SEASONAL INFECTIVITY OF ASTER LEAFHOPPERS IN CARROT

Kenneth E. Frost\textsuperscript{1} and Russell L. Groves\textsuperscript{2}

Principal Investigators
\textsuperscript{1}Research Assistant, Department of Plant Pathology, University of Wisconsin-Madison
\textsuperscript{2}Assistant Professor and Entomology Extension Specialist, Department of Entomology, University of Wisconsin-Madison

Collaborators
Randy Van Haren - President and Lead Pest Management Specialist, Pest Pros, Inc., Plainfield, WI 54966

Problem Defined:
Each year, Wisconsin growers produce carrots on an average of 4200 acres grossing over $6 million dollars in revenues (USDA-NASS, 2007 annual bulletin). Unfortunately, carrot fields are threatened annually by the occurrence of aster yellows phytoplasma (AYp), which is obligately transmitted by the aster leafhopper (\textit{Macrosteles quadrilineatus} Forbes). Current control practices strictly utilize insecticide sprays that target the aster leafhopper. Spray timing is guided by an aster yellows index that is based on the proportion of infective leafhoppers present in a field at a given point in time. Crop scouting and molecular diagnostic tools have decreased the inherent lag between finding inoculative leafhoppers and prescribed sprays. However, yield losses of 5-20\% resulting from AYp are still commonplace (1). A more comprehensive and sustainable, multi-tactic control strategy is warranted to lower inoculum pressure in the areas surrounding susceptible crops. The management of off-crop habitats in ways that minimize the persistence and decrease the accumulation of AYp inoculum in the local environment has the potential to contribute to the sustainability of carrot production primarily through reductions in pesticide.

Research Objectives:
The primary focus of this research is to improve our knowledge of where leafhoppers acquire the pathogen, when they move into susceptible fields, and when they spread the pathogen to crops. Specifically, our focus has been to 1) accurately identify primary inoculum sources of AYp of greatest epidemiological significance in non-crop habitats surrounding carrot fields and 2) to compare the genetic structure of the population of AYp from reservoir hosts to that within carrot and to determine if genotype variability relates to either prevalence or infectivity potential of the pathogen. Ultimately, this project will provide accurate, new information about the relative importance of AYp sources in the habitat surrounding carrot fields. This information can then be used to evaluate the local AYp risk and, in turn, management practices can be developed to decrease the accumulation and local persistence of the pathogen.

Background Information:
The spring migration of leafhoppers from the Gulf-states to Wisconsin has been documented to influence the potential for aster yellows epidemics in carrot (2, 3). However, it has been suggested that the dispersal of migrant leafhoppers (after arrival) and locally produced...
leafhoppers influence the likelihood of an AYP epidemic in a given year. The mixed age-structure of developing leafhopper populations acquire the pathogen from local plant sources, and subsequently transmit AYP to carrot. In severe years, local AYP sources are thought to greatly contribute to epidemic development in Wisconsin carrot fields (4). Additional data that supports the proposition that AYP is acquired local plant sources and is subsequently spread to carrot comes from a 14-year survey of aster leafhopper infectivity conducted by Pest Pros, Inc. of Plainfield, Wisconsin. These data show that the period of highest leafhopper AYP infectivity coincides with the emergence of the locally produced aster leafhoppers (Figure 1).

Other recent studies have shown that the spatial arrangement of host and non-host plant species influence epidemic severity (5, 6). Zhou’s (2002) modeling demonstrated that AYP acquisition and transmission and AYP latent period within host plant and leafhopper are important factors that influence epidemic development. There is evidence that all of these factors are influenced by strain variability. For example, different AYP strains are transmitted by the leafhopper at different rates (7, 8). To our knowledge there have been no studies to characterize the genetic diversity of AYP in Wisconsin susceptible crops. In addition, the genetic relationships associated with ability to cause disease on carrot and to survive within reservoir hosts has not been examined. This area of study is very important considering the long history of carrot cultivation in Wisconsin.

Our ongoing research is designed to generate significant, new information about the factors that influence geographic movement and spread of aster yellows in carrot fields, which can be used to develop a multidisciplinary approach for the management of AYP in the carrot producing regions of Wisconsin.

**Preliminary Data Collected:**

**Fall 2007**

**Methods:** Our research plan relies on our ability to differentiate AYP strains that exist in the environment and preliminary studies were conducted to determine if multiple AYP strains were present in the Wisconsin carrot crop. In the fall of 2007, we sampled carrot plants displaying symptoms typical of aster yellows (i.e. stunting, yellowing and reddening, twisting, distortion of flowers, bushy or broom-like growth, stunted and malformed roots) from 8 fields in the central sands of Wisconsin. Molecular methods originally developed to distinguish different AYP strains in lettuce (9) were used to differentiate AYP strains in carrot. In lettuce, different strain types produce different symptoms and we are current evaluating symptoms produced by the
different strain types in carrot.

**Results:** Ninety-four percent of the symptomatic carrot plants tested positive for AYp. Six of the seven AYp strain types previously described in Ohio were present in Wisconsin. The predominant strain types that were found in carrot were AY-WB (32%), a previously uncharacterized AY strain (27%) and AY-BD2 (15%). In leafhoppers, the predominant strain types were AY-WB (44%), a previously uncharacterized strain (28%) and AY-S (15%). The proportions of strain types found in carrot fields were significantly correlated to the proportion of strain types found in aster leafhoppers collected throughout the summer of 2007.

**Discussion:** Our survey revealed a great degree of variability in the populations of aster yellows phytoplasma present in Wisconsin carrot fields (Figure 2). The relative abundance of AYp strain types extracted from carrot varied by location. In half of the carrot fields sampled, a previously uncharacterized AYp strain represented ≥40% of the strain types present. The proportion of strain types found at one carrot field did not always correspond to strain types found at other locations. However, some locations had strain type profiles that were highly correlated with the profiles of other locations. These results suggest the possibility that local factors may influence the distribution and spread of aster yellows. The implications of these data suggest that a better characterization of the strain differences is warranted to improve our understanding of the epidemiology of aster yellows in Wisconsin and the relative importance of in-bound inoculum in migratory insects.

**Summer 2008**

**Methods:** In 2008, we again sampled carrot plants displaying symptoms typical of aster yellows from 8 fields in the central sands of Wisconsin. Plants from each field were sampled on 4 different dates beginning in June and ending in late August. Aster leafhoppers were also sampled from each of the fields throughout the summer 2008. AYp was extracted from all of the samples and, once again, molecular methods were used to differentiate AYp strains in carrot. We also collected several AYp isolates that are being maintained in the greenhouse for insect transmission studies.

**Expected Results:** Primer-typing of AYp isolates and data analysis from our 2008 sampling again indicated varied AYp genotypes present in carrot fields associated with specific symptoms present in those fields. New in 2008, we are attempting to determine if the AYp genotype profile changes throughout the growing season which could be indicative that the aster leafhopper is selectively transmitting predominant AYp strains. It might also indicate that the sources of AYp or inhabiting leafhopper populations change throughout the growing season.
Studies Planned for 2009 and 2010: Over the next 2 summers we plan to tackle 2 objectives:

**Objective 1:** Accurately identify the primary reservoir hosts of AYP in habitats surrounding carrot fields and determine which have the greatest epidemiological importance as potential inoculum sources.

We plan to select several fields for more comprehensive studies and at each of the study sites we would like to measure: 1) *plant species abundance in non-crop areas*, 2) *AYp prevalence in non-crop plant species*, 3) *vector phenology in crop and in non-crop areas*, and 4) *aster yellows incidence in Carrot*.

**Expected outcomes:** Results from these studies will indicate the species that are present in the field margins and the likelihood that a plant species will be infected with AYP. We will initially focus efforts on plant species that have previously be implicated as AYP reservoirs (Table 1). From these data we will calculate an index for each field edge by multiplying the relative abundance of a weed species times the estimated prevalence of AYP within that species. This index will be used to assess a field’s AYP risk in terms of inoculum potential. Leafhopper population size data will be used to estimate vector visitation on the non-crop species at the field edge scale providing an accurate estimate of a field’s AYP risk.

**Objective 2:** I) Compare the genetic structure of the population of AYP isolates collected from reservoir hosts and within affected carrot, and II) determine if AYP variability relates to either disease prevalence or infectivity (virulence) of the pathogen.

From our sampling at each of the study sites we will characterize *AYP variability* in non-crop hosts and within affected carrot. We have also started a collection of primer-typed AYP isolates that are maintained *in planta* and will be used to measure leafhopper *transmission efficiency*.

**Expected outcomes:** I) Results from these studies will indicate if AYP genotypes present in carrot fields correspond with the predominant genotypes present in off-crop habitats. In addition, we will determine if AYP genotypes are associated with specific non-crop hosts. II) Results will indicate if differences in leafhopper transmission efficiency correspond with particular AYP genetic variants. In particular, we will find out if the aster leafhopper is selectively transmitting AYP genotypes, or predominant strains. This data will provide a better understanding of the importance of AYP variability in relation to the distribution and spread of AYP in the carrot agroecosystem and will aid in the development of management strategies to mitigate AYP risk.

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<thead>
<tr>
<th>Family</th>
<th>Genus (species)</th>
<th>Common Name</th>
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<tr>
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<td></td>
<td><em>Daucus carota</em></td>
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Table 1. Common weed species implicated by Schultz (1979) as reservoirs of the AYP in Wisconsin.
References: