INFLUENCE OF MAIZE MOSAIC VIRUS AND HOST PLANT GENOTYPES ON THE BIO-ECOLOGY OF *PEREGRINUS MAIDIS*

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

ENTOMOLOGY

MARCH 2012

By

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ACKNOWLEDGEMENTS

I would like to express a special thanks to my advisor Dr. Alberto Bressan and my committee members Drs. Mark G. Wright and James L. Brewbaker for their continued guidance, encouragement, and support throughout this work. Thank you all for the opportunity to do this research and thank you for sharing some good laughs, interesting conversations, and your knowledge.

I would like to thank Mark Ladao and Tyler Dacuy for his assistance with the rearing and sample preparations for ELISA tests. Thanks to the Waimanalo farm crew for all their help in the field research. Special thanks to my fellow lab mates, especially Shizu and Darlene, for their help and making work enjoyable. Thanks to Devin, Josh, Pikky, and Adam for sharing quality beer time to help relieve the stresses of graduate school. Also, thanks to my fellow Entomology students for their help and friendship during my time this time. Thank you to Cori and Linda for all your help in the PEPS office and thank you PEPS faculty for your guidance and support. Thanks to my fellow CTAHR Club friends for all your support and help with our many events and community services. Allene, Erica, and Vera, thanks for all your support, words of encouragement, awesome stories, and funny jokes. Lastly, thank you Ihi and Koa for all that you have done; greatly appreciate it.

I dedicate this thesis to my mom, who was diagnosed with cancer in 2009, but never wanted me stop regardless of her health. She is definitely an inspiration in my life and is now cancer free. I also dedicate this thesis to my grandparents, Kumao and Matsuno Higashi, for always bestowing on me the importance of education.

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Chapter 1

A Short Review: Maize mosaic virus and its planthopper vector *Peregrinus maidis*.

Vector Biology and Ecology

The corn planthopper, *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae), was first observed and described in Hawaii by Perkins in 1892 (Zimmerman 1948) where it is believed to have been accidently introduced in the early 1880's (Giffard, 1922). *Peregrinus maidis* is regarded as a serious economic pest in many tropical and sub-tropical corn and sorghum growing regions around the world (Brewbaker, 2003; Metcalf, 1943; Singh & Seetharama, 2008). Crop loss may result from either direct feeding damage. Overwhelming number of planthoppers feeding on a single plant results in a condition often referred as "hopper burn" (Takara & Nishida, 1983). Corn plants with hopperburn show extended leaf necrosis, reduced plant vigor, stunting, and are predisposed to moisture stress (Backus et al., 2005; Singh & Seetharama, 2008). High infestation levels on young corn plants can lead to early plant death or to significant yield losses (Brewbaker, 1981; Nishida, 1978). Two economically important corn diseases are vectored by *P. maidis*, *Maize stripe virus* (Tsai, 1982) and *Maize mosaic virus* (MMV) (Nault & Knoke 1981), however, only MMV is currently spread in Hawaii.

Although a corn specialist, *P. maidis* has shown to be oligophagous. Namba and Higa (1971) examined the survival of *P. maidis* of various alternative grass hosts that include napiergrass (*Eleusine indica*), vareygrass (*Paspalum urvillei*), sugarcane (*Saccharum officinarum*), sourgrass (*Trichachne insularis*), Californiagrass (*Brachiaria mutica*), Job's tears (*Coix lacryma-jobi*), panagolagrass (*Digitaria decumbens*), and nutgrass (*Cyperus rotundus*). In addition, Tsai (1996) showed that *P. maidis* was able to survive on itch grass (*Rottboellia exaltata*), gama grass (*Tripsacum dactyloides*), goose grass (*Eleusine indica*), and barnyard grass (*Echinochloa crusgalli*). However, these alternative hosts were not suitable for oviposition.

Planthopper females usually lay eggs within the midribs of corn leaves on both young and older corn plants. Females go through a pre-oviposition period of 3-6 days followed by an oviposition period of 11-48 days (Tsai & Wilson, 1986). A female can lay between 400-800 eggs and as much as 20 eggs laid per day (Singh & Seetharama, 2008). There are five nymphal developmental stages (instars) which takes an average 3-6 days between each stage at a temperature range of 21-27°C (Tsai & Wilson, 1986). First instars begin to feed soon after hatching and display a cryptic behavior, hiding between leaf sheaths and leaf folds within the whorl of young corn plants.

As with other delphacid planthoppers (Denno & Roderick, 1990), *P. maidis*, exhibit two distinct morphologies at stage of adulthood, marcopters (long winged form) and brachypters (short wing form). This dimorphism is observed in both sexes. Macropterous form allows the hopper to escape from unfavorable conditions but lack high reproductive ability. The brachypterous form, on the other hand, cannot fly very efficiently but are strong jumpers and are better suited for higher reproductive output. Generally, the production of winged form can be associated with changes in host plant quality and planthopper density (Denno & Roderick, 1990). For instance, previous work conducted by Denno et al. (1985), illustrated that crowding favored the production of macropters in the planthoppers *Prokelisia* marginata, a relative to *P. maidis* (Van Duzee, 1897), but this effect was mitigated when host plants received high level of nitrogen fertilization. Because of its morphological traits, planthoppers are very efficient in colonize and exploit host plants in a changing environmental condition.

Maize mosaic virus

Maize mosaic virus (MMV) is a *nucleorhabdovirus* of the family *Rhabdoviridae*. Members of this virus family are important pathogens to vertebrates, invertebrates, and plants (Jackson et al., 2005). In general, the transmission of rhabdoviruses by insect vectors is highly specific. Plant-infecting rhabdoviruses replicate into their vectors. (Hogenhout, 2003).

Like other *Rhabdoviruses*, MMV virons are bullet-shaped or bacilliform measuring in 224 nm x 68 nm is size (Falk & Tsai, 1985). MMV contain a monocistronic, single stranded, negative-sense RNA (Ammar et. a., 2009; Reed et. al., 2005). The genomic RNA is about 12,100 nucleotides in length and contains 6 core genes: N, G, M, P, L and P3 (Reed et. al., 2005). It is encased in a lipid envelope derived from host nuclear or cellular membrane. The Nucleocapsid (N) protein has an important structural and functional role in the encapsulation of the genomic RNA, viral assembly and replication (Ammar et. a., 2009). The Glycoprotein (G) is presumed to play an important role in receptor mediated edocytosis (Redinbaugh & Hogenhout, 2005; Jackson et. al. 2005). The matrix protein (M) connects the G protein and envelope to the ribonucleocapsid core. Polymerase-associated phosphoprotein (P) and the multifunctional polymerase (L) play important roles in virus replication and gene expression (Jackson et al., 2005).

MMV is an important disease to corn production in Hawaii (Brewbaker, 1981; Brewbaker, 2003) and other corn growing regions such as Puerto Rico, Mexico, Belize, Guatemala, Nicaragua, Tanzania, Malagasy, Mauritius, Guam, Fiji, Australia, and the United States (Singh & Seetharama, 2008). It was first reported on corn in Hawaii as early as 1914 (Kunkel, 1921). Infection causes acute chlorosis to the leaf veins, sheaths, stalk, and ear husks. Infection also leads to stunted plant growth (Herold, 1972). These symptoms typically occurs 7-

14 days post inoculation (Ammar et al., 1987). These effects can lead to serious economic loss due to plant death or poor ear development, especially if infection occurs early in plant development. Because of the severity of this virus, Brewbaker (1979) suggested that the collapse of the Mayan civilization of Central America was in association with epidemics of *Maize mosaic virus*.

Virus-Vector Interaction

Peregrinus maidis is the only known vector of MMV. Kunkel (1922) was first to report the vector transmission of MMV. The virus is acquired by nymphs and is transmitted in a persistant-propagative manner (Carter, 1941; Falk & Tsai, 1985). Because the virus replicates within its planthopper vector (Ammar & Hogenhout, 2004; Hogenhout et al., 2008), MMV is transmitted throughout the insects life span.

The infection route of MMV within *P. maidis* has been recently investigated. Ammar and Hogenhout (2008) illustrated that the virus disseminates to the salivary glands via the nervous system, instead of through the hemolymph as originally thought. In short, the virus first accumulates along the cells forming the anterior part of the midgut and anterior diverticulum, travels to the esophagus, and through the neural tissues, then infecting the salivary glands. The virus progressively spreads probably through the neural tissue and further throughout other insect's organs like the skeletal muscles, fat tissues, hindgut, and the epidermis (Ammar & Hogenhout, 2008; Ammar et al., 2009). Although virus infection is also found throughout germ cells of both males and females, offspring are not infected. MMV requires 3-4 weeks to reach the salivary glands prior to transmission (latent period) (Ammar & Hogenhout, 2008). In general, the longer a vector feeds on an infected plant the higher the rates of acquisition, however this does not appear to be the case for MMV, since the transmission does not increase with access feeding time longer than 1 week (Ammar & Hogenhout, 2008).

In fact, only a proportion (25-30%) of planthoppers that feed on MMV-infected plants become vectors of MMV (Ammar & Hogenhout, 2008; Carter, 1941). The low infection rates are caused by barriers that hinder virus replication and movement within the insect and thus, limiting virus transmission (Hogenhout et al., 2003; Whitfield et al., 2011). Transmission of *P. maidis* injected with MMV directly into the haemocoel lead to a much higher transmission rates (over 90%) then *P. maidis* that acquired the virus orally from infected corn. This suggests the planthoppers midgut acts as the primary barrier to virus invasion (Hogenhout et. al., 2003; Hogenhout et. al., 2008). Viral infection requires specific interactions with receptors (protein) located in the gut epithelium and the viruses' glycoprotein (G) (Ammar et al., 2009; Hogenhout et al., 2008). The planthopper also has active immune responses active against pathogen's invasion (Whitfield et al., 2011).

Corn Production in Hawaii:

Corn for seed production is a vital part of Hawaii's agriculture economy. Ideal year round growing conditions allow 3 to 4 crop cycles in a single year. According to a recent report by the National Agricultural Statistics Service (Sept. 2011), Hawaii's seed crop industry is currently valued at $224 million, a 74% increase from $44 million in 2001. Hawaii's corn seed industry has increased their production acreage of 56% (from 3,100 to 7,100 acres) and seed production by 53% (from 5,600,000 to 12,000,000 pounds). This rapid expansion and production has led to elevated concerns about pest and disease outbreaks.

Continuous corn plantings and high fertilizer inputs promote large *P. maidis* populations and, consequently high incidences of MMV. Continuous insecticide applications are used to control *P. maidis* populations and suppress MMV infection. However, this practice results in increased production costs, risks of insecticide resistances, and concerns for public health and worker safety. The use of parasitoids for biological control would be desirable, however, parasitism from parasitic wasps such as *Aranagrus optabilis* Perkins, *Paranagrus frequens* Perkins, *Paranagrus flaveolus* Waterhouse, *Pseudogonatopus hospes* Perkins, *Ootetranhchus beatus* Perkins, and *Haplogonatopus vitiensis* Perkins were found to have limited success in Hawaii (Napompeth, 1973; Olmi, 1984; Wilson, 1980; Zimmerman, 1948). Mirid egg predators such as *Cyrtorhinus lividipennis* Reuter, *Conocephalus saltator* (Saussure), *Cyrtorhinus mundulus* (Breddin), and *Tytthus mundulus* (Breddin) were also not successful in Hawaii (Fullaway & Kraussn, 1945; Napompeth, 1973; Verma, 1955). Currently, there are no other means of control. Together, both MMV and *P. maidis* threatens the economic sustainability of Hawaii's growing corn seed industry.

MMV Resistant Corn and gene *Mv*

Inheritable resistant to MMV was identified through an intensive tropical maize breeding program in large effort by Dr. James Brewbaker (College of Tropical Agriculture & Human Resources; University of Hawaii) and colleagues. Many inbred parent stocks, susceptible MMV, have been converted to incorporate MMV resistance. Resistant germoplasm seems to have originated from Caribbean and Cuban flint material (Brewbaker, 1981; Ming et al., 1997; Vega et al., 1995).

Though molecular mapping techniques, Ming et al. (1997) was able to locate a major gene conferring MMV resistance on chromosome 3. This resistance is denoted as gene "*Mv*". This resistance is characterized as monogenic with a co-dominate gene action (Ming et. al.; 1997). With co-dominance, alleles from each parent line, recessive and dominant, are equally expressed in the hybrid genotype. Hence, under field conditions, hybrid (heterozygote; *Mv/mv*) corn lines show varying levels of phenotypic resistance under sever epiphytotics (Brewbaker, 1979; Brewbaker, 1981; Brewbaker & Josue, 2007; Brewbaker et al., 1991; Ming et al., 1997). However, MMV resistant corn lines (*Mv* dominant) can show 5-15% MMV infection, but they do not show symptoms of severe stunting as the susceptible lines. Therefore, Brewbaker (1981) has regarded this resistance as not immunity but rather a high level of resistance to MMV infection.

Conversions of many sweet corn varieties and field corn inbreds were achieved through five or more backcrosses followed by several generations of sib and self-pollinations to achieve near isogenic lines (Brewbaker, 1981; Brewbaker, 2003; Brewbaker et al., 1991; Ming et al., 1997), with much of the screening conducted under a high field pressure of MMV. Many of the Hawaiian inbred corn cultivars are currently integrated with this resistance (Brewbaker, 1981; Brewbaker, 2003) and many of the resistant sweet corn varieties are being utilized by local farmers.

Summary of Chapters:

There is a considerable need to develop alternative management strategies that will reduce number of insecticide applications and enhance sustainable of corn production in Hawaii. Successful Integrated Pest Management programs require adequate knowledge of the ecological and biological interactions between the pest and pathogen involved. As described earlier in this chapter, both biology and the transmission characteristics of MMV have been well studied. However, there is no information on how MMV infection influences the biology and behavior of its planthopper vector. Knowledge on the epidemiology of MMV and corn resistance to MMV may help design future control strategies.

Chapter two focuses on the effects of MMV on *P. maidis* development, longevity, fecundity, and morphology. Wing dimorphism is an important factor in virus and vector epidemiology. In chapter three, I determine if planthopper wing form or gender are factors linked with virus acquisition. I therein compare the rates of MMV acquisition between field collected and greenhouse reared planthoppers. In the same chapter I examine the population dynamics of planthoppers on corn plants with and without symptoms of MMV.

The use of resistant varieties still can be considered as one of the most cost-effective and environmentally friendly pest and disease control option (Brewbaker, 1981; Dintinger et al., 2005; Redinbaugh et al., 2004). Although resistance to MMV has been conferred through field studies and genetic mapping, there is no knowledge on the mechanisms of this resistance. It is unclear if the resistance is directed toward the planthopper or toward the corn virus. Chapter four means to investigate whether or not MMV resistance had an effect on the life history, virus acquisition, and preference of *P. maidis*. The significance of these ecological and biological finding are discussed in each chapter in the light of developing a more sustainable options for MMV and corn planthopper management.

References:

Ammar ED, Gingery RE & Nault LR (1987) Interactions between Maize mosaic virus and Maize stripe viruses in their insect vector, Peregrinus-maidis, and in Maize. Phytopathology 77: 1051-1056.

Ammar ED & Hogenhout SA (2004) Immunofluorescence localization by confocal microscopy of Maize mosaic virus (Rhabdoviridae) in organs and tissues of its planthopper vector Peregrinus maidis. Phytopathology 94: S4-S4.

Ammar ED & Hogenhout SA (2008) A neurotropic route for Maize mosaic virus (*Rhabdoviridae*) in its planthopper vector *Peregrinus maidis*. Virus Res 131: 77-85. doi:10.1016/j.virusres.2007.08.010.

Ammar ED, Tsai CW, Whitfield AE, Redinbaugh MG & Hogenhout SA (2009) Cellular and molecular aspects of Rhabdovirus interactions with insect and plant hosts. Ann Rev Entomol 54: 447-468. doi:10.1146/annurev.ento.54.110807.090454.

Backus EA, Serrano MS & Ranger CM (2005) Mechanism of hopperburn: An overview of insect taxonomy, behavior, and physiology. Ann Rev Entomol 50: 125-151. doi:doi:10.1146/annurev.ento.49.061802.123310.

Brewbaker J (1979) Diseases of maize in the wet lowland tropics and the collapse of the Classic Maya civilization. Econ Bot 33: 101-118. doi:10.1007/bf02858277.

Brewbaker JL (1981) Resistance to maize mosaic virus: Virus and viruslike diseases of maize in the United States (ed. by DT Gordon, Knoke J.K., Scott G.E.) Southern Coop Ser Bull 247, p. 7.

Brewbaker JL, ed. (2003) Corn production in the tropics: The Hawaii experience. College of Tropical Agriculture and Human Resources, University of Hawaii Manoa, Honolulu, Hawaii, USA.

Brewbaker JL & Josue AD (2007) Registration of 27 Maize parental inbred lines resistant to Maize mosaic virus. Crop Sci. 47: 459-461. doi:10.2135/cropsci2005.11.0407.

Brewbaker JL, Kim SK & Logrono ML (1991) Resistance of tropical maize inbreds to major virus and virus-like disease. Maydica: 257-265.

Carter W (1941) *Peregrinus maidis* Ashmed, and the transmission of corn mosaic. Annl Entomol Soc Am 34: 5.

Denno RF, Douglass LW & Jacobs D (1985) Crowding and host plant nutrition – Environmental deterimants of wing-form in *Prokelisia-marginata*. Ecology 66: 1588-1596.

Denno RF & Roderick GK (1990) Population biology of planthoppers. Ann Rev Entomol 35: 489-520.

Dintinger J, Boissot N, Chiroleu F, Hamon P & Reynaud B (2005) Evaluation of maize inbreds for maize stripe virus and maize mosaic virus resistance: Disease progress in relation to time and the cumulative number of planthoppers. Phytopathology 95: 600-607.

Falk BW & Tsai JH (1985) Serological detection and evidence for multiplication of Maize mosaic-virus in the planthopper *Pereginus-maidis*. Phytopathology 75: 852-855.

Fullaway DT & Kraussn L (1945) Common Insects of Hawaii. Tongg Pub. Co. , Honolulu.

Giffard WM (1922) The distribution and island endemism of Hawaiian Delphacidae (Homoptera) with additional lists of their food plants. Proc. Hawaiian Entomol. Soc. 5: 103-118.

Herold R (1972) Maize mosaic virus. CMI/AAB Description of Plant Viruses #94.

Hogenhout SA, Ammar E-D, Whitfield AE & Redinbaugh MG (2008) Insect vector interactions with persistently transmitted viruses. Ann Rev of Phytopathol 46: 327-359. doi:doi:10.1146/annurev.phyto.022508.092135.

Hogenhout SA, Redinbaugh MG & Ammar E-D (2003) Plant and animal rhabdovirus host range: a bug's view. Trends in Microbiol 11: 264-271. doi:10.1016/s0966-842x(03)00120-3.

Jackson AO, Dietzgen RG, Goodin MM, Bragg JN & Deng M (2005) Biology of plant rhabdoviruses. Ann Rev Phytopathol 43: 623-660. doi:10.1146/annurev.phyto.43.011205.141136.

Kunkel LO (1921) A possible causative agent for the mosaic disease of corn. Hawaii Sugar Plant Assoc. Bot. Ser. 3: 14.

Kunkel LO (1922) Insect transmission of yellow stripe disease. Hawaiian Planters' Rec 26: 58-64.

Metcalf ZP (1943) General catalogue of the Hemiptera, fasc. IV. Fulgoroidea, part 3, Araeopidae (Delphacidae). Smith College, Northhamptom, Mass.: 553.

Ming R, Brewbaker JL, Pratt RC, Musket TA & McMullen MD (1997) Molecular mapping of a major gene conferring resistance to maize mosaic virus. Theor Appl Gen 95: 271-275. doi:10.1007/s001220050559.

Namba R & Higa SY (1971) Host plant studies of the corn planthopper, *Peregrinus maidis* (Ashmead), in Hawaii. Proc Hawaiian Entomol Soc 21: 105-108.

Napompeth B (1973) Ecology and population dynamics of the corn planthopper, *Peregrinus maidis* (Ashmead) (Homoptera: Delphacidae) in Hawaii. Ph. D Thesis University of Hawaii Honolulu, HI.

National Agriculture Statistics Service. 2011. Statistics of Hawaii Agriculture. U.S. Department of Agriculture, Honolulu, HI.

Nault LR & Knoke JK (1981) Maize vectors: page 77-84 in Virus and viruslike diseases of Maize in the United States. (ed. by DT Gordon, JK Knoke & GE Scott) South Coop Ser Bull 274, pp. 218.

Nishida T (1978) Management of the corn planthopper in Hawaii. Plant Protection Bull. 26: 5-9.

Olmi M (1984) A revision of the Dryinidae (Hymenoptera). Amer Entomol Inst 37: 1-1913.

Redinbaugh M & Hogenhout S (2005) Plant Rhabdoviruses: In The world of Rhabdoviruses (ed. by Z Fu) Springer Heidelberg, Berlin pp. 143-163.

Redinbaugh MG, Jones MW & E. GR (2004) The genetics of virus resistance in maize (*Zea Mays L.*). Maydica: 183-190.

Reed SE, Tsai CW, Willie KJ, Redinbaugh MG & Hogenhout SA (2005) Shotgun sequencing of the negative-sense RNA genome of the rhabdovirus Maize mosaic virus. J Virol Meth 129: 91-96. doi:10.1016/j.jviromet.2005.05.013.

Singh BU & Seetharama N (2008) Host plant interactions of the corn planthopper, *Peregrinus maidis* Ashm. (Homoptera: Delphacidae) in maize and sorghum agroecosystems. Arthropod-Plant Inter 2:163-196. doi:10.1007/s11829-007-9026-z.

Takara J & Nishida T (1983) Spatial distribution of the migrants of the corn delphacid, *Peregrinus maidis* (Ashmead) (Homoptera:Delphacidae) in cornfields. Proc Hawaiian Entomol Soc 24: 7.

Tsai JH (1996) Development and oviposition of *Peregrinus maidis* (Homoptera: Delphacidae) on various host plants. Florida Entomol 79: 19-26.

Tsai JH & Zitter TA (1982) Characteristics of Maize stripe virus transmission by the corn delphacid. J Econ Entomol 75: 397-400.

Tsai JH & Wilson SW (1986) Biology of *Peregrinus-maidis* with descriptions of immature stages (Homoptera, Delphacidae). Annl Entomol Soc Am 79: 395-401.

Van Duzee EP (1897) A preliminary review of the North American Delphacidae. Bull Buffalo Soc Nat Sci Buffalo, N. Y. pp. 225-261.

Vega FE, Barbosa P, Kuosell HL, Fisher DB & Nelsen TC (1995) Effect of feeding on healthy and diseased corn plants on a vector and on a non-vector insect. Experientia 51: 293-299.

Verma JS (1955) Biological studies to explain the failure of *Cyrtorhinus mundulus* (Breddin) as an egg-predator of *Peregrinus maidis* (Ashmead) in Hawaii. Proc Hawaii Entomol Soc 15: 623–634.

Whitfield AE, Rotenberg D, Aritua V & Hogenhout SA (2011) Analysis of expressed sequence tags from Maize mosaic rhabdovirus-infected gut tissues of *Peregrinus maidis* reveals the presence of key components of insect innate immunity. Insect Mol Bio 20: 225-242. doi:10.1111/j.1365-2583.2010.01060.x.

Wilson SW (1980) The planthoppers or Fulgoroidea of Illinois with information on the biology of selected species. Ph.D. Thesis Southern Illinois University at Carbondale, Illinois, USA, p. 465pp.

Zimmerman EC (1948) Insects of Hawaii. University of Hawaii Press, Honolulu, Hawaii, USA.

Chapter 2

Influence of *Maize mosaic virus* (Rhabdoviridae, Nucleorhabdovirus) on the fitness and wing morphology of its planthopper vector, *Peregrinus maidis* (Hemiptera, Delphacidae).

Abstract

Maize mosaic virus (MMV; *Rhabdoviridae*) is a plant *nucleorhabdovirus* transmitted in a persistent-propagative manner by the corn planthopper, *Peregrinus maidis*. The fitness of *P. maidis* developing on healthy or MMV-infected corn leaves from either young (less than 30 days old) or old (more than 58 days old) corn plants were examined. Since MMV is acquired at a low rate, the planthoppers that developed from the infected leaves were segregated into 2 categories as determined by ELISA tests; MMV-infected, and MMV-exposed. Planthoppers that developed on healthy leaves were deemed “unexposed” and used as a control. Mean developmental time of MMV-infected and unexposed planthoppers were not significant different; however, MMV-exposed planthoppers developed slightly, yet significantly slower than the previous two categories. Development on younger leaves was significantly faster compared to that on older leaves. Longevity was not significantly different. However, unexposed planthoppers were more fecund then both MMV-exposed and MMV-infected planthoppers, which displayed a similar reduced fecundity. Since the abundance of wing forms may vary in response to host-plant physiology, the influence of MMV on the production of macropters and brachypters was examined. The proportion macropters produced on young healthy and MMV-infected leaves were similar, but a significantly higher proportion macropters were produced on older MMV-infected leaves compared to healthy leaves of similar age. Our findings suggest MMV may indirectly influence the dispersal of the corn planthopper through host-plant infection.

Introduction

Many plant viruses are exclusively transmitted by arthropod vectors as their means of spreading to new hosts. Therefore, much of the fitness of plant viruses depends upon their compatibility with the vector(s) and sustaining specific interactions (Power, 2000). To maximize transmission rates, plant viruses may influence the biology and behavior of their vector(s)

(Bosque-Pérez and Eigenbrode, 2011; Maramorosch and Jensen, 1963). It is believed that plant pathogenic viruses may manipulate their arthropod vectors as a result of the direct interaction of the virus with the insect. In addition, viruses may also indirectly influence their vectors as a result of the host plant infection, leading to induced changes in plant attractiveness to the vector or changes in vector performance. Studies that have focused on the direct effects of a plant virus have often demonstrated positive effects on the fitness of their insect vectors (Belliure et al., 2005; Fereres et al., 1989; Inoue and Sakurai, 2006; Miller and Coon, 1964). Conversely, research that focused on the indirect effects have revealed a variety of interactions on the vector's fitness that vary according to the virus-vector-host plant combination and range from positive (Belliure et al., 2005; Maris et al., 2004b) to negative (DeAngelis et al., 1993; Jensen, 1959; Khan and Saxena, 1985).

Vector-virus interactions may also depend on the level of intimacy between a plant virus and its insect vector(s), which is related to the mode of transmission (Power, 1992; Power & Flecker, 2003). Insect-vectored plant viruses are divided into four broad categories based on the transmission mode. Non- and semi-persistent viruses are short lived within their insect vectors and viral load is lost after acquisition in a very short amount of time. These associations are considered to have low intimacy levels since non- and semi-persistent viruses do not enter into insect body, instead are retained extracellularly, in the stylet or foregut (Ng & Falk, 2006). Non-persistent viruses have been shown to have either positive (Hunt and Nault 1990, Mayer et al. 2002) or negative (Lowe and Strong 1963, Ellsbury et al. 1985, Khan and Saxena 1985) effects on their insect vector's fitness. Circulative and propagative plant viruses are retained for a long time within their insect vectors, and are assumed to involve higher levels of direct interaction. In general, persistent viruses invade the vector's gut tissues, enter the hemocoel, and then infect the salivary glands before being transmitted to new host plants. Since circulative plant viruses do not replicate within the vector's body, it has been suggested that their effects on the vector's fitness are probably mainly indirect, whereas the effects of propagative plant viruses, which replicate within the vector's tissues, may be both direct and indirect (Sisterson, 2009). Sometimes prolonged feeding on virus-infected plants may lead to detrimental effects on vector performance, such as reduced life expectancy, fecundity, population growth, and increase mortality (Castle & Berger, 1993; Cohen et al., 1987; Rubinstein & Czosnek, 1997; Donaldson & Gratton, 2007). However, other studies have reported beneficial effects to vector's biology with increased longevity, survivorship, and faster development (Belliure et al., 2005; Stumpf & Kennedy, 2007; Hodge & Powell, 2010). Overall the outcome of these interactions are difficult to be generalized and predict probably because plant viruses and their arthropod vectors are very diverse and have a variety of interactions (Cohen et al., 1987).

Members of the virus family *Rhabdoviridae* are known to infect vertebrates, invertebrates, and plants. In general, the transmission of rhabdoviruses by insect vectors is highly specific. Plant-infecting rhabdoviruses replicate in their insect vectors but are not vertically transmitted to their offspring (Hogenhout, 2003). *Maize mosaic virus* (MMV) **(***Rhabdoviridae: Nucleorhabdovirus*) is an economically important plant pathogen of corn (*Zea mays* L.) (Brewbaker, 2003). MMV is transmitted exclusively by the corn planthopper, *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) in a persistent-propagative manner (Ammar & Nault, 2002; Falk & Tsai, 1985). The infection route of MMV within *P. maidis* has been recently investigated. Ammar and Hogenhout (2008) demonstrated that the virus disseminates to the salivary glands via the neural tissue, instead of through hemolymph as originally thought. MMV progressively infects many organs and tissues, reaching high concentrations. However, approximately only 25-30% of planthoppers that feed on MMV-infected plants can vector the virus (Ammar & Hogenhout, 2008). The low infection rates are caused by barriers that hinder virus replication and movement within the insect, thus limiting transmission (Hogenhout, 2003; Whitfield et al., 2011). Since virus injection directly into the haemocoel led to higher transmission rate of MMV than by oral acquisition, it has been suggested the planthopper's midgut is a primary barrier for MMV acquisition (Hogenhout et. al., 2003; Ammar et. al., 2008).

Plant viruses may influence the occurrence of different wing morphs of their insect vectors. For instance, it has been shown that aphids colonizing virus-infected plants produce a greater number of winged morphs (alate) than those on healthy plants, which is believed to enhance the fitness of the virus by increasing the migration potential of their vectors (Blua & Perring, 1992; Gildow, 1980; Hodge & Powell, 2010). Several species of planthoppers have dimorphic wing forms called brachypters and macropters (Tsai & Wilson, 1986). The brachypters have short wings and are not effective flyers whereas the macropters are better adapted for long distance migration, having fully developed wings (Denno & Roderick, 1990). However, unlike the macropters, brachypters are typically more fecund, and therefore better suited to exploit the host plant's resources (Denno & Roderick, 1990). Generally, the production of winged form can be associated with changes in host plant quality and planthopper density (Denno & Roderick, 1990). For instance, previous research conducted by Denno et al. (1985), illustrated that crowding favored the production of macropters in the planthopper *Prokelisia marginata* (Van Duzee, 1897). However, this effect is mitigated when host plants received high levels of nitrogen fertilization. I have observed similar patterns for *P. maidis* colonizing corn fields (unpublished data).

Here, the developmental time, the fecundity, and longevity of planthoppers that developed on MMV-infected versus healthy corn leaves were compared. Furthermore, I examined how plant age (young versus old) may influence the ratio of macropters to brachypters. I took advantage of the fact that only a portion of planthoppers become infected while feeding from MMV-infected corn plants (Falk and Tsai, 1985; Ammar and Hogenhout, 2008). The direct effects of MMV infection was distinguished from the additive indirect effects by determining the infection status of the planthoppers, contrasting the fitness parameters of "exposed" planthoppers - that in spite of feeding on infected plants did not acquire the virus - from the "infected" planthoppers - that by feeding on infected plants were able to acquire the virus.

Material and Methods

Experimental Design

Planthopper development on leaf cuttings from 2 different ages of corn plants that were either healthy or MMV-infected was examined. Young and old corn plants were less than 30 days old and more than 58 days old, respectively. Infected plants were produced by allowing planthoppers, after a 14 days acquisition access period (AAP), a 7-day inoculation access period (IAP) to transmit the virus to 7 day old corn seedlings. MMV young and old plants were used after 12-14 days and 58 days post-inoculation, respectively. Only leaves that expressed symptoms of MMV infection such as chlorotic streaking of the leaf veins and stunting of the internodes were used (Herold, 1972).

Immediately upon adult emergence, planthoppers were provided with only healthy plant leaves of similar age for feeding and oviposition for their entire adult lifespan. We used the basal portion of the leaves, which are typically colonized by the planthoppers in the field. To assess influence of MMV on development, fecundity and longevity, planthoppers used in the experiments were divided into 3 categories: planthoppers that developed on MMV-infected leaves and tested positive for MMV by ELISA assays were classified as “infected”; planthoppers that developed on MMV-infected leaves but tested negative by ELISA assays, having escaped virus infection, were categorized as “exposed”; and planthoppers that developed on non-infected corn leaves were considered “unexposed”.

Insect rearing

A colony of *P. maidis* was established from adults collected from a corn field located at the Waimanalo Research Station (College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Oahu, HI). Planthoppers were maintained on potted corn seedlings, cv. Golden Cross Bantam (GCB) within mesh-ventilated insect rearing cages (356 mm x 356 mm x 610 mm) (BioQuip Products Inc.; Rancho Dominguez, CA, USA) in a greenhouse with an average temperate of 26 ± 3°C and natural light conditions. The colony was maintained by placing new potted GCB seedlings inside the rearing cages every 3-4 weeks.

Production of MMV infected plants

A MMV-infected corn seedling was obtained from a corn field at Waimanalo Research Station (College of Tropical Agriculture and Human Resources, University of Hawaii, Oahu, HI). Infection by MMV was confirmed by testing symptomatic leaf samples with an ELISA test . The seedling was transplanted into a 25 cm diameter circular pot, and maintained in the greenhouse. First and second instar nymphs were transferred onto infected the infected plant for an acquisition access period (AAP) of 14 days. These planthoppers were then transferred to one-week old GCB corn seedlings for a 7-day inoculation access period (IAP). Symptoms of MMV generally appeared after 7-14 days post inoculation and were used for the experiments as previously described or to maintain a source of MMV-infected plants in the greenhouse.

Production of first instar nymphs

Planthopper females were transferred from the rearing colony to 14 day-old GCB corn seedlings enclosed within a cylindrical cage (130 mm diameter x 300 mm tall). Females were allowed to oviposit on seedlings for a period of 24 hr. and were removed thereafter. Seedlings were maintained for another 6-7 days. Leaves bearing the eggs were detached from the plants, cut into small pieces of approximately 80 mm in length, and were placed into 150 x 15 mm Petri dishes containing moist filter paper layered at the bottom of the dish. Nymphs that hatched within 24 hr. from the onset of the hatching were used for the experiments.

Development, longevity, and fecundity

First instar nymphs were individually placed into small glass tubes (12 x 75 mm) (Fisher Scientific, Pittsburgh, PA, USA) containing either healthy or MMV-infected corn leaf cuttings with the midrib. To avoid rapid dehydration, each leaf cutting was embedded on the bottom of each tube into 1 ml of 1.2% agar (Fisher Scientific, Pittsburgh, PA, USA). A dental cotton swab (38 x 10 mm) was used to cover the top of each tube. Tubes were maintained in a growth chamber (VWR International, Randor, PA, USA; Model 2015) set at a temperature of 26°C and with a photoperiod of 12h : 12h (L:D). Nymphs were transferred to new tubes containing fresh healthy or MMV-infected leaf cuttings every 3-5 days. Tubes were inspected daily and examined for the presence of exuviae to confirm molting. The dates of each molt, duration of stadia, mortality, sex, and adult morphology at completion of the development (macropters or brachypters) were recorded.

Planthoppers that developed from either healthy or MMV-infected corn leaves were transferred as male and female pairs into new glass tubes (13 x 100 mm) (Fisher Scientific, Pittsburgh, PA, USA) containing only healthy leaf cuttings that were prepared as previously reported. Planthopper pairs were transferred every two days to new tubes containing fresh healthy leaves. The number of eggs laid per leaf was counted under a dissecting microscope. The average number of eggs laid per female per day, the total number of eggs laid per female life span, and adult longevity were determined. Dead planthoppers were individually placed in 1.5 ml centrifuge tubes and stored in -20°C until processed for MMV detection.

MMV detection

Double Antibody Sandwich (DAS) ELISA was used to detect MMV in planthoppers. Planthoppers that developed on MMV-infected leaves, and tested positive for MMV, were deemed as “infected”. Conversely, planthoppers that developed on MMV-infected leaves but tested negative for MMV were categorized as “exposed,” having escaped virus infection. The ELISA test was conducted following manufacture protocol (AGDIA; Indiana, USA). Absorbance was measured with a microplate reader (BioRad, Hercules, CA, USA) at a wavelength of 405nm. Samples were considered positive for MMV if absorbance values exceeded the mean negative control readings by 2.5 times. Negative control samples were prepared from non-infected planthoppers collected from the rearing colony. MMV-injected planthoppers that successfully transmitted MMV to corn seedlings were used as positive control.

Data Analysis

Data on developmental time, adult longevity, and fecundity were analyzed using a mixed model analysis of variance (PROC MIXED, SAS Software 9.2; SAS Institute, 2008, Cary, N.C.).

For the development time, main effects were plant age (young vs. old), and planthopper infection status (healthy vs. MMV-exposed vs. MMV-infected). The interaction of the main effects was included in the model. Replications were designated as a random factor. For adult longevity, the main effects were planthoppper infection status, and morphology (macropters vs. brachypters), and and the model included an interaction term. Replications were designated as a random factor. Because of the low numbers of infected females obtained, data for the fecundity was pooled across all 3 replicates and only the main effect of planthopper infection status was compared. When significance was obtained at the level $a < 0.05$, means were separated with a Tukey's test . The data expressing the proportion of nymphal mortality were arcsine square root transformed. Reported means are from non-transformed data. Contingency tables were used compare the proportion of wing forms produced across the different treatments and analyzed using a χ^2-square test of independence (PROC FREQ, SAS Software 9.2).

Results

MMV Infection

The planthoppers used in our experiments were individually tested by ELISA assay for MMV infection. Out of 215 planthoppers that developed from MMV-infected leaves, 59 tested positive for MMV (27%). The proportion of MMV-infected planthoppers that developed on young (31/101; 31%) and older MMV-infected leaves (28/114; 25%) were not significantly diferent ($\chi^2 = 1.011$; df = 1; $P > 0.05$). Infection rates of macropterous males, macropterous females, brachypterous males, and brachypterous females were 21% (15/21), 30% (12/40), 31% (31/32) and 30% (22/73), respectively. The proportion of MMV infection did not differ by wing morphology: macropters (32/105; 30%) and brachypters (27/110; 26%) or by sex: males (25/102;

25%) and females (34/113; 30%); (χ^2 = 0.949; df = 1; P > 0.05) and (χ^2 = 0.838; df = 1; P > 0.05), respectively.

Development

To ascertain the direct effect of MMV infection on *P. maidis* fitness, planthoppers that developed from MMV-infected corn leaves were segregated into 2 categories, MMV-infected and MMV-exposed, as determined by ELISA tests. These categories were compared with unexposed planthoppers, which developed on healthy leaves.

ANOVA showed that both the effects of host plant age ($F_{1, 425}$ = 37.25; P < 0.0001) and insect infection status ($F_{2, 425}$ = 4.67; P < 0.0039) had a significant effect on planthopper development while the interaction between both effects was not significant (P > 0.05). Table 2.1 shows the mean developmental time of *P. maidis* developing for the various treatments. Planthoppers on older leaves took significantly longer to develop into adults than those developing from younger plant leaves (Table 2.1). MMV-exposed planthoppers took longer to develop into adults than unexposed (P = 0.009) and MMV-infected planthoppers (P = 0.0238; Figure 2.1).

Nymphal mortality

Nymphal mortality did not differ significantly between plants of different age (young vs. old), plant infection status (healthy vs. MMV-infected plants (P > 0.05). Similarly, nymphs developing on MMV-infected leaves tended to experience higher mortality than nymphs developing on healthy plant leaves (19% and 16%, respectively) but was not significantly different. There were fewer nymphs dying on young healthy leaves then on MMV-infected leaves of similar age, (11% and 16%, respectively), however this was not significantly different.

Nymphs developing on older healthy and MMV-infected leaves had similar nymphal mortality (21% and 22%, respectively).

Adult longevity and fecundity

There were no significant differences in mean adult longevity between unexposed, MMV-exposed, and MMV-infected adults ($P > 0.05$; Figure 2.2; Table 2.2). There was, however, a significant difference in the mean longevity between brachypters and macropters ($F_{1, 371} = 24.00$; $P < 0.001$). In fact, macropters (46.3 ± 1.35 days) tend lived longer than brachypters (38.2 ± 1.14 days). The interaction between insect infection status and morphology was also significant ($F_{2, 371} = 3.05$; $P = 0.048$; Figure 2.2; Table 2.2), however, this is likely due to the lower longevity observed for brachypters exposed to MMV. There were no statistical differences between the longevity of MMV-exposed MMV-infected and unexposed brachypters (Figures 2.2 & 2.3).

A significant difference was detected in mean fecundity (eggs laid per day) among the three levels of insect infection status ($F_{2, 175} = 4.72$; $P = 0.01$). Both MMV-exposed ($P = 0.03$) and MMV-infected ($P = 0.04$) females laid significantly less eggs per day than unexposed females (Figure 2.4). Similar outcomes were observed when the average total eggs laid per female were compared ($F_{2, 175} = 5.73$; $P = 0.004$). Figure 2.5 illustrates the average number of eggs laid over time by unexposed, MMV-exposed, and MMV-infected females. During the first 15 days egg laying trends for all exposure levels were quite similar (Figure 2.5). After day 17, both MMV-exposed and MMV-infected females laid fewer eggs than unexposed females. MMV-infected females stopped laying eggs (~ 60 days), before MMV-exposed (~ 65 days) and unexposed (~ 68 days) females (Figure 2.5).

Morphology

There was a significant difference in the proportion of macropters reared on young healthy, young MMV-infected, old healthy, and old MMV-infected corn leaves ($\chi^2 = 54.27$; df = 3; $P < 0.001$; Figure 2.6). Although the proportion of macropters produced on young healthy (22%) and MMV-infected leaves (31%) were statistically not significant ($P > 0.05$; Figure 2.6A) the proportion of macropters reared from older MMV-infected corn leaves was 69% compared to only 41% reared from healthy corn leaves of the same age ($\chi^2 = 17.01$; df = 1; $P < 0.001$; Figure 2.6B). Since MMV infection was not associated with wing morphology, and both macropters and brachypters experienced similar infection rates, our data suggest that increase numbers of macropers observed on the old plants result from indirect effects of plant infection rather than a direct virus-planthopper interaction.

Discussion

To dissect the effects of MMV on the fitness of *P. maidis,* planthoppers that developed on MMV-infected leaves were segregated based on their infection status. Planthoppers that tested positive for MMV were deemed "infected" whereas those that tested negative were categorized as "exposed". MMV was detected by ELISA assays in 27% of the tested planthoppers. The infection rate obtained in our study is similar to the results obtained in previous research (Ammar & Hogenhout 2008), and shows that only a portion of planthoppers that feed on infected corn plants acquire MMV. Furthermore, in our experiments, MMV infection was not associated to a specific sex or wing morphology.

Our results show MMV infection had little direct influence on the planthopper's fitness, although infection resulted with a slight reduction in fecundity (18%) to exposed planthoppers, suggesting an indirect effect. Other studies that used different virus-vector combinations have shown increase in the fitness of vectors that develop on virus-infected plants. These studies suggest that an increased concentration of free amino acids and/or suppression of host plant defenses may increase vector's fitness (Belliure et al., 2005; Fereres et al., 1989; Jimenez-Martinez et al., 2004; Maris et al., 2004a; Srinivasan & Alvarez, 2007; Weibull et al., 1990).

Although not infected with virus, MMV-exposed planthoppers took longer to mature into adults than to unexposed and MMV-infected planthoppers, which both required very similar developmental times. This pattern was consistently observed for planthoppers developing on young and older plant tissues. Recently, Whitfield et al. (2011) analyzed the expressed sequence tags (EST) from MMV-infected gut tissues of the corn planthopper. They found that many of the ESTs identified had homology to proteins involved with the pathogens recognition and with innate immunity. Importantly, the analysis of the expression levels of 4 ESTs belonging to putative innate immune-response genes, such as autophagy-specific gene 3 phosphoinositol-4 5-bisphosphate-3-kinase, Jun N-terminal kinase, and tripeptidyl peptidase were down-regulated in MMV-infected guts compared to guts from unexposed planthoppers (Whitefield et al, 2011). Here, I suggest that the observed delay in the development time of MMV-exposed planthoppers may result from a cost of mounting an immune response to overcome MMV invasion. This effect may be attenuated for MMV-infected planthoppers, because viral infection suppresses the host's immune response (Whitfield et al. 2011). Testing the expression level of immune response-related genes on MMV-exposed and MMV-infected planthoppers may provide further insights.

After adult emergence, all of the planthoppers used in our experiments were provided with healthy plant leaves to feed on. This experimental setting mimics the biology of planthoppers in the field where they vacate MMV-infected plants from which they have developed for the young healthy plants. On average, macropters lived longer than brachypters; interestingly, differences in longevity between macropters and brachypters averaged to 15, 9, and 4 days for MMV-exposed, MMV-infected, and unexposed planthoppers, respectively. The average longevity of macropters did not significantly differ among the three planthopper virus exposure categories, though MMV-exposed and infected macropters lived slightly longer than the unexposed macropters.

The average fecundity of both MMV-infected and exposed planthoppers was similar and was significantly less than unexposed planthoppers. A lower reproductive output would reduce vector populations in the field and therefore reduce the rate of new infected corn plants, but this is countered by the fact that not all, or even the majority, of *P. maidis* become infected with MMV. This inefficient infection rate allows for the development of healthy vectors (from exposed females) to maintain population numbers because of their higher fecundity. Ammar and Hogenhout (2008) detected MMV infection among the nerves and visceral muscles associated with female's ovarioles and oviduct but not the ovaries themselves. This infection could explain the reduction in eggs laid by infected females, however, does not account for the reduction in fecundity in MMV-exposed females. There was no evidence of MMV being transmitted sexually as MMV rarely tested positive for both male and female mating pairs.

The physiological status of the plant is an important factor in wing morph determination in planthoppers (Denno & Roderick, 1990). Exposure to environmental cues during early to middle instars may trigger planthoppers to develop into a particular morph (Denno & Roderick, 1990; Iwanaga & Tojo, 1986; Kisimoto, 1965; Zera & Denno, 1997). In our experiment, the proportion of macropters increased from 22% to 41% when planthopper developed on young and old healthy corn leaves, respectively. Whereas the proportion of macropters increased from 31%

to 69% when planthopper developed on young and old MMV-infected corn leaves, respectively. Therefore, suggesting that MMV may influence the frequency of macropters in a plant-age related manner.

Because MMV infection was not associated with wing morphology and that both macropters and brachypters experienced similar infection rates, our data suggest that increases numbers of macropters observed on the older leaves result from indirect effects of plant infection rather than a direct virus-planthopper interaction. Therefore, MMV may influence the dispersal the corn planthopper by influencing the production of wing morphs indirectly through host-plant infection. Figure 7 proposes a life cycle of *P. maidis* in the presence or absence of MMV in corn fields. Infected macropters migrate into and colonize young corn fields. Some viruliferous planthoppers, beside colonizing and reproducing on the host plant, inoculate the seedlings with MMV. When nymphs emerge, they immediately start feeding and developing on the infected plant. On young MMV-infected plants, a large proportion of planthoppers mature into brachypters (Figures 2.6 & 2.7). Brachypters have limited dispersal activity but are highly fecund and therefore promote host colonization. MMV-infected plants age earlier than healthy plants and the production of macropters is accelerated (Figure 2.6 & 2.7). The latter effect promotes the dispersal of the planthoppers and in turn, MMV.

It has been proposed that co-evolution between pathogen and vector might result in selection pressure favoring non-deleterious or beneficial relationships (Bosque-Pérez & Eigenbrode, 2011; Elliot et al., 2003; Power, 1992). For MMV and its planthopper vector, this may be the case. Our results show MMV infection of *P. maidis* has minimal effect on the insects, but this was not the case for MMV-exposed *P. maidis*. If MMV infection is less detrimental to *P. maidis* than only exposure, then why infection rates are relatively low? Insect gut barriers and immune response that inhibit or limit the effects of virus invasion may have evolved as the result of selection pressures on *P. maidis* from interaction with MMV. There may be a constant struggle between selection against virulence and the viruses' ability to maintain specific interactions with it vector (Power, 2000). Regardless, the inefficient infection rates of this virus and vector system allows for balance between virus transmission and vector fitness. Since MMV infection is present within the neural tissues and the brain, it is likely that MMV will have an effect on *P. maidis* behavior as well. Future work should investigate possible differences in feeding behavior and host plant selection between infected, unexposed, and MMV-exposed planthoppers.

In summary, MMV infection of its vector is not severe and most effects on the insect seem to be indirect. I have proposed reduced fitness among exposed planthoppers may be due to interactions involving the insect immune response to MMV. Wing dimorphism is an important factor in virus and vector epidemiology. MMV is able to exploit this indirectly by changes to host-plant physiology. Although MMV propagates within its vector and concentration increases over time (Ammar & Hogenhout, 2008), minimal direct effects on the fitness parameters were observed. However, infection of the neural tissues suggests possible direct influence in vector behavior. It would be insightful to characterize and compare the changes in feeding patterns for MMV-infected and exposed planthoppers.

Figures

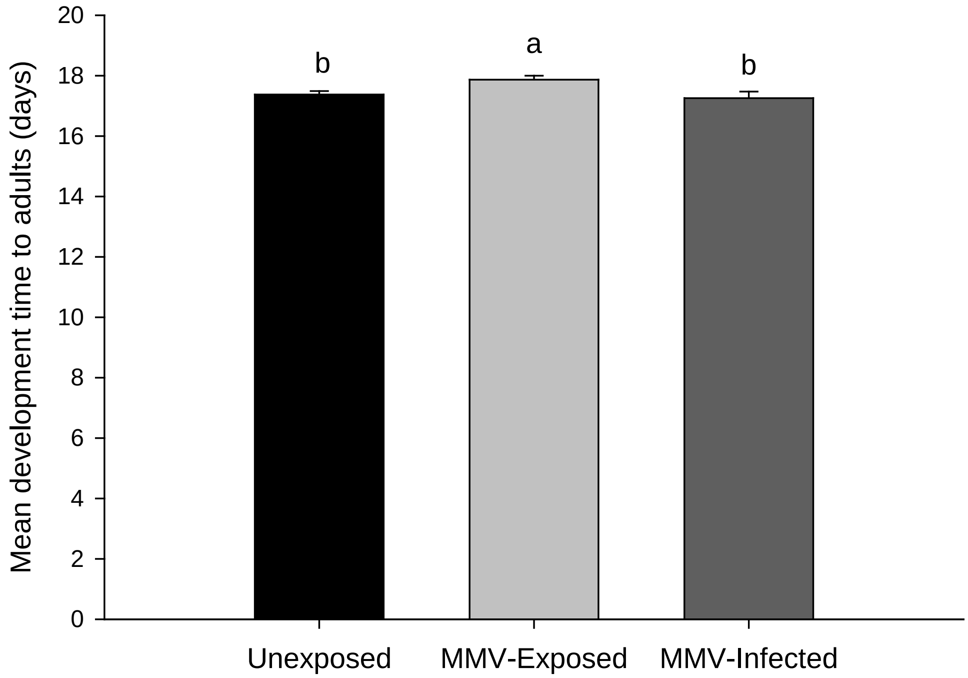

Figure 2.1. Average developmental time (days ± SE) of *Peregrinus maidis* nymphs reared on healthy and MMV-infected corn leaves. Planthoppers feeding on MMV-infected corn leaves were segregated by those that became infected with MMV ("MMV-Infected") and those that did not ("MMV-Exposed"). Planthopper that were reared on healthy plants were deemed "unexposed". Effects with the same letter indicate no significant difference.

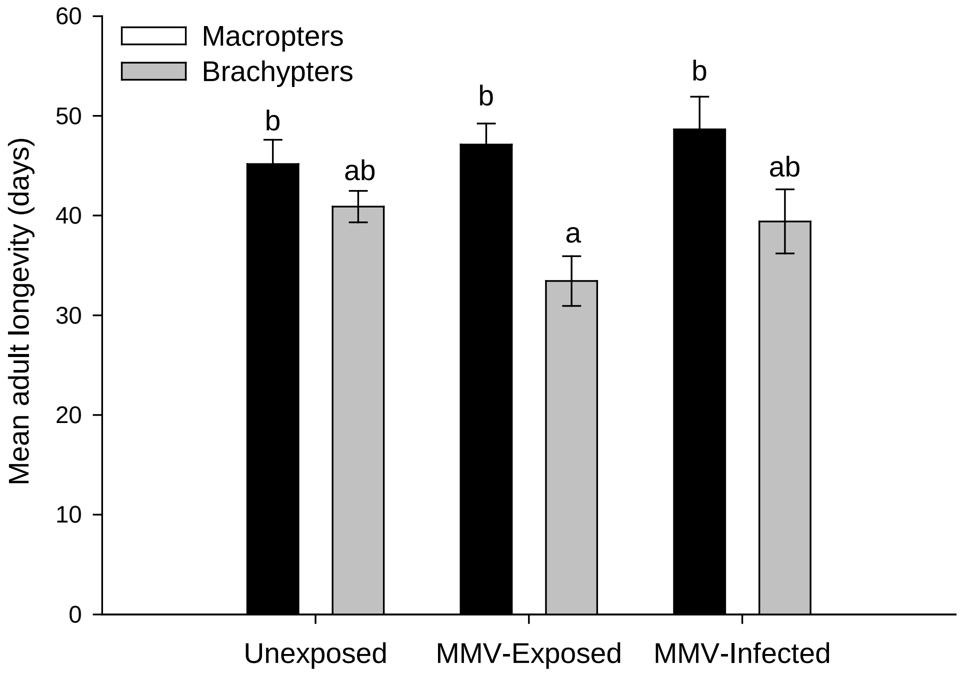

Figure 2.2. The average longevity (days ± SE) of unexposed, MMV-exposed, and MMV-infected macropters (black bars) and brachypters (grey bars) of *Peregrinus maidis* planthoppers. Effects with the same letter indicate no significant difference.

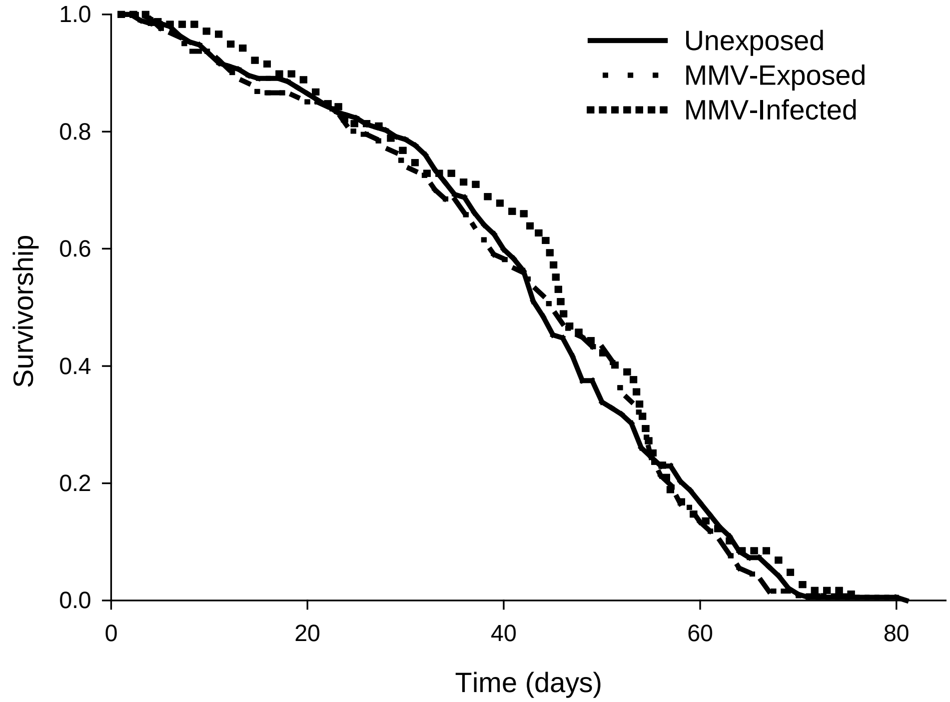

Figure 2.3. Survivorship curves of unexposed (solid line), MMV-exposed (dashed line), and MMV-infected (dotted line) *Peregrinus maidis*.

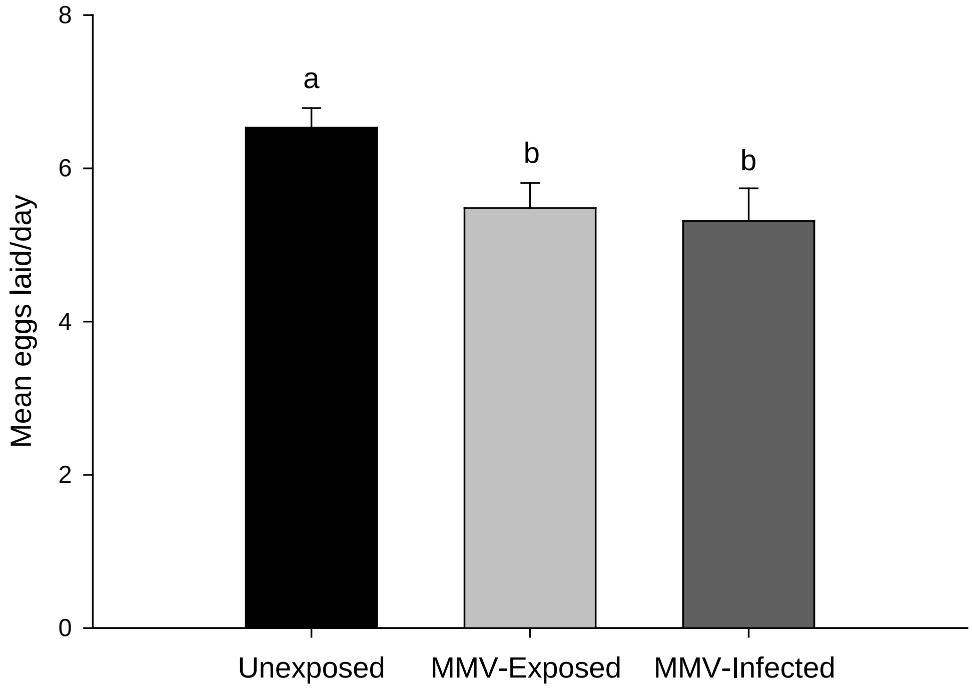

Figure 2.4. Average fecundity (eggs laid/day ± SE) of unexposed, MMV-exposed, and MMV-infected *Peregrinus maidis*. Effects with the same letter indicate no significant difference.

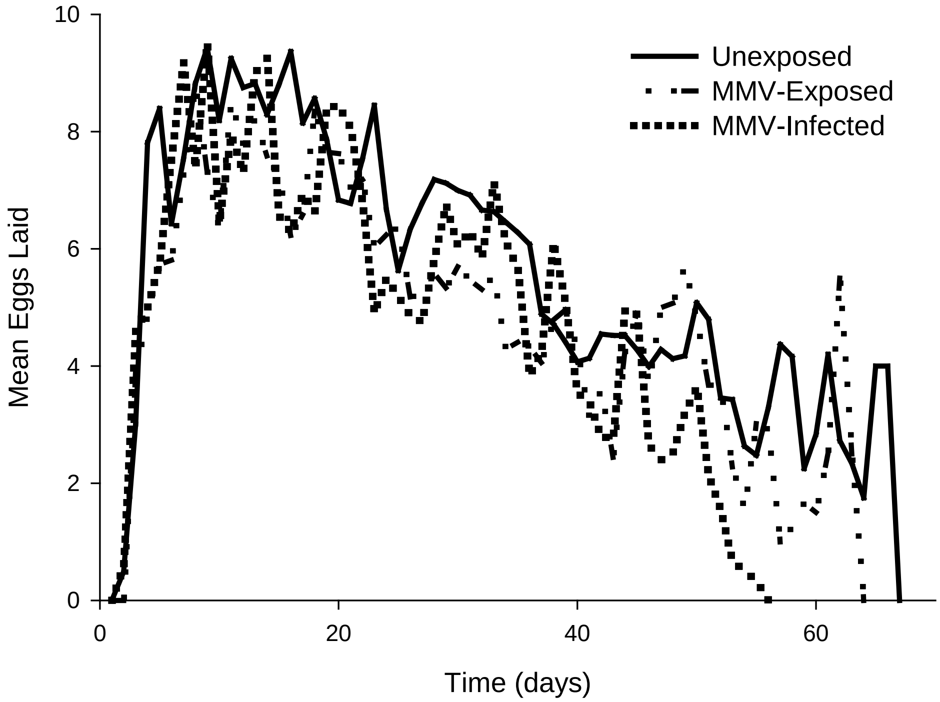

Figure 2.5. Average pattern of eggs laid by unexposed (solid line), MMV-exposed (dashed line), and MMV-infected (dotted line) *Peregrinus maidis* females.

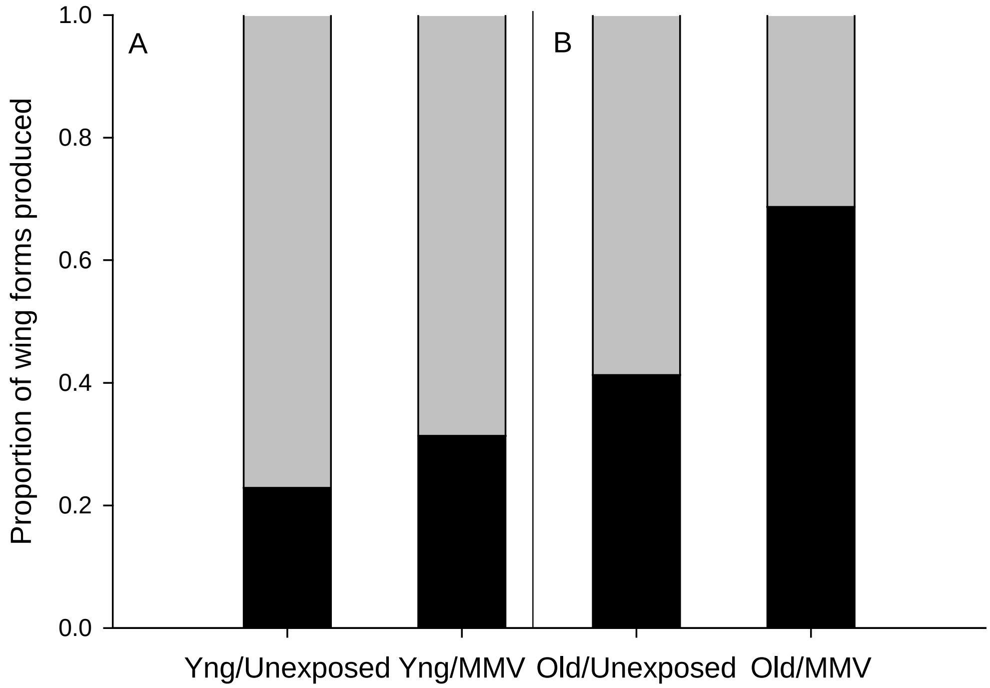

Figure 2.6. Proportions of macropters and brachypters of *Peregrinus maidis* produced from healthy and MMV-infected corn leaves of young (A) and old (B) corn plants.

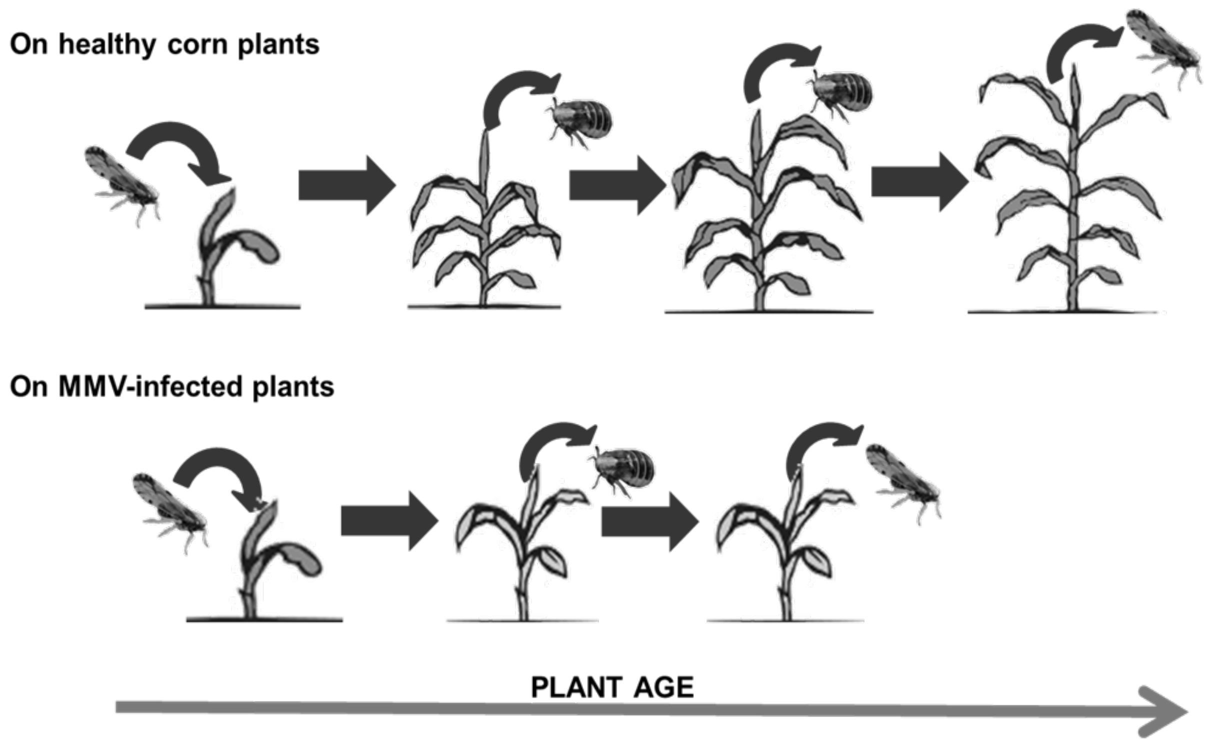

Figure 2.7. Proposed life cycle of *Peregrinus maidis* in the presence or absence of MMV in a corn planting cycle. Changes in host plant physiology due to MMV infection indirectly influence a higher production of macropters.

Tables

Table 2.1. Developmental time of *Peregrinus maidis* from healthy or MMV-infected corn leaves of different ages.

Instar	YOUNG PLANTS[i]						OLD PLANTS					
	n	Unexposed (Mean±SE)	*n*	MMV-Exposed (Mean±SE)	*n*	MMV-Infected (Mean±SE)	*n*	Unexposed (Mean±SE)	*n*	MMV-Exposed (Mean±SE)	*n*	MMV-Infected (Mean±SE)
1st		3.75±0.10a		3.63±0.08a		3.65±0.15a		4.48±0.08a		4.57±0.09a		4.43±0.18a
2nd		2.93±0.07a		3.11±0.09a		2.81±0.11a		2.96±0.06a		3.06±0.08a		3.11±0.12a
3rd		2.75±0.06b		2.90±0.07ab		2.84±0.10ab		2.86±0.06ab		3.08±0.07a		2.93±0.16ab
4th		3.25±0.06a		3.13±0.06a		3.19±0.07a		3.17±0.06a		3.08±0.08a		3.25±0.11a
5th		4.25±0.09a		4.44±0.09a		4.13±0.12a		4.34±0.07a		4.53±0.09a		4.18±0.12a
Total	106	16.99±0.35cd	70	17.30±0.37bd	31	16.52±0.43cd	110	17.88±0.35b	115	18.57±0.36a	28	17.77±0.44ab

Rows with the same letter indicate no significant difference using Tukey-Kramer test at the $P < 0.05$ level.

[i] indicates planthopper development on young plant leaves significantly shorter than old plant leaves.

Table 2.2. Average nymphal mortality (%) of *Peregrinus maidis* developing on young and old, healthy and MMV-infected plants

Plant Age	Plant Status	Mean Mortality % (±SE)	P-Value
YOUNG	*Healthy*	10.8 ± 0.225	P > 0.05
	MMV-infected	16.3 ± 0.562	
OLD	*Healthy*	21.4 ± 0.183	
	MMV-infected	22.3 ± 0.749	

Percent data arcsine transformed. No significant difference was detected at $P < 0.05$ level.

Table 2.3. Summary of ANOVA for *Peregrinus maidis* longevity.

Effect	DF	Longevity (days) F Value	Longevity (days) P Value
Insect status†	*2*	0.68	0.5050
Insect morphology‡	*1*	24.00	<.0001
Interaction	*2*	3.05	0.0485

Significance was determined at the $P < 0.05$ level.
† Comparison of unexposed, MMV-exposed, and MMV-infected *P. maidis*.
‡ Macropterous versus brachypterous wing forms.

References

Ammar ED & Hogenhout SA (2008) A neurotropic route for Maize mosaic virus (*Rhabdoviridae*) in its planthopper vector *Peregrinus maidis*. Virus Research 131: 77-85. doi:10.1016/j.virusres.2007.08.010.

Ammar ED & Nault LR (2002) Virus transmission by leafhoppers, planthoppers and treehoppers (Auchenorrhyncha, Homoptera). Adv. Bot. Res. 36: 141-167.

Belliure B, Janssen A, Maris PC, Peters D & Sabelis MW (2005) Herbivore arthropods benefit from vectoring plant viruses. Ecology Letters 8: 70-79.

Blua MJ & Perring TM (1992) Alatae production and population increase of aphid vectors on virus-infected host plants. Oecologia 92: 65-70.

Bosque-Pérez NA & Eigenbrode SD (2011) The influence of virus-induced changes in plants on aphid vectors: Insights from luteovirus pathosystems. Virus Research 159: 201-205. doi:10.1016/j.virusres.2011.04.020.

Brewbaker JL, ed. (2003) Corn Production in the Tropics: The Hawaii Experience. College of Tropical Agriculture and Human Resources, University of Hawaii Manoa, Honolulu, Hawaii, USA.

Castle S & Berger P (1993) Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship. Entomol. Exp. Appl. 69: 51-60. doi:10.1007/bf02380673.

Cohen S, Duffus JE & Berlinger MJ (1987) Epidemiological studies on whitefly-transmitted viruses in California and Israel. BARD Report Project No. I.

DeAngelis JD, Sether DM & Rossignol PA (1993) Survival, development, and reproduction in western flower thrips (Thysanoptera: Thripidae) exposed to Impatiens necrotic spot virus. Environ. Entomol. 22: 1308-1312.

Denno RF & Roderick GK (1990) Population biology of planthoppers. Annu. Rev. of Entomol. 35: 489-520.

Donaldson JR & Gratton C (2007) Antagonistic Effects of Soybean Viruses on Soybean Aphid Performance. Environ. Entomol. 36: 918-925. doi:10.1603/0046-225x(2007)36[918:aeosvo]2.0.co;2.

Elliot SL, Adler FR & Sabelis MW (2003) How virulent should a parasite be to its vector? Ecology 84: 2568-2574.

Ellsbury, MM, Pratt RG, Knight, WE (1985) Effects of single and combined infection of Arrowleaf clover with Bean yellow mosaic virus and a *Phytophthora sp*. on reproduction and colonization by Pea Aphids (Homoptera: Aphididae). Environ. Entomol. 14(3): 356-359.

Falk BW & Tsai JH (1985) Serological detection and evidence for multiplication of Maize mosaic-virus in the planthopper *Pereginus-maidis*. Phytopathology 75: 852-855.

Fereres A, Lister RM, Araya JE & Foster JE (1989) Development and reproduction of the English grain aphid (Homoptera: Aphididae) on wheat cultivars infected with Barley yellow dwarf virus. Environ. Entomol. 18: 388-393.

Gildow FE (1980) Increased production of alatae by aphids reared on Oats infected with Barley yellow dwarf virus. Ann. Entomol. Soc. Am. 73: 343-347.

Herold R (1972) Maize mosaic virus. CMI/AAB Description of Plant Viruses #94.

Hodge S & Powell G (2010) Conditional facilitation of an aphid vector, *Acyrthosiphon pisum*, by the plant athogen, *Pea enation mosaic virus*. J. of Insect Sci. 10: 1-14. doi:10.1673/031.010.14115.

Hogenhout SA (2003) Plant and animal rhabdovirus host range: a bug's view, Vol. 11: Trends Microbiol. 11: 264-267.

Hunt RE & Nault LR (1990) Influence of Life History of Grasses and Maize Chlorotic Dwarf Virus on the Biotic Potential of the Leafhopper Graminella nigrifrons (Homoptera: Cicadellidae). Environ Entomol 19: 76-84.

Inoue T & Sakurai T (2006) Infection of Tomato spotted wilt virus (TSWV) shortens the life span of thelytokous *Thrips tabaci* (Thysanoptera: Thripidae). App. Entomol. and Zool. 41: 239-246.

Iwanaga K & Tojo S (1986) Effects of juvenile hormone and rearing density on wing dimorphism and oocyte development in the brown planthopper, *Nilaparvata lugens*. J. Insect Physiol. 32: 585.

Jensen DD (1959) A plant virus lethal to its insect vector. Virology 8: 164-175. doi:Doi: 10.1016/0042-6822(59)90002-9.

Jimenez-Martinez ES, Bosque-Perez NA, Berger PH, Zemetra RS, Ding HJ & Eigenbrode SD (2004) Life History of the bird cherry-oat aphid, *Rhopalosiphum padi* (Homoptera: Aphididae), on transgenic and untransformed wheat challenged with Barley yellow dwarf virus. Environ. Entomol. 33: 1207.

Khan ZR & Saxena RC (1985) Behavior and biology of *Nephotettix virescens* (Homoptera: Cicadellidae) on tungro virus-infected rice plants: Epidemiology implications. Environ. Entomol. 14: 297-304.

Kisimoto R (1965) Studies on the polymorphism and its role playing in the population growth of the brown planthopper, *Nilaparvata lugens* Stal. Bull. Shikoku Agric. Exp. Stn. 13:1-106

Lowe S, Strong FE (1963) The unsuitability of some viruliferous plants as hosts for the Green peach aphid, *Myzus persicae*. J. Econ. Entomol. 56(3): 308-309.

Maramorosch K & Jensen DD (1963) Harmful and beneficial rffects of plant viruses in insects. Ann. Rev. Microbiol. 17: 495-530. doi:doi:10.1146/annurev.mi.17.100163.002431.

Maris PC, Joosten NN, Goldbach RW & Peters D (2004) Tomato spotted wilt virus infection improves host suitability for its vector *Frankliniella occidentalis*. Phytopathology 94: 706-711. doi:10.1094/phyto.2004.94.7.706.

Mayer RT, Inbar M, McKenzie CL, Shatters R, Borowicz V, Albrecht U, Powell CA & Doostdar H (2002) Multitrophic interactions of the silverleaf whitefly, host plants, competing herbivores, and phytopathogens. Arch Insect Biochem Physiol 51: 151-169. doi:10.1002/arch.10065.

Miller JW & Coon BF (1964) The effect of Barley yellow dwarf virus on the biology of its vector the English grain aphid, *Macrosiphum granarium*. J. Econ. Entomol. 57: 970-974.

Ng JCK & Falk BW (2006) Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. Ann. Rev. Phytopathol. 44: 183-212. doi:doi:10.1146/annurev.phyto.44.070505.143325.

Power AG (1992) Patterns of virulence and benevolence in insect-borne pathogens of plants. Crit. Rev. Plant Sci. 11: 351-372.

Power AG (2000) Insect transmission of plant viruses: a constraint on virus variability. Curr. Opin. Plant Biol. 3: 336-340. doi:10.1016/s1369-5266(00)00090-x.

Power, A. G., and A. S. Flecker. 2003. Virus specificity in disease systems: are species redundant? Pages 330–346 in P. Kareiva and S. A. Levin, eds. The importance of species: perspectives on expendability and triage. Princeton University Press, Princeton, N.J.

Rubinstein G & Czosnek H (1997) Long-term association of tomato yellow leaf curl virus with its whitefly vector *Bemisia tabaci*: effect on the insect transmission capacity, longevity and fecundity. J. Gen. Virol. 78: 7.

Sisterson MS (2009) Transmission of insect-vectored pathogens: Effects of vector fitness as a function of infectivity status. Environ. Entomol. 38: 345-355.

Srinivasan R & Alvarez JM (2007) Effect of mixed viral Iinfections (potato virus Y–potato leafroll virus) on biology and preference of vectors *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae). J. Econ. Entomol. 100: 646-655. doi:10.1603/0022 0493(2007)100[646:eomvip]2.0.co;2.

Stumpf CF & Kennedy GG (2007) Effects of tomato spotted wilt virus isolates, host plants, and temperature on survival, size, and development time of *Frankliniella occidentalis*. Entomol. Exp. Appl. 123: 139-147. doi:10.1111/j.1570-7458.2007.00541.x.

Tsai JH & Wilson SW (1986) Biology of *Peregrinus-maidis* with descriptions of immature stages (Homoptera: Delphacidae). Ann. Entolmol. Soc. Am. 79: 395-401.

Van Duzee EP (1897) A preliminary review of the North American Delphacidae. Bull. Buffalo Soc. Nat. Sci. 5: 225-261.

Weibull J, Ronquist F & Brishammar S (1990) Free amino acid composition of leaf exudates and phloem sap: A comparative study in Oats and Barley. Plant Physiol. 92: 222-226.

Whitfield AE, Rotenberg D, Aritua V & Hogenhout SA (2011) Analysis of expressed sequence tags from Maize mosaic rhabdovirus-infected gut tissues of *Peregrinus maidis* reveals the presence of key components of insect innate immunity. Insect Molec. Bio. 20: 225-242. doi:10.1111/j.1365-2583.2010.01060.x.

Zera AJ & Denno RF (1997) Physiology and ecology of dispersal polymorphism in insects. Ann. Rev. Entomol. 42: 207-230. doi:doi:10.1146/annurev.ento.42.1.207.

Chapter 3

Infection rates and comparative population dynamics of *Peregrinus maidis* on *Maize mosaic virus* symptomatic and non-symptomatic corn plants.

Abstract

Wing dimorphism is an important factor to consider in the epidemiology of plant viruses since virus transmission critically depends on the ability of the vectors to disperse to suitable plant hosts. The corn planthopper, *Peregrinus maidis* (Hemiptera: Delphacidae), has two distinct wing forms. Brachypters have short wings and are not efficient dispersers whereas macropters have long wings and are capable dispersers. In this chapter, the population dynamics of *P. maidis* on infected and non-infected plants was monitored throughout a cycle of corn production. Also, the rates of *Maize mosaic virus* (MMV) acquisition and transmission *P*, *maidis* relative to wing morphology and gender among field-collected and a laboratory colony of *P. maidis* were compared. Results from the field experiment showed that the population dynamics of *P. maidis* differed between plants that showed symptoms of MMV infection at 28 days after planting (DAP; early infection), 49 (DAP; late infection), and non-symptomatic plants. Early infected plants typically harbored less planthoppers, majority of which were brachypters. The opposite was observed on non-symptomatic plants. These differences may likely be the consequence of change in host plant physiology cause by the effects of MMV infection. No association was found between macropterous males, macropterous females, brachyterous males, and brachyterous females in relation to MMV acquisition or transmission. However, MMV incidence was higher for females may be explained by different feeding behaviors among gender.

Introduction

Maize mosaic virus (MMV) (*Rhabdoviridae*: *Nucleorhabdovirus*) causes disease of corn (*Zea Mays* L.) in Hawaii as well as many tropical and sub-tropical corn growing regions worldwide (Brewbaker, 2003; Singh & Seetharama, 2008). Susceptible corn plants infected with MMV express symptoms of dwarfing of internodes and husks, chlorotic striations (mosaic) between the leaf veins, the leaf sheath and the stalk (Herold 1972). Early infection can lead to plant death. MMV is transmitted to the plant hosts by its insect vector the corn planthopper, *Peregrinus maidis* (Ashmead) (Hogenhout et al., 2008). Transmission is highly specific and occurs following a persistent-propagative interaction (Ammar & Nault, 2002; Falk & Tsai, 1985).

Like most planthoppers (Hemiptera: Delphacidae), *P. maidis* exhibits two distinct wing forms, brachypters and macropters (Tsai & Wilson, 1986). Macropters have fully developed wings and are better adapted for dispersal, whereas brachypters have short wings not efficient dispersers (Denno & Roderick, 1990). However, unlike the macropters, brachypters are typically more fecund, and therefore better suited to exploit the host plant's resources (Denno & Roderick, 1990). Generally, the production of planthopper winged forms varies in response to changes in host plant quality and planthopper density (Denno & Roderick, 1990). For instance, poor plant host nutrition or over-crowding has been shown to favor the development of macropterous planthoppers; conversely, the majority of the planthoppers develop into brachypters on nutritionally high quality plants (Denno et al., 1985; Denno & Roderick, 1992). The regulation in wing-form production allows planthoppers to locally persist in favorable environments but to also escape unfavorable conditions, making them suitable colonizers of transient (non-persistent) habitats, including several agro-ecosystems (Denno et al., 1996; Zera & Denno, 1997). For instance, *P. maidis* alternates production of macropters and brachypters at different stages of the corn growth cycle. Macropters migrate into and colonize corn fields at early stages of corn growth. Because of the early stage of plants and high nitrogen inputs typically applied to corn plantings, the new generation of planthoppers develops mostly into brachypters. As the plant matures and the resource become poor, the proportions of macropters in the colony increase to facilitate dispersal onto higher quality corn plants.

Although poorly studied, wing dimorphism of insect vectors is an important factor to consider in the epidemiology of plant viruses. In fact, virus transmission critically depends on the ability of the vector to disperse to suitable plant hosts. It has been demonstrated that some plant viruses may indirectly increase the production insect vectors' winged morphs as a result of the host plant infection. For example, aphids colonizing virus-infected plants produce a larger numbers of winged morphs (alate) than those on healthy plants. This effect is believed to enhance the fitness of the virus by increasing the dispersal potential of their vectors (Gildow, 1980; Blua & Perring, 1992; Hodge & Powell, 2010). Because of their dispersal capability, it is likely that the spread of MMV heavily depends on macropters rather than brachypters. Thus, it would be advantageous for MMV to infect a larger number of macropterous planthoppers over brachypters. Infection rates between males and females may also be an important factor to consider as each sex may differ in feeding and dispersal behavior (Denno & Roderick, 1990).

Previously (chapter 2), I examined the influence of MMV on the biology and wing dimorphism of *P. maidis*. The major outcomes from that work demonstrated that MMV plant infection indirectly promoted a larger production of macropters at the cost of brachypters at a late stage of the plant infection. Additionally, MMV did not associate more frequently with a specific planthopper wing morphotype. However, in that study, plantoppers were individually reared on leaf cuttings within isolated culture tubes, which is unlikely to replicate natural condition of insects in the field. Here, I compared the rates of MMV acquisition and transmission by *P. maidis* relative to its' wing morphology and gender by using field-collected planthoppers on MMV-infected plants, as well as a laboratory-maintained colony of planthoppers that developed on MMV-infected plants. In addition, I examined the population dynamics of *P. maidis* on infected and non-infected (not expressing symptoms of MMV infection) plants throughout a cycle of corn production.

Material and Methods

Population Dynamics

Field Experiment

Fields of an MMV susceptible sweet corn cultivar, Golden Cross Bantam (GCB), were planted approximately every 8 weeks since August 2009 at the Waimanalo Research Station of the College of Tropical Agriculture and Human Resources, CTAHR, (University of Hawaii at Manoa, HI). Each plot consisted of four 36.58 m long rows with an inter-row spacing of 0.46 m. This temporal planting scheme favored an increase in MMV incidences (Ming et al., 1997) as a likely consequence of the continuous movement of infectious planthoppers from the old to the younger corn planting. I estimated that the overall incidence, across all plots, ranged between 60-70% of MMV-infected corn plants by visual observations.

In March of 2010, a field trail was planted adjacent to these continuous-cycle fields. Seeds of the MMV-susceptible sweet corn variety, "Obsession" (Seminis Vegetable Seeds, Inc.), were sown along 7 rows 42.7 m long, spaced approximately 0.21 m apart, using a hand planter. The inter-row spacing was 0.75 m. Prior to planting, the field was treated with pre-plant herbicide (Eradicane, Gowan Company) and pre-emergence herbicide (Prowl H20, BASF) for weed control. Atrazine (AAtrex 4L; Syngenta) was used as a post-emergent and applied at 4-5 weeks after planting. The field was fertilized (16: 16: 16, N: P: K) at 560 kg / ha before planting and side dressed with urea at 336 kg / ha at approximately 5 weeks after planting. Plants were watered by drip irrigation. One week after planting 400 plants were randomly tagged at their base using colored ribbon and each plant was labeled individually with a number for identification. Planthopper counts were only done on tagged plants, and the same plants were used to monitor MMV infection.

Planthopper sampling

The number of adult planthoppers colonizing each tagged corn plant was counted starting from 1 week after planting and continued at weekly intervals for 10 weeks (March 2010 – June 2010). Because the architecture of corn plants changes with growth stage, planthoppers were sampled in four locations of the plant, which in preliminary observations, harbored the majority of planthoppers: 1) the leaf sheath at the base of the plant, 2) the whorl, 3) the base of the tassel 4) and the ear husks. Planthopper counts within and around the ear husks and tassel were made at the later stage of corn plant maturation, when the reproductive organs developed. Counts in the whorls were conducted up to approximately 56 DAP, after that time tassel emerged. Counts on the lower leaf sheath were made throughout sampling period.

Incidence of MMV

All corn plants used for planthopper counts were visually inspected for MMV symptoms at weekly intervals from one week after plant emergence. The number of MMV-infected plants was recorded on the basis of MMV symptoms (mosaic striations of leaf veins and the stunting of internodes, Herold 1972). The date at which MMV infection was first noticed was also recorded. Plant infection level was used to delineate three “treatments” based on the time the symptoms become apparent. Thus, plants showing symptoms of MMV infection at 28 days after planting (DAP) were considered as early infection. Plants showing symptoms of MMV infection at 49 DAP were considered as late infection. Early and late infected plants were compared to plants that showed no symptoms of MMV infection.

Infection Rates

Field collections and greenhouse rearing

Peregrinus maidis adults were sampled on MMV-infected plants using an aspirator, from the continuous corn plots plantings at seven different occasions throughout 2009-2010. Planthoppers were transferred into collection tubes then brought to the laboratory and transferred onto healthy GCB corn seedlings within a mesh-ventilated insect rearing cage (356 mm x 356 mm x 610 mm) (BioQuip Products Inc.; Rancho Dominguez, CA, USA) in a greenhouse with an average temperate of 26 ± 3°C and natural light conditions.

A planthopper colony was established in February of 2009 with insects collected from healthy corn plants in a field located at the Waimanalo Research Station. Planthoppers were maintained on MMV infected GCB corn plants as described above. MMV-infected planthoppers were produced by transferring first instar nymphs to MMV-infected corn plants, where they were allowed to feed for their entire developmental time (18 days).

Planthoppers were segregated into four groups: brachypterous males, brachypterous females, macropterous males, and macropterous females. Individual planthoppers were then confined onto a 1-week-old potted GCB corn seedling enclosed within a cylindrical cage (130 mm diameter x 300 mm tall) for an inoculation access period (IAP) of 7 days. After this period, planthoppers were collected into1.5 ml centrifuge tubes and stored at -20°C. Test plants used for transmission were sprayed with acephate (Ortho®, The Scotts Company LLC) and maintained for another 2-3 weeks to allow for MMV symptom development. At that time, the newest extended leaf was collected from each seedling and stored at -20°C until tested by ELISA.

Detection of MMV

Double Antibody Sandwich (DAS) ELISA was used to detect MMV in individual planthoppers and leaf samples. The ELISA test was conducted following manufacture protocol (AGDIA; Indiana, USA). Absorbance was measured with a microplate reader (BioRad, Hercules, CA, USA) at a wavelength of 405nm. Samples were considered positive for MMV if absorbance values exceeded the mean negative control readings by 2.5 times. Negative control samples were prepared from non-infected planthoppers collected from the rearing colony. MMV-injected planthoppers that successfully transmitted MMV to corn seedlings were used as positive control.

Data Analysis

The average number of adult planthoppers per corn plant showing symptoms of MMV infection early in the crop cycle (28 DAP), late in the crop cycle (49 DAP), or on non-symptomatic plants, were compared over time using repeated measures ANOVA (PROC GLM) (SAS Software 9.2; SAS Institute, 2008, Cary, N.C.). Repeated measures ANOVA were used because the same plants were sampled throughout the planting cycle. Tukey's test was conducted to compare the mean estimates among treatments. Within the model, pre-planned contrasts were conducted to compare the numbers of macropters and brachypters among the 3 treatments.

Infection rates of planthoppers infected by MMV were segregated by 4 categories: brachypterous males, brachypterous females, macropterous males, and macropterous females for both field collected and greenhouse reared planthoppers. Data were arcsine [sqrt(proportion)] transformed to compare the infection rates between field collected and laboratory reared planthoppers using mixed model ANOVA (PROC MIXED; SAS Institute 2008). Reported means are from non-transformed data. A contingency table was also used to compare infection rates between field and greenhouse MMV-infected planthoppers and analyzed by a chi-square goodness of fit test.

