Factors Influencing Aster Leafhopper (Hemiptera: Cicadellidae) Abundance and Aster Yellows Phytoplasma Infectivity in Wisconsin Carrot Fields

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ABSTRACT In Wisconsin, vegetable crops are threatened annually by infection of the aster yellows phytoplasma (AYp), the causal agent of aster yellows (AY) disease, vectored by the aster leafhopper, *Macrosteles quadrilineatus* Forbes. Aster leafhopper abundance and infectivity are influenced by processes operating across different temporal and spatial scales. We applied a multilevel modeling approach to partition variance in multifield, multiyear, pest scouting data sets containing temporal and spatial covariates associated with aster leafhopper abundance and infectivity. Our intent was to evaluate the relative importance of temporal and spatial covariates to infer the relevant scale at which ecological processes are driving AY epidemics and identify periods of elevated risk for AYp spread. The relative amount of aster leafhopper variability among and within years (39%) exceeded estimates of variation among farm locations and fields (7%). Similarly, time covariates explained the largest amount of variation of aster leafhopper infectivity (50%). Leafhopper abundance has been decreasing since 2001 and reached its minimum in 2010. The average seasonal pattern indicated that periods of above average abundance occurred between 11 June and 1 August. Annual infectivity appears to oscillate around an average value of 2% and seasonal periods of above average infectivity occur between 19 May and 15 July. The coincidence of the expected periods of high leafhopper abundance and infectivity increases our knowledge of when the insect moves into susceptible crop fields and when it spreads the pathogen to susceptible crops, representing a seasonal interval during which management of the insect can be focused.

KEY WORDS *Macrosteles quadrilineatus*, *aster* yellows phytoplasma, aster yellows, insect migration, variance component analysis

Aster yellows (AY) is a widespread disease of plants caused by the aster yellows phytoplasma (AYp), a small, wall-less prokaryotic organism that is currently placed in the provisional genus '*Candidatus* Phytoplasma' (Lee et al. 2000, IRPCM Phytoplasma/Spiroplasma working team – Phytoplasma taxonomy group 2004). The AYp has an extensive and diverse host range infecting over 350 plant species including many common vegetable, ornamental, and agronomically important field crops, and several noncrop plant species (Kunkel 1926, Chiykowski 1965, Chiykowski and Chapman 1965, Chiykowski 1967, Westdal and Richardson 1969, Peterson 1973, Lee et al. 1998, Lee et al. 2000, Lee et al. 2003, Hollingsworth et al. 2008). Plant-to-plant spread of AYp in the field generally occurs as a result of transmission by more than 24 leafhopper species (Mahr 1989, Christensen et al. 2005). However, the aster leafhopper, *Macrosteles quadrilineatus* Forbes, is considered to be the most important vector of the AYp because of its prevalence in susceptible, midwestern crops (Drake and Chapman 1965, Hoy et al. 1992).

The AYp is persistently transmitted by the aster leafhopper and both nymphs and adults can acquire the pathogen. Once infected, an individual aster leafhopper can remain infective for the remainder of its life. The aster leafhopper is a polyphagous insect species that uses over 300 different plant species for food, oviposition, and shelter (Wallis 1962, Peterson 1973), many of which are susceptible to AYp infection. Aster leafhopper host plant species can be classified into two primary groups based on utilization patterns to include: 1) feeding hosts, or 2) feeding and reproductive hosts. Other factors such as plant community composition (Lee and Robinson 1958, Wallis 1962, Schultz 1979), plant physiological state (Peterson 1973), season and geographic location (Lee and Robinson 1958, Wallis 1962, Peterson 1973) can also affect host preferences of aster leafhopper in the field. In Wisconsin, cultivated grains are hosts for overwintering eggs and

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also serve as early season feeding and reproductive hosts for the aster leafhopper (Drake and Chapman 1965). In addition to grain crops, the aster leafhopper feeds upon and is moderately abundant in mixed broadleaf weeds and grasses that border crop fields (Schultz 1979).

Each spring, the aster leafhopper migrates from the Gulf Coast states to the upper midwest (Chiykowski and Chapman 1965). The aster leafhopper generally migrates in a south to north direction but, in flight, leafhopper movement is greatly influenced by synoptic weather systems, making it difficult to predict when and where the aster leafhopper will arrive. The migratory behavior together with the mode of pathogen transmission by the aster leafhopper enables the insect to acquire and transmit the pathogen over great distances. Large numbers of migrating aster leafhoppers have been reported to influence the potential for AY epidemics in vegetable crops grown in Wisconsin and in other midwestern states (Chiykowski 1965, Chiykowski and Chapman 1965, Drake and Chapman 1965, Chapman 1973, Hoy et al. 1992). The severity of AY outbreaks is thought to be directly related to the infectivity and the abundance of aster leafhoppers immigrating into a susceptible crop (Chapman 1971).

In Wisconsin, AY management has focused primarily on controlling the insect vector, the aster leafhopper. The aster yellows index (AYI), was developed as a risk assessment tool to enumerate the maximum allowable numbers of infectious leafhoppers and define periods in the growing season when protection of a susceptible crop was most needed (Chapman 1971, 1973). Simply, the AYI metric is the product of aster leafhopper (relative) abundance and infectivity. Insecticide sprays are then recommended if the AYI exceeds an allowable threshold that is based on the relative susceptibility of the crop to infection by AYp. Originally, the AYI was calculated using an infectivity estimate determined from a series of early season (migratory) leafhopper collections and bioassays on susceptible Chinese aster, *Callistephus chinensis* (L.) Nees. This infectivity estimate was used for the entire growing season, whereas aster leafhopper abundance was determined weekly, or more frequently, for each field throughout the summer (Mahr et al. 1993).

Following observations that aster leafhopper abundance and infectivity in and around carrot (*Daucus carota* L.) fields was dynamic in time and space (Mahr et al. 1993), efforts were made to estimate infectivity for each field throughout the summer to obtain a more site and time-specific AYI. In many pathogen–disease systems, including the aster yellows patho-system in Wisconsin, contemporary tools for pathogen detection (i.e., nucleic acid based detection methods) have been adopted to estimate the infection frequencies (Bloomquist and Kirkpatrick 2002, Munyaneza et al. 2010). However, even with the availability of contemporary tools, significant annual and site-specific variation of pathogen detection in the insect vector frequently occurs. In most cases, the relationship between pathogen presence in the vector and the vector's ability to successfully transmit the pathogen is not known. In turn, many producers avoid risk of pathogen spread by using inexpensive, prophylactic applications of pyrethroid insecticides, a management practice that circumvents the utility of the AYI. An improved understanding the factors that influence variation in aster leafhopper abundance and infectivity will further improve aster yellows management.

Environmental processes that drive plant disease epidemics occur at multiple temporal and spatial scales. For example, large (temporal and spatial) scale climate patterns may influence the risk for fusarium head blight (FHB) development in the midwestern United States (Kriss et al. 2012). However, smaller scale weather fluctuations such as a short-term dry period around wheat at anthesis can counteract the overall impact of a generally wet year by reducing the number of primary fungal infections leading to reduced FHB severity (De Wolf et al. 2003, Kriss et al. 2012). Investigating the patterns of aster leafhopper abundance or infectivity variation across different temporal and spatial scales will provide insight into the processes that drive the variation in annual AY epidemics. For example, if aster leafhopper migration was important for producing variation in the aster leafhopper infectivity, then it might be expected that interannual variation of aster leafhopper infectivity would be high relative to intra-annual variation. In addition, an improved understanding of variation across different temporal and spatial scales can also inform future sampling strategies (Wheatley and Johnson 2009). For instance, if interannual variation of aster leafhopper infectivity was comparatively large relative to intra-annual variation, then repeated sampling within a season would explain very little about aster leafhopper infectivity. Unfortunately, experiments that manipulate environmental processes across multiple spatial and temporal scales (simultaneously) are difficult to perform. Yet, compiled data from observational studies that include spatial and temporal information at multiple scales offer an opportunity to obtain information about the scale at which ecological factors, contributing to abundance and infectivity variation, occur (Magnuson 1990, Sagarin and Pauchard 2010).

Here we present an approach for parsing sources of variation in Poisson-distributed count data, similar to the method described by Duffy et al. (2010) that examined binomial data. Specifically, we applied this approach to analyze a multiyear, multilocation data set of aster leafhopper abundance (2001–2011) obtained from pest scouting records in Wisconsin carrot fields. We also used a similar approach to examine sources of variation associated with aster leafhopper infectivity (1994–2008) collected from similar fields, locations, and years. For each data set, we quantified the variance components associated with annual, seasonal, and geographic variability. The primary goal of this study was to identify the scale of the processes that drive AY epidemics and identify periods of time in the growing season when crop protection is most needed. Our specific objectives in this study were to 1) evaluate the relative importance of time (i.e., year and

calendar date) and space (i.e., farm and field) in explaining the variability observed in aster leafhopper abundance and AYp-infectivity; and 2) identify periods of time in the growing season where aster leafhopper abundance and infectivity was above and below average, corresponding to periods of elevated or low risk, respectively.

Materials and Methods

Aster Leafhopper Abundance. Field sampling was conducted using sweep nets in commercial carrot fields to monitor the relative abundance of aster leafhopper in specific areas of Wisconsin from 2001 through 2011. In total, 237 fields were sampled over the 11-yr span of this survey resulting in an average of 31 fields per year with multiple fields resampled in successive years because of crop rotation practices. The fields were clustered geographically into six distinct growing regions in the Central Plain, the Western Upland, and Eastern Ridge ecoregions of Wisconsin. The approximate distance among fields ranged from 0.1 to 15 km within a farm and 15–200 km among farms. In Wisconsin, carrots are direct seeded in mid-April through early June and a cover crop (e.g., oats, wheat, or rye) is concomitantly established to prevent wind damage to the developing carrot crop during seedling emergence. Carrot seedlings typically emerge in late-May and early June and the crop is usually harvested from late August through mid-November, depending on the growing season.

In all years, aster leafhopper monitoring began before carrot emergence, usually in mid-May, and terminated 1 to 3 wk before carrot harvest, and no later than 20 September for all field sites in all years. Early sample dates, those before 25 May, occurred primarily in rye, wheat, or oat because the carrot crop does not typically emerge until after that date. At each location, the relative abundance of leafhopper adults associated with the carrot canopy was determined by standard sweep net sampling along two to 18 transects extending into the carrot crop toward the middle of the field. Twenty-five to 100 pendulum sweeps per transect were conducted using a standard sweep net (38 cm diameter) and all aster leafhopper stadia were counted. Counts were enumerated as adult aster leafhoppers per 25 sweeps. Decimals, occurring when >25 sweeps were conducted, were rounded to the nearest integer. Fields were sampled weekly unless weather or grower management did not allow for sampling.

Aster Leafhopper Collections and AYp Infectivity. Aster leafhopper infectivity was monitored using a transmission bioassay and, for the commercial carrot production area of Wisconsin, records of infectivity were available from 1994 through 2008. In total, infectivity was estimated from among 378 aster leafhopper populations; ≈25 populations of which were from multiple geographic locations and several dates throughout each growing season.

When possible, >200 leafhoppers were collected in sweep nets and placed onto oat seedlings for transport back to the laboratory for transmission bioassays. Typically, 204 leafhoppers were placed in pairs onto 102 Chinese aster (*Callistephus chinensis*) plants and insects were allowed to feed for a 48-h inoculation access period (IAP). Disease symptoms were assessed after a 2-wk incubation period and percent infectivity was calculated as:

$$\text{infectivity} = \text{number of diseased plants/total number of leafhoppers}$$

The total number of leafhoppers was used as the denominator because infectivity levels are often low and a diseased plant was more likely because of a single infective leafhopper rather than the presence of two infective leafhoppers on the same plant.

Statistical Analysis. *Factors Contributing to Variation of Aster Leafhopper Abundance.* A generalized linear mixed modeling (GLMM) approach based on Poisson regression (log-link) with random intercepts was used to examine the relative importance of year, week, farm, and field on the abundance of the aster leafhopper (Pinheiro and Bates 2000, Madden et al. 2002, Nita et al. 2008, Bolker et al. 2009). The multilevel model (Gelman and Hill 2007) had the following form:

$$Y_{i(abcd)} \approx \text{Poisson}(\mu_{i[abcd]}) \quad \text{(model 1)}$$

$$g(\mu_{i(abcd)}) = \log_e(\mu_{i(abcd)}) = X\beta + \log(effort) + \varepsilon_a + \varepsilon_b + \varepsilon_c + \varepsilon_d$$

$$\varepsilon_a \approx N(0, \sigma^2_a)$$

$$\varepsilon_b \approx N(0, \sigma^2_b)$$

$$\varepsilon_c \approx N(0, \sigma^2_c)$$

$$\varepsilon_d \approx N(0, \sigma^2_d),$$

where $Y_{i(abcd)}$ was the total aster leafhopper count for a field and the total aster leafhopper count for a field was offset by the number of transects walked (or sampling effort) in each field. The regression coefficient for the offset term $\log(effort)$, by definition, was constrained to one. The fixed effects term, β, represented the model intercept and was interpreted as the statewide seasonal average aster leafhopper abundance in carrot fields. ε_a, ε_b, ε_c, and ε_d were the random effects (or intercepts) for year, day, farm, and field, respectively. They represented the variance components associated with the temporal and spatial "blocks" of this model. The variance components of aster leafhopper abundance were quantified on the aster leafhopper count given by $g(\mu_{i[abcd]})$ and variance components were assessed in terms of variances (or standard deviations) on the latent, or $\log_e$ scale of the model.

Mixed-effects models, in general, are used because they associate random effects to observations sharing the same level of a classification factor. Thus, mixed-effects models are useful because they can accurately represent the covariance structure that exists among samples when repeated measurements are taken at the same location or time (Pinheiro and Bates 2000); essentially we are assuming all observations from a given source (or subject) are correlated. Often, when

research emphasis is placed on estimating fixed regression coefficients, random effects are included in a model to account for the covariance among sample groupings before estimating the regression coefficients. However, in our case, the variance–covariance structure itself was of interest and all factors in our analyses were considered random because the primary goal was to examine the nature of different spatial and temporal levels from which the data are presumed to have come (Pinheiro and Bates 2000, Baayen et al. 2008, Nita et al. 2008).

Model 1 represents the case in which the variance can be divided into separate components for year (ε_a), week (ε_b), farm (ε_c), and field within farm (ε_c), and where the magnitude of the variance components (i.e., σ^2_a, σ^2_b, σ^2_c, or σ^2_d) could be interpreted as a measure of the relative importance of the different spatial and temporal factors associated with aster leafhopper count. There are many ways that a GLMM can be defined to examine the interactions among different combinations of covariate groupings and to quantify their associated variances (Duffy et al. 2010). In our case we were not interested in the contributions of specific years as much as we were interested in the variation because of year. In addition, we were more interested in the day-to-day variability of aster leafhopper abundance than the variability of aster leafhopper abundance on a specific day. The flexibility of the GLMM allowed us to specify random variables for year (ε_a) and day (ε_b) and model that variability as $\varepsilon_a + \varepsilon_b$ (i.e., $\sigma^2_a + \sigma^2_b$). We could also examine the variability of a specific day by including a day–year interaction term, ε_{ab} (or σ^2_{ab}), in the model. Thus, a $\sigma^2_{ab} > 0$ implies that annual aster leafhopper abundance varies interactively with calendar day and the variability among day–year pairs would be more adequately modeled as $\varepsilon_a + \varepsilon_b + \varepsilon_{ab}$ (i.e., $\sigma^2_a + \sigma^2_b + \sigma^2_{ab}$). Our biological interpretation of this approach is analogous to that of regression analyses where both intercept and slope are allowed to vary among treatments. Estimates of the variability of each grouping (i.e., year, day, and year–day) are obtained and the relative amount of variability described by each level of grouping is reflective of the importance of each factor. Important terms are those that describe larger amounts of variability. Applied specifically to insect abundance data, ε_a is an estimate of the annual variation of aster leafhopper abundance, ε_b is an estimate of variation in seasonal aster leafhopper abundance (or phenology), and ε_{ab} is an estimate of how aster leafhopper phenology varies interactively among years. Thus, the inclusion of different "interaction terms" as random effects in the GLMM leads to numerous ways to partition the variances associated with aster leafhopper count, providing insight about the underlying biology and spatial or temporal scales at which processes important for aster leafhopper population dynamics are occurring (Duffy et al. 2010).

In our data set, the temporal and spatial grouping of covariates occurs at different scales. For example, year and day represent a different spatial grain size. Studying the patterns of aster leafhopper abundance variation at different temporal scales can provide insight about the scale of the underlying ecological processes driving aster leafhopper prevalence (Levin 1992, Wheatley and Johnson 2009). Large variation of aster leafhopper abundance among years, relative to other sources, might suggest that aster leafhopper numbers are influenced by climatic or biological factors (i.e., El Niño and La Niña cycles, winter mortality, or early generation survivorship at southerly latitudes). In contrast, large variation within years might be better explained by processes such as differences in grower management or synoptic weather events. Similarly, insights can be gained by examining variation occurring among and within geographic location. For example, large variation of aster leafhopper abundance among geographic locations might imply that the local habitat (i.e., noncrop reproductive host plants) surrounding crop a field is important. Alternatively, small variations in aster leafhopper abundance among geographic locations might suggest larger scale processes, occurring across all locations, drive insect abundance (i.e., mean annual temperatures).

In general, a full model that included all the random effects of interest was constructed and Akaike information criterion (AIC) and likelihood ratio tests (LRT) were used to evaluate if the inclusion of random effects parameters were justified in the model. Parameter estimates for a selected submodel are reported in the text (Table A1 contains parameter estimates for the full model and various submodels). All models were fit using the *glmer* (lme4: version 0.999375–39; Bates et al. 2011) function in the lme4 package of R (lme4: version 0.999375–39; Bates et al. 2011, R version 2.15.0; R Development Core Team 2012), which allows for the analysis of crossed classified data as crossed random effects (Pinhero and Bates 2000, Baayen et al. 2008).

Factors Contributing to Variation of Aster Leafhopper Infectivity. A linear mixed modeling (LMM) approach, similar to the GLMM approach previously used for aster leafhopper abundance, was used to examine the relative importance of year, farm and calendar day, on aster leafhopper infectivity. In this data set, calendar day, corresponded to weekly estimates of leafhopper infectivity and sample dates were represented as Julian date at the mid-point of the sample week. Again, the multilevel model representing a simple case for describing infectivity had the form:

$$Y_{i(abc)} = \mu + \varepsilon_a + \varepsilon_b + \varepsilon_c + \varepsilon_{i(resid)} \quad \text{(model 2)}$$

$$\varepsilon_a \approx N(0, \sigma^2_a)$$

$$\varepsilon_b \approx N(0, \sigma^2_b)$$

$$\varepsilon_c \approx N(0, \sigma^2_c)$$

$$\varepsilon_{i(resid)} \approx N(0, \sigma^2_r),$$

where $Y_{i(abc)}$ was the estimated aster leafhopper infectivity – the square root-transformed proportion of leafhoppers able to transmit the AYp. As described above, this LMM was also extended to examine the interactions among the random effects terms, partition-

ing the variance of aster leafhopper infectivity among known spatial and temporal "blocks," providing insight about the scales at which processes important for influencing variation of aster leafhopper infectivity are operating. Models again were fit using the *lmer* function (lme4: version 0.999375–39; Bates et al. 2011) and AIC and LRT were used to evaluate if the inclusion of random effects parameters were justified in the model (See Table A2 for parameter estimate of the full model).

Model Diagnostics. The variance assumptions of regression analysis are often made for statistical purposes. For example, if there is not constant variance, standard errors may be biased leading to unreliable statistical tests. Trends occurring in the model residuals would violate the assumption of independent response variables and often are a result of erroneous model structure. However, identifying trends in the residuals may reveal useful biological patterns and may imply the pattern of a trend which can be directly fit in subsequent modeling efforts. The results we present here are to emphasize the utility of visually examining the possible patterns of variation in data (i.e., residuals). A more direct modeling of these data trends was subsequently performed (Frost et al. 2012), but was not the focus of this paper.

A series of residual plots were used to assess the assumptions of the random effects model and determine if the errors in the model predictions behave in the same way within each level of grouping in the data. For mixed effects models, there are several different types of residuals that can be obtained because of the different group levels of the model and each type of residual is useful for evaluating model assumptions (Pinheiro and Bates 2000, Nobre and Singer 2007). Here, we focus on plots of the conditional modes of the random effects versus temporal (group) indices because we were interested in the temporal aspects relating to pathogen transmission. Thus, we present plots examining the conditional modes of the random effects for the population expected values for year, ordered by year, and calendar day, ordered by day to demonstrate the temporal trends of insect abundance and infectivity data, among and within year. Conditional modes of the random effects for the various levels of the models were extracted using the *ranef* function and plotted using the *qplot* function of the *ggplot2* package (Wickham 2009). Trend lines were generated using a generalized additive model.

Correlations Among Years, Farms, and Fields. The inclusion of random effects into a regression model has an effect on the structure of the model's variance-covariance matrix (Zuur et al. 2009). If the mixed effects regression models are specified appropriately, the GLMM and LMM framework can be used to examine these induced correlations among farms within year or year-week groups (Zuur et al. 2009; See example *Appendix* B). For example, a GLMM used to examine correlations among farms within years can be formulated as:

$$\mu_{p(y)} = \exp(\beta_1 x_1 + \beta_2 x2 + \beta_3 x_3 + \varepsilon_{p[y]})$$

$$\varepsilon_{p(y)} \approx N(0, \Sigma_p)$$

Here β_1 through β_3 are fixed effects for the mean leafhopper abundance of farm 1 through farm 3 and the design matrix uses dummy variables (i.e., 0 or 1) to represent the farm category. The random variable, $\varepsilon_{p(y)}$, is independent, normally distributed, and its (symmetric) covariance matrix is:

$$\Sigma_p = \begin{matrix} \sigma_1^2 & \rho_1\sigma_1\sigma_2 & \rho_2\sigma_1\sigma_3 \\ \rho_1\sigma_1\sigma_2 & \sigma_2^2 & \rho_3\sigma_2\sigma_3 \\ \rho_2\sigma_1\sigma_3 & \rho_3\sigma_2\sigma_3 & \sigma_3^2 \end{matrix}$$

which accounts for correlations (i.e., $\rho_1 - \rho_3$) of farms within each year. In a similar way, the GLMM can be formulated to examine correlations of farms within years, weeks or year–week combinations. In turn, these correlations allow us to examine if similarities exist among locations that may be important for insect abundance. As in Duffy et al. (2010), we assessed the distribution of the ρ estimate using parametric bootstrapping (100 bootstrap estimates). However, we did not try to imply the significance of the correlation based on bootstrapping because ρ was explicitly defined by fitting data to the GLMM (or LMM). The correlations obtained from the variance-covariance matrices were used to calculate (Euclidean) distance matrices. The *hclust* function was used to conduct hierarchical clustering of the distance matrices and produce dendrograms for visualization of correlative relationships among variables.

Outside of the GLMM context, the correlation of aster leafhopper abundance among field combinations within a farm was also examined for all farms in all years. For example, we were interested to know if aster leafhopper counts from two fields, on the same farm and sampled at the same time, would be similar. Because of the large number of correlation coefficients produced in this analysis, we chose to graphically visualize the distributions of the coefficients using density plots. The density plots were produced using the *stat density* function in the *ggplot2* package (Wickham 2009), which uses kernel density estimation and densities were scaled to one within each farm. After visual examination, the *lm* function was used to conduct an analysis of variance (ANOVA) to examine the effect of farm and year on within farm correlations among fields. The marginal sum of squares was used to evaluate the importance of factors in the ANOVA model.

Results

Quantitative Variability in Aster Leafhopper Abundance. Aster leafhopper count data for an individual field on a sample date was variable, ranging from zero to 60 aster leafhoppers per 25 sweeps over the 11-yr interval, and included a large number of occasions when no aster leafhopper were caught. Data such as these traditionally have been log-transformed and analyzed using ordinary linear models with normal errors. However, plots of the field variance versus the field mean suggested that the assumption of constant variance was not valid and revealed that the variance, although not equal to the mean, appeared to be pro-

Table 1. Variance estimates for aster leafhopper abundance and infectivity from best fitting GLMM or LMM, respectively

Variance component	Variance estimate[a]	% of total variance
Abundance		
Year × week × field[b]	0.924	28.0
Year × week × farm	0.892	26.2
Year	0.860	24.3
Day	0.478	7.5
Year × day	0.452	6.7
Farm	0.431	6.1
Field	0.193	1.2
Infectivity		
Year	0.047	31.0
Year × week	0.033	15.4
Week	0.017	4.0
Residual	0.054	49.7

[a] Reported as a standard deviation; percent of total variance calculated using variances (i.e., σ^2).
[b] Corresponds to observation level, or residual variability.

portional to the mean. This property of the data were more consistent with Poisson-distributed data and we used a GLMM, Poisson family (log-link), to examine the aster leafhopper count data.

The average aster leafhopper abundance for all fields in all years was estimated by model 1 to be 0.44 (95% CI: 0.23–0.85) aster leafhoppers per 25 sweeps. The temporal "blocks" of the GLMM described a larger proportion of aster leafhopper count variability (Table 1). For example, year, day, and the day × year interaction terms accounted for ≈39% of the aster leafhopper count variability, whereas farm and field accounted for 7% of the total aster leafhopper count variability. When temporal blocks were allowed to interact with spatial blocks, the largest proportion of aster leafhopper count variation was described at shorter temporal scales. For example, the day × year × farm term described 26% of the total variation of aster leafhopper counts and the day × year × field term, which corresponded to the observation level (or residual) variability, was estimated to be 0.924 (reported as σ), ≈28% of the total variance. The remainder of the random effects terms that we examined did not account for a large proportion of aster leafhopper count variance and were excluded from the final model.

Aster Leafhopper Abundance Model Diagnostics. Plots of the conditional modes of year random effects, ordered by year, indicated that aster leafhopper abundance has been decreasing since 2001 and reached its minimum in 2010 (Fig. 1A). In addition, the seasonal (or within-year) pattern that resulted from plotting the day conditional modes, ordered by calendar day, indicated that periods of above average aster leafhopper abundance occurred between 11 June and 1 August, representing a seasonal "window" during which higher aster leafhopper abundance occurs (Fig. 1B). These plots indicated there was a pattern among years and within years that could be more directly modeled, which was the focus of our second paper (Frost et al. 2012).

Quantitative Variability of Aster Leafhopper Infectivity. Over 15 yr of measurement, aster leafhopper infectivity ranged between 0 and 14%. These data were bounded (i.e., by 0 and 100%) and a histogram of infectivity indicated that the data were not normally distributed. Therefore, the infectivity data were square root-transformed before regression analysis. The average infectivity estimated by model 2 was 1.9% (95% CI: 1.2%–2.9) although we would predict the average annual infectivity to fall between 0.2 and 5.6% (i.e., from supplemental Table A1: $0.139 \pm 2^*\sqrt{[0.014^2 + 0.047^2]}$). Similar to aster leafhopper abundance, farm (or location) did not explain a large amount of the variability in aster leafhopper infectivity (Table 1). However, year, week, and year × week groups described ≈50% aster leafhopper infectivity variability, whereas the remaining 50% of the variability could not be attributed to a known factor in our data set (i.e., residual variance).

Aster Leafhopper Infectivity Model Diagnostics. The largest proportion of variance was explained by year and, therefore, we plotted the conditional modes of the random effects for the population expected values of year, ordered by year (Fig. 2A). This plot suggested that annual aster leafhopper infectivity oscillated 2% among years, although more data would be necessary to estimate the periodicity of infectivity. Plots of the week conditional modes of the random effects, ordered by week, indicated that periods of above average aster leafhopper infectivity occurred between 19 May and 15 July (Fig. 2B). Again, these plots indicated that among year and within year seasonal patterns of aster leafhopper infectivity that could be more directly modeled (Frost et al. 2012).

Correlations of Aster Leafhopper Abundance. Correlations of aster leafhopper abundance among years ranged from −0.73 to 0.88 with no distinct grouping that emerged within years (dendrogram not shown). In addition, correlations were plotted versus the lag between years with no apparent association occurring (not shown). All farms were positively correlated within year groupings (Table 2) and correlation coefficients ranged from 0.59 to 0.95, suggesting that the effect of year on aster leafhopper abundance was consistent among farms. Within year-week correlation coefficients ranged from −0.17 to 0.40, suggesting the aster leafhopper abundance estimates among farms, at this shorter time scale, were less correlated (Table 3). The similarity of farms at these different scales can be visualized using correlation as a distance measure to produce dendrograms. Based on hierarchical clustering of the correlation coefficients, farms generally formed two branches and appeared to group by (similar) geographic location within year and year–week scales (Fig. 3). This clustering of farms may also be partially explained by similarities in habitat characteristics in the landscape surrounding the farms.

To determine if aster leafhopper counts from two fields at the same farm would be similar, we initially used density plots to examine the distribution of correlation coefficients of aster leafhopper abundance among field (within farm) combinations. On average,

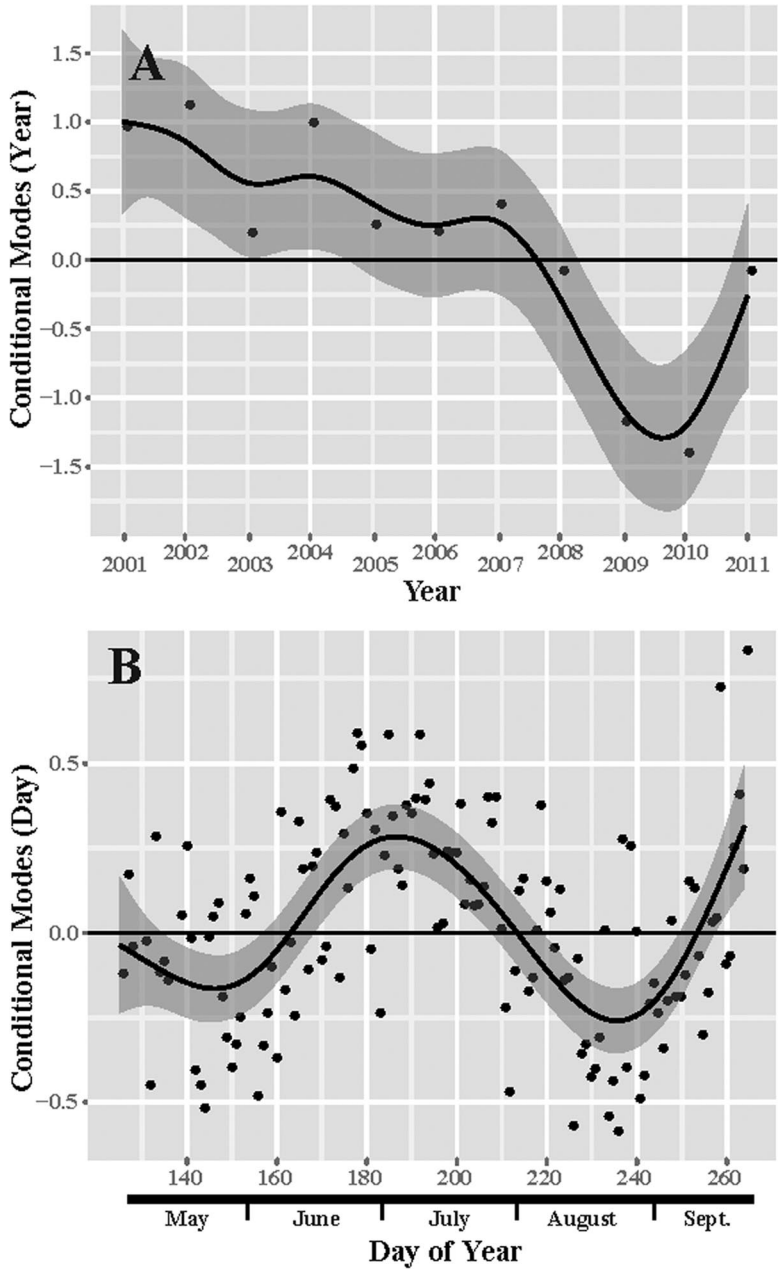

Fig. 1. Conditional modes, or predictions at the population level given the random effects, for A) annual aster leafhopper abundance, ordered by year and B) seasonal aster leafhopper abundance, ordered by day. Generalized additive models were used to add smoothed curves to each plot to examine annual and seasonal trends of aster leafhopper abundance.

the distributions of correlation coefficients among field combinations were approximately normal for all farms in all years (not shown). The correlation coefficients of field combinations for a farm varied interactively with year (year × farm effect: $F = 4.8$; df = 27, 1495; $P < 0.001$) and a general interpretation for the main effects of year ($F = 17.0$; df = 10, 1495; $P < 0.001$) and farm ($F = 3.7$; df = 3, 1495; $P = 0.011$) was not possible.

Correlations of Aster Leafhopper Infectivity. Aster leafhopper infectivity among all farms was positively correlated within year (Table 4) and remained positively correlated within year–week combinations (Table 5). Unlike aster leafhopper abundance, farms did not cluster by geographic location based on correlations within a year. However, within year–week groupings, farms formed two clusters possibly based on geographic location. Specifically, farms in southern

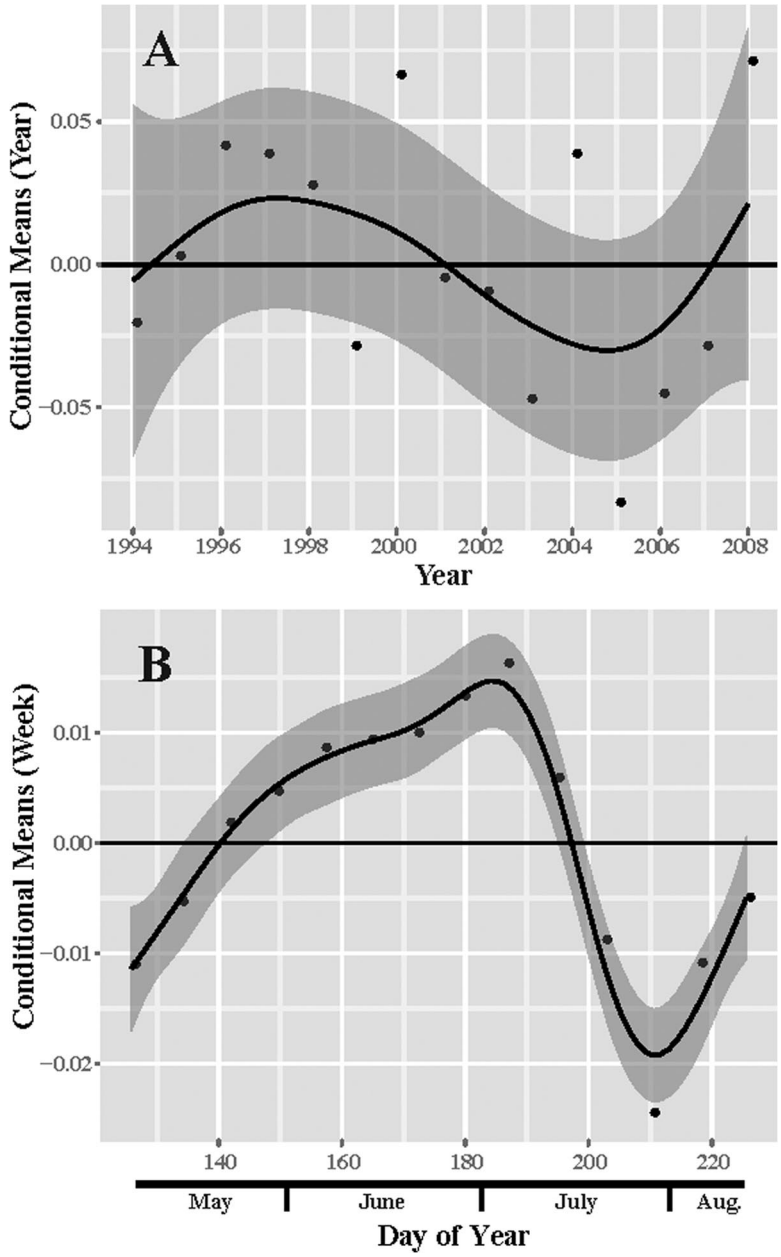

Fig. 2. Conditional modes, or predictions at the population level given the random effects, for A) annual aster leafhopper infectivity, ordered by year and B) seasonal aster leafhopper infectivity, ordered by day. Generalized additive models were used to add smoothed curves to each plot to examine annual and seasonal trends of aster leafhopper infectivity.

Table 2. Correlation of aster leafhopper abundance among farms within year groups

Location	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Farm 2	0.59				
Farm 3	0.89	0.89			
Farm 4	0.89	0.72	0.91		
Farm 5	0.80	0.80	0.92	0.85	
Farm 6	0.95	0.65	0.89	0.87	0.70

Table 3. Correlation of aster leafhopper abundance among farms within year–week groups

Year	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Farm 2	−0.17				
Farm 3	0.18	0.22			
Farm 4	0.05	0.01	0.03		
Farm 5	0.03	0.40	0.23	0.00	
Farm 6	0.40	−0.09	0.16	−0.04	0.05

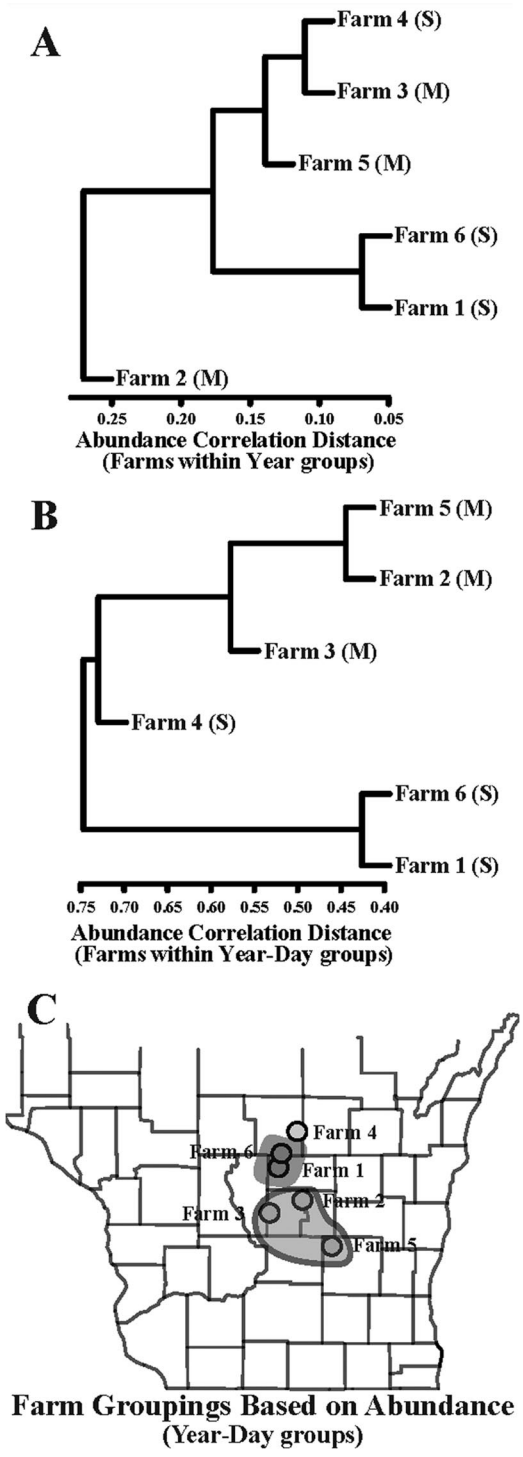

Fig. 3. Dendrograms for visualizing the correlative relationships of aster leafhopper abundance among farms within A) year and B) year-day groups. Farms with predominantly sandy soils and with a soil organic fraction exceeding 65% are denoted as (S) and (M), respectively. C) Approximate geographic locations of farms sampled for aster leafhopper abundance and groupings implied by dendrograms (Similar farms shaded similarly).

Wisconsin grouped together and away from farms in central Wisconsin (Fig. 4).

Discussion

A successful disease control program relies on a detailed understanding of the critical factors that directly influence epidemic development. For plant pathogens spread by arthropods, insect vector abundance and transmission capability, or infectivity, have been reported as important factors that influence plant disease severity in a given growing season (Chapman 1971, 1973; Madden et al. 2000; Jeger et al. 2004). In this paper, we present an approach for examining factors affecting variation in observed insect abundance and infectivity and apply this approach to a long-term observational data set. In our approach, the importance of factors contributing to aster leafhopper abundance and infectivity variation was determined by a factor's relative contribution to the explanation of total variation.

We found that geographic location, farm or field alone, was not a factor that contributed (significantly) largely to the observed variation of insect abundance, relative to other sources of variation. However, aster leafhopper abundance varied greatly among years. Immigration of the aster leafhopper, presumably from the Gulf states in early spring (Chiykowski and Chapman 1965) and later from the central and northern Great Plains (Hoy et al. 1992), has long been considered the principle source for infectious aster leafhoppers in susceptible carrot. The trajectory of air movement and position of cold fronts could affect the geographic extent of adult insect arrival (e.g., depositions zones) (Hurd 1920, Huff 1963, Westbrook and Isard 1999, Zhu et al. 2006) and it would be expected that major synoptic weather events, which occur over larger geographic extents, could lead to low variability of insect abundance at larger spatial scales (i.e., scales larger than the extent of our observational study). Leafhopper abundance also varied greatly at the smaller temporal and spatial scales, within years and farms. For example, >50% of aster leafhopper count variability was described by the interaction terms of day × year × farm and day × year × field. Synoptic weather and wind patterns also occur at these shorter time-scales and are known to correlate with leafhopper influxes (Chiykowski 1965, Hoy et al. 1992, Huff 1963), which could have influenced weekly aster leafhopper abundances. However, this result may be more indicative of unique crop production practices implemented on different carrot fields at these shorter time scales.

Similar to aster leafhopper abundance, temporal factors accounted for the largest proportion of the variability of aster leafhopper infectivity, which was dominated by the among year variance component (31%). Farm (or location) was not a factor that contributed largely to infectivity. Taken together, the results of our variance component analysis of aster leafhopper abundance and infectivity are consistent with the hypothesis that the aster leafhopper immi-

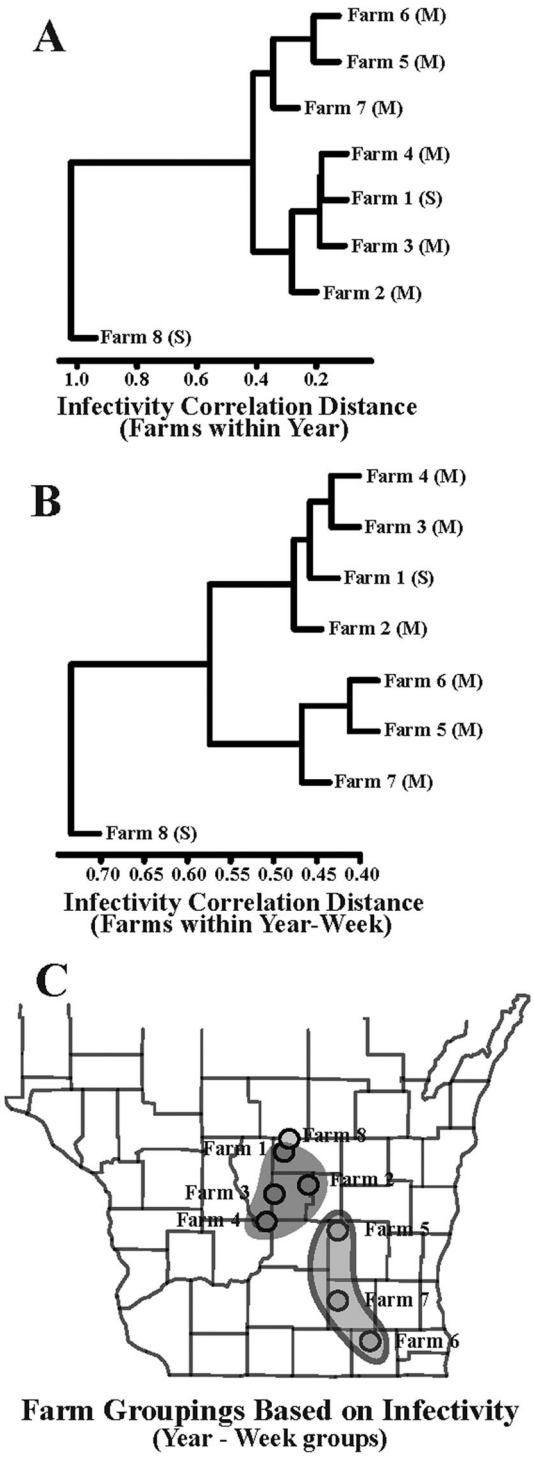

Fig. 4. Dendrograms for visualizing the correlative relationships of aster leafhopper infectivity among farms sampled within A) year and B) year-week groups. Farms with predominantly sandy soils and with a soil organic fraction exceeding 65% are denoted as (S) and (M), respectively. C) Approximate geographic locations of farms and groupings implied by dendrograms (Similar farms shaded similarly).

gration contributes, in part, to the annual risk of AY epidemics in Wisconsin.

Nearly 50% of the aster leafhopper infectivity variation could not be partitioned to a known factor in our data set. This large residual variability could be because of multiple causes, such as AYp strain variability (Lee et al. 2003, Zhang et al. 2004, Frost et al. 2008) or misidentification of the AYp as the cause of the disease symptoms observed in the bioassay plants. In this study, infectivity was determined using infectivity bioassays and examines only those insects in the field that are already able to transmit. Currently, it is common to determine the percentage of insects that are carrying a pathogen by using polymerase chain reaction (PCR) assays and the seasonal pattern of pathogen detections in their insect vectors has been documented for numerous pathosystems (Beanland et al. 1999, Bloomquist and Kirkpatrick 2002, Munyaneza et al. 2010, Bressan et al. 2011). Although PCR can specifically detect the presence of a pathogen, the relationship between a PCR detection and transmission capability of an individual insect is rarely known. Further documentation of the seasonal pattern of infectious insect vectors and research examining the relationship between PCR detection and transmission capability of the insect is necessary to provide accurate pest management recommendations. A comparison of PCR detections and percent capable vectors from the same field population of leafhoppers may provide information about the importance of local inoculum in the environment because insects acquiring AYp locally would be less likely to pass through the necessary latent period before being assayed (or controlled). For example, field populations of leafhoppers, in mid-June through mid-July, exist as a mixture of migratory insects arriving from distant locations and insects that overwinter or migrants, after arrival, that acquire AYp in the local landscape. The migratory insects that have acquired at distant locations would be more likely to have passed through the approximate 2-wk latency period before their arrival in the field. Leafhoppers acquiring AYp locally would be less likely to have passed through a requisite latency period and would not yet be infectious, primarily because the distance needed to travel is less and would take less time. For this reason, we hypothesize that one possible explanation for the difference between the percentage of infectious individuals and PCR-positive detections may result from the inoculum contribution of the local environment.

Although the analysis did not directly describe the temporal patterns of aster leafhopper abundance, plots examining the conditional modes of the year random effects indicated that average annual aster leafhopper abundance decreased through the interval 2001–2010. We cannot explain why aster leafhopper abundance steadily decreased over this period of time, although periodic or quasi-periodic climate patterns, such as the diurnal temperature cycles associated with El Niño and La Niña, or synoptic weather patterns could potentially impact overwintering survival and seasonal insect phenology in southern latitudes

Table 4. Correlation of aster leafhopper infectivity among farms within year groups

Location	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7
Farm 2	0.64						
Farm 3	0.74	0.63					
Farm 4	0.74	0.64	0.73				
Farm 5	0.67	0.58	0.66	0.67			
Farm 6	0.63	0.53	0.62	0.63	0.71		
Farm 7	0.52	0.44	0.51	0.52	0.57	0.60	
Farm 8	0.08	0.03	0.08	0.08	−0.00	−0.03	0.01

(Westbrook et al. 1997, Diffenbaugh et al. 2008, Morey et al. 2012), seasonal migratory cues (Carlson et al. 1992, Isard and Irwin 1993), and weather patterns conducive to leafhopper transport and dispersion (Huff 1963, Carlson et al. 1992, Westbrook and Isard 1999). In addition, the magnitude of migrating aster leafhopper population and its trajectory from the aster leafhopper source regions is likely to be affected by the among year variation in the abundance of small grains acreage planted in the source regions and the location of those acres with respect to seasonal wind and weather patterns.

We observed that, on average, there is a period of elevated aster leafhopper abundance between 11 June and 1 August in Wisconsin. This observation is consistent with previous reports of aster leafhopper phenology in Wisconsin and in the midwest (Hoy et al. 1992, Mahr et al. 1993). In Wisconsin, aster leafhopper overwinter as eggs (Drake and Chapman 1965) and eclosion and subsequent development of aster leafhopper to the adult stage is linearly related to temperature (Jensen 1981, Mahr 1989). It is typical to accumulate enough thermal units by 11 June for aster leafhopper to have developed into winged adults. Thus, the above average aster leafhopper captures observed around 11 June may, in part, be because of the emergence of the local leafhopper population. This seasonal pattern of leafhopper abundance, elevated in mid-to-late June through mid-July followed by a decline in late-July to August, also has been observed in other temperate regions of the United States and Europe for different leafhopper species. For example, *Circulifer tenellus* (Baker) (beet leafhopper), *Psammotettix alienus* (Dahib), and *Graphocephala atropunctata* (Signoret) (blue-green sharpshooter) abundances all peak in June followed by a decline in late July and August (Lindblad and Areno 2002, Munyaneza et al. 2010, Gruber and Daugherty 2012). It may be that these leafhoppers all overwinter, or diapause, in the same life stage (i.e., eggs) and have to develop through a similar number of stadia (i.e., four to five) leading to a reasonably synchronous emergence as adults among species.

To our knowledge, this is the first large systematic study that reports over a decade of insect infectivity estimates. Because these data were collected from among and within 14 growing seasons, we were able to examine the inter- and intra-annual variability of aster leafhopper infectivity. Although highly variable among years, the average natural infectivity was estimated as 2% and we would predict infectivity to range between 0.2 and 5.6% for any given year. In addition, there may be long-term trends of annual infectivity which, if modeled, could help to anticipate high infectivity years. However, more years of data are necessary to determine if the periodicity of natural leafhopper infectivity occurs and to quantify such oscillations (i.e., wavelengths, amplitudes). Gruber and Daugherty (2012) reported seasonal data on the proportion of *G. atropunctata* that transmitted *Xylella fastidiosa* from two historical data sets and concluded that infectivity was either constant or increasing exponentially over the season. In contrast, we found that natural infectivity of aster leafhopper increased early in the season, from mid-May through late-June, and then decreased in mid-July. Thus, periods of above average natural infectivity typically occurred between 19 May and 15 July. Taken together, the coincidence of the expected periods of high leafhopper abundance and infectivity represent a potential 'treatment window' in which management of the insect could be focused.

The landscape surrounding each farm (or farm location) can influence aster leafhopper abundance or infectivity because each location supports a unique composition of predominant plant species. For example, the aster leafhopper uses over 300 different plant species for food, oviposition, and shelter (Wallis 1962, Peterson 1973) and many of these are species are susceptible to AYp infection (Kunkel 1926, Chi-

Table 5. Correlation of aster leafhopper infectivity among farms within year–week groups

Location	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7
Farm 2	0.33						
Farm 3	0.39	0.30					
Farm 4	0.33	0.39	0.40				
Farm 5	0.34	0.17	0.30	0.20			
Farm 6	0.25	0.19	0.25	0.23	0.43		
Farm 7	0.23	0.19	0.18	0.20	0.26	0.40	
Farm 8	0.06	0.06	0.07	0.15	0.01	0.01	0.02

ykowski 1965, Chiykowski and Chapman 1965, Chiykowski 1967, Westdal and Richardson 1969, Peterson 1973, Lee et al. 1998, Lee et al. 2000, Lee et al. 2003, Hollingsworth et al. 2008). Thus, the species composition surrounding each farm likely influences the reproductive capability of the aster leafhopper and/or the prevalence of AYp in the local environment. The habitats surrounding farms may also interact with seasonal weather to further influence leafhopper development and infectivity. It is known that the development of the aster leafhopper, under the same temperature conditions, occurs more slowly on *C. chinensis* than it does on either oats or barley (Mahr 1989). Therefore, the observed farm-to-farm variability of leafhopper phenology may be the result of local weather operating similarly at all farms and times (years and weeks), but operating interactively with the local landscape (different hosts). It is therefore interesting that carrot farms appeared to group differently at the year and year-week scales based on the observed correlations in leafhopper abundance. In contrast, farms tended to group similarly at the year and year–week scales based on the correlation in leafhopper infectivity. Thus the local landscape might affect leafhopper abundance more than it affects infectivity within a growing season.

Studying the pattern of variation and correlations across different temporal and spatial scales is informative for developing future sampling strategies (Wheatley and Johnson 2009, Zuur et al. 2009, Sagarin and Pauchard 2010). For aster leafhopper infectivity, the largest amount of variation was observed in among year samples. In turn, successive, in-season sampling for aster leafhopper infectivity may provide little additional information to explain risk for disease development. This is, perhaps, why the earlier AYI proposed and implemented by R. K. Chapman was a successful AY management tool (Chapman 1973). In addition, farm location, although not a large contributor to variation of infectivity, may be important if we wish to quantify farm-to-farm variation of infectivity. For example, based on our correlation analysis in Wisconsin, it would be best to determine infectivity for farms located in the southern and central parts of the state to maximize the among location variability. Aster leafhopper abundance varied at the smaller scales, within year and among fields. In addition, the correlation of aster leafhopper abundance among fields and within farms varied interactively with year. The practical application of this outcome suggests that scouting to determine aster leafhopper abundance for a specific field and date combination will remain necessary for accurate, site-specific insect management recommendations, even if two fields occur at the same farm location.

Compiled data from observational studies are useful to obtain information about the scale at which ecological factors contributing to aster leafhopper abundance and infectivity occur, because manipulating environmental processes across multiple spatial and temporal scales is difficult. Unfortunately, data collected over long periods of time are at risk of problems associated with accuracy and precision (Manly 1998); long-term data sets are often collected by multiple individuals and for multiple purposes. In our case, the consistency with how the data were collected helped to reduce some of the inherent variability often associated with long-term datasets. For research purposes, it is often necessary to obtain representative samples for all possible conditions that may occur in the biological system of interest. The value of long-term data sets is that they provide a relevant range of observations from the systems we wish to describe (Magnuson 1990). For example, large data sets usually include data points from observations during aberrant or extreme environmental conditions.

In the future, the availability and integrity of large pest scouting data sets will likely increase as agricultural data collection becomes less demanding and data storage becomes less expensive using commercialized software (Sagarin and Pauchard 2010). Several efforts are currently being made to streamline data collection efforts (i.e., Ag Connections, Inc., Murray, KY, scoutpro.org), but similar to other areas of biology, methods will still need to be developed to thoroughly examine these data (Sagarin and Pauchard 2010). The methods presented in this paper may be applicable for analyzing other multiyear, multilocation observational pest scouting data sets to reveal patterns of variability driving plant disease or pest epidemics. Applied specifically to the aster yellows disease system, this methodology improves our understanding of the spatial and temporal patterns of variation of aster leafhopper abundance, informs efforts to directly model the seasonal patterns of leafhopper abundance and infectivity to deduce AY risk, and further increases our knowledge of when the insect moves into susceptible crop fields and when it spreads the pathogen to susceptible crops.

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References Cited

Baayen, R. H., D. J. Davidson, and D. M. Bates. 2008. Mixed-effects modeling with crossed random effects for subjects and items. J. Mem. Lang. 59: 390–412.

Bates, D., M. Maechler, and B. Bolker. 2011. lme4: linear mixed-effects models using S4 classes. R package version 0.999375–42. (http://CRAN.R-project.org/package=lme4).

Beanland, L., C. W. Hoy, S. A. Miller, and L. R. Nault. 1999. Leafhopper (Homoptera: Cicadellidae) transmission of aster yellows phytoplasma: does gender matter? Environ. Entomol. 28: 1101–1106.

Bloomquist, C. L., and B. C. Kirkpatrick. 2002. Frequency and seasonal distribution of pear psylla infected with the pear decline phytoplasma in California pear orchards. Phytopathology 92: 1218–1226.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M.H.H. Stevens, and J. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24: 127–137.

Bressan, A., F.J.M. Garcia, and E. Boudon-Padieu. 2011. The prevalence of '*Candidatus* Arsenophonus phytopathogenicus' infecting the plant hopper *Pentastiridius leporinus* (Hemiptera: Cixiidae) increase nonlinearly with the population abundance in sugar beet fields. Environ. Entomol. 40: 1345–1352.

Carlson, J. D., M. E. Whalon, D. A. Landis, and S. H. Gage. 1992. Springtime weather patterns coincident with long-distance migration of potato leafhopper into Michigan. Agric. For. Meteorol. 59: 183–206.

Chapman, R. K. 1971. Prediction of aster leafhopper migrations and the resultant aster yellows problem in Wisconsin. Proc. North Cent. Branch Entomol. Soc. Am. 26: 96–98.

Chapman, R. K. 1973. Integrated control of aster yellows. Proc. North Cent. Branch Entomol. Soc. Am. 28: 71–92.

Chiykowski, L. N. 1965. The reaction of barley varieties to aster yellows virus. Can. J. Bot. 43: 373–378.

Chiykowski, L. N. 1967. Reaction of some wheat varieties to aster yellows virus. Can. J. Plant Sci. 47: 149–151.

Chiykowski, L. N., and R. K. Chapman. 1965. Migration of the six-spotted leafhopper in central North America. Wis. Agric. Exp. Stn. Res. Bull. 261: 21–45.

Christensen, N. M., K. B. Axelsen, M. Nicolaisen, and A. Schultz. 2005. Phytoplasmas and their interactions with hosts. Trends Plant Sci. 10: 526–535.

De Wolf, E. D., L. V. Madden, and P. E. Lipps. 2003. Risk assessment models for wheat fusarium head blight epidemics based on within-season weather data. Phytopathology 93: 428–435.

Diffenbaugh, N. S., C. H. Krupke, M. A. White, and C. E. Alexander. 2008. Global warming presents new challenges for maize pest management. Environ. Res. Lett. 3: 044007.

Drake, D. C., and R. K. Chapman. 1965. Evidence for long distance migration of the six-spotted leafhopper in Wisconsin. Wis. Agric. Exp. Stn. Res. Bull. 261: 1–20.

Duffy, M. A., C. E. Caceres, S. R. Hall, A. J. Tessier, and A. R. Ives. 2010. Temporal, spatial, and between-host comparisons of patterns of parasitism in lake zooplankton. Ecology 91: 3322–3331.

Frost, K. E., C. L. Groves, and R. L. Groves. 2008. Detection of multiple strains of the aster yellows phytoplasma in Wisconsin carrot fields [(abstr.)]. Phytopathology 98: S55.

Frost, K. E., P. D. Esker, R. van Haren, L. Kotolski, and R. L. Groves. 2013. Seasonal pattern of aster leafhopper (Hemiptera: Cicadellidae) abundance and aster yellows phytoplasma infectivity in Wisconsin carrot fields. Environ. Entomol. 42: 491–502.

Gelman, A., and J. Hill. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, Cambridge, United Kingdom.

Gruber, B. R., and M. P. Daugherty. 2012. Understanding the effects of multiple sources of seasonality on the risk of pathogen spread to vineyards: vector pressure, natural infectivity, and host recovery. Plant Pathol. 62: 194–204. Doi: 10.1111/j. 1365-3059.2012.02611.x. pp. 11.

Hollingsworth, C. R., L. M. Atkinson, D. A. Samac, J. E. Larsen, C. D. Motteberg, M. D. Abrahamson, P. Glogoza, and I. V. MacRae. 2008. Region and field level distributions of aster yellows phytoplasma in small grain crops. Plant Dis. 92: 623–630.

Hoy, C. W., S. E. Heady, and T. A. Koch. 1992. Species composition, phenology, and possible origins of leafhoppers (Cicadellidae) in Ohio vegetable crops. J. Econ. Entomol. 85: 2336–2343.

Huff, F. A. 1963. Relation between leafhopper influxes and synoptic weather conditions. J. Appl. Meteorol. 2: 39–43.

Hurd, W. E. 1920. Influence of the wind on the movements of insects. Mon. Weather Rev. 48: 94–98.

IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma taxonomy group. 2004. '*Candidatus* Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. Int. J. Syst. Evol. Microbiol. 54: 1243–1255.

Isard, S. A., and M. E. Irwin. 1993. A strategy for studying the long-distance aerial movement of insects. J. Agric. Entomol. 10: 283–297.

Jeger, M. J., J. Holt, F. van den Bosch, and L. V. Madden. 2004. Epidemiology of insect-transmitted plant viruses: modeling disease dynamics and control interventions. Physiol. Entomol. 29: 291–304.

Jensen, J. O. 1981. Management of the aster leafhopper and aster yellows in Wisconsin. Ph.D. thesis, University of Wisconsin, Madison.

Kriss, A. B., P. A. Paul, and L. V. Madden. 2012. Variability in fusarium head blight epidemics in relation to global climate fluctuations as represented by the El Niño-Southern Oscillation and other atmospheric patterns. Phytopathology 102: 55–64.

Kunkel, L. O. 1926. Studies on aster yellows. Am. J. Bot. 13: 646–705.

Lee, I.-M., D. E. Gundersen-Rindal, and A. Bertaccini. 1998. Phytoplasma: ecology and genomic diversity. Phytopathology 88: 1359–1366.

Lee, I.-M., R. E. Davis, and D. E. Gunderson. 2000. Phytoplasma: phytopathogenic mollicutes. Annu. Rev. Microbiol. 54: 221–255.

Lee, I.-M., M. Martini, K. D. Bottner, R. A. Dane, M. C. Black, and N. Troxclair. 2003. Ecological implications from a molecular analysis of phytoplasmas involved in an aster yellows epidemic in various crops in Texas. Phytopathology 93: 1368–1377.

Lee, P. E., and A. G. Robinson. 1958. Studies on the six-spotted leafhopper *Macrosteles fascifrons* (Stal) and aster yellows in Manitoba. Can. J. Plant Sci. 38: 320–327.

Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology 73: 1943–1967.

Lindblad, M., and P. Areno. 2002. Temporal and spatial population dynamics of *Psammotettix alienus*, a vector of wheat dwarf virus. Int. J. Pest Manage. 48: 233–238.

Madden, L. V., M. J. Jeger, and F. van den Bosch. 2000. A theoretical assessment of the effects of vector-virus transmission mechanism on plant disease epidemics. Phytopathology 90: 576–594.

Madden, L. V., W. W. Turechek, and M. Nita. 2002. Evaluation of generalized linear mixed models for analyzing disease incidence data obtained by designed experiments. Plant Dis. 86: 316–325.

Magnuson, J. T. 1990. Long-term ecological research and the invisible present. Bioscience 40: 495–501.

Mahr, S.E.R. 1989. Development and aster yellows-infectivity of the aster leafhopper. Ph.D. thesis, University of Wisconsin, Madison.

Mahr, S.E.R., J. A. Wyman, and R. K. Chapman. 1993. Variability in aster yellows infectivity of local populations of the aster leafhopper (Homoptera: Cicadellidae) in Wisconsin. J. Econ. Entomol. 86: 1522–1526.

Manly, B.F.J. 1998. Sampling and modelling of insect populations, pp 3–20. *In* J. Baumgartner, P. Brandmayr, and

B.F.J. Manly (eds.), Population and community ecology for insect management and conservation. A.A. Balkema Publishers, Brookfield, VT.

Morey, A. C., W. D. Hutchison, R. C. Venette, and E. C. Burkness. 2012. Cold hardiness of *Helicoverpa zea* (Lepidoptera: Noctuidae) pupae. Environ. Entomol. 41: 172–179.

Munyaneza, J. E., J. M. Crosslin, J. E. Upton, and J. L. Buchman. 2010. Incidence of the beet leafhopper-transmitted virescence agent phytoplasma in local populations of the beet leafhopper, *Circulifer tenellus,* in Washington State. J. Insect Sci. 10: 18.

Nita, M., M. A. Ellis, and L. V. Madden. 2008. Variation in disease incidence of phomopsis cane and leaf spot of grape in commercial vineyards in Ohio. Plant Dis. 92: 1053–1061.

Nobre, J. S., and J. M. Singer. 2007. Residual analysis for linear mixed models. Biom. J. 49: 863–875.

Peterson, A. G. 1973. Host plant and aster leafhopper relationships. Proc. North Cent. Branch Entomol. Soc. Am. 28: 66–70.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-plus. Springer New York, Inc., New York.

R Development Core Team. 2012. R: a language and environment for statistical computing, version 2.15.0. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/).

Sagarin, R., and A. Pauchard. 2010. Observational approaches in ecology open new ground in a changing world. Front. Ecol. Environ. 8: 379–386.

Schultz, G. A. 1979. Epidemiology of the aster leafhopper and aster yellows in relation to disease control. Ph.D. thesis, University of Wisconsin, Madison.

Wallis, R. L. 1962. Host plant preference of the six-spotted leafhopper. J. Econ. Entomol. 55: 998–999.

Westbrook, J. K., and S. A. Isard. 1999. Atmospheric scales of biotic dispersal. Agric. For. Meteorol. 97: 263–274.

Westbrook, J. K., W. W. Wolf, P. D. Lingren, J. R. Raulston, J. D. Lopez, Jr., J. H. Matis, R. S. Eyster, J. F. Esquivel, and P. G. Schleider. 1997. Early-season migratory flights of corn earworm (Lepidoptera: Noctuidae). Environ. Entomol. 26: 12–20.

Westdal, P. H., and H. P. Richardson. 1969. The susceptibility of cereals and wild oats to an isolate of the aster yellows pathogen. Can. J. Bot. 47: 755–760.

Wheatley, M., and C. Johnson. 2009. Factors limiting our understanding of ecological scale. Ecol. Complex. 6: 150–159.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer, New York. (http://had.co.nz/ggplot2/book).

Zhang, J., S. A. Hogenhout, L. R. Nault, C. W. Hoy, and S. A. Miller. 2004. Molecular and symptom analyses of phytoplasma strains from lettuce reveal a diverse population. Phytopathology 94: 842–849.

Zhu, M., E. B. Radcliffe, D. W. Ragsdale, I. V. MacRae, and M. W. Seeley. 2006. Low-level jet streams associated with spring aphid migration and current season spread of potato viruses in the U.S. northern Great Plains. Agric. For. Meteorol. 138: 192–202.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer Science + Business Media, LLC, New York.

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