding.[47] By targeting pesticide applications to align with peak abundance of infected leafhoppers, the number of applications required to control the disease may decline, which would result in increased profits for small-scale vegetable farmers and a reduction in non-target impacts.[48] Our results also imply that leafhopper diagnostics could begin two weeks after peak leafhopper abundance is detected in celery fields. From a biological viewpoint, the relationship between the abundance of infected and uninfected individuals within a population of

aster leafhoppers is not well understood and may depend on the latency of aster yellows phytoplasma within the vector and host plant.[44] The applicability of our decision support tool is likely most useful for pathogens transmitted in a persistent manner and where the transmission from the vector to crop is delayed relative to non-persistently transmitted pathogens. The lag between detection of pathogens in the vector to transmission to the crop allows management actions to occur before much of plant infection occurs. If pathogen transmission to plants take place in a short period of time (e.g. a single insertion of mouthparts), while diagnostics may reduce overall disease transmission, due to the time between sampling and information delivery to farmers, there could be significant crop infection occurring.

In the spatial analysis we determined that celery fields located within a 2.5 km radius have similar infectivity patterns, meaning that our diagnostic efforts can eliminate multiple samples originating from celery fields located near each other without losing relevant information. Aster leafhoppers stay in a relatively small geographic area when ideal hosts are available at the end of their spring migration.[49] They reproduce and feed until host plant quality declines which signals the need for dispersal.[49] Since they have many host plants, the availability of ideal hosts in a small area is relatively high therefore leafhoppers are likely to travel short distances. This may explain why spatial patterns were similar in celery fields that were nearby. In carrots, the lack of spatial correlation may be due to the greater distance among fields (1.6 – 30.6 km between fields). The differences in aster leafhopper temporal patterns between the two cropping systems is interesting and could be due to variations in the establishment of plants. For instance, celery seedlings are transplanted from greenhouses while carrots are direct seeded.[31,32] Our results indicate that celery seedlings likely provide an early season host for aster yellows phytoplasma infected leafhoppers which may later prefer and move to direct seeded carrots. This relationship may be driven by the

palatability of the host plants, which is known to mediate insect populations, including leafhoppers.[19,50] For example, as plants mature they may become less palatable which may influence shifts in insect populations to a more palatable resource.[51,52] In addition, infected insect vectors may demonstrate behavioral differences when compared to uninfected individuals and these behavioral differences may influence the presence of infected individuals in certain crops.[53–55] Regardless of the mechanism behind the observed pattern, our results demonstrate the need for crop specific understanding of aster leafhopper management.

While fee-based phytoplasma testing is available at many plant diagnostic laboratories, these are focused on testing plant materials and not phytoplasma vectors (Szendrei Z, pers. Comm.). Currently our aster leafhopper decision support tool is available to farmers in Michigan and is conducted only by our laboratory. In order to increase its sustainability and availability in a broader geographic range, commercial diagnostic laboratories that can process leafhoppers in 24 h will need to become involved. This will also mean a necessary change in funding structure, with a move away from the current grant funded effort to a per-sample processing fee. Thus far, the large volume of samples processed by our laboratory seemed an impediment for adoption by commercial laboratories (Szendrei, Z. pers. Comm). With our current results reporting on the spatiotemporal patterns in the aster yellows system, we could meaningfully reduce the number of samples needed from the field, which could make the diagnostics more appealing to adoption by commercial laboratories.

5 Conclusion

Decision support tools deliver time sensitive information to farmers through the integration of pest monitoring, weather/computer modeling, and alert systems.[1,3] The use of these tools can

lead to reductions in pesticide applications on farms, increases in beneficial insects, and increased farmer profits.[1,2] Our decision support tool used a novel combination of scouting by crop consultants, rapid disease diagnostics, and a web-based text messaging system to provide county-level recommendations for pest management. While we cannot identify a causal link between our decision support tool and the reductions of the infected population, the patterns we observed have important practical outcomes. If such decision support tools are implemented on a large enough scale, they may have the potential to minimize yield loss and decrease the incidence of and potential for disease over time. These tools should be implemented alongside existing control measures for aster yellows phytoplasma rather than in isolation, given that evidence indicates cultural measures, including weed control, can reduce disease incidence.[47]

APPENDIX

Supplementary Tables for Chapter 2

Table S2.1. The number of commercial celery farms and fields for aster leafhopper collections during the 2014 – 2019 growing seasons in Michigan. A '1' indicates a sampled field in a year. A '0' indicates that the field was not sampled.

Farm	Field	2014	2015	2016	2017	2018	2019
Farm 1	Field 1	1	0	0	0	0	0
Farm 1	Field 2	1	1	1	1	1	1
Farm 1	Field 3	1	1	1	1	1	1
Farm 1	Field 4	0	0	0	0	1	1
Farm 1	Field 5	0	0	0	0	1	0
Farm 1	Field 6	0	0	0	0	0	1
Farm 1	Field 7	0	0	0	0	0	1
Farm 2	Field 8	1	0	0	0	1	0
Farm 2	Field 9	0	1	1	1	1	1
Farm 2	Field 10	0	0	0	0	1	0
Farm 2	Field 11	0	0	0	0	1	0
Farm 2	Field 12	0	0	0	0	1	0
Farm 2	Field 13	0	0	0	0	0	1
Farm 2	Field 14	0	0	0	0	0	1
Farm 2	Field 15	0	0	0	0	0	1
Farm 2	Field 16	0	0	0	0	0	1
Farm 3	Field 17	1	1	1	0	1	0
Farm 4	Field 18	1	1	0	0	0	0
Farm 5	Field 19	1	1	1	1	1	1
Farm 5	Field 20	0	1	1	1	1	1
Farm 5	Field 21	0	0	0	0	0	1
Farm 6	Field 22	1	0	0	0	0	0
Farm 6	Field 23	1	0	0	0	1	0
Farm 6	Field 24	1	0	0	1	1	0
Farm 6	Field 25	0	1	1	1	1	1
Farm 6	Field 26	0	0	1	0	0	0
Farm 6	Field 27	0	0	0	1	0	0
Farm 6	Field 28	0	0	0	0	1	0
Farm 6	Field 29	0	0	0	0	1	0
Farm 6	Field 30	0	0	0	0	1	1
Farm 6	Field 31	0	0	0	0	1	0
Farm 6	Field 32	0	0	0	0	1	0
Farm 7	Field 33	1	0	0	1	1	0
Farm 7	Field 34	0	1	1	1	0	0
Farm 7	Field 35	0	0	0	0	1	0
Farm 8	Field 36	0	0	1	0	0	0
Farm 9	Field 37	0	0	1	0	0	0
Farm 9	Field 38	0	0	0	0	1	0
Farm 10	Field 39	0	1	1	1	1	1
Farm 10	Field 40	0	0	0	0	1	1
Totals		**11**	**10**	**12**	**11**	**25**	**17**

Table S2.2. The number of commercial carrot farms and fields for aster leafhopper collections during the 2014 – 2019 growing seasons. A '1' indicates a sampled field in a year. A '0' indicates that the field was not sampled.

Farm	Field	2014	2015	2016	2017	2018	2019
Farm 1	Field 1	1	0	0	0	1	0
Farm 2	Field 2	1	1	0	0	0	0
Farm 3	Field 3	1	0	0	0	0	0
Farm 4	Field 4	1	1	0	1	1	1
Farm 5	Field 5	1	0	0	0	1	1
Farm 5	Field 6	0	0	0	0	0	1
Farm 5	Field 7	0	0	0	0	0	1
Farm 5	Field 8	0	0	0	0	0	1
Farm 5	Field 9	1	0	0	0	0	0
Farm 5	Field 10	0	0	0	0	1	0
Farm 6	Field 11	1	1	1	0	0	0
Farm 7	Field 12	0	1	0	0	0	0
Farm 8	Field 13	0	1	0	0	0	0
Farm 9	Field 14	0	0	1	0	0	1
Farm 9	Field 15	0	0	0	0	0	1
Farm 9	Field 16	0	0	0	0	1	0
Farm 9	Field 17	1	0	0	0	0	0
Farm 10	Field 18	0	0	0	1	0	0
Farm 11	Field 19	1	0	0	1	0	0
Farm 12	Field 20	0	0	0	1	0	0
Totals		**9**	**5**	**2**	**4**	**5**	**7**

Table S2.3. Weekly mean densities of aster leafhoppers (leafhoppers per 100 sweeps) collected throughout the 2014–2019 growing seasons in Michigan. Leafhoppers were collected with sweep nets from commercial carrot and celery farms.

Week	Carrot			Celery		
	N[1]	Total[2]	Mean[3]	N[1]	Total[2]	Mean[3]
20	NA	NA	NA	1	2.67	2.67
21	NA	NA	NA	6	5.56	0.93 ± 0.43
22	3	3.94	1.31 ± 0.68	36	156.43	4.35 ± 0.64
23	4	20.91	5.23 ± 0.75	36	79.92	2.22 ± 0.35
24	3	33.29	11.10 ± 10.29	46	197.09	4.28 ± 0.72
25	6	12.22	2.04 ± 0.92	27	42.56	1.58 ± 0.28
26	4	19.84	4.96 ± 2.77	21	42.54	2.03 ± 0.55
27	6	29.18	4.86 ± 2.67	16	23.36	1.46 ± 0.33
28	14	58.06	4.15 ± 1.34	21	53.96	2.57 ± 0.71
29	5	34.33	6.87 ± 5.42	16	35.77	2.24 ± 0.94
30	9	32.06	3.56 ± 1.54	29	94.83	3.27 ± 0.67
31	16	177.35	11.08 ± 3.03	9	49.78	5.53 ± 1.96
32	4	20.29	5.07 ± 2.57	10	55.19	5.52 ± 2.72
33	5	10.63	2.13 ± 1.18	4	6.54	1.63 ± 0.45
34	4	9.48	2.37 ± 1.40	4	7.34	1.83 ± 0.82

[1]: Number of samples taken across years per sampling week.
[2]: Total number of leafhoppers per 100 sweeps summed across years.
[3]: Mean number of leafhoppers per 100 sweeps averaged across years.

Table S2.4. Weekly mean abundances of aster yellows phytoplasma infected aster leafhoppers collected throughout the 2014–2019 growing seasons in Michigan. Leafhoppers were collected with sweep nets from commercial carrot and celery farms and identified as infected using qPCR based diagnostic methods.

Week	Carrot			Celery		
	N[1]	Total[2]	Mean[3]	N[1]	Total[2]	Mean[3]
20	NA	NA	NA	1	0.00	0.00
21	NA	NA	NA	6	1.00	0.17 ± 0.17
22	3	0.00	0.00	36	4.00	0.11 ± 0.07
23	4	0.00	0.00	36	4.00	0.11 ± 0.07
24	3	0.00	0.00	46	11.00	0.24 ± 0.11
25	6	1.00	0.17 ± 0.17	27	10.00	0.37 ± 0.15
26	4	8.00	2.00 ± 1.22	21	13.00	0.62 ± 0.30
27	6	10.00	1.67 ± 1.05	16	2.00	0.13 ± 0.09
28	14	15.00	1.07 ± 0.73	21	7.00	0.33 ± 0.20
29	5	0.00	0.00	16	4.00	0.25 ± 0.17
30	9	0.00	0.00	29	2.00	0.07 ± 0.05
31	16	4.00	0.25 ± 0.14	9	0.00	0.00
32	4	0.00	0.00	10	0.00	0.00
33	5	1.00	0.20 ± 0.20	4	1.00	0.25 ± 0.25
34	4	0.00	0.00	4	1.00	0.25 ± 0.25

[1]: Number of samples taken across years per sampling week.
[2]: Total number of infected leafhoppers summed across years.
[3]: Mean number of infected leafhoppers averaged across years.

Supplementary Figures for Chapter 2

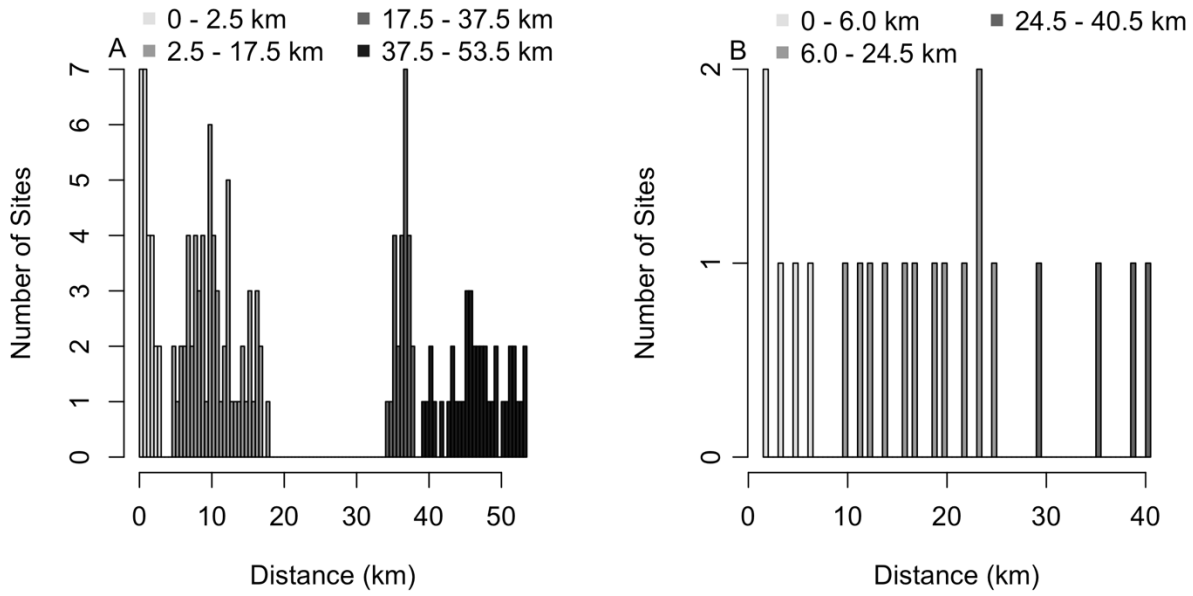

Figure S2.1. Spatial autocorrelation analysis (Moran's I) indicating the number of sites within each distance band for (A) celery and (B) carrot sites.

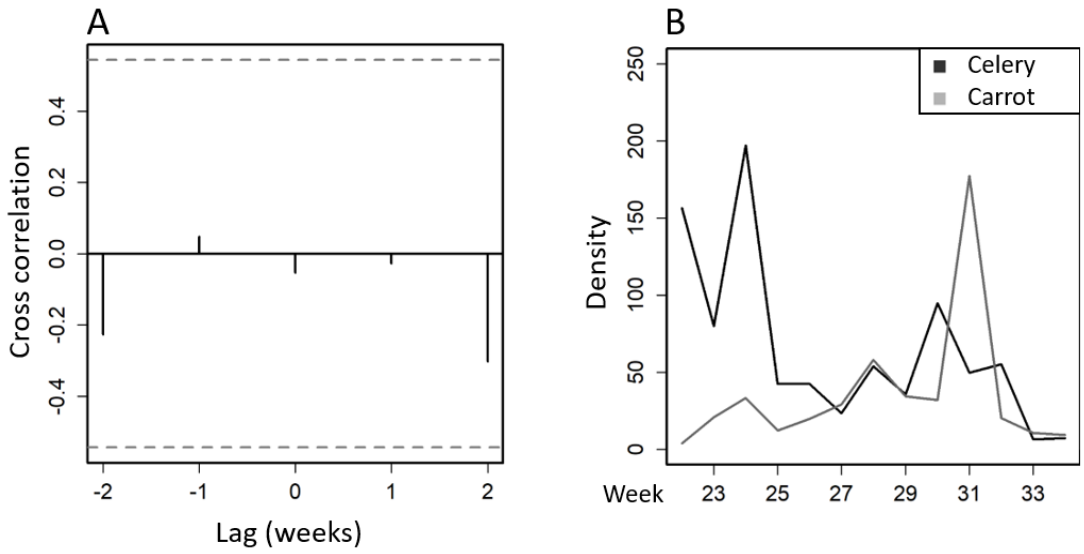

Figure S2.2. Temporal relationship between aster leafhopper populations in celery and carrot using cross correlation analysis for aster leafhopper density (leafhoppers per 100 sweeps) in celery and carrot fields in Michigan during the 2014–2019 growing seasons. Dotted lines indicate 95% confidence interval and each lag represents a week. (A) No correlation was found between leafhopper densities between the two crops. (B) Density of aster leafhoppers plotted by week across the season in Michigan celery and carrot fields. The weeks on the x-axis correspond with the weeks of the calendar year.

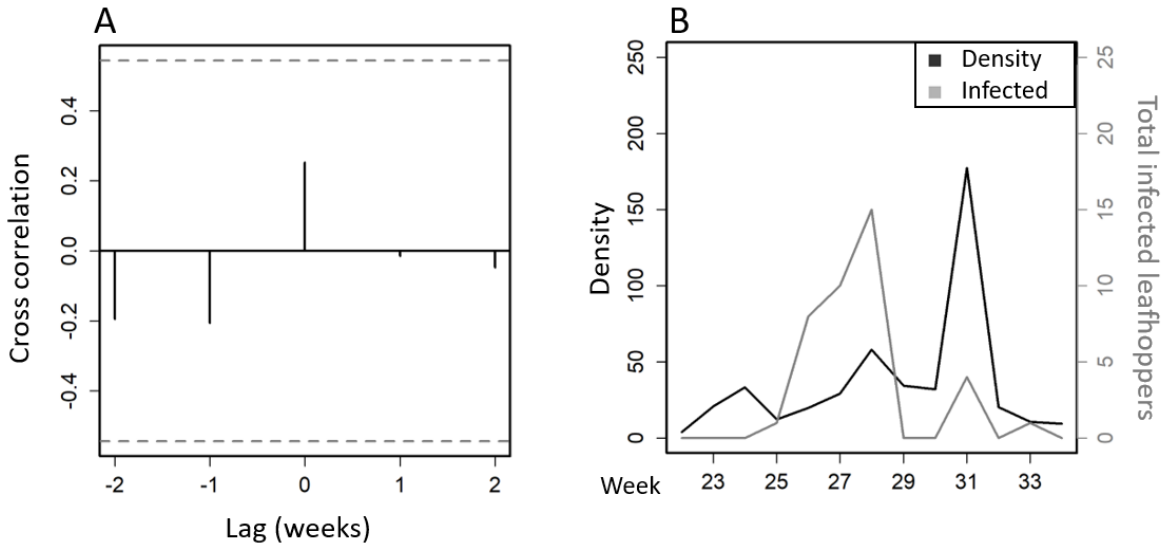

Figure S2.3. Cross correlation analysis for aster leafhopper density (leafhoppers per 100 sweeps) and the number of aster yellows phytoplasma infected leafhoppers in carrot fields in Michigan during the 2014–2019 growing seasons. Dotted lines indicate 95% confidence interval and each lag represents a week. (A) No correlation was found between leafhopper density and infectivity in carrots. (B) Density of aster leafhoppers and total number of aster yellows phytoplasma infected leafhoppers plotted by week across the season in Michigan carrot fields. The weeks on the x-axis correspond with the weeks of the calendar year.

Supplementary Information for Chapter 2

Text messaging and infectivity follow up discussion

During the growing season in celery, there is a natural increase in abundance peaking in late June – early July followed by a decline in abundance within the crop. [29] This also occurs in carrots, but at a slower rate, with more leafhoppers being found within fields during August (personal observation, P. Stillson). After the leafhoppers reach peak abundance in celery, they move from the field to the edge for the remainder of the season, likely due to an increase in preferred plants in the edge (see chapter 3, and Jubenville 2015). [29] In addition, weather may contribute to fluctuations in abundance, but in this chapter, we did not investigate weather's effects on abundance or infectivity during the season.

Beyond these variables, we were unable to provide controls due to the nature of this research and the ethical implications of not providing infectivity reports to all farmers. In order to rectify this in future studies that may follow up on this research, I suggest comparing two groups of farmers: those consenting to not receive the text messages and those that receive infectivity text messages. This study could then be used to improve our understanding of how the text messaging tool works. Alternatively, all farmers could still receive the text messages, if a comparable control were to be used, such as comparing infectivity between commercial and organic farms. At this time, we have only worked with one farm that used organic management, and only one of their fields was organic (two collections in 2019).

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LITERATURE CITED

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CHAPTER 3: Identifying leafhopper targets for controlling aster yellows in carrots and celery

1 Introduction

Aster yellows phytoplasma (*Candidatus* Phytoplasma sp.) is an insect-vectored plant pathogen[1] which causes a variety of symptoms including yellowing, virescence, phyllody, witch's broom, and ultimately premature death.[2] Even when infected crops reach harvest, they are often unmarketable.[1,3] Aster yellows has been reported to reduce yields by 10%[4] and is one of the most widespread phytoplasmas, affecting 14 vegetable crops across various plant families.[5–7] It is vectored by at least 24 leafhoppers,[8] which must acquire the phytoplasma from the environment by feeding on infected plants,[1] since phytoplasmas are rarely transovarial.[1,9] Not all leafhoppers can transmit aster yellows, which may be associated with a narrow diet breadth where the leafhoppers do not feed on the infected plant or do not feed on the phloem of the infected plant.[7] If a leafhopper feeds on an infected plant, the phytoplasma must successfully migrate to the salivary glands before transmission is possible.[10,11] Polyphagous leafhoppers can acquire phytoplasma from crops or weedy host plants and then spread it among susceptible crop fields or between the field and field edge.[7]

Movement of phytoplasmas in agroecosystems is primarily facilitated by polyphagous leafhoppers feeding locally on infected host plants,[1] and seasonal migrations of some leafhopper species from overwintering to summer habitats.[12–14] In North America, the main vector of aster yellows phytoplasma is the migratory *Macrosteles quadrilineatus* – the aster leafhopper (Forbes; Hemiptera: Cicadellidae), a polyphagous species with over 300 host plants,[15] and a broad geographic distribution.[12] *Macrosteles quadrilineatus* may move between different crops, between

fields, and into field edges to feed on grasses and weeds.[16] This movement among various host plants can increase the chances of other leafhopper vectors acquiring aster yellows.[1,17] Currently, *M. quadrilineatus* is the focus for controlling aster yellows phytoplasma for vegetable farmers in the Midwest, USA. However, agroecosystems can have diverse leafhopper communities. These leafhopper vectors may then create disease reservoirs in the field edge, especially in perennial weeds that can be a source of infection every year.[1,18,19] The identity and vector status of these other leafhopper taxa is understudied and may be important for developing sustainable management methods for aster yellows.

To investigate if additional leafhopper species are important aster yellows vectors, we collected leafhoppers from commercial celery and carrot farms in Michigan during the 2018 and 2019 growing seasons. We collected leafhoppers from the crops and the field edges using sweep nets, identified the leafhoppers to the lowest taxonomic level possible using DNA barcoding, conducted molecular diagnostics to determine if they contained phytoplasma, and compared leafhopper species abundances in the different crops and locations.

2 Materials and methods

2.1 Study system

Leafhoppers were collected using sweep nets (38 cm diameter aerial net) from mid-May through early August in the 2018 and 2019 growing seasons. All farms surveyed (Fig 3.1) were large-scale commercial operations, managed with synthetic pesticides. Sweep net samples were taken between 11:00 and 14:00 on clear days when insecticides had not been recently applied.

In 2018, leafhoppers were collected three times from the field edge (June 26, July 10, and August 1) from one celery farm and weekly from inside seven celery and five carrot fields (n = 36

collections). In 2019, collections from both within the fields and from the edges were conducted weekly at ten celery and seven carrot farms (n = 226 collections). A minimum of 100 sweeps from inside the crop fields were taken from randomly chosen sites, approximately >10 m into the field, away from the field edge. The 'field edge' consisted of naturally occurring vegetation around crop fields, along driveways, or along wooded edges (Fig 3.2A, B). In both years, sweeps were taken within randomly selected 5 m sections of the field edge; the total number of sweeps varied by field edge due to the variability in the amount of vegetation available for sweeping (200–500 sweeps/field). After collection, all leafhoppers were transported in a cooler from the field to the laboratory, where they were stored at -20°C.

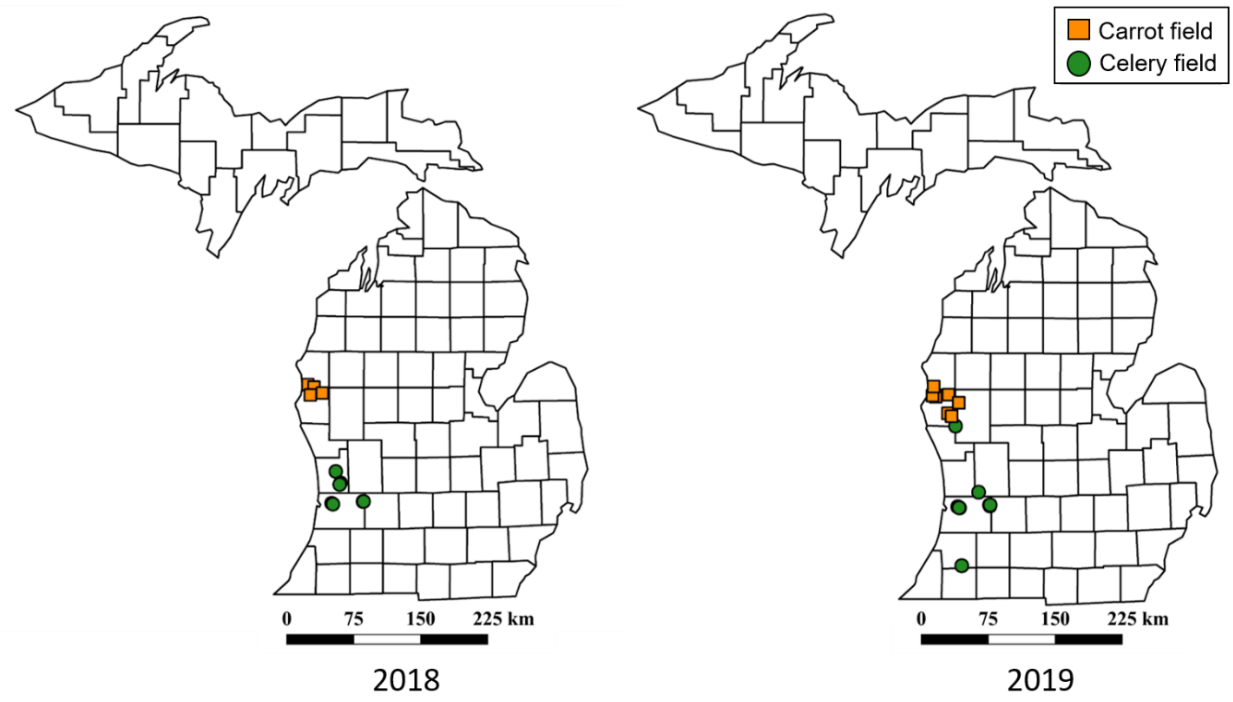

Figure 3.1. Map of collections sites from Michigan, USA. Symbols indicate locations of celery and carrot fields where leafhoppers were collected in 2018 and 2019. Leafhoppers were collected using sweep nets and transported to the laboratory for identification and to determine phytoplasma infectivity.

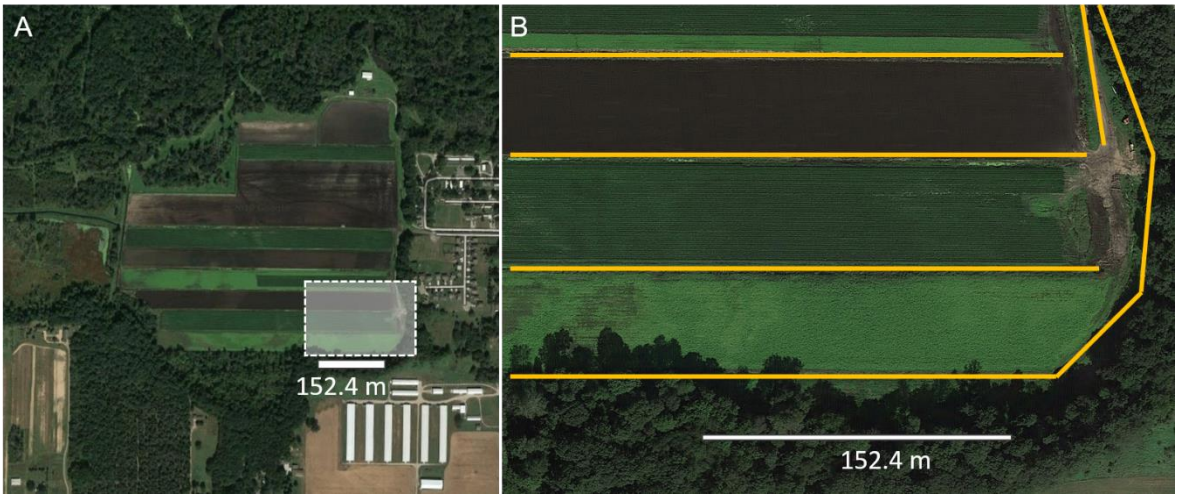

Figure 3.2. Leafhoppers were collected in celery and carrot field edges in Michigan in 2018 and 2019. (A) Aerial view of a celery field with boxed area magnified in B. (B) The surveyed field edge types are indicated by the yellow lines, consisting of vegetation between adjacent crop fields and edges between fields and non-agricultural vegetation, including weedy herbaceous plants growing along roads or paths adjacent to fields, or plants naturally growing along wooded edges.

2.2 Leafhopper identification and phytoplasma detection

In the laboratory, leafhoppers were sorted into groups upon arrival from the field: *M. quadrilineatus*, *Empoasca fabae* – the potato leafhopper (Harris), and other leafhoppers grouped based on morphological similarities. *Macrosteles quadrilineatus* and *E. fabae* were sight identified and were placed into homogenization tubes for DNA extraction. All *M. quadrilineatus* (n = 2,883) DNA was extracted following Demeuse et al.;[20] modifications to this protocol included individually extracting DNA from each leafhopper and eluting DNA in 50 µl EB elution buffer (Qiagen).

To identify the other leafhopper species by DNA barcoding, we used a modified Dellaporta DNA extraction to minimize DNA fragmentation.[21] Leafhoppers (n = 2,166) were placed individually in 2 ml homogenization tubes (Sarstedt, Nümbrecht, Germany), along with 3 homogenization beads (2.3 mm diameter, zirconia/silica; BioSpec Products, Bartlesville, OK, USA), and 400 µl Dellaporta buffer (1 ml of 100 mM Tris, pH 8.0, 1 ml of 500 mM EDTA, 1.25

ml 500 mM NaCl, 10 µl β-mercaptoethanol and 6.75 ml of Ultrapure water). Leafhoppers were homogenized for 10 s at 4.0 m/s (FastPrep-24, MP Biomedicals, Irvine, CA, USA). Afterwards, 52.8 µl 10% SDS was added, samples were vortexed then incubated at 65°C for 10 min. After incubation, 128 µl 5 M potassium acetate was added. Samples were vortexed then centrifuged for 10 min at 15,000 rcf. Supernatant was removed and placed in a clean 1.7 ml centrifuge tube. Afterwards, 240 µl cold isopropanol was added to the supernatant and the samples were incubated at room temperature for 5 min. Samples were mixed by gentle inversion. Samples were placed in a -20°C freezer for 1 h and then centrifuged in a 4°C refrigerated centrifuge (Centrifuge 5810 R, Eppendorf, Hamburg, Germany) for 20 min at 15,200 rcf. Supernatant was removed and 800 µl 70% ethanol was added to the pelleted DNA. Samples were again mixed by gentle inversion and then placed back in the refrigerated centrifuge for 10 min. The supernatant was removed, and pellets allowed to air dry. Pellets were suspended in 50 µl EB elution buffer (Qiagen).

We used PCR to amplify the cytochrome c oxidase subunit I (COI) gene using the Ron and Nancy primer set (Thermo Fisher Scientific, Waltham, MA, USA),[22] cleaned the PCR product with QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA), and submitted the DNA to Michigan State University's Research Technology Support Facility (RTSF) for Sanger sequencing. The sequences were compared to the National Center for Biotechnology Information genomic database (NCBI), and the leafhoppers were identified based on sequence match and were also compared to previously identified, morphologically similar, specimens in the Albert J. Cook Arthropod Research Collection at Michigan State University and on Bug Guide.[23] After identification, leafhoppers were divided into commonly collected (≥ 50 leafhoppers collected) or rare (< 50 leafhoppers collected) species. For future reference, one adult specimen of each morphological group was pinned, or one nymph was preserved in 70% ethanol. Voucher

specimens were stored in the Albert J. Cook Arthropod Research Collection, Michigan State University (voucher number: 2019-09).

2.3 Detection of phytoplasma

All leafhoppers were evaluated for the presence of aster yellows phytoplasma with a TaqMan assay,[20] using universal phytoplasma primers and probe (Thermo Fisher Scientific).[24] For *M. quadrilineatus*, we used a cycle threshold (Ct) value < 32 to determine positives, as established for our regular diagnostic work for farmers.[20] All non-*M. quadrilineatus* with Ct-values ≤ 40, were also tested using conventional PCR with P3/P7 universal phytoplasma primers[25] to verify the presence of phytoplasma. The PCR products were run on a 1% agarose gel precast with GelRed (Biotium, Fremont, CA, USA) for 1 h at 90 V. Bands were visualized with a UV transilluminator (Bioolympics, Thousand Oaks, CA, USA). In addition, we searched the literature to determine which of the collected leafhoppers are known vectors for aster yellows phytoplasma or other phytoplasmas, or if there are other members of the genus that are phytoplasma vectors. Vector status for leafhoppers found through the literature search was determined through transmission studies where leafhoppers inoculated healthy test plants or inoculated sucrose solutions. We then compared the collected leafhopper species to this dataset.

2.4 Data analysis

To identify which leafhopper species may be feeding on the crops, or moving between the crops and field edge, we determined if there were differences in leafhopper species abundance between species found in both locations and crops. We used a generalized linear model, where crop type and field location were fixed factors. Differences among means of tested factors were determined with post-hoc pairwise comparison (Tukey's HSD: $\alpha = 0.05$; function = 'emmeans', package = 'emmeans').[26] The total number of leafhoppers per 100 sweeps was used for each of

the most abundant leafhopper species (genera or species ≥ 50 leafhoppers collected). Leafhoppers per 100 sweeps was used to standardize leafhopper densities across collections with different numbers of sweeps. We performed separate statistical analyses for celery and carrot.

To determine if there were differences in the number of infected *M. quadrilineatus* between the crop and field edge, we used a generalized linear model, where field location (inside or outside field) was used as a fixed factor. Differences among means of tested factors was again determined with post-hoc pairwise comparison (Tukey's HSD: $\alpha = 0.05$). In addition, this was also done using crop (carrot or celery) as a fixed factor. All statistical analyses were conducted in R v.3.6.0.[27]

3 Results

In total, we collected 5,049 leafhoppers from celery and carrot fields and their field edges combined during the 2018 and 2019 growing seasons. We identified 25 genera and 14 species, with an additional 16 morphotypes identified to family level (Cicadellidae; Table 3.1, Fig 3.3A-Z). Eight genera and four species represented 94% of collected leafhoppers (Table 3.1). The most abundant species were *M. quadrilineatus* (57%) and *E. fabae* (23%).

Table 3.1. Leafhoppers collected from commercial celery and carrot farms in Michigan, USA, from 2018 to 2019. Field edges were defined as areas bordering the crop field or between adjacent fields where crops were not growing. Fractions indicate the number of individuals that generated cycle thresholds (Ct ≤ 40) in a real-time PCR with universal phytoplasma primers[24] out of the total number of individuals collected.

Genera/Species	Celery field	Celery edge	Carrot field	Carrot edge	2018 total	2019 total	Ct-value or range
Agallia sp.	2	7	10	24	5	38	-
Aphrodes bicinctus	0	49	0	29	9	69	-
Athysanus argentarius	0	2	0	6	0	8	-
Balclutha sp.	1	29	½	23	1/21	34	36.97
Colladonus clitellarius	1	0	0	2	1	2	-
Commellus sp.	0	0	0	2	0	2	-
Cuerna sp.	0	0	1	2	0	3	-
Diplocolenus subg. *Verdanus*	0	0	0	32	0	32	-
Doratura stylata	0	0	0	1/191	0	1/191	34.93
Draeculacephala sp.	0	1/23	3	45	1	1/70	39.90
Elymana inornata	0	0	0	2	0	2	-
Empoasca fabae	6/235	6/418	409	75	11/304	5/833	25.20 – 40.00
Endria inimica	0	0	0	6	0	6	-
Erythroneura sp.	1	0	0	0	1	0	-
Forcipata loca	0	8	0	1	0	9	-
Graphocephala sp.	0	1/1	27	1	1/28	1	40.00
Idiocerus raphus	3	1	0	1	3	2	-
Idiocerus sp.	0	1/11	0	0	1/10	1	36.51
Jikradia olitoria	0	6	0	2	0	8	-
Latalus sp.	0	2	2	4/135	0	4/139	35.39 – 37.03
Macrosteles quadrilineatus	1/447	3/582	7/1423	1/431	3/707	9/2176	17.56 – 31.73
Neokolla hieroglyphica	0	0	41	5	0	46	-
Norvellina sp.	1	1	0	0	1	1	-
Paraphlepsius sp.	0	11	5	2	2	16	-
Psammotettix lividellus	8	1/187	1	18	0	1/214	36.93
Scaphytopius sp.	2	10	½	4	¼	14	39.30
Unknown Cicadellidae	0	5	6	24	4	31	-
Total leafhoppers collected	701	1353	1932	1063	1101	3948	

Figure 3.3. Leafhopper species collected from Michigan, USA, celery and carrot farms from 2018 to 2019. (A) *Agallia* sp., (B) *Aphrodes bicinctus*, (C) *Athysanus argentarius*, (D) *Balclutha* sp., ® *Colladonus clitellarius*, (F) *Commellus* sp., (G) *Cuerna* sp.*, (H) *Diplocolenus* subg. *Verdanus*, (I) *Doratura stylata*, (J) *Draeculacephala* sp., (K) *Elymana inornata*, (L) *Empoasca fabae*, (M) *Endria inimica*, (N) *Erythroneura* sp., (O) *Forcipata loca*, (P) *Graphocephala* sp., (Q) *Idiocerus raphus*, ® *Idiocerus* sp., (S) *Jikradia olitoria*, (T) *Latalus* sp., (U) *Macrosteles quadrilineatus*, (V) *Neokolla hieroglyphica**, (W) *Norvellina* sp., (X) *Paraphlepsius* sp., (Y) *Psammotettix lividellus*, (Z) *Scaphytopius* sp. Note: * indicates that only nymphs were collected, all other leafhoppers were collected as adults or as both adults and nymphs. See acknowledgements for photo credits.

3.1 Celery collections

We collected 2,054 leafhoppers from 2018 and 2019 from celery farms, with 701 leafhoppers (34% of the total) collected from within the celery fields and 1,353 (66%) from the field edge. A total of 18 genera and 9 species were identified. *Macrosteles quadrilineatus* (50%), *E. fabae* (32%), and *Psammotettix lividellus* (Zetterstedt; 9%) were the most abundant leafhopper taxa (≥ 50 individuals collected of each). *Erythroneura* sp. (Fitch) was only found in celery fields but not in field edges. When comparing the abundances of the eight most abundant leafhopper taxa within and outside celery fields, they were all predominantly found in the field edge. *Macrosteles quadrilineatus* was 1.65 times more abundant in celery field edges than within the field (p-value ≤ 0.01), similarly, *E. fabae* was 2.23 times more abundant in field edges than within celery fields (p-value = 0.03; Fig 3.4). *Psammotettix lividellus* and *Balclutha* sp. (Kirkaldy) were both found primarily outside celery fields with 1.13 (p-value = 0.97) and 1.21 (p-value = 0.97) times greater abundances in the edge respectively than in the celery field. Three other taxa – *Latalus* sp. (DeLong & Sleesman), *Aphrodes bicinctus* (Schrank), and *Draeculacephala* sp. (Ball) – were found only in the field edge (Fig 3.5C, D). *Doratura stylata* (Boheman) was absent from celery fields and edge collections.

3.2 Carrot collections

We collected 2,995 leafhoppers from carrot farms in 2018 and 2019, with 1,932 leafhoppers (65%) collected from within carrot fields and 1,063 (35%) from the field edges. A total of 23 genera and 13 species were identified. The most abundant leafhopper taxa were *M. quadrilineatus* (62%), *E. fabae* (16%), *D. stylata* (6%), and *Latalus* sp. (5%) (≥ 50 individuals collected for each). Leafhoppers found only in carrots included *Commellus* sp. (Osborn & Ball), *Cuerna* sp. (Melichar), *Diplocolenus* subg. *Verdanus* (Oman), *Doratura stylata*, *Elymana inornata*

(Van Duzee), *Endria inimica* (Say), and *Neokolla hieroglyphica* (Say). When comparing the abundances of the eight most abundant leafhopper taxa within and around carrot fields, *M. quadrilineatus* had 1.75 times greater abundance within the carrot fields than in the field edge (p-value ≤ 0.01), *E. fabae* had 4.20 times greater abundance within the field (p-value = 0.99; Fig 3.4), as did *P. lividellus* with 1.44 times greater abundance in the field (p-value = 0.99) than in the field edge. Conversely, *Latalus* sp., *Balclutha* sp., and *Draeculacephala* sp. had greater abundances within the field edges than in the carrot fields, with 3.00 (p-value = 0.97), 2.00 (p-value = 0.99), and 1.94 (p-value = 0.78) times more leafhoppers collected respectively. Two other taxa – *A. bicinctus*, and *D. stylata* – were only found in the carrot field edge (Fig 3.5A, B).

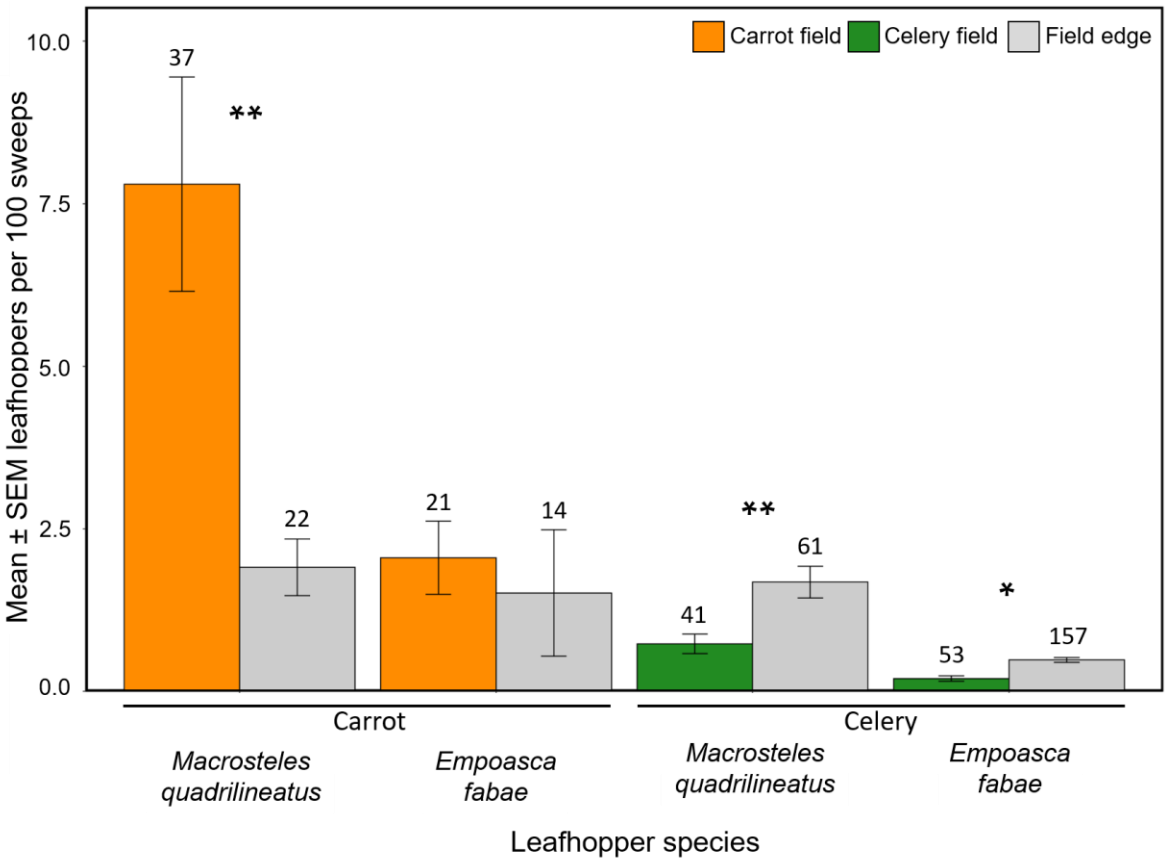

Figure 3.4. Mean ± SEM *Macrosteles quadrilineatus* and *Empoasca fabae* abundance in commercial carrot and celery fields and field edges in 2018 and 2019. Numbers above bars indicate the number of individuals collected for each species and location. Asterisks indicate statistically significant differences between field and edge collections for each taxa (Tukey's HSD; * p-value ≤ 0.05, ** p-value ≤ 0.01).

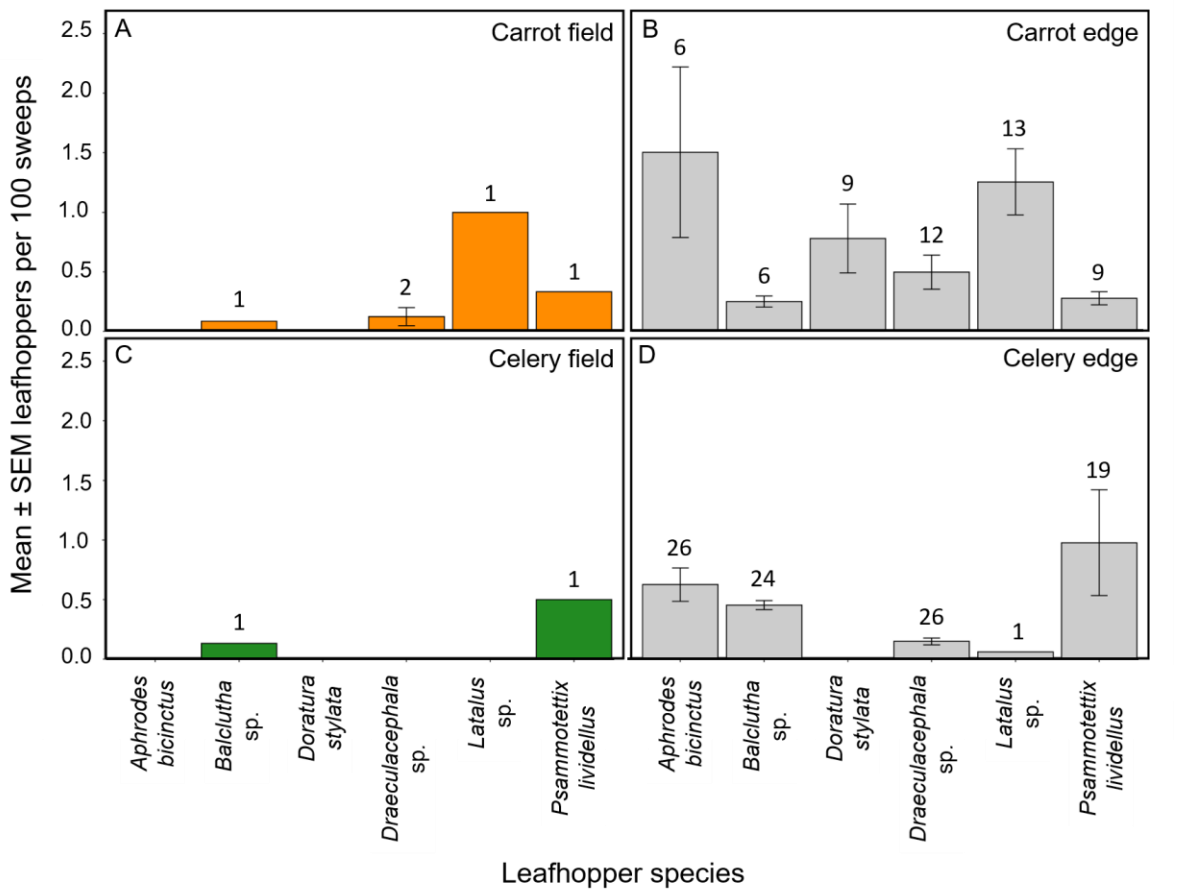

Figure 3.5. Mean ± SEM of the eight most abundant leafhopper species in celery and carrot fields and field edges (≥ 50 individuals collected); excluding *Macrosteles quadrilineatus* and *Empoasca fabae*. Leafhoppers were collected from commercial carrot (A, B) and celery (C, D) farms in Michigan in 2018 and 2019. Numbers above bars indicate the number of leafhoppers collected for each crop and location.

3.3 Phytoplasma infectivity

Across the two study years, 12 *M. quadrilineatus* tested positive for aster yellows using the Ct-value threshold of 32 typically used in our detection assay.[20] Twenty-seven individuals from nine other taxa had Ct-values between 25.2 and 40: 16 *E. fabae*, 4 *Latalus* sp., 1 *Balclutha* sp., 1 *Draeculacephala* sp., 1 *D. stylata*, 1 *Graphocephala* sp. (Van Duzee), 1 *Idiocerus* sp. (Lewis), 1 *P. lividellus*, and 1 *Scaphytopius* sp. (Ball; Table 3.1). One *E. fabae* tested positive for aster yellows phytoplasma using P3/P7 primers, while all the other leafhoppers were negative for aster yellows with this primer set.

In addition, we found three known aster yellows phytoplasma vectors in our collections including *A. bicinctus*, *Athysanus argentarius* (Metcalf), and *E. inimica* but none of these produced Ct-values $\leq$ 40. Of the leafhoppers that were identified to genus, *Agallia* sp. (Curtis), *Paraphlepsius* sp. (Baker), and *Scaphytopius* sp. may potentially be vectors since there are aster yellows vectors in these genera. Of those we identified to species, *Colladonus clitellarius* (Say), *E. inornata*, and *N. hieroglyphica* while not known to transmit aster yellows, other species in their genera are aster yellows vectors (Table 3.2, 3.3).

There was no difference in the number of infected *M. quadrilineatus* between crops (p-value = 0.61) or between the crop field and the field edge (p-value = 0.67).

Table 3.2. Known leafhopper vectors of aster yellows phytoplasma or other phytoplasmas for the species collected in this study. Phytoplasma abbreviations are AWB = alfalfa witches broom, AshY = ash yellows, AYp = aster yellows, Cp = clover phyllody, CYE = clover yellow edge, EastX = Eastern X, EAYp = European aster yellows, GFD = Grape flavescence doree, NAGVY = North American grapevine yellows IIIB, Sp = stolbur, SGP = strawberry green petal. *Diplocolenus* subg. *Verdanus*, *Doratura stylata*, *Forcipata loca*, and *Idiocerus raphus* were omitted as there is no record of whether they or their congeners vector phytoplasmas.

Species	Vectors AYp	Vectors other phytoplasmas	Congener vectors AYp	Congener vectors other phytoplasmas	References
Aphrodes bicinctus	Yes	EAYp, Sp, SGP, Cp, CYE	-	*A. albifrons*	8, 28, 29
Athysanus argentarius	Yes	-	-	-	30
Colladonus clitellarius	-†	EastX, AshY	*C. geminatus*, *C. montanus montanus*	*C. geminatus*, *C. montanus montanus*	8, 31–36
Elymana inornata	-	-	*E. sulphurella*	*E. virescens*	37–39
Empoasca fabae	-	-	-	*E. decipiens*, *E. papayae*	40–42
Endria inimica	Yes	-	-	-	43
Jikradia olitoria	-	N/AGVY	-	-	44
Macrosteles quadrilineatus	Yes	EAYp, Sp, Cp	*M. sexnotatus*	*M. cirstata*, *M. laevis*, *M. quadripunctulatus*, *M. sexnotatus*, *M. striifrons*, *M. viridigriseus*	19, 28, 45–59
Neokolla hieroglyphica	-	AWB	*N. severini*	*N. confluens*, *N. severini*	8, 50, 60
Psammotettix lividellus	-	GFD	-	*P. cephalotes*, *P. striatus*	8, 18, 61

†: "-" indicates that no data is available about whether the species or members of the genus can vector AYp or other phytoplasmas

Table 3.3. Known leafhopper vectors of aster yellows phytoplasma or other phytoplasmas for the genera collected in this study. *Commellus* sp., *Draeculacephala* sp., *Erythroneura* sp., and *Graphocephala* sp. were omitted as there is no record of whether species in these genera vector phytoplasmas.

Genus	Vectors AYp	Vectors other phytoplasmas	References
Agallia sp.	*A. constricta*	-	62, 63
Balclutha sp.	-†	*B. punctata*	64
Cuerna sp.	-	*C. septentrionalis*	60
Latalus sp.	-	*Latalus* sp.	65
Norvellina sp.	-	*N. seminuda*	45
Paraphlepsius sp.	*P. apertinus, P. irroratus*	*P. irroratus*	8, 31, 63, 66
Scaphytopius sp.	*S. acutus acutus*, *S. acutus delongi*	*S. acutus acutus, S. acutus delongi*, *S. magdalensis*	8, 31, 35, 67–72

†: "-" indicates that no data is available about whether the species or members of the genus can vector AYp or other phytoplasmas.

4 Discussion

Our leafhopper survey confirmed that *M. quadrilineatus* is the primary leafhopper vector of aster yellows phytoplasma in Michigan celery and carrot agroecosystems, which is consistent with findings from Ohio[12] and Wisconsin[73] carrot fields, and is the first study to confirm this in Midwestern celery fields. While other leafhopper species reside in and near these crops, we did not find strong evidence that they contribute to phytoplasma infections within these crops. Additionally, we determined that the leafhopper communities were different between the two cropping systems with the field edges characterized by a greater diversity of species than the crop fields.

With aster yellows phytoplasma's wide host plant range,[5] it is essential to identify its leafhopper vectors. Our results indicated that across both celery and carrot cropping systems, *M. quadrilineatus* was the most abundant species, and although carrots overall had more diversity in leafhopper taxa, the edges of both crops were comparable in leafhopper abundance and composition. The known aster yellows phytoplasma vectors collected were *A. bicinctus*, *A. argentarius*, and *E. inimica* (only in carrot) which were all found in the field edge and are known to feed on grasses, cereals, and clover.[30,43,74] We also collected *Scaphytopius* sp. from both cropping systems and while they are likely to be *Scaphytopius acutus* (Say), a known vector of aster yellows,[75] we did not find strong evidence that this leafhopper is vectoring phytoplasma (Ct = 39.3, n = 1). Unlike some of the other leafhopper species in our collections, *Scaphytopius* sp. was found in both carrot and celery fields and field edges, indicating that it is likely to frequently move to new host plants. *Doratura stylata* and *Latalus* sp. had the lowest Ct-values (Ct = 34.93, n = 1; Ct = 35.39–37.03, n = 4), besides *M. quadrilineatus* and *E. fabae*. While *D. stylata* has not been reported in the literature as a phytoplasma vector, *Latalus* sp. has been reported as a vector,

but we were unable to verify the real-time PCR findings with conventional PCR and sequencing. High Ct-values can potentially result when non-vector leafhoppers feed on an infected plant and the phytoplasma is present in the digestive tract.[76] Because of this, the only way to confirm new vectors is through transmission assays which involve having suspected vectors feed on phytoplasma infected plants to acquire the pathogen, followed with them inoculating healthy plants or a sucrose solution.[43,75,77] If the disease is detected in the plant, after a latency period lasting up to a month, or in the sucrose solution after inoculation, then the leafhopper is a vector for the phytoplasma.[43,75,77] Nevertheless, real-time PCR is known to be more sensitive for aster yellows detection than conventional PCR;[20] thus we cannot exclude the possibility that some of the leafhoppers with high Ct-values are in the early stages of infection.

We verified one *E. fabae* with conventional PCR and sequencing as containing aster yellows phytoplasma. *Empoasca fabae* has previously been detected with a strain of aster yellows, although the authors did not determine vector status.[78] Two other *Empoasca* spp. Are known phytoplasma vectors: *Empoasca papayae* (Oman) vectors Papaya Bunchy Top associated with *Candidatus* Phytoplasma aurantifolia,[40,42,79] and *Empoasca decipiens* (Paoli) vectors chrysanthemum yellows phytoplasma, which is closely related to aster yellows (Table 3.2).[41]

Although we hypothesized that field edges may be disease reservoirs and a source of infection for the crops, our findings indicate that the edge may not be the primary source of phytoplasma infection. Since more *M. quadrilineatus* were positive for aster yellows in samples collected from within crop fields, compared to field edges, this could indicate that aster yellows phytoplasma is brought into the field by migrating *M. quadrilineatus* that later move from the crop to the field edge.[28,29] In the field edge, infected *M. quadrilineatus* can infect plants which may become disease reservoirs and sources of aster yellows phytoplasma for other leafhoppers.

Based on our findings, *M. quadrilineatus* had higher abundance in carrot fields compared to celery which could be due to differences in the management of the two crops. For example, celery is transplanted in the spring, while carrots are direct seeded, thus celery is available earlier in the season for *M. quadrilineatus* colonization than carrots. Carrots are grown in counties North of the celery producing area (Fig 3.1), accentuating the difference in developmental stages between the two crops. The management intensity of the two crops is also different with more frequent insecticide applications in celery compared to carrots (personal observation, Z. Szendrei). This is likely due to the direct damage to celery stems and leaves by annually occurring pests such as caterpillars and aphids and the relatively higher value of celery compared to carrots ($19.5 million and $14.5 million respectively in Michigan in 2018).[80]

Areas surrounding crop fields such as field edges can play an important role in the lifecycle of vectored pathogens not only for creating disease reservoirs but by managing vector populations using trap crops.[81] Difference in host plant preference could be used to attract *M. quadrilineatus* away from crops to trap crops planted in field edges. For trap crops to work effectively, we need to identify plants that are more attractive to *M. quadrilineatus* compared to the vegetable crops.[82] By planting trap crops we may be able to mostly contain the leafhoppers in the field edge, especially when crops are in their most susceptible developmental stages. It will also be important to screen for aster yellows resistant hosts, from which phytoplasma cannot be acquired and transmitted to other plants. Since other leafhopper species are likely not as important in aster yellows transmission, focusing on *M. quadrilineatus* behavioral management could potentially be an effective and sustainable strategy to reduce aster yellows' economic impact. In addition, farmers may implement other control measures, such as mowing weedy field margins, thus reducing potential alternative hosts for *M. quadrilineatus*. These management strategies can also be paired

with diagnostics-based support tools that inform growers about leafhopper infectivity.[83] By utilizing multiple methods for management, farmers will be able to better control aster yellows phytoplasma in a sustainable way.

5 Conclusions

Insect-vectored plant pathogens are challenging to manage with sustainable methods, especially when both the vector and pathogen have wide host ranges. Here, we made an important first step by confirming that *M. quadrilineatus* is an important vector of aster yellows and that *E. fabae* may potentially be another vector in celery and carrot agroecosystems. The next step will be to conduct transmission tests to determine if *E. fabae* can vector aster yellows, as they are often abundant in aster yellows susceptible crops, many of which also have *M. quadrilineatus*.[84,85] Both leafhoppers are found in the field and field edges of celery and carrot fields, and if *E. fabae* can vector aster yellows, limiting where the leafhoppers can acquire the pathogen by using disease resistant trap crops will minimize phytoplasma prevalence.

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CHAPTER 4: Conclusions and future directions

In this thesis, I have discussed potential strategies for controlling *M. quadrilineatus* and aster yellows phytoplasma through a pathogen-based decision support tool (Chapter 2). I have also confirmed that *M. quadrilineatus* is the main vector of aster yellows in these celery and carrot agroecosystems and identified other leafhopper species that may contribute to transmission (Chapter 3). Our knowledge of phytoplasma transmission has greatly increased in recent years,[1–3] but management strategies are lacking. The research presented in this thesis helps outline potential strategies for controlling *M. quadrilineatus* and helps to identify possible vectors of disease transmission in celery and carrot agroecosystems to aid in managing aster yellows phytoplasma.

In Chapter 2, my research focused on determining if the Vegetable Entomology Laboratory's decision support tool, designed to target aster yellows phytoplasma infected *M. quadrilineatus*, improved management of infected leafhoppers within celery and carrot farms. Decision support tools have become an important component of IPM (Integrated Pest Management) and in recent years, have become more common with the advancement of internet based programs and mobile devices[4–6] and identifying if these tools work as they are designed to is crucial to proper pest management. Besides verifying the efficacy of the support tool, I identified patterns in *M. quadrilineatus* abundance and infectivity during the growing season and found temporal trends between infectivity across carrot and celery systems. Additionally, a spatial component was analyzed to identify how far from the collection origin are infected leafhopper results useful.

The results from this chapter have useful implications for management of aster yellows in various cropping systems. We determine that our decision support tool worked to control infected

M. quadrilineatus populations in celery and carrot systems and similar tools could be applied to other aster yellows susceptible cropping systems as well as to other pathosystems where the pathogen is latent for several weeks before it is transmittable. We also determined that in celery systems, a high abundance of *M. quadrilineatus* proceeds an increase in infectivity by two weeks, demonstrating that a high abundance of leafhoppers does indicate infected leafhoppers are present in the crop. We also found that in our system infectivity in celery proceeds that in carrots, indicating that management in carrots should take place after the disease has been detected in celery, not before. Additionally, our spatial findings indicate that if an infected leafhopper is found at a celery farm, farmers should manage all of their fields, not just the field that the leafhopper was found at. This decreases the amount of sampling farmers need to do and will maintain the same level control. Both the support tool and these temporal and spatial findings provide Michigan farmers with additional guidelines for managing *M. quadrilineatus*.

In Chapter 3, my research focused on identifying vectors of aster yellows phytoplasma in the crop field and the field edges of celery and carrot agroecosystems. This research determined that although there are aster yellows vectors in the edges of both cropping systems, these vectors are likely not contributing to the spread of aster yellows within the crops. Instead, *M. quadrilineatus* is likely the primary vector and moves the pathogen to the edge, infecting the other leafhoppers. As molecular diagnostics have become less expensive over the past few decades, studies have focused on investigating new vectors to improve disease management and limit transmission. While these studies are becoming more common,[7–10] my research is novel as it takes into account both known pest species and non-pest species and compares their populations across two distinct susceptible cropping systems.

This study provided important foundational research in managing aster yellows in celery and carrots. I identified that other leafhopper species in these crops are likely not contributing to the spread of aster yellows as most non-*M. quadrilineatus* that had Ct-values ≤ 40 were in the edge while infected *M. quadrilineatus* were found within the fields, thus we need to focus management on *M. quadrilineatus.* This may be possible using trap crops[11] that *M. quadrilineatus* prefer to vegetable crops. Beyond this, the trap crops need to be resistant or immune to aster yellows to prevent creating a disease reservoir that may increase the likelihood of other leafhopper species becoming vectors. In addition, *E. fabe* was identified as a potential aster yellows vector. Because of its high abundance in the same susceptible crops as *M. quadrilineatus*. *Empoasca fabae* is likely acquiring the pathogen from reservoirs that *M. quadrilineatus* creates and so identifying if it can vector the disease through transmission tests is essential to determine if this pest also needs to be managed to control aster yellows outbreaks.

Overall, this thesis was the first to explore the use of a decision support tool to manage *M. quadrilineatus* that also controlled aster yellows phytoplasma. It was also the first to explore the leafhopper species diversity within Michigan celery and carrot agroecosystems to determine whether other vectors are present and contributing to the spread of aster yellows in these crops.

APPENDIX

Record of Deposition of Voucher Specimens

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2019-09

Author: Patrick T. Stillson

Title of thesis: Biology and management of leafhoppers and aster yellows phytoplasma in Michigan celery and carrot agroecosystems

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Table S4.1. Voucher specimens deposited at the Albert J. Cook Arthropod Research Collection (Michigan State University).

Family	Genus-Species	Life Stage	Quantity	Preservation
Cicadellidae	*Agallia quadripunctata*	Adult	1	Pinned
Cicadellidae	*Aphrodes bicinctus*	Adult	1	Pinned
Cicadellidae	*Aphrodes bicinctus*	Nymph	1	Ethanol
Cicadellidae	*Athysanus argentarius*	Adult	1	Pinned
Cicadellidae	*Balclutha* sp.	Adult	1	Pinned
Cicadellidae	*Colladonus clitellarius*	Adult	1	Pinned
Cicadellidae	*Commellus* sp.	Adult	1	Pinned
Cicadellidae	*Cuerna* sp.	Nymph	1	Ethanol
Cicadellidae	*Diplocolenus* subg. *Verdanus*	Adult	1	Pinned
Cicadellidae	*Doratura stylata*	Adult	1	Pinned
Cicadellidae	*Doratura stylata*	Nymph	1	Ethanol
Cicadellidae	*Draeculacephala* sp.	Adult	1	Pinned
Cicadellidae	*Draeculacephala* sp.	Nymph	1	Ethanol
Cicadellidae	*Elymana inornata*	Adult	1	Pinned
Cicadellidae	*Empoasca fabae*	Adult	1	Pinned
Cicadellidae	*Endria inimica*	Adult	1	Pinned
Cicadellidae	*Endria inimica*	Nymph	1	Ethanol
Cicadellidae	*Erythroneura* sp.	Adult	0	Fig S4.1
Cicadellidae	*Forcipata loca*	Adult	1	Pinned
Cicadellidae	*Graphocephala* sp.	Adult	1	Pinned
Cicadellidae	*Idiocerus raphus*	Adult	1	Pinned
Cicadellidae	*Jikradia olitoria*	Adult	1	Pinned

Table S4.1. (cont'd)

Family	Genus-Species	Life Stage	Quantity	Preservation
Cicadellidae	*Latalus* sp.	Adult	1	Pinned
Cicadellidae	*Macrosteles quadrilineatus*	Adult	20	Pinned
Cicadellidae	*Neokolla hieroglyphica*	Nymph	1	Ethanol
Cicadellidae	*Norvellina* sp.	Adult	1	Pinned
Cicadellidae	*Paraphlepsius* sp.	Adult	1	Pinned
Cicadellidae	*Paraphlepsius* sp.	Nymph	1	Ethanol
Cicadellidae	*Psammotettix lividellus*	Adult	1	Pinned
Cicadellidae	*Scaphytopius* sp.	Adult	1	Pinned
Cicadellidae	Morphotype 1	Nymph	1	Ethanol
Cicadellidae	Morphotype 2	Nymph	1	Ethanol
Cicadellidae	Morphotype 3	Nymph	1	Ethanol
Cicadellidae	Morphotype 4	Nymph	1	Ethanol
Cicadellidae	Morphotype 5	Adult	1	Pinned
Cicadellidae	Morphotype 6	Adult	1	Pinned
Cicadellidae	Morphotype 7	Adult	1	Pinned
Cicadellidae	Morphotype 8	Adult	1	Pinned
Cicadellidae	Morphotype 9	Adult	1	Pinned
Cicadellidae	Morphotype 10	Adult	1	Pinned
Cicadellidae	Morphotype 11	Adult	1	Pinned
Cicadellidae	Morphotype 12	Adult	1	Pinned
Cicadellidae	Morphotype 13	Adult	1	Pinned
Cicadellidae	Morphotype 14	Adult	1	Pinned
Cicadellidae	Morphotype 15	Adult	0	Fig S4.2
Cicadellidae	Morphotype 16	Adult	0	Fig S4.3

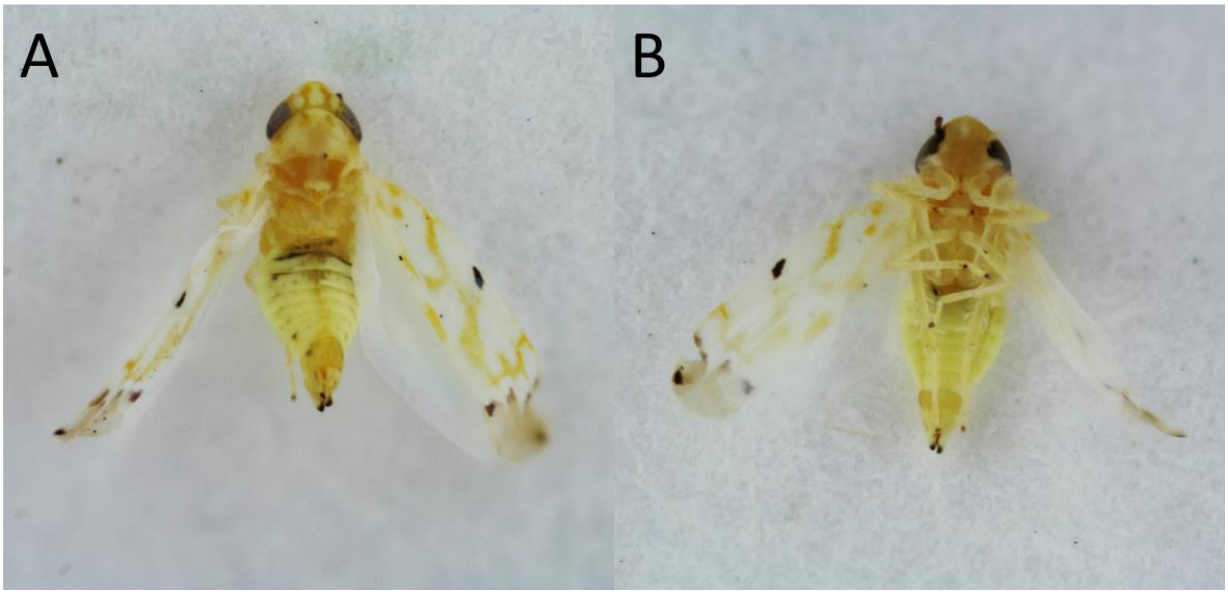

Figure S4.1. *Erythroneura* sp. voucher specimen. Dorsal (A) and ventral (B) view.

Figure S4.2. Morphotype 15 voucher specimen. Dorsal view.

Figure S4.3. Morphotype 16 voucher specimen. Dorsal (A) and lateral (B) view.

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