Results

Population dynamics

The experimental field sustained a high incidence of MMV, with 55% of plants (220 out of 400) expressing symptoms of MMV infection. Of the 400 tagged plants, 50 developed MMV symptoms by 28 DAP, 112 by 48 DAP and 187 plants showed no symptoms of MMV infection. The cumulative average number of planthoppers on early, late, and non-symptomatic plants was 5.0, 8.4, and 9.5 planthoppers per plant respectively. The average number of adult planthoppers per plant across the ten sampling dates varied significantly between early-infected (showing symptoms of MMV infection by 28 DAP), late infected (showing symptoms of MMV infection by 49 DAP), and non-symptomatic plants ($F_{2,349} = 15.26$; $P < 0.0001$; Figure 3.1).

Early infected plants harbored significantly more planthoppers only at 28 DAP than late-infected and non-symptomatic plants, ($F_{1,\ 349} = 10.12$; $P = 0.002$) and ($F_{1,\ 349} = 5.90$; $P = 0.016$), respectively (Figure 3.1). However, early infected plants harbored significantly less planthoppers then late infected plants at 49 and 56 DAP ($F_{1,\ 349} = 12.44$; $P = 0.0005$) and ($F_{1,\ 349} = 9.77$; $P =$

0.002) and consistently harbored significantly less planthoppers than non-symptomatic plants between 49 and 70 DAP (49 DAP: $F_{1, 349} = 25.77$; $P < 0.0001$; 56 DAP: $F_{1, 349} = 20.12$; $P < 0.0001$; 63 DAP: $F_{1, 349} = 11.90$; $P < 0.0006$; 70 DAP: $F_{1, 349} = 8.05$; $P = 0.005$). Late-infected plants also averaged less planthoppers than non-symptomatic plants, but only at 63 DAP were these differences significantly lower ($F_{1, 349} = 4.79$; $P = 0.0293$; Figure 3.1).

Figure 3.2 illustrates the changes in the average number of macropters and brachypters present on early-infected, late-infected, and non-symptomatic plants over the entire crop cycle. Overall, the average number of brachypters per plant did not differ significantly between early infection, late infection, and non-symptomatic plants ($F_{2, 345} = 0.17$; $P = 0.8464$), with the exception for 28 DAP where the average number of brachypters on early infected plants peaked and was significantly greater than on late infected and non-symptomatic plants, ($F_{1, 345} = 24.93$; $P < 0.0001$) and ($F_{1, 345} = 17.34$; $P < 0.0001$), respectively (Figure 3.2B). After 28 DAP, the number of brachypters on early infected plants declined rapidly compared to late infected and non-symptomatic plants which both peaked between 35 - 49 DAP. At 49 DAP, the average number of brachypters on early infected plants was significantly less than non-symptomatic plants ($F_{1, 345} = 4.32$; $P = 0.0385$).

The average number of macropters differed significantly between early infection, late infection, and non-symptomatic plants ($F_{2, 345} = 28.07$; $P < 0.0001$). The average number of macropters recorded on early infected plants was significantly less than on non-symptomatic plants from 35 through 70 DAP (35 DAP: $F_{1, 345} = 4.77$; $P = 0.0297$; 42 DAP: $F_{1, 345} = 4.24$; $P = 0.0402$; 49 DAP: $F_{1, 345} = 36.61$; $P < 0.0001$; 56 DAP: $F_{1, 345} = 17.24$; $P < 0.0001$; 63 DAP: $F_{1, 345} = 14.65$; $P = 0.0002$; 70 DAP: $F_{1, 345} = 8.01$; $P = 0.0049$) and were significantly less than late-infected plants on DAP 35 and from 49 through 63 DAP (35 DAP: $F_{1, 345} =4.12$; $P = 0.0432$; 49

DAP: $F_{1, 345}$ = 18.26; P < 0.0001; 56 DAP: $F_{1, 345}$ = 7.99; P = 0.0050; 63 DAP: $F_{1, 345}$ = 7.22; P = 0.0075). Macropters peaked at 56 DAP for all three treatments (Figure 3.2A). Figure 3.3 shows the changes in the proportion of macropters and brachypters over the planting cycle.

Infection Rates

Results of ANOVA showed that wing morphology, gender, and their statistical interaction had no significant effect on infection and transmission rates of both field collected or greenhouse reared planthoppers (P > 0.05; Table 3.1; Figure 3.4). However, the number of greenhouse reared females tested positive for MMV infection was significantly more than males (χ^2 = 4.596; d.f. =1; P < 0.032). The infection rates of field collected planthoppers averaged 27% and ranged between 17% to 40% (Table 3.2). The infection rates of planthoppers reared on MMV infected corn plants under greenhouse conditions was 32% and ranged between 13% to 45% (Table 3.3).

Discussion

There was a distinct difference in the population dynamics of *P. maidis* on plants that expressed symptoms of MMV infection at early (28 DAP) or late (49 DAP) stages of corn growth, and on plants that did not show symptoms of MMV infection. Overall, non-symptomatic plants harbored twice the number of planthoppers than early infected plants. Early infected plants had higher planthopper numbers when symptoms of virus infection became apparent (28 DAP), but declined thereafter as plants aged, suggesting that MMV infection made host plants less favorable for planthopper development. This observed peak in numbers was associated with an early occurrence of brachypters, while the temporal pattern of macropters across the three treatments followed a similar trend over the course of the planting cycle, each peaking at 56 DAP.

The differences in planthopper densities may likely be the consequence of changes in host plant physiology and time of which infection occurred. Virus infection of young plants compromises normal plant growth, causing severe stunting and poor ear and tassel development. A reduction in plant size and the premature decline in nutritional quality may limit the colonization rate, and planthopper population growth. A smaller, stunted plant may have less suitable oviposition sites. A poor nutritional resource can trigger macropter development and thus promote planthopper emigration. Previously, I have demonstrated that planthoppers reared on MMV-infected corn leaves produce a larger proportion of macropters in contrast to planthoppers that were reared on healthy leaves of plants of similar age. Denno and co-workers (1985, 1994, & 1996) have illustrated how plant physiological status affects the production of planthopper brachypters and macropters in great detail.

Multiple generations of planthoppers may occur within a single cropping cycle. Macropters are responsible for colonizing and laying eggs on young corn seedlings, producing the first generation of planthoppers that predominantly mature into brachypters, although some macropters may occur. However the second generation of planthoppers largely consists of macropters. On plants showing early symptoms of infection, first generation brachypter populations are sustained for a longer period of time (~21 days) than on late infected (~7 days) and symptomless plants (zero days) (Figure 3.3). This might be the result of two factors: 1) infection on young corn plants initially improves plant host quality (Castle & Berger, 1993; Maris et at., 2004) and favors a larger production of brachypters or 2) the planthoppers that mature into macropters immediately disperse from infected plants. Several studies have suggested that pathogen infection increases the concentration of free amino acids and/or suppression of host plant defenses, improving host quality and increasing vector fitness (Belliure et al., 2005; Fereres et al., 1989; Jimenez-Martinez et al., 2004; Maris et al., 2004; Srinivasan & Alvarez, 2007; Weibull et al., 1990). Results obtained through this work further suggest that population dynamics change according to plant infection status. During the early stages of plant infection, changes in host plant physiology may favor a larger numbers of brachypters although at a later stage of infection, the declining host plant quality elicits the dispersal of vectors. As seen in chapter 2, there is no obvious increase of macropters on young MMV-infected corn plants; however, there was a 28% increase in macropter production on MMV-infected corn plant than on healthy plants of similar age.

Vector behavior is an important factor that may account for lower numbers of macropters observed on infected plants. By nature, macropters are more likely to disperse from their original host plant from which they developed on than brachypters. Possible differences in gender behavior may also exist although it would be very difficult to observe directly in the field. Through a mathematical model, Sisterson (2008) predicted that a preference to feed on healthy plants could influence dispersal activity and increase rates of secondary pathogen spread by the vector. However, feeding preference for infected plants would limit vector dispersal and decrease secondary spread (Sisterson, 2008). It would be interesting to investigate whether preferences differ between wing forms and/or gender and also whether or not if preferences change following virus infection by conducting choice test assays.

In this study, the acquisition and transmission rates of MMV by brachypterous females, brachypterous males, macropterous females, and macropterous males were similar, thus suggesting that MMV does not favor infection of a particular combination of planthopper wing morphotype and gender. Similar results were obtained by Dhileepan et al. (2006) and Ridley et al. (2008) who demonstrated that the acquisition rates of *Fiji disease virus* (*Fijivirus*) between the different wing forms and gender of the planthopper *Perkinsiella saccharicida* did not differ significantly. These results were also consistent with data reported in chapter 2 of this thesis. With the exception of field collected macropterous females (Figure 3.4), females tended to have a higher incidence of MMV then males. This tendency could be explained by differences in gender behavior. Females likely spend more time feeding then males in effort to support egg production and as a result females are exposed to MMV then males. On the other hand, males may be dividing their efforts between feeding and searching for mates (Denno, 1994), thus subsequently incurring lower MMV infection. Similar previous studies also showed no differences in plant virus incidence between planthopper genders (Arneodo et al., 2002; Dhileepan et al., 2006; Ornaghi et al., 1999; Ridley et al., 2008).

The overall virus acquisition rates of greenhouse-reared planthoppers and field collected planthoppers were not different (mean of 32% and 27%, respectively). Though, the infection rates of field collected and greenhouse-reared plathoppers were highly variable across sampling date. Ammar and Hogenhout (2008) demonstrated that acquisition access periods longer than 1 week on MMV infected plants did not lead to an increase of MMV-viruliferous planthoppers. Although field planthoppers were collected from virus infected plants, it is possible that not all of them developed on those plants; instead some may have landed after migration from neighboring plant hosts. Therefore immigration events would be a source of variation and may be a reason for acquisition rates being slightly lower for field than greenhouse assays.

MMV transmission rates between greenhouse-reared planthoppers and field collected ones did not differ (mean 30% and 25%, respectively). However, not all of the MMV-infected planthoppers did transmit the virus. In fact, out of 317 planthoppers from greenhouse reared plants that were tested, 6 planthoppers (2%) failed to transmit MMV to their test plants. Similarly, 3 planthoppers out of 225 planthoppers from field collections (1%) failed to transmit MMV to their test plants. These finding are consistent with those of Falk & Tsai (1985) and Ammar and Hogenhout (2008) that support the existence of midgut and salivary gland barriers to virus movement within the insect, which are important factors that limit transmission efficiency (Ammar et al., 2009; Hogenhout et al., 2003). However, it seems that the salivary gland barriers is less efficient in preventing virus movement than midgut, since majority of planthoppers that do get infected, transmit MMV. In the current study, no association could be made between failure in transmission and planthopper wing morphology or gender since a very low number of planthoppers failed to transmit MMV.

The study of virus-vector interactions is important for understanding the factors underlying disease epidemics and the improvement of control measures. Wing dimorphism is strongly correlated with planthopper population biology and virus epidemiology. Although it would be advantageous for MMV to preferentially infect macropters, in this study, there was no association between virus acquisition and planthopper morphology; however females seemed to have higher infection rates. The changes in the population dynamics of *P. maidis* on infected plants likely reflects changes in host plant quality. Immigration and emigration events are important factors to consider and make interpretation of the results challenging. However, to my understanding this is one of the few studies that have quantified the population dynamics of vectors in relation to infected plants in a field setting.

Figures

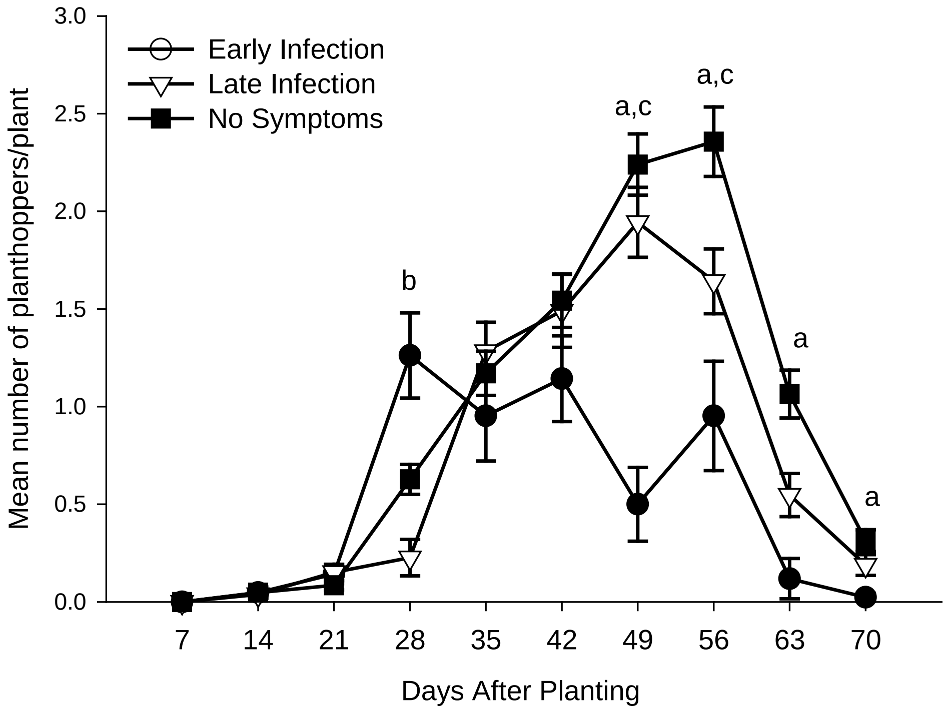

Figure 3.1. Mean number (± SE) of *P. maidis* adults counted on plants that showed MMV virus symptoms early (28 DAP; circle), those that show virus symptoms late (49 DAP; triangle), and symptomless plants (square) throughout the field experiment. Letters above each week describe significant differences; a represents early infection significantly less than plants with no symptoms; b represents late infection and no symptoms significantly less than early infection; c represents early infection significantly less than late infection.

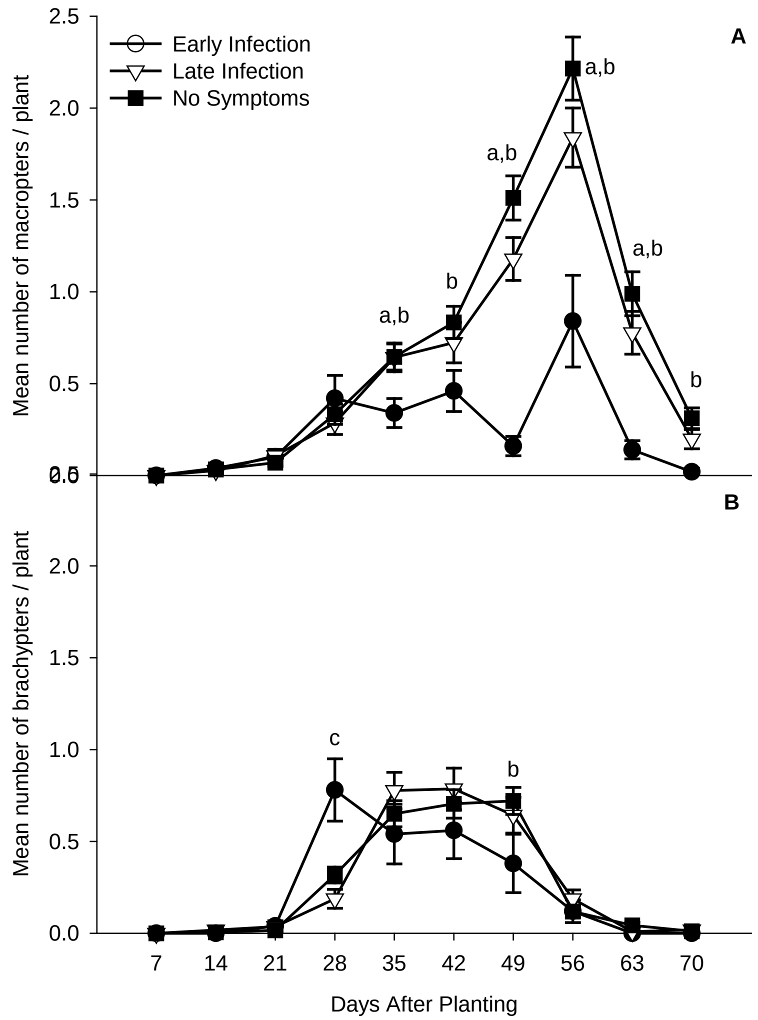

Figure 3.2. Mean number (± SE) of *P. maidis* A) macropters and B) brachypters counted on plants that showed MMV virus symptoms early (28 DAP; circle), those that show virus symptoms late (49 DAP; triangle), and symptomless plants (square) throughout the field experiment. Letters above each week represents significance; a represents early infection significantly less than late infection; b represents early infection significantly less than plants showing no symptoms; c represents early infection significantly greater than plants showing no symptoms of MMV infection and late infected plants.

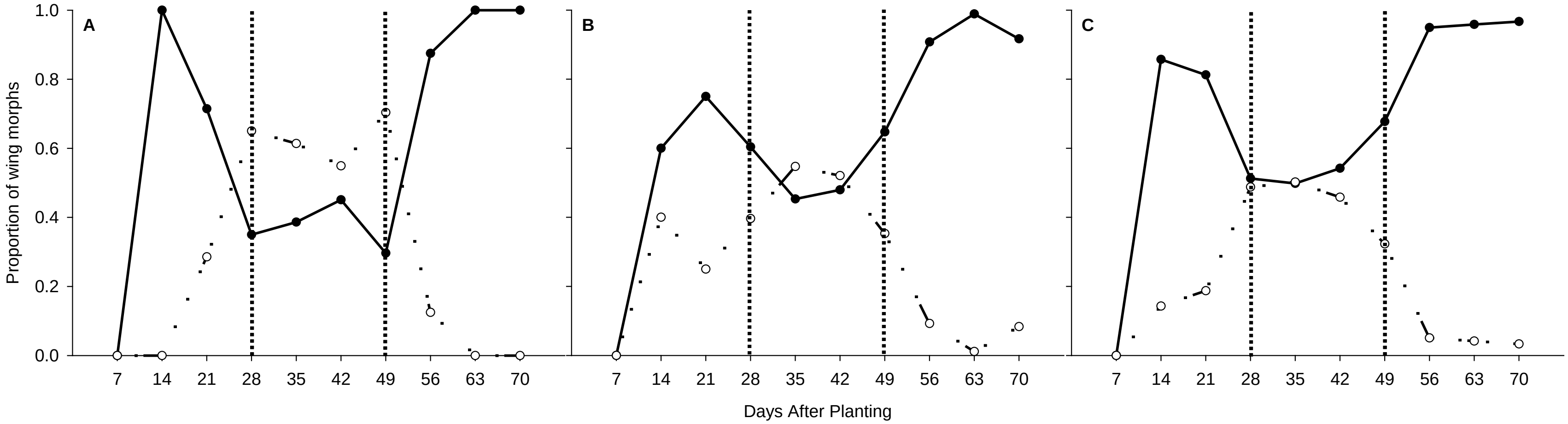

Figure 3.3. Changes in the proportion of macropters (solid line) and brachypters (dashed line) from planthopper counts on plants showing symptoms of MMV infection at A) 28 DAP (early infected plants), B) 49 DAP (late infected plants) and C) symptomless plants throughout the field experiment. Vertical dotted lines indicated infection dates.

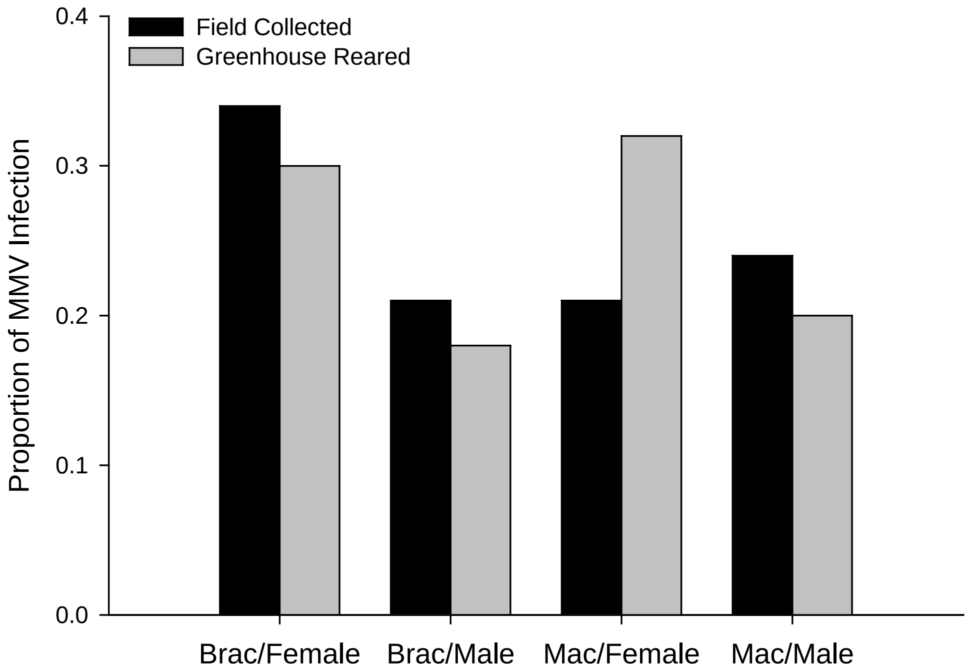

Figure 3.4. Proportion of *P. maidis* tested positive for MMV infection by ELISA separated by wing and gender combination. Grey bars represent greenhouse reared planthoppers and black bars represent field collected planthoppers.

Tables

Table 3.1. The effects of wing morphology (macropters vs brachypters) and gender (males vs females) on MMV infection rates in field collected and greenhouse reared *Peregrinus maidis,* compared by ANOVA.

Effect	Lab Reared			Field Collected		
	df	F-Value	P-Value	df	F-Value	P-Value
Wing morphology	*1*	0.09	0.77	*1*	0.35	0.55
Gender	*1*	3.71	0.06	*1*	1.03	0.32
Wing morphology x Gender	*1*	0.06	0.81	*1*	1.81	0.19

Table 3.2. Percentage of MMV-infected *Peregrinus maidis* sampled from field corn plants.

Collection no.	Collection date	*N*	Brachypter males	Brachypter females	Macropter males	Macropter females	Overall
			%	%	%	%	%
1	17-Dec-2009	20	na	na	0	20	20
2	23-Feb-2010	35	9	3	6	9	26
3	15-Mar-2010	30	7	7	7	20	40
4	21-Apr-2010	37	8	8	14	3	32
5	12-Jul-2010	36	8	3	6	3	19
6	4-Aug-2010	37	5	11	5	8	30
7	15-Oct-2010	30	8	7	0	3	17
Total		225					
Mean %			7	6	6	8	27

Adult planthoppers were collected form field MMV-infected corn plants and brought back into the lab for testing. No significant difference in MMV acquisition between wing forms at the $P < 0.05$ level. na, not applicable

Table 3.3. Percentage of MMV acquisition among various adult forms of *P. maidis* reared on MMV-infected plants.

Sample no.	Sample date	*N*	Brachypter males	Brachypter females	Macropter males	Macropter females	Overall
			%	%	%	%	%
1	9-Nov-2009	24	0	0	8	4	13
2	31-Mar-2010	20	0	25	0	0	25
3	20-May-2010	41	10	12	7	12	41
4	12-Jul-2010	40	10	3	0	8	20
5	30-Aug-2010	40	5	8	5	8	25
6	30-Sep-2010	40	10	18	8	8	43
7	30-Nov-2010	40	5	10	10	20	45
8	20-Dec-2010	36	6	14	6	8	33
9	6-Jan-2011	36	6	6	6	11	33
Total		317					
Mean %			6	10	6	10	32

Planthoppers were continuously reared on potted MMV-infected corn plants (GCB).
No significant difference in MMV acquisition between wing forms at the $P < 0.05$ level. na, not applicable

References:

Ammar ED & Nault LR (2002) Virus transmission by leafhoppers, planthoppers and treehoppers (Auchenorrhyncha, Homoptera). Adv Bot Res 36:141-167.

Ammar ED & Hogenhout SA (2008) A neurotropic route for *Maize mosaic virus* (Rhabdoviridae) in its planthopper vector *Peregrinus maidis*. Virus Res 131: 77-85. doi:10.1016/j.virusres.2007.08.010.

Ammar ED, Tsai CW, Whitfield AE, Redinbaugh MG & Hogenhout SA (2009) Cellular and molecular aspects of Rhabdovirus interactions with insect and plant hosts. Ann Rev Entomol 54: 447-468. doi:10.1146/annurev.ento.54.110807.090454.

Arneodo JD, Guzman FA, Conci LR, Laguna IG & Truol GA (2002) Transmission features of Mai de Río Cuarto virus in wheat by its planthopper vector *Delphacodes kuscheli*. Ann Appl Bio 141: 195-200. doi:10.1111/j.1744-7348.2002.tb00212.x.

Belliure B, Janssen A, Maris PC, Peters D & Sabelis MW (2005) Herbivore arthropods benefit from vectoring plant viruses. Ecology Letters 8: 70-79.

Blua MJ & Perring TM (1992) Alatae production and population increase of aphid vectors on virus-infected host plants. Oecologia 92: 65-70.

Brewbaker JL, ed. (2003) Corn production in the tropics: The Hawaii Experience. College of Tropical Agriculture and Human Resources, University of Hawaii Manoa, Honolulu, Hawaii, USA.

Castle SJ, Berger PH (1993) Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship. Entomol Exp Appl 69: 51–60.

Denno R (1994) The evolution of dispersal polymorphisms in insects: The influence of habitats, host plants and mates. Res Pop Ecol 36: 127-135. doi:10.1007/bf02514927.

Denno RE, Roderick GK, Peterson MA, Huberty AF, Dobel HG, Eubanks MD, Losey JE & Langellotto GA (1996) Habitat persistence underlies intraspecific variation in the dispersal strategies of planthoppers. Ecol Monogr 66: 389-408.

Denno RF, Douglass LW & Jacobs D (1985) Crowding and host plant nutrition – Environmental deterimants of wing-form in *Prokelisia-marginata*. Ecology 66: 1588-1596.

Denno RF & Roderick GK (1990) Population biology of planthoppers. Ann Rev Entomol 35: 489-520.

Denno RF & Roderick GK (1992) Density-related dispersal in planthoppers – Effects of interspecific crowding. Ecology 73: 1323-1334.

Dhileepan K, Croft BJ, Ridley AW, James AP & Raghu S (2006) Susceptibility of source plants to Sugarcane Fiji disease virus influences the acquisition and transmission of the virus by the planthopper vector *Perkinsiella saccharicida*. J AppEntomol 130: 67-71. doi:10.1111/j.1439-0418.2005.01023.x.

Falk BW & Tsai JH (1985) Serological detection and evidence for multiplication of Maize mosaic-virus in the planthopper *Pereginus-maidis*. Phytopathology 75: 852-855.

Fereres A, Lister RM, Araya JE & Foster JE (1989) Development and reproduction of the English grain aphid (Homoptera: Aphididae) on wheat cultivars infected with Barley yellow dwarf virus. Environ. Entomol. 18: 388-393.

Gildow FE (1980) Increased production of alatae by aphids reared on Oats infected with Barley yellow dwarf virus. Ann. Entomol. Soc. Am. 73: 343-347.

Herold R (1972) Maize mosaic virus. CMI/AAB Description of Plant Viruses #94.

Hodge S & Powell G (2010) Conditional facilitation of an aphid vector, *Acyrthosiphon pisum*, by the plant athogen, *Pea enation mosaic virus*. J. of Insect Sci. 10: 1-14. doi:10.1673/031.010.14115.

Hogenhout SA, Ammar E-D, Whitfield AE & Redinbaugh MG (2008) Insect vector interactions with persistently transmitted viruses. Ann Rev Phytopathol 46: 327-359. doi:doi:10.1146/annurev.phyto.022508.092135.

Hogenhout SA, Redinbaugh MG & Ammar E-D (2003) Plant and animal rhabdovirus host range: a bug's view. Trends Microbiol 11: 264-271. doi:10.1016/s0966-842x(03)00120-3.

Jimenez-Martinez ES, Bosque-Perez NA, Berger PH, Zemetra RS, Ding HJ & Eigenbrode SD (2004) Life History of the bird cherry-oat aphid, *Rhopalosiphum padi* (Homoptera: Aphididae), on transgenic and untransformed wheat challenged with Barley yellow dwarf virus. Environ. Entomol. 33: 1207.

Maris PC, Joosten NN, Goldbach RW & Peters D (2004) Tomato spotted wilt virus infection improves host suitability for its vector *Frankliniella occidentalis*. Phytopathology 94: 706-711. doi:10.1094/phyto.2004.94.7.706.

Ming R, Brewbaker JL, Pratt RC, Musket TA & McMullen MD (1997) Molecular mapping of a major gene conferring resistance to maize mosaic virus. Theor Appl Genetics 95: 271-275. doi:10.1007/s001220050559.

Ornaghi JA, March GJ, Boito GT, Marinelli A, Beviacqua JE, Giuggia J & Lenardon SL (1999) Infectivity in natural populations of *Delphacodes kuscheli* vector of "Mal Rio Cuarto" virus. Maydica 44: 219-223.

Ridley AW, Dhileepan K, Walter GH, Johnson KN & Croft BJ (2008) Variation in acquisition of *Fiji Disease Virus* by *Perkinsiella saccharicida* (Hemiptera: Delphacidae). J Econ Entomol 101: 17-22. doi:10.1603/0022-0493(2008)101[17:viaofd]2.0.co;2.

Singh BU & Seetharama N (2008) Host plant interactions of the corn planthopper, *Peregrinus maidis* Ashm. (Homoptera: Delphacidae) in maize and sorghum agroecosystems. Arthropod-Plant Inter 2: 163-196. doi:10.1007/s11829-007-9026-z.

Sisterson MS (2008) Effects of insect-vector preference for healthy or infected plants on pathogen spread: Insights from a model. J Econ Entomol 101: 1-8. doi:10.1603/0022-0493(2008)101[1:eoipfh]2.0.co;2.

Srinivasan R & Alvarez JM (2007) Effect of mixed viral Iinfections (potato virus Y–potato leafroll virus) on biology and preference of vectors *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae). J. Econ. Entomol. 100: 646-655. doi:10.1603/0022 0493(2007)100[646:eomvip]2.0.co;2.

Tsai JH & Wilson SW (1986) Biology of *Peregrinus maidis* with descriptions of immature stages (Homoptera: Delphacidae). Ann Entomol Soc Am 79: 395-401.

Weibull J, Ronquist F & Brishammar S (1990) Free amino acid composition of leaf exudates and phloem sap: A comparative study in Oats and Barley. Plant Physiol. 92: 222-226.

Zera AJ & Denno RF (1997) Physiology and Ecology of dispersal polymorphism in insects. Ann Rev Entomol 42: 207-230. doi:doi:10.1146/annurev.ento.42.1.207.

Chapter 4

Influence of the plant resistance gene *Mv* on the fitness of the corn planthopper, *Peregrinus maidis,* and acquisition rates of *Maize mosaic virus*.

Abstract

The use of crops resistant to pests and pathogens is a cost-effective approach to pest management. A corn breeding program conducted in Hawaii has identified a source of inheritable resistance to *Maize mosaic virus* (*Rhabdoviridae*: *Nucleorhabdovirus*). This resistance has been characterized to be controlled by one gene named "*Mv*", which has a co-dominant action. To date, it has not been studied if the resistance associated with gene *Mv* is directed only toward MMV or if it may also affect the insect vector, the corn planthopper *Peregrinus maidis*. Therefore, I examined the life history of *P. maidis* and its ability to acquire MMV on near isogenic lines homozygous dominant (*Mv*/*Mv*), recessive (*mv*/*mv*) and heterozygous (*Mv/mv*) for gene *Mv*. A field trail was also conducted to observe differences in planthopper colonization between those corn genotypes. Field observations revealed lower densities of *P. maidis* on *Mv* dominant corn lines, than recessive lines and their hybrids (*Mv/mv*). Additionally, dominant genotypes had a lower incidence of MMV infected plants in the field. Acquisition rates of MMV by the planthoppers that fed on *Mv* dominant and heterozygous corn genotypes were lower than 20%, whereas the acquisition rates on *Mv* recessive corn genotypes were greater than 30%. However, in laboratory assays gene *Mv* had little to no effect on the development, longevity and fecundity on *P. maidis.* This evidence suggest that gene *Mv* may be functioning against the virus, possibly reducing virus replication or movement within the plant host, thus limiting virus acquisition, but does not influence the fitness of the planthopper vector.

Introduction

In Hawaii, corn (*Zea mays* L., maize) is commonly grown for sweet corn and parent-seed production. The production of commercial sweet corn has remained fairly consistent in terms of yield and price, with an annual farm value estimated at US$ 1.23 million (NASS, 2011). The production of corn produced for breeding purpose by seed-technology companies has grown to become a vital part of Hawaii's agriculture economy. Ideal year round growing conditions allow three to four crop cycles per year. According to the National Agricultural Statistics Service (Sept. 2011), Hawaii's seed crop industry is currently valued at US$ 224 million, a 74% increase from US$ 44 million in 2001. Hawaii's corn seed industry has increased in production area by 56% (from 3,100 to 7,100 acres) and seed production by 53% (from 5,600,000 to 12,000,000 pounds). This rapid expansion and intensification in corn crop production has led to increased concerns about pest and disease outbreaks.

The corn planthopper, *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae), is considered to be a pest of corn (*Zea mays* L.) in many tropical and sub-tropical corn growing regions around the world, including Hawaii (Metcalf 1943; Namba and Higa 1970; Singh and Rana 1992; Tsai 1996; Singh and Seetharama 2008). High densities of *P. maidis* planthoppers on corn plants can cause "hopperburn" through their feeding activity (Brewbaker, 2003; Takara & Nishida, 1983). Symptoms of "hopperburn" are leaf chlorosis followed by necrosis, reduced plant vigor, and plant stunting (Backus et al., 2005). In addition, *P. maidis* vectors two corn viruses, *Maize mosaic virus* (MMV, *Rhabdoviridae*) and *Maize stripe virus* (MStV, *Tenuivirus*) (Ammar et al., 1987). Both MMV and MStV are transmitted in a persistent and propagative manner (Ammar and Hogenhout 2008). In Hawaii, continuous corn plantings promote large populations of *P. maidis,* and consequently high incidences of MMV, leading to significant yield losses (Brewbaker 1981; Singh and Seetharama 2008).

For sweet corn and seed nurseries, chemical control is extensively used to manage *P. maidis* populations and reduce the spread of *Maize mosaic virus* (MMV). Several insecticide applications may be implemented to reduce pest pressure. This can be an expensive and hazardous practice and includes the risk of insecticide resistance build-up. The use of parasitoids as biological control agents against *P. maidis* is an attractive management option, however, parasitism rates were found to be very low in corn fields in Hawaii (Napompeth 1973). The identification and use of genetically virus or insect resistant lines, through conventional plant breeding program, is considered to be a sustainable, cost-effective and environmentally friendly practice for control of pests and diseases (Brewbaker, 1981; Dintinger et al., 2005; Redinbaugh et al., 2004)

Extensive research has been conducted to identify sources of corn virus resistances and to incorporate them into commercial corn inbred lines (Balint-Kurti & Johal, 2008; Brewbaker et al., 1991; Redinbaugh et al., 2004). Many programs have focused on improving resistance toward economically important corn viruses such as *Maize dwarf mosaic virus* (MDMV, *Potyviridae*), *Sugarcane mosaic virus* (SCMV, *Potyviridae*), *Maize mosaic virus, Maize streak virus* (MSV, *Geminiviridae*), *Maize chlorotic dwarf virus* (MCDV, *Waikavirus*), *Maize stripe virus* (MStV, *Tenuivirus*), *Maize rayado fino virus, Maize rough dwarf virus, Maize chlorotic mottle virus*, and *Maize Rio Cuarto virus* as well as important emerging corn viruses such as *High plains virus* and *Maize fine streak virus* (Redinbaugh et al., 2004).

In Hawaii, Dr. James L. Brewbaker (College of Tropical Agriculture & Human Resources; University of Hawaii) and colleagues implemented an intensive tropical corn breeding program to identify inheritable resistance against MMV. A source of resistance to MMV was identified in the germplasm originated from Caribbean and Cuban flint material (Brewbaker, 1981; Ming et al., 1997; Vega et al., 1995). Though molecular mapping techniques, Ming et al. (1997) localized the gene "*Mv*" conferring resistance to MMV on chromosome 3. This resistance has been characterized as being monogenic and having a co-dominant gene action (Ming et. al. 1997). Thus, alleles from each parent that are recessive (*mv/mv*) and dominant (*Mv/Mv*) for gene *Mv* are equally expressed in the hybrid genotype (*Mv/mv*). Hence, under field conditions, hybrid corn lines show varying levels of phenotypic resistance under severe pressure of MMV infection (Brewbaker, 1979; Brewbaker, 1981; Brewbaker & Josue, 2007; Brewbaker et al., 1991; Ming et al., 1997). Homozygous dominant corn lines (*Mv*/*Mv*) can show 5-15% MMV infection in fields with high pressure for virus infection, but symptoms are not severe as observed on susceptible lines. Therefore, Brewbaker (1981) has regarded this resistance as a high level of "tolerance" to MMV infection. To date, many important inbred parent stocks have been converted to incorporate MMV tolerance. Conversions were achieved through five or more backcrosses followed by several generations of sib and self-pollinations to achieve near isogenic lines (Brewbaker, 1981; Brewbaker, 2003; Brewbaker et al., 1991; Ming et al., 1997), with the screening conducted under field pressures of MMV. Many of the Hawaiian sweet corn cultivars are currently integrated with this high tolerance (Brewbaker, 1981).

Although this tolerance to MMV has been confirmed through field studies and genetic mapping, there is no information on the mechanisms of gene *Mv*. Increasing our understanding of MMV tolerance in relation to gene *Mv* may be particularly important to improve the management of MMV and *P. maidis*. Since field screening for MMV resistance was conducted under variable populations of planthoppers and virus pressure, it is unclear if the mode of action is directed toward MMV or toward the planthopper. Thus it is important to identify effects derived from this tolerance that would limit vector populations or alternatively reduce MMV transmission. A reduction in vector feeding activity and low acquisition rates may in turn reduce the spread of MMV. The objectives of this study is to determine if the presence of gene *Mv* has an effect on the life history of *P. maidis* and to compare MMV acquisition rates among *P. maidis* feeding on resistant and susceptible corn inbred lines. A field trail was also conducted to observe any differences in planthopper colonization between *Mv* recessive and resistant material.

Material and Methods

Plant genotype

Six corn inbred lines and one variety of sweet corn were used in our experiments. Corn inbreds consisted of two series of near isogenic corn lines (Mo17 and B73) homozygous recessive (m*v/mv)*, homozygous dominant (Hi60 and Hi47) (*Mv*/*Mv*) for gene *Mv* and, their corresponding hybrid crosses (Hi60 x Mo17 and Hi47 x B73) heterozygous for gene *Mv* (*Mv/mv*) (Table 1). In addition, the hybrid sweet corn Golden Cross Bantam (GCB), also homozygous recessive (*mv/mv)*, was used (Table 1). Inbreds Hi60 and Hi47 are *Mv* conversions of their parent inbreds Mo17 and B73 respectively, meaning that MMV resistance was incorporated into these inbreds by multiple back crossing events to create a near isogenic line (Brewbaker, 1981). Both B73 and Mo17 are temperate inbreds that are known to be susceptible to MMV infection as well as the sweet corn variety Golden Cross Bantam (Brewbaker, 1981), which was used as a control.

These six corn lines along with GCB were grown and maintained in a greenhouse as potted corn seedlings. Three corn seeds were sown into 12.7 cm by 15.2 cm circular pots containing Sunshine Soil Mix no.4 (Sun Grow Horticulture Canada Ltd.). Plants were watered daily and fertilized once a month with Miracle Grow (NPK 24-8-8). Four week-old plants at V4-V5 growth stage were used for laboratory experiments. The same selection of genetic material was used for the field assay.

Laboratory assays

Insect rearing

A colony of *P. maidis* was established from adults collected from a corn field located at the Waimanalo Research Station (College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Oahu, HI). Planthoppers were maintained on potted corn seedlings, cv. Golden Cross Bantam (GCB) within mesh-ventilated insect rearing cages (356 mm x 356 mm x 610 mm) (BioQuip Products Inc.; Rancho Dominguez, CA, USA) in a greenhouse with an average temperate of 26 ± 3°C and natural light conditions. The colony was maintained by replacing new potted GCB seedlings inside the rearing cages every 3-4 weeks.

Production of first instar nymphs

Planthopper females were transferred from the rearing colony to 14 day-old GCB corn seedlings enclosed within a cylindrical cage (130 mm diameter x 300 mm tall). Females were allowed to oviposit for a period of 24 h and were then removed. Seedlings were maintained for another 6-7 days. Thereafter, leaves bearing the eggs were detached from the plants, were sectioned into small pieces of approximately 80 mm in length, and were placed into 150 x 15 mm Petri dishes containing moist filter paper. Nymphs that hatched within 24 h from the onset of the hatching were used for the experiments.

Development, longevity, and fecundity

First instar nymphs were individually placed into small glass tubes (12 x 75 mm) (Fisher Scientific, Pittsburgh, PA, USA) containing leaf cuttings from either one of the seven corn lines previously described (Table 1). To avoid rapid dehydration, each leaf cutting was embedded on the bottom of each tube into 1 ml of 1.2% agar (Fisher Scientific, Pittsburgh, PA, USA). A dental cotton swab (38 x 10 mm) was used to close the top of each tube. Tubes were maintained in a growth chamber (VWR International, Randor, PA, USA; Model 2015) set at a temperature of 26°C and with a photoperiod of 12 h : 12 h (L:D). Nymphs were transferred to new tubes containing fresh healthy leaf cuttings every 3-5 days. Tubes were inspected daily and examined for the presence of exuviae. Mortality was also recorded at that time. The dates of each molt, duration of stadia, mortality, sex, and adult morphology at completion of the development (macropters or brachypters) were recorded. To examine the effect of corn genotype on longevity and fecundity, matured planthoppers were transferred as male and female pairs into new glass tubes (13 x 100 mm) (Fisher Scientific, Pittsburgh, PA, USA). Planthopper pairs were transferred every two days to new tubes containing fresh healthy leaves. The number of eggs laid per leaf was counted using a dissecting microscope. The average number of eggs laid per female per day, the total number of eggs laid per female life span, and adult longevity were determined.

MMV acquisition assay

Two-week old corn seedlings of seven corn lines (Hi60, Mo17, Hi60 x Mo17, Hi47, B73, Hi47 x B73 and GCB) were infected with MMV. Five male adult planthoppers were injected as nymphs with clarified MMV extract prepared from MMV infected leaves (Ammar et al., 2005), were transferred to each of the seven corn lines for an inoculation access period (IAP) of 7 days. Planthoppers and corn seedlings were contained within cylindrical cages (130 mm diameter x 300 mm tall). Two-three weeks post inoculation, plants that showed symptoms of MMV were used for acquisition assays. Thirty 1st instar nymphs were transferred to the infected plants and were allowed to feed throughout their entire development, which took about 18 days. Adult planthoppers were collected from each of the seven infected corn genotypes and stored at -80°C until further processing for MMV detection by ELISA.

Double Antibody Sandwich (DAS) ELISA was used to detect MMV in planthoppers. The ELISA test was conducted following manufacture protocol (AGDIA; Indiana,USA). Absorbance was measured with a microplate reader (BioRad, Hercules, CA, USA) at a wavelength of 405nm. Samples were considered positive for MMV if absorbance values exceeded the mean negative control readings by 2.5 times. Negative control samples were prepared from non-infected, unexposed planthoppers collected from the rearing colony. MMV-injected planthoppers that successfully transmitted MMV to corn seedlings were used as positive control.

Field assay

Experiment design

A field trail was conducted at the Waimanalo Research Station (University of Hawaii, Oahu) in March 2010. The seven corn inbreds (Table 1) were planted in a randomized complete block design with four replications for each inbred. Twenty seeds of each inbred were sown along 4.57 m long rows, spaced approximately 0.21 m apart, using a manual hand planter. The inter-row spacing was 0.75 m and 0.91 m spacing between each plot. The entire experimental plot measured approximately 21.24 m x 2.74 m. Prior to planting, the field was treated with pre-plant herbicide (Eradicane, Gowan Company) and pre-emergence herbicide (Prowl H20, BASF) for weed control. Atrazine (AAtrex 4L; Syngenta) was used as a post-emergent and applied at 4-5 weeks after planting. The field was fertilized (16 :16 : 16; N : P : K) at 560 kg / ha before planting and with urea at 336 kg / ha at approximately 5 weeks after planting. Plants were watered by drip irrigation. Plant stand counts were recorded at 1week after planting and 15 plants of each row were randomly tagged at their base using colored ribbon and identified as plants 1 through 15.

Planthopper sampling

The number of adult planthoppers colonizing each tagged corn plant was counted once a week for 10 weeks (March 2010 – June 2010). Because the architecture of corn plants changes with age, planthoppers were sampled in four locations on the plant: 1) the lower leaf sheath at the base of the plant, 2) the whorl, 3) the base of the tassel, and 4) the ear husks. Planthopper counts within and around the ear husks and tassel were made at the later stage of corn plant maturation, when the reproductive organs developed. Counts in the whorls were conducted up to approximately 56 DAP, after that time tassel emerged. Counts on the lower leaf sheath were made throughout sampling times.

MMV incidence

All corn plants used for planthopper counts were visually inspected for MMV symptoms at weekly intervals from one week after plant emergence. The number of MMV-infected plants was recorded on the basis of MMV symptoms (mosaic striations of leaf veins and the stunting of internodes, Herold 1972). The date of which MMV infection was first noticed was recorded.

Data analysis

Planthopper development, longevity, and fecundity

Because of limitations in available greenhouse space, corn inbreds were split into 2 trials and tested separately. The first trial consisted of the near isogenic lines Hi47, B73, and their hybrid Hi47 x B73, and GCB. The second trial consisted of the near isogenic lines Hi60, Mo17, and their hybrid Hi60 x Mo17, and GCB. Mean developmental time, mean longevity, and mean fecundity (avg. eggs laid / day) were compared using mixed model ANOVA (PROC MIXED, SAS Institute). The model was constructed to examine corn genotype as main effect and trial was designated as random factor. Tukey's test was used to compare the mean values among corn inbreds when significance was found at the $P < 0.05$ level. The pre-developmental mortality, expressed as proportion of dead nymphs, were arcsine square-root [arcsine(proportion dead)] transformed. Reported means are from non-transformed data.

MMV Acquisition

Rates of MMV acquisition by *P. maidis* that developed on the different corn lines were compared using ANOVA (PROC MIXED; SAS Institute). Inbred was selected as main effect, replicates were selected as random factor. The following pre-planned contrasts were performed to compare the effects of corn genotype; homozygous dominant genotype (*Mv/Mv*) vs. homozygous recessive genotype (*mv/mv*); homozygous dominant genotype (*Mv/Mv*) vs. heterozygous genotype (*Mv/mv*); heterozygous genotype (*Mv/mv*) vs. homozygous recessive (*mv/mv*); homozygous dominant genotype vs. GCB; heterozygous genotype (*Mv/mv*) vs. GCB; homozygous recessive genotypes (*mv/mv*) vs. GCB.. Data on the proportion of infected planthoppers were arcsine [sqrt(proportion infected)] transformed for analysis. Reported means are from non-transformed data.

Field Experiment

The number of *P. maidis* adults per plant over time was analyzed using repeated-measures ANOVA (PROC GLM, SAS Software 9.2; SAS Institute, 2008, Cary, N.C.). A repeated measures design was used because the same plants were sampled throughout the planting season. The model was constructed to examine the effect of inbred sampled over time. Block was designated as a random factor and plant as the repeated subject. When significance was found at the $P < 0.05$ level, Tukey's test was used to compare the mean values among corn inbreds. Within the model, pre-planned contrasts were conducted: homozygous dominant genotype vs. homozygous recessive genotype; homozygous dominant genotype vs. heterozygous genotype; heterozygous genotype vs. homozygous recessive; homozygous dominant genotype vs. GCB; heterozygous genotype vs. GCB; homozygous recessive genotypes vs. GCB. The proportion of plants showing MMV virus symptoms were arcsin transformed [sqrt(proportion infected)] and analyzed using mixed model ANOVA (PROC MIXED, SAS Institute). Reported means are from non-transformed data.

Results

Laboratory Assay

Development, longevity, and fecundity

There was a significant difference detected in mean developmental time of *P. maidis* feeding on the leaves of the different corn genotypes ($F_{3,\,503} = 14.88$; $P < 0.001$; Table 4.2). Planthopper development on *Mv* dominant, recessive and heterozygous corn was significantly shorter than on GCB corn plants ($P < 0.001$). However, there was no significant difference in mean developmental time between *Mv* dominant, heterozygous, and recessive corn genotypes (*P*

> 0.05). Developmental time between instars differed significantly between the four inbred genotypes ($P < 0.05$; Table 4.2). Nymphs developing on GCB consistently took longer between successive molts compared to the other corn genotypes.

Nymphal mortality observed between genotypes was not significantly different (ANOVA, $P > 0.05$). No significant difference ($P > 0.05$) was observed for mean longevity of adults reared on the different corn genotypes (Table 4.2). The survivorship curves of *P. maidis* exposed to *Mv* dominant, heterozygous, recessive, and GCB were comparable (Figure 4.4). However, mean fecundity (eggs laid / female / day) did significantly differ between genotypes ($F_{3, 200} = 5.52$; $P = 0.0012$; Table 4.2). Fecundity on GCB sweet corn was statistically similar to *Mv* dominant, heterozygous, and recessive genotypes. However, fecundity on *Mv* heterozygous genotype was significantly lower in comparison to *Mv* dominant and recessive genotypes.

MMV acquisition

Acquisition efficiencies for MMV differed across inbreds ($F_{6, 20} = 14.66$; $P < 0.001$). The highest proportion of MMV acquisition was observed on GCB and the lowest on inbred Hi47 (Figure 4.1). Virus acquisition rates between *Mv* dominant (Hi47 vs. Hi60), *Mv* heterozygous (Hi47xB73 vs. Hi60xMo17), and *Mv* recessive (B73 vs. Mo17) inbreds, were not statistically different from one another ($P > 0.05$; Figure 4.1). The effect of genotype on the acquisition of MMV by *P. maidis*, was further analyzed by planned contrasts. Planthoppers that develop on GCB plants showed a significantly higher rate of MMV infection than planthoppers that developed from *Mv* dominant and heterozygous genotypes ($F_{1, 20} = 45.72$; $P < 0.001$) and ($F_{1, 20} = 38.82$; $P < 0.001$), respectively. There was no significant difference between GCB and *Mv* recessive genotypes ($P > 0.05$; Figure 4.2). Acquisition rates did not significantly differ between planthoppers developing on *Mv* dominant and heterozygous genotypes. However, MMV acquisition was significantly lower for planthoppers developing on *Mv* dominant and heterozygous genotypes compared to planthoppers developing on *Mv* recessive genotypes ($F_{1,\,20}$ = 38.86; $P < 0.001$) and ($F_{1,\,20}$ = 31.17; $P < 0.001$), respectively.

Field assay

Planthopper counts

The average number of planthoppers in corn plots increased over time, peaking between 42 days after planting (DAP) and 56 DAP with an average of less than 3 adult planthoppers per plant, and steadily declining thereafter (Figure 4.3). The number of adult planthoppers per plant significantly differed between corn inbreds ($F_{3,\,373}$ = 9.39; $P < 0.001$). Inbred Hi47xB73 (heterozygous for gene *Mv*), consistently harbored more planthoppers than the other corn types and GCB. The effect of corn genotype on the abundance of planthopper was further analyzed using planned contrasts (Table 4.3). Results revealed that corn genotype heterozygous for gene *Mv* had a significantly larger number of planthoppers than both corn genotypes dominant for gene *Mv* and GCB plants, but was not different from corn genotypes recessive for gene *Mv*. Planthopper density on GCB plants also differed significantly between *Mv* dominant and recessive plants (Table 4.3).

Figure 4.3 shows the mean planthopper counts on the near isogenic corn lines dominant, heterozygous, recessive corn lines for gene *Mv* and GCB over time. Planthopper density was significantly greater on plants heterozygous for gene *Mv* versus *Mv* dominant plants at several sampling times (28 DAP: $F_{1,\,397}$ = 9.22; P = 0.0026; 35 DAP: $F_{1,\,397}$ = 16.80; $P < 0.0001$; 42 DAP: $F_{1,\,397}$ = 12.12; P = 0.0006; 49 DAP: $F_{1,\,397}$ = 17.44; $P < 0.0001$) and GCB plants at multiple sampling times (28 DAP: $F_{1, 397}$ = 4.84; P = 0.0285; 35 DAP: $F_{1, 397}$ = 4.50; P = 0.0346; 49 DAP: $F_{1, 397}$ = 20.99; P < 0.0001; 56 DAP: $F_{1, 397}$ = 7.88; P = 0.0053; 63 DAP: $F_{1, 397}$ = 5.75; P = 0.0170; 70 DAP: $F_{1, 397}$ = 5.36; P = 0.0211), respectively. There were significantly less planthoppers on GCB plants than *Mv* recessive plants at weeks 7 and 8 (49 DAP: $F_{1, 397}$ = 12.49; P = 0.0005; 56 DAP: $F_{1, 397}$ = 9.54; P = 0.0022) and *Mv* dominant plant at weeks 8, 9, and 10 (56 DAP: $F_{1, 397}$ = 4.74; P = 0.0300; 63 DAP: $F_{1, 397}$ = 15.08; P = 0.0001; 70 DAP: $F_{1, 397}$ = 11.74; P = 0.0007), respectively. Corn plants recessive for gene *Mv* harbored significantly more planthoppers than corn with genotype dominant for gene *Mv* only at 49 DAP ($F_{1, 397}$ = 8.34; P = 0.0041), but harbored significantly less planthoppers per plant at 63 DAP ($F_{1, 397}$ = 9.37; P = 0.0024) and 10 ($F_{1, 397}$ = 9.93; P = 0.0018).

MMV incidence

The incidence of MMV in the field trial was relatively high with 53% of plants (212 out of 400) expressing symptoms of MMV infection. At the end of the plant growth-cycle (70 DAP) the proportion of corn plants showing symptoms of MMV infection was significantly different among the different corn genotypes ($F_{3, 24}$ = 3.63; P = 0.027; Figure 4.4). By contrast comparison, GCB corn had a significantly higher incidence of MMV compared to corn plants with genotype dominant for gene *Mv*, from 42 DAP to 70 DAP (42 DAP: $F_{1, 24}$ = 4.31; P = 0.049; 49 DAP: $F_{1, 24}$ = 10.22; P = 0.004; 56 DAP: $F_{1, 24}$ = 7.09; P = 0.014; 63 DAP: $F_{1, 24}$ = 10.27; P = 0.0038; 70 DAP: $F_{1, 24}$ = 10.27; P = 0.004; Figure 4.4). Similarly, GCB corn also had a significantly higher proportion of MMV infected plants compared to *Mv* recessive plants between 49 DAP and 70 DAP (49 DAP: $F_{1, 24}$ = 4.46; P = 0.045; 56 DAP: $F_{1, 24}$ = 4.57; P = 0.043; 63 DAP: $F_{1, 24}$ = 4.85; P = 0.038; 70 DAP: $F_{1, 24}$ = 4.85; P = 0.038).

The proportion of corn plants showing MMV symptoms also differed significantly between corn inbreds ($F_{6, 21}$ = 7.05; P = 0.0003; Figure 4.5). Inbred Hi47 and hybrid Hi47xB73 had significantly less plants then GCB showing symptoms for MMV infection. Although sharing the same dominant genotype (*Mv/Mv*), inbred Hi47 had significantly less MMV infected plants compared to inbred Hi60 ($F_{1, 21}$ = 8.32; P = 0.01; Figure 4.5). Similarly, hybrid Hi47xB73 had significantly less MMV infection compared to hybrid Hi60xMo17, both with *Mv* heterozygous genotype ($F_{1, 21}$ = 14.15; P = 0.001). Conversely, both *Mv* recessive inbreds, B73 and Mo17, has similar infection rates (Figure 4.5).

Discussion

The population of planthoppers sampled in the field assay averaged at less than three adults per plant at peak population numbers. In Hawaii, this is considered to be a very low density of planthoppers on *Z. mays* plants. However, the incidence of MMV in the field was high. This suggests that planthoppers may be actively moving between plant hosts. As discussed in chapter 3, macropters are more likely to disperse from their original plant host from which they developed; however, MMV transmission rates were not different between macropter and brachypters.

Inbreds homozygous dominant for gene *Mv* had disease incidence of approximately 40%, about 11, 17 and 34% lower than inbreds homozygous recessive for gene *Mv*, heterozygous for gene *Mv* and GCB, respectively. Hi47 corn lines had a much lower infection rate than inbred Hi60 corn lines. However, inbreds B73 and Mo17, both recessive for gene *Mv* (*mv/mv*), had similar disease rates. These results confirm previous field observation on the resistance to MMV associated with gene *Mv* (Ming et al., 1997; Brewbaker et al., 1991; Dintinger et al., 2005; Jin et al., 1998; Martin et al., 1999; Ndjiondjop et al., 1999; Sharp et al., 2002). The differences observed between inbreds Hi47 and Hi60 suggest the latter inbred is more tolerant to MMV infection than the former. Similar differences in incidence of MMV effects were observed in heterozygous inbreds as well. These differences may be attributed to inconsistent planthopper and MMV pressure in the field during resistance screening, and backcrossing or other genetic factors that may affect the tolerance level to MMV.

Corn genotypes heterozygous for gene *Mv* consistently harbored more planthoppers throughout the crop planting, and the corn genotypes homozygous dominant for gene *Mv*, harbored fewer planthoppers than homozygous recessive genotypes. The incidence of MMV was greater on *Mv* heterozygous than *Mv* recessive corn genotypes. The greater abundance of planthoppers on the hybrid crosses may be associated with plant size. Since these plants tend to be taller than their parent lines they may provide more available living space, thus able to attract and support higher densities of developing planthoppers. Little differences in planthopper densities were observed between inbreds of the same genotype (Hi47 and Hi60; B73 and Mo17; Hi47xB73 and Hi60xMo17). It seems that planthoppers had a lower preference for plants with an *Mv* dominant genotype.

Several species of planthoppers have dimorphic wing forms called brachypters and macropters (Denno & Roderick, 1990; Zera & Denno, 1997). Macropters are better adapted for dispersal having fully developed wings, whereas brachypters have short wings and are not effective flyers. However, unlike the macropters, brachypters are typically more fecund, and therefore a better suited to exploit the host plant’s resources (Denno & Roderick, 1990). Denno and Roderick (1990) and Denno et al., (1996) have shown that planthopper wing morphs can shift in response to host plant quality. As the quality or host plant nutrition declines, more macropters are produced for their dispersal capability. A previous study (Chapter 2) focusing on the effect of MMV on the biology of *P. maidis,* illustrated that MMV has a strong indirect influence on the production of macropters. This interaction may facilitate the spread of the virus. Despite having a higher incidence for MMV, GCB plants typically harbored fewer planthoppers than the other inbreds tested. I suggest the rapid decline in planthopper numbers observed on GCB plants 42 DAP (Figure 4.3) is likely the result of the severe symptoms of MMV infection, that led to a larger production of macropters.

Parejarearn et al. (1984) found that *Nilaparvata lugens* (Delphacidae) showed little preference toward *Rice ragged stunt virus* (RSV) resistant varieties. A field study by Dhileepan et al. (2003) showed that *Fiji disease virus* (*Fijivirus*) incidence reflected resistances ratings and highly susceptible sugarcane cultivars were most preferred by field populations of *Perkinsiella saccharicida* (Delphacidae). Low colonization responses by insect vectors on resistant corn cultivars have also been reported (Dintinger et al., 2005; Singh & Seetharama, 2008). It has been suggested that higher vector preference toward susceptible varieties may be due to the expression of specific stimulants that promote increased feeding activity in susceptible plants (Cook & Denno, 1994; Dhileepan & Croft, 2003). However, this may not be the case here as heterozygous *Mv* genotyes harbored higher densities then susceptible genotypes.

Crop resistance to insects alters the relationship of an insect pest with its plant host, thus leading to a changed abundance of insects in the field. There are different types of resistance (e.g. antibiosis or antixenosis) which affect the relationship between the insect and plant differently (Kennedy, 1976). Several studies have illustrated negative effects to insect pests feeding on insect resistant crop varieties. A noticeable reduction in performance was observed when the brown planthopper, *Nilaparvata lugens,* was reared on resistance wild rice species *Oryza officinalis* (Hu et al., 2010). Kennedy & Schaefers (1974) observed that a strong resistance to the raspberry aphid, *Amphorophora agathonica*, in red raspberry had a negative impact on aphid preference and performance. Maris et al. (2003) found that *Frankliniella occidentalis* feeding on resistant pepper plants (*Capsicum* hybirds) had a reduced fecundity. Parejarearn et al. (1984) also observed a reduced longevity and fecundity on these resistant varieties and suggested that resistant was acting at the level of virus transmission but not to virus multiplication. In my study the performance of planthoppers across the various corn genotypes was found to be very similar. Although a few statistical differences among the performance parameters were detected, I do not consider it to be biologically significant since the magnitudes of differences is quite small. This implies that gene *Mv* does not influence planthopper fitness and does not have a deterrent effect on *P. maidis* feeding. In fact, planthoppers on *Mv* recessive corn lines typically performed better than on GCB sweet corn, which is also highly susceptible to MMV. Reports of plant resistance against *P. maidis* are scarce (Singh & Seetharama, 2008). However, resistance to *P. maidis* has been observed in sorghum (*Sorghum bicolor*) (Chandra Shekar et al., 1993a; Chandra Shekar et al., 1993b). Chandra Shekar (1991; 1993a) have reported differences in settling preferences, increase mortality, longer nymphal development, and a 5-fold reduction in fecundity in *P. maidis* on resistant genotypes of sorghum compared with a susceptible hybrid variety. A study on the feeding behavior *P. maidis* conducted by Fisk (1980) showed that settling behavior and feeding was negatively affected when *P. maidis* were exposed to mixtures of phenolic acids and their esters on artificial media at concentrations similar to those occurring in young sorghum plants. These assays were also correlated with a reduction in the ability of *P. maidis* to locate and feed on the phloem tissues. No studies, at least to our knowledge, have illustrated similar effects of *P. maidis* on corn.

Results obtained from the acquisition assays yielded significant differences among the corn genotypes. Planthoppers that developed on *Mv* dominant and heterozygous corn genotypes had the lowest incidence of MMV, acquiring the MMV at rates below 20%. In contrast, planthoppers that developed on GCB and *Mv* recessive corn plants acquired MMV at rates above 30%. These results suggest that gene *Mv* affects the rate of virus acquisition. Our results are similar to those of Dhileepan et al. (2006) and Ridley (2008). These authors showed that *Fiji disease virus* (FDV, *Fijivirus*) was acquired on infected sugarcane by *P. saccharicida* at a lower rates on a resistant cultivar (WD1 or WD2) than compared to acquisition from a susceptible (NCo310) or mixed (susceptible and resistant) cultivar source. Low acquisition rates may prove to be a useful characteristic in controlling MMV in corn cropping systems. The resistant inbreds will have a lower chance of becoming infected and will be poorer source of the virus, thus, reducing the spread of MMV. A reduction of virus acquisition observed in *Mv* homozygous dominant inbreds may be due to altered feeding behavior of *P. maidis* (Dintinger et al., 2005). The uses of electrical penetration graphs monitoring have helped to investigate the impact of resistance on disease transmission. Chang & Ota (1978) showed that the feeding patterns of the planthopper *Perkinsiella saccharicida* (Hemiptera: Dephacidae) were more active in phloem ingestion on *Fiji disease virus* susceptible sugarcane cultivars versus those that were resistant. Dintinger et al. (2005) reported that the feeding behavior of *P. maidis* on MMV resistant corn lines was significantly disturbed in comparison to inbreds with lesser or no resistance.

Much of the evidence presented here supports the hypothesis that gene *Mv* is acting at the level of the virus, possibly reducing virus replication (Lapidot et al., 2001) and/or movement within the plant host, thus limiting virus acquisition. Further studies need to be done to understand the mechanisms of the *Mv* resistance gene. It would be interesting for future studies to investigate the population dynamics of *P. maidis* in the field planted with various resistant genotypes versus a mono-crop of all resistant genotypes. This may demonstrate how *P. maidis* populations, as well as MMV incidence, are suppressed across several cropping cycles.

Resistant varieties can be used as a cost-effective approach to pest management. Economic benefits can occur by limiting insecticide applications and in turn, help increase the population of beneficial species, promoting a more sustainable agroecosystem. The introduction of gene *Mv* into sweet corn varieties have drastically helped reduced the spread of MMV in Hawaii, which was considered as a very serious problem since the 1940's (Brewbaker, 2003). Many, if not all, of Hawaii sweet corn farmers grow MMV resistant sweet corn. To date there is no evidence that MMV has developed resistance breaking-strains. A possible application for corn produced for breading, is the use of MMV resistant inbreds as a valuable barriers to the virus. Since infestations by the migrant forms *P. maidis* are usually highest at the border regions of the fields (Takara & Nishida, 1983), resistant inbreds could be deployed strategically as border material of corn breeding nurseries or production fields and help prevent incursion and further spread of MMV within the field.

Figures

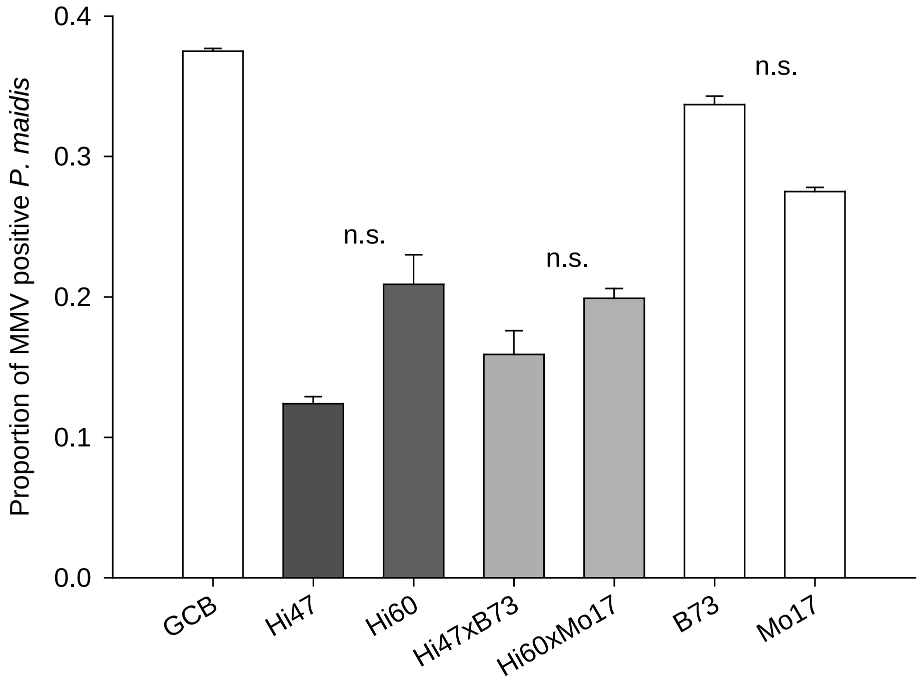

Figure 4.1. Proportion (± SE) of *P. maidis* positive for MMV infection that developed on different inbred; Hi47 & Hi60 (*Mv* dominant; dark grey bars), B73 & Mo17 (*Mv* recessive; white bars), Hi47xB73 & Hi60xMo17 (heterozygous *Mv*; light grey bars), and GCB (susceptible sweet corn; black bar). "n.s." located above and between columns indicates no significant difference between means for inbreds.

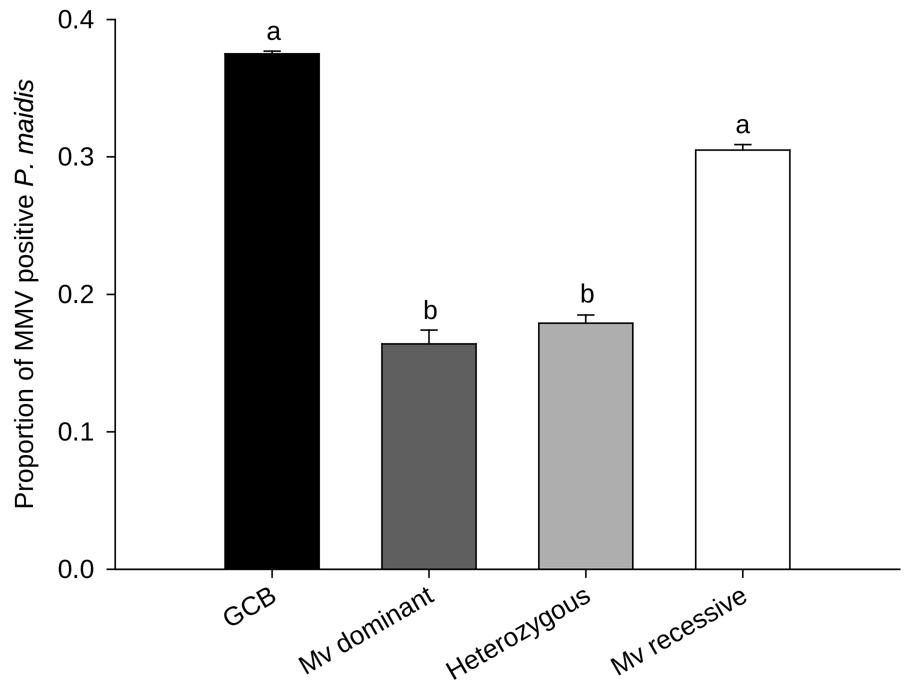

Figure 4.2. Proportion (± SE) of *P. maidis* positive for MMV acquisition that developed on genotypes; homozygous *Mv* dominant (Hi47 & Hi60; dark grey bar), homozygous *Mv* recessive (B73 & Mo17; white bar), heterozygous *Mv* (Hi47xB73 & Hi60xMo17; light grey bar), and susceptible sweet corn (GCB; black bar). Same letters above each bar represents no significant difference.

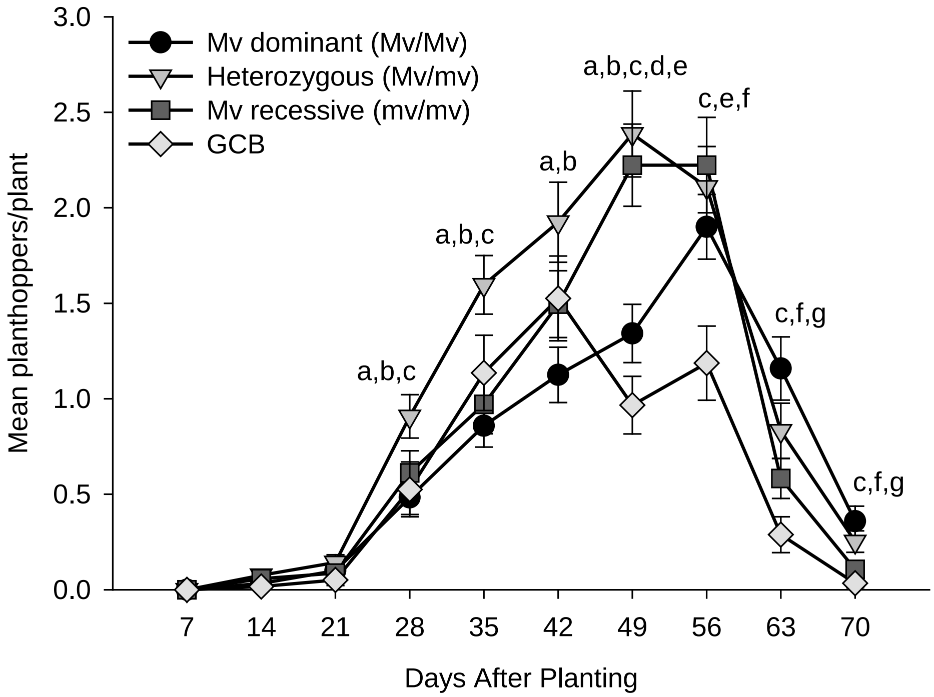

Figure 4.3. Average (± SE) planthoppers found on homozygous *Mv* dominant (Hi47 and Hi60; circle), homozygous *Mv* recessive (B73 and Mo17; square), heterozygous *Mv* (Hi47xB73 and Hi60xMo17; triangle), and susceptible sweet corn (GCB; diamond) genotypes at each sampling week. Letters above the data points for each sampling week indicate significant differences as follows: a: heterozygous *Mv* greater than *Mv* dominant; b: heterozygous *Mv* greater than *Mv* recessive; c: heterozygous *Mv* greater than GCB, d: *Mv* recessive greater than *Mv* dominant; e: *Mv* recessive greater than GCB; f: *Mv* dominant greater than GCB; g: *Mv* dominant greater than *Mv* recessive.

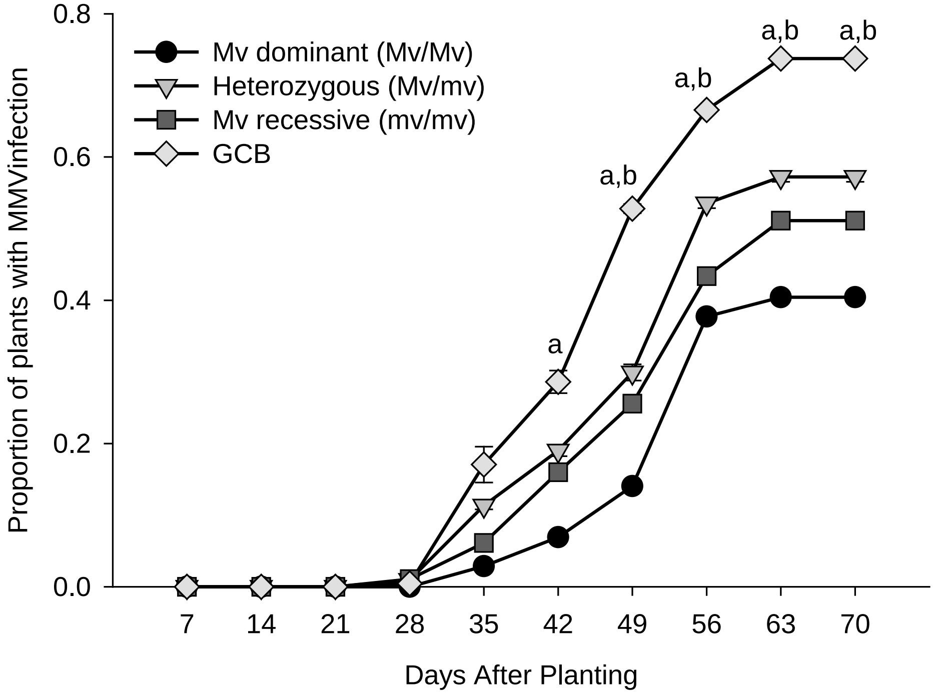

Figure 4.4. Proportion (± SE) of corn plants exhibiting symptoms of MMV observed between corn genotype; homozygous *Mv* dominant (Hi47 and Hi60; circle), homozygous *Mv* recessive (B73 and Mo17; square), heterozygous *Mv* (Hi47xB73 and Hi60xMo17; triangle), and susceptible sweet corn (GCB; diamond). Letters above each sampling week represents significance. a represents GCB greater than *Mv* dominant; b represents GCB greater than *Mv* recessive.

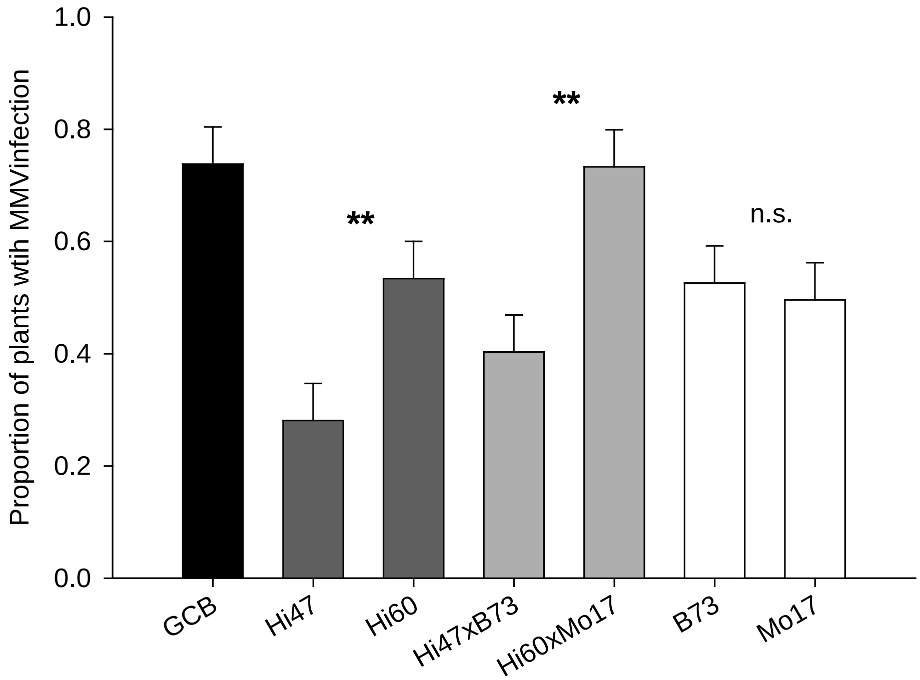

Figure 4.5. Proportion (± SE) of corn plants exhibiting symptoms of MMV observed between each corn inbred; Hi47 & Hi60 (*Mv* dominant; dark grey bars), B73 & Mo17 (*Mv* recessive; white bars), Hi47xB73 & Hi60xMo17 (heterozygous *Mv*; light grey bars), and GCB (susceptible sweet corn; black bars). Asterisks located above and between columns indicated a significant difference between the two inbreds. Inbred Hi47 and hybrid Hi47xB73 had significantly less MMV infected plants then GCB.

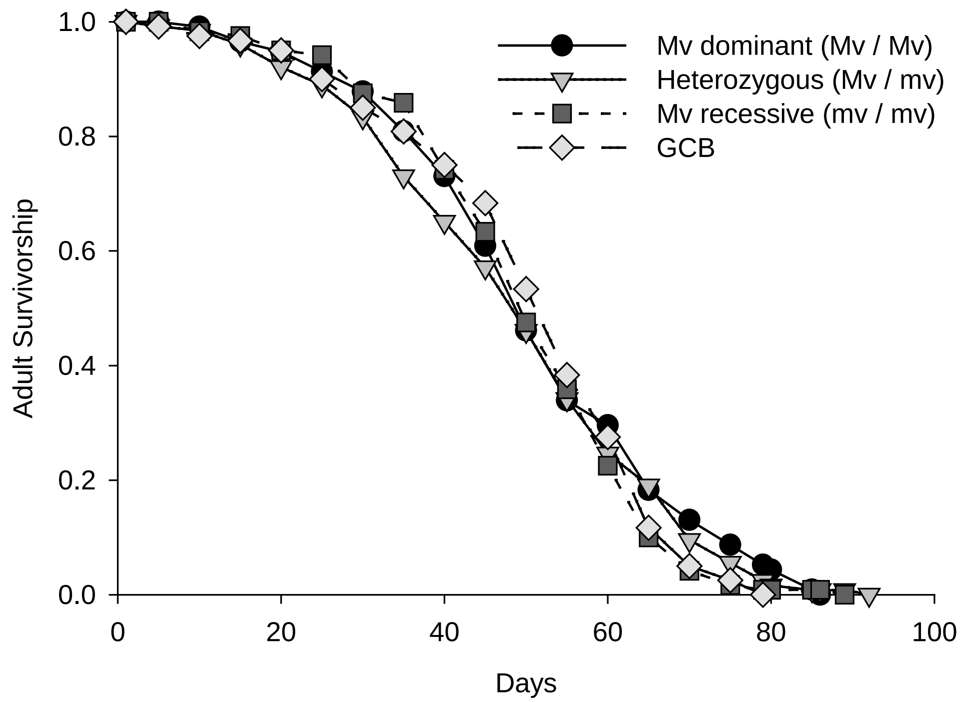

Figure 4.6. Cumulative survivorship (%) of *Peregrinus maidis* reared on the different corn genotypes; *Mv* dominant (Hi47 & Hi60; circle), homozygous *Mv* recessive (B73 & Mo17; square), heterozygous *Mv* (Hi47xB73 & Hi60xMo17; triangle), and susceptible sweet corn (GCB; diamond).

Tables

Table 4.1. List of corn inbred lines and their genotypes.

Inbred	**Origin**	**Genotype**
B73	Iowa; Iowa Stiff Stalk Synthetic	*Mv* recessive (*mv*/*mv*)
Mo17	Missouri; 187-2 x C103	*Mv* recessive (*mv*/*mv*)
Hi60	Hawaii conversion of Mo17	*Mv* dominant (*Mv*/*Mv*)
Hi47	Hawaii conversion of B73	*Mv* dominant (*Mv*/*Mv*)
Hi60 x Mo17	Hybrid cross of parents	*Mv* Heterozygous(*Mv*/*mv*)
Hi47 x B73	Hybrid cross of parents	*Mv* Heterozygous(*Mv*/*mv*)
Golden Cross Bantam (GCB)	Sweet corn var.	*Mv* recessive (*mv*/*mv*)

Inbreds with “Hi” denotes *Mv* conversion by multiple backcrosses to achieve near isogenic line.
Mv dominant = homozygous dominant (*Mv/Mv*) gene.
Mv Heterozygous = (*Mv/mv*)
Mv recessive = homozygous recessive (*mv/mv*) gene.

Table 4.2. Development, adult longevity, and fecundity (mean ±SE) of *Peregrinus maidis* on *Mv* dominant, heterozygous *Mv*, *Mv* recessive, and GCB corn plants.

Parameters	*(n)*	**GCB** Mean ± SE	*(n)*	***Mv* Dominant** Mean ± SE	*(n)*	***Mv* Heterozygous** Mean ± SE	*(n)*	***Mv* Recessive** Mean ± SE
Development Time (days)								
First Instar	136	4.0 ± 0.2 (a)	133	3.6 ± 0.2 (b)	145	3.6 ± 0.2 (b)	134	3.8 ± 0.0 (ab)
Second Instar	131	3.1 ± 0.1 (a)	128	2.8 ± 0.1 (b)	142	2.9 ± 0.1 (ab)	132	2.8 ± 0.1 (b)
Third Instar	130	2.8 ± 0.1 (a)	128	2.6 ± 0.1 (b)	140	2.9 ± 0.1 (a)	129	2.7 ± 0.1 (ab)
Forth Instar	126	3.2 ± 0.2 (a)	126	2.9 ± 0.2 (b)	140	3.1 ± 0.2 (ab)	126	3.0 ± 0.2 (ab)
Fifth Instar	124	4.5 ± 0.2 (a)	124	4.3 ± 0.1 (ab)	137	4.2 ± 0.1 (b)	123	4.1 ± 0.1 (b)
Total	124	17.7 ± 0.2(a)	124	16.3 ± 0.2(b)	137	16.6 ± 0.2 (b)	123	16.2 ± 0.2 (b)
Nymphal Mortality		17% (a)		11% (a)		12% (a)		16% (a)
Adult Longevity (days)	120	47.9 ± 3.7 (a)	115	48.8 ± 3.7 (a)	126	47.1 ± 3.7 (a)	120	48.1 ± 3.7 (a)
Fecundity (Avg. eggs/♀/Day)	51	6.5 ± 0.2 (ab)	40	7.8 ± 0.4 (a)	58	6.0 ± 0.3 (b)	56	7.0 ± 0.4 (a)

Mixed model ANOVA (PROC MIXED) was used to test the main effect of genotype.
Means within the same rows sharing the same letter are not significantly different ($P > 0.05$, Tukey-Kramer test).

Table 4.3. Results of the contrasts from repeated measures ANOVA (PROC GLM) on the average *P. maidis* counted per plant on *Mv* dominant, heterozygous *Mv*, and *Mv* recessive corn genotypes.

Contrast	d.f.	F - Value	P -Value
Mv dominant vs. *Mv* recessive	1, 397	1.06	0.3046
Mv dominant vs. heterozygous *Mv*	1, 397	23.72	< 0.0001
heterozygous *Mv* vs. *Mv* recessive	1, 397	12.56	0.0004
Mv dominant vs. GCB	1, 397	5.02	0.0256
Heterozygous *Mv* vs. GCB	1, 397	38.37	< 0.0001
Mv recessive vs. GCB	1, 397	8.99	0.0029

Significance was determined at the $a < 0.05$ level.

References:

Ammar ED, Gingery RE & Nault LR (1987) Interactions between Maize mosaic virus and Maize stripe viruses in their insect vector, *Peregrinus-maidis*, d in Maize. Phytopathology 77: 1051-1056.

Ammar ED, Gomez-Luengo RG, Gordon DT & Hogenhout SA (2005) Characterization of Maize Iranian mosaic virus and comparison with Hawaiian and other isolates of Maize mosaic virus (*Rhabdoviridae*). Phytopathology 153: 129-136.

Ammar ED & Hogenhout SA (2008) A neurotropic route for Maize mosaic virus (*Rhabdoviridae*) in its planthopper vector *Peregrinus maidis*. Virus Res 131: 77-85. doi:10.1016/j.virusres.2007.08.010.

Backus EA, Serrano MS & Ranger CM (2005) Mechanism of hopperburn: An overview of insect taxonomy, behavior, and physiology. Ann. Rev. Entomol. 50: 125-151. doi:doi:10.1146/annurev.ento.49.061802.123310.

Balint-Kurti PJ & Johal GS (2008) Maize Disease Resistance: In *Handbook of Maize: Its Biology* (ed. by JL Bennetzen & SC Hake) Springer, New York, pp. 229-250.

Brewbaker J (1979) Diseases of maize in the wet lowland tropics and the collapse of the Classic Maya civilization. Econ Bot 33: 101-118. doi:10.1007/bf02858277.

Brewbaker JL (1981) Resistance to Maize mosaic virus: In *Virus and viruslike diseases of maize in the United States* (ed. by DT Gordon, Knoke J.K., Scott G.E.) Ohio Agric Res Dev Cent, Ohio, Southern Coop Ser Bull 247, pp 145-151.

Brewbaker JL, ed. (2003) Corn production in the tropics: The Hawaii experience. College of Tropical Agriculture and Human Resources, University of Hawaii Manoa, Honolulu, Hawaii, USA.

Brewbaker JL & Josue AD (2007) Registration of 27 Maize parental inbred lines resistant to Maize mosaic virus. Crop Sci. 47: 459-461. doi:10.2135/cropsci2005.11.0407.

Brewbaker JL, Kim SK & Logrono ML (1991) Resistance of tropical maize inbreds to major virus and virus-like disease. Maydica: 257-265.

Chandra Shekar BM (1991) Mechanisms of resisance on sorghum to shoot bug, *Peregrinus maidis* (Ashmead) (Homoptera:Delphacidae). M.Sc. Thesis, Andhra Pradesh Agriculture University, Hyderabad, India, 106pp.

Chandra Shekar BM, Reddy KD, Singh BU & Reddy DDR (1993a) Antixenosis component of resistance to corn planthopper, *Peregrinus maidis* (Ashmead) in sorghum. Insect. Sci. Appl. 14:77-84

Chandra Shekar BM, Singh BU, Reddy KD & Reddy DDR (1993b) Antibiosis component of resistance in sorghum to corn planthopper, *Peregrinus maidis* (Ashmead) (Homoptera:Delphacidae). Insect Sci Appl 14: 559-569.

Chang VCS & Ota AK (1978) Feeding activities of *Perkinsiella* leafhoppers and Fiji disease resistance of Sugarcane. J Econ Entomol 71: 297-300.

Cook AG & Denno RF (1994) Planthopper/plant interactions: feeding behavior, plant nutrition, plant defense, and host plant specialization: In *Planthoppers - Their Ecology and Management* (ed. by RF Denno & TJ Perfect) Chapman & Hall, New York, pp. 114-139.

Denno RF & Roderick GK (1990) Population biology of planthoppers. Ann Rev Entomol 35: 489-520.

Denno RE, Roderick GK, Peterson MA, Huberty AF, Dobel HG, Eubanks MD, Losey JE & Langellotto GA (1996) Habitat persistence underlies intraspecific variation in the dispersal strategies of planthoppers. Ecol Monogr 66: 389-408.

Dhileepan K & Croft BJ (2003) Resistance to Fiji disease in Sugar Cane: Role of cultivar preference by planthopper vector *Perkinsiella saccharicida* (Homoptera: Delphacidae) J Econ Entomol 96: 148-155.

Dhileepan K, Croft BJ, Ridley AW, James AP & Raghu S (2006) Susceