NON CROP SOURCES OF CMV AND IMPLICATIONS FOR MANAGEMENT

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Abstract. Cucumber mosaic virus (CMV) is a tripartite, positive-sense RNA plant virus in the genus Cucumovirus, family Bromoviridae. This virus is one of the most economically important viruses affecting several crop plant species and it has one of the widest host ranges among the characterized viruses. Recently, snap bean (Phaseolus vulgaris L.) and pepper (Capsicum annuum L.) crops in Wisconsin have experienced significant increases in incidence and crop losses associated with infection of this virus. This increase has anecdotally been linked to the recent introduction and establishment of the soybean aphid (Aphis glycines Matsumura) in the upper Midwest region. Presumably, the unique population biology and dispersal of this species has changed both the spatial arrangement and temporal movement patterns previously observed with respect to CMV in the region. We have begun studies to compare the genetic diversity of virus populations present in affected crops over years, within and among locations, between affected crops, and finally within dispersing aphid vectors. CMV has three RNAs and six open reading frames (ORF’s). Reassortment between RNAs and recombination in the 3’ and 5’ nontranslated regions and between ORFs 3a and 3b have been demonstrated to contribute to CMV evolution. To complete these diversity studies, we will examine sequence heterogeneity in all six regions of the genome.

Project Description: This is the second year of a project that was initiated in the 2007 growing season and supported by the University of Wisconsin’s, College of Agriculture through Hatch Formula 142-Funds. This project has a high probability of success both in terms of generating significant new information regarding the epidemiology of nonpersistent virus diseases in Wisconsin vegetable production and in providing practical guidance towards management of this pathosystem. Elucidation of the genetic diversity
of the virus complex in Wisconsin will aid our understanding of the mechanism of spread of these virus diseases. Outlined objectives address gaps in our present understanding that must be filled if we are to develop a management plan to address not only the disease potential but to best manage vector species largely responsible for movement within snap bean industry. More detailed information on the seasonal patterns of vector dispersal, host plant use, and dispersal within and from various crop types in the agricultural landscape will help to accurately time and focus management efforts to reduce areawide vector populations.

**Background and Rationale.** Wisconsin continues to be a leader in the production of processing snap beans (*Phaseolus vulgaris* L.) in the US averaging over 68,000 production acres through the interval 1997 to 2006 (National Agricultural Statistics Service, 2006). Total farm gate value of processing snap beans in the state have equally increased averaging 32.5 M dollars per year over the same interval. However, production and revenue increases over this period have been offset by recent losses due to significant increases in a novel complex of plant viruses including cucumber mosaic virus (CMV), alfalfa mosaic virus (AMV), clover yellow vein virus (CIYVV), bean yellow mosaic virus (BYMV), bean common mosaic virus (BCMV), and perhaps others not well documented at this time (German et al. 2004). Epidemics of these viruses in processing snap beans have become prevalent over much of the northern US in recent years and all are transmitted by dispersing aphid vectors (German, et al. 2004, Grau et al. 2002, Larsen et al. 2002, Nault 2003, Nault et al. 2004).

While some of these viruses had been previously noted to affect processing snap beans, their occurrence was considered intermittent and affected fields were distributed discretely in specific areas (Delahaut et al. 2001). Beginning in 2000, significant increases in virus infections, in particular both CMV and AMV, were noted throughout many processing snap bean production areas in eastern and south-central Wisconsin. Both of these viruses have very broad host ranges and are transmitted in a non-persistent manner by several aphid vector species. The host range of CMV includes over 800 plant species including both crop and non-crop weed species (Palukaitis et al. 1992) and is transmitted by over 60 species of aphids (Raccah, 1985, Raccah et al. 1986, Irwin, 1994, Dixon, 1998). Alfalfa mosaic virus has a less broad host range infecting nearly 100 plant species in 21 plant families and is transmitted by 14 aphid vectors (Brunt et al. 1996, Nault 1997).

Coincident with the increasing incidence of viruses statewide, the soybean aphid (*Aphis glycines* Matsumura) was also first observed in Wisconsin in July of 2000. Populations of this insect rapidly dispersed across several North Central states and have infested millions of soybean acres in the region (Landis et al. 2003). The soybean aphid has been implicated in the recent increases in CMV, AMV, and other problematic viruses in processing snap beans due to the recent population increases and the documented competence of this insect species as a vector of nonpersistent viruses (Hill et al. 2001, Alleman 2002). The temporal patterns of virus increase in susceptible snap beans are also consistent with the unique dispersal biology of this highly mobile insect. Specifically, dramatic increases in virus incidence appear to follow in the wake of mid-
season dispersal flights of soybean aphid often resulting from overcrowding of aperous (non-winged) individuals developing on soybean through the early summer months. In some years, however, significant annual dispersal in this vector species has not always been observed, yet virus incidence has not been fully abated.

Although significant new information has been developed recently to describe soybean aphid seasonal dispersal, it’s competence as a virus vector, and the timing of virus increase in susceptible, processing snap bean crops, limited information exists to document the primary inoculum sources where these viruses are acquired. Knowledge of which vector(s) species transmit these viruses to processing snap beans in Wisconsin, where they acquire the viral pathogens, when they move into fields, and when they spread the pathogen to snap beans is critical to understanding and managing the spread of these viral diseases. This project is directed at further enhancing our present understanding of the epidemiology of problematic bean viruses in affected areas of Wisconsin with a focus on factors that influence virus geographical distribution and spread.

**Research Objectives:**

1) To identify and characterize the seasonal abundance of the primary aphid vectors of CMV and AMV among perennial crops in the agricultural landscape.

2) To compare the genetic structure of the population of CMV and AMV isolates collected from virus-affected, susceptible succulent bean plantings, dispersing insect vectors, and potential reservoir hosts.

**Experimental Approach and Preliminary Results:**

1) To identify and characterize the seasonal abundance of the primary aphid vectors of CMV and AMV among perennial crops in the agricultural landscape.

The seasonal dispersal dynamics of aphid vector species has been compared among virus-affected processing snap bean fields and affected pepper fields. The results of these studies have begun to provide further insight into the temporal and spatial patterns of aphid movements into and among different habitats relative to the onset of symptom expression in susceptible beans. Aphid trapping began in early May and will be monitored through September to cover both early and late plantings using the North Central Region’s, Aphid Suction Trap Network. Vertical traps contain an approximately 1.5-L, clear plastic container affixed to the bottom of wire-framed, suction trap. The top of traps are positioned approximately 5 m above the soil surface and a 0.5-L of a mixture of propylene glycol and water is checked (and replaced as needed) weekly throughout the season at the time all alatae are collected. Trapped alatae (winged aphids) are removed from the solution, counted, and held in 95% ethyl alcohol for later identification and virus detection.
The presence of CMV in a subsample of weekly vectors captured in pan traps within traps is just underway using immuno-capture(IC) RT-PCR (Schneider and Roossinck 2001). Based on genomic information, available genomic primers have been used to investigate the incidence of CMV infection present in alatae captured within the different locations and at different collection times. In the future, attempts will be made to directly compare the genotype profiles of detected viruses in each trap to the timing of vector flights and rapidly increasing incidence in susceptible, processing bean crops.

2) To compare the genetic structure of the population of CMV and AMV isolates collected from virus-affected, susceptible succulent bean plantings, dispersing insect vectors, and potential reservoir hosts.

Studies have also been initiated to ascertain the genetic diversity within the virus affected regions of central and eastern Wisconsin among CMV populations. We predict that by completing this objective we should be able to group CMV via host association and identify the primary vector species and reservoir hosts which may serve as inoculum sources for the spread of viruses in Wisconsin. A subset of the total strains isolated during the course of these proposed studies have been examined in these analyses. Previously isolated strains, representing geographically distinct locations (California, New York and Wisconsin) and different host groups have been included as reference control strains.

Symptomatic plants (N=100 / location) were collected from affected snap bean and pepper crops and initially confirmed to be CMV-infected by DAS-ELISA in September, 2007. A subset of 10, CMV-infected pepper plants were selected from each of 3 locations (Locations = G, V, and A) in Adams County in central Wisconsin and an additional 3 locations (Locations = W, M, and N) of CMV-infected snap beans in Waushara, Green Lake, and Manitowoc Counties, respectively (Figure 1). Total RNA was extracted from DAS-ELISA positive, CMV-infected plants and reverse-transcriptase polymerase chain reaction (RT-PCR) performed according to Lin et. al (2004). Specific PCR primer pairs were used to amplify different regions of the CMV genome representing 6 ORF’s: (1) ORF 1a (1089 bp), (2) ORF 2a (653 bp), (3) ORF 2b (370 bp), (4) ORF M (842 bp), (5) ORF CP (678 bp), and (6) ORF 3’ NTR (315 bp). Sequence analyses were performed to determine the relative similarity / dissimilarity of CMV strains collected both within and among affected vegetable crops (e.g. snap beans and peppers).

Sequence heterogeneity is being investigated in all six genomic regions between two susceptible crop species at these selected field locations in Wisconsin. To date, the subset of plant samples representing CMV-affected snap bean and pepper illustrate some within and among field variability in the population of CMV strains collected (Figures 2 & 3). These data suggest either (1) significant within field selection may be occurring within
affected crops, (2) differing aphid vector species may be influencing the strain composition present in affected fields, or (3) primary inoculum sources outside fields may be numerous and support a similar range of strain variation in non-crop sources. Future directions will continue to include the seasonal detection and characterization of CMV strain diversity in: 1) non-colonizing aphid vectors collected from the North Central Region’s, Soybean Aphid Suction Trap Network (http://www.ncipmc.org/traps), 2) perennial feed and forage crops species (e.g. alfalfa, clover, etc.) as well as non-crop weed species previously documented as naturally infected with CMV surrounding affected crop fields.

References.


