Research Article

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A novel plant pathogen management tool for vector management

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Abstract

BACKGROUND: Decision support systems often focus on insect control due to direct damage. However, when insects vector plant pathogens, these decision support systems must be tailored to disease management. However, a decision system that links diagnosticians to vector management is lacking and complicated by patterns of insect abundance over space and time. Here, we describe an approach that integrated monitoring of an insect pest (aster leafhopper; *Macrosteles quadrilineatus*, Forbes) that vectors aster yellows phytoplasma (*Candidatus* Phytoplasma spp.), with rapid disease diagnostics and web-based text messaging in two crops, carrots and celery.

RESULTS: From 2014–2019, a total of 8,343 aster leafhoppers were collected, 99 of these were infected with phytoplasma. Text messaging reduced the number of infected leafhoppers. When we compared infected leafhopper density across crops, their temporal patterns were most similar at a 2-week delay. Comparisons within crop indicated that in celery uninfected and infected leafhopper density was most similar at a 2-week delay, but there was no similar pattern in carrots. Leafhopper density and infectivity were not similar beyond individual farms.

CONCLUSION: Our results suggest that farmers should account for these temporal and spatial patterns when managing leafhoppers infected with aster yellows phytoplasma to improve pest management. By combining extensive monitoring, with rapid disease diagnostics, and text messaging, we demonstrate the value of our decision support tool.

Supporting information may be found in the online version of this article.

Keywords: aster leafhopper; aster yellows phytoplasma; decision support systems; insect vectors; disease 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, which perform direct damage to plants 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 decision 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. 1).

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.[14–16] 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, creating a mixture of infected and uninfected insects due to 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*

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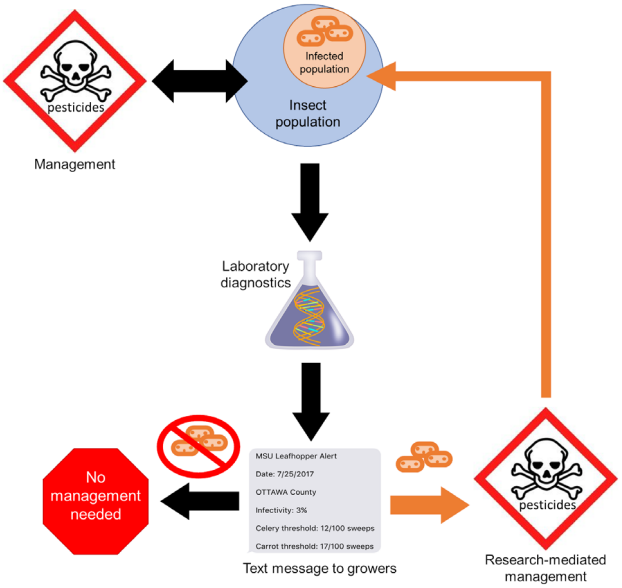

Figure 1 Vectored disease management. Aster yellows phytoplasma is transmitted by aster leafhoppers to carrots and celery. The economic damage is caused by the phytoplasma; therefore, it is important to assess the proportion of the leafhopper population which is infected with the disease. Leafhopper 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* 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.

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 change farmer management from 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 in order to understand the relationship between infected and uninfected vector populations, we examined temporal and spatial patterns. Our results 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 2–3 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 8 weeks before transplanting into fields, and farmers continue to transplant weekly for approximately 2 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 consistent 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 synthetic 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; Tables S1 and S2).

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 fields 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 S1 – S2). 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

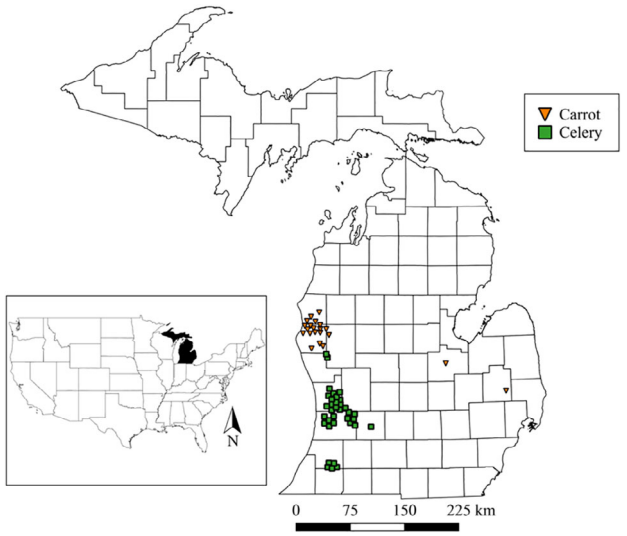

Figure 2 Collection locations. Map of Michigan, USA, symbols indicate the locations of commercial carrot and celery fields where aster leafhoppers were collected from 2014–2019. Leafhoppers were collected using sweep nets and were transported to the laboratory to determine aster yellows infectivity.

leafhoppers and all other leafhoppers. Leafhoppers not identified as aster leafhoppers were excluded from subsequent analyses.

2.3 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^{-1} (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 one to two leafhoppers and 200 µL for samples with three leafhoppers). Varying elution buffer volumes were used to standardize the DNA concentration across samples. Final DNA concentrations ranged from 0.50–350 ng μL^{-1}. 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]

2.4 Disseminating information

2.4.1 *Infectivity threshold calculations*

Action thresholds 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) and (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) and (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.

2.4.2 *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. 1).

2.5 Statistical analysis

2.5.1 *Text messaging and infectivity*

To identify if 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.

2.5.2 *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 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 S3 – S4) (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 S3). 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 were 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) and (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) and (6). Cross correlation values found at each lag (four 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 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 2 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 examined if populations of uninfected leafhoppers were most similar to the infected population when delayed for up to 2 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) and (6). The value for 'n' varied by cropping system with 15 and 13 sampling weeks for celery and carrot, respectively, (Tables S4).

2.5.3 *Spatial variation in leafhopper populations*

To identify if 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 seven 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 fields within a farm, and these coordinates were placed within one distance band (Fig. S1). 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 8343 aster leafhoppers were collected, and 99 infected leafhoppers were detected (Tables S3 – S4). In carrot and celery, there were 1870 and 6473 leafhoppers and 39 (2.09%) and 60 (0.93%) infected individuals, respectively, which was similar to other studies that detected 0.09% to 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 ($\chi^2 = 6.63$, df = 2, *P*-value = 0.06; Fig. 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 ($\chi^2 = 6.63$, 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 ($\chi^2 = 6.63$, df = 2, *P*-value = 0.02).

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 ($\chi^2 = 5.75$, df = 1, *P*-value = 0.02; Fig. 4(a)). However, no difference was found between the mean abundance of infected leafhoppers when comparisons were made between the two crops ($\chi^2 = 0.26$, df = 2, *P*-value = 0.61; Fig. 4(b)).

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. S2A, B). However, the population of infected leafhoppers in carrot was lagging by 2 weeks compared to the population in celery (r = 0.79, *P*-value = 0.004; Fig. 5(a)) 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. 5(b)).

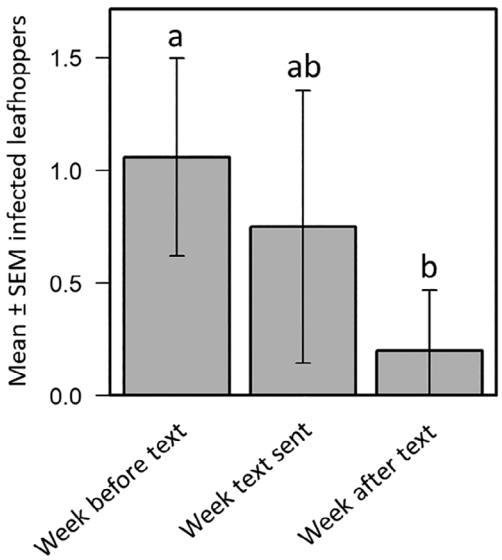

Figure 3 Infectivity after text messages. Abundance (mean ± SEM) of aster yellows phytoplasma infected leafhoppers during the 2016–2019 growing seasons by the number of weeks since farmers received a text message indicating leafhopper infectivity. Text messages were sent to inform stakeholders of the 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.

3.4 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. 6 (a)). Patterns of infected leafhopper abundance across weeks 22–34 were similar to the pattern of uninfected leafhoppers across weeks 20–32 (Fig. 6(b)), indicating that the population pattern of infected individuals was similar to that of uninfected individuals but at a 2-week delay. No temporal relationship was found between the density of uninfected and infected leafhoppers in carrot (Fig. S3A, B).

3.4.1 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 ≤0.001). Celery fields >2.5 km apart, however, did not correlate strongly in leafhopper density or infectivity (Fig. 7(a), S1A). When evaluated, carrot fields within 6 km were 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. 7(b), S1B). No correlation was observed in the infected leafhopper population between carrot fields at any distance (Fig. 7(b), S1B).

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. 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 2 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 takes place in a short period of time (*e.g.* a single

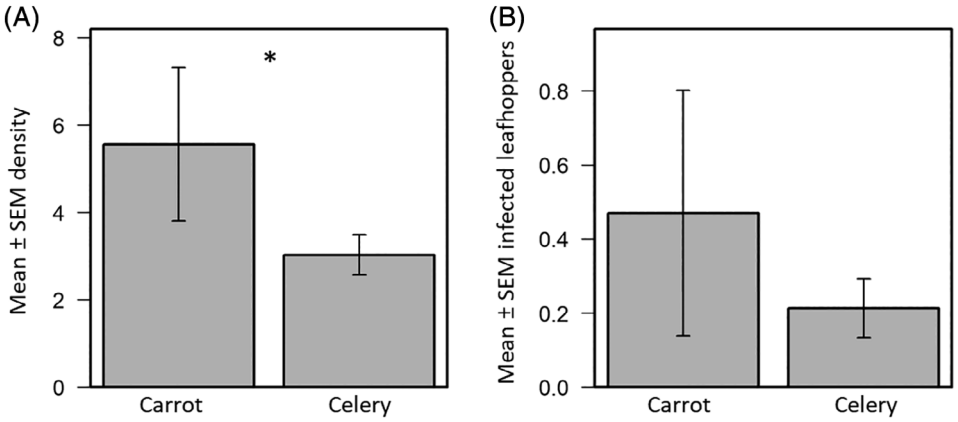

Figure 4 Abundance and infectivity between crops. (A) Mean ± SEM aster leafhopper density (leafhoppers per 100 sweeps) and (B) mean ± SEM abundance of aster leafhoppers infected with aster yellows phytoplasma in carrots and celery. Leafhoppers were collected with sweep nets from commercial celery and 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.

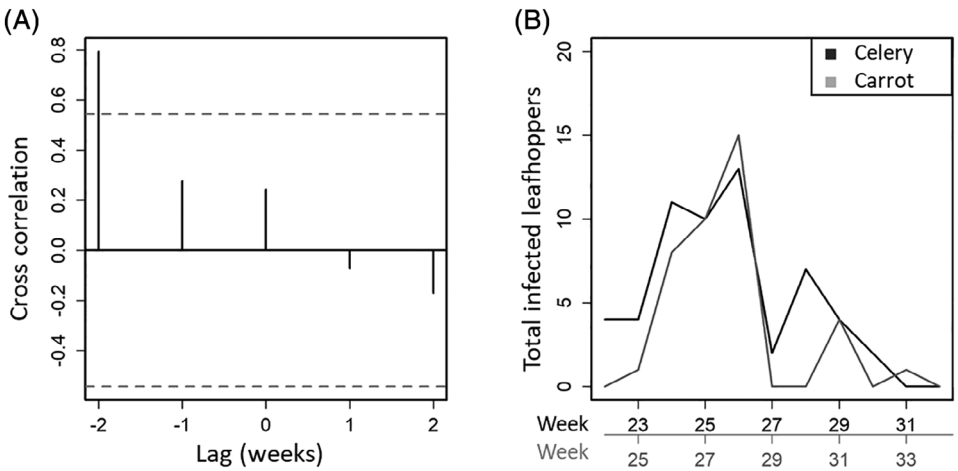

Figure 5 Temporal relationship between aster leafhopper populations in celery and carrot. Cross correlation analysis of the abundance of aster yellows phytoplasma infected aster leafhoppers in celery and carrot fields in Michigan from 2014 to 2019. Dotted lines indicate a 95% confidence interval and 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 2 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 2 weeks to illustrate the 2-week lag that was identified in the cross correlation.

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 (1.6–38.6 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.

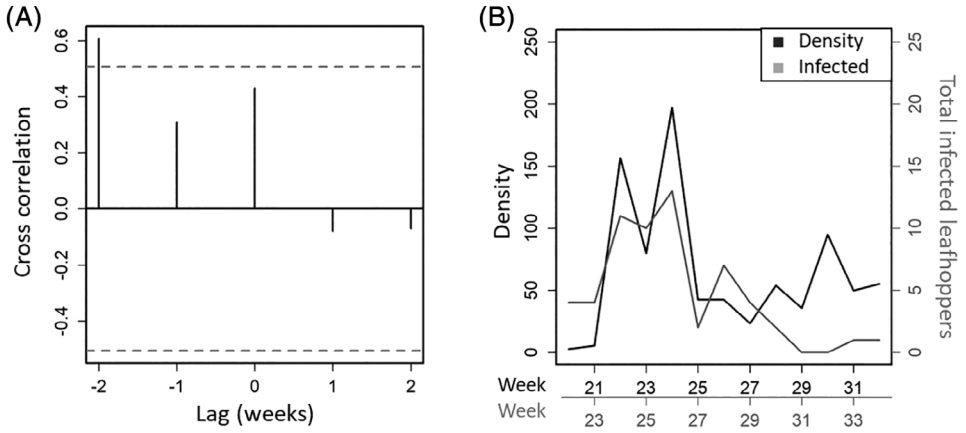

Figure 6 Temporal relationship between aster leafhopper density and infectivity. Cross correlation analysis of the density of aster leafhoppers (leafhoppers per 100 sweeps) and the abundance of aster yellows phytoplasma infected aster leafhoppers in celery fields in Michigan from 2014 to 2019. Dotted 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 2 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 2 weeks to illustrate the two-week lag that was identified in the cross-correlation.

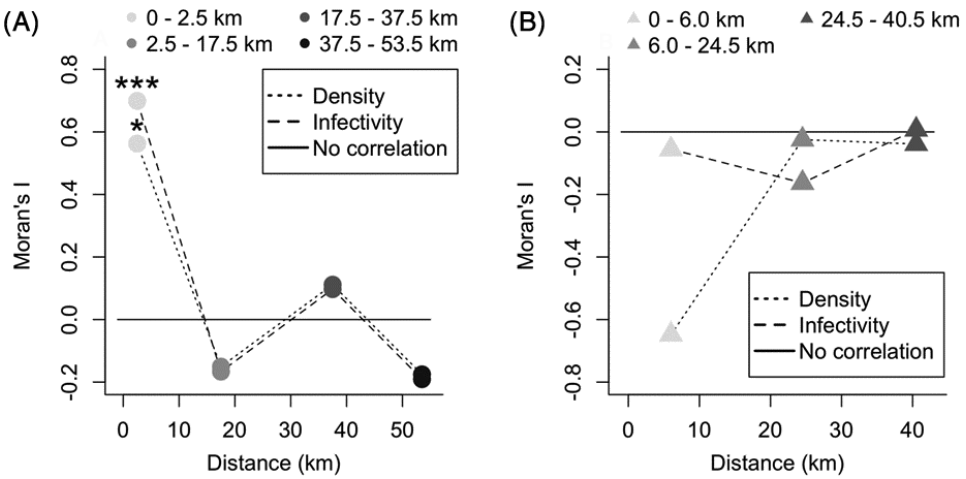

Figure 7 Spatial variation in leafhopper populations. Correlation of leafhopper density and infectivity between sampling sites as a function of distance bands split by (A) celery and (B) carrot. (A) Celery sampling sites within 2.5 km were similar in leafhopper density and infectivity. However, no correlation was found between sites beyond 2.5 km apart for either density or infectivity. (B) Carrot sites within 6 km were 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.

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, 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]

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AUTHOR CONTRIBUTIONS

PTS and ZS collected samples and assisted in processing. PTS, EHB, and JGI analyzed and interpreted the data. PTS ZS, EHB wrote the manuscript. All authors contributed to editing and reviewing the manuscripts and gave final approval for publication. The authors have declared that no competing interests exist.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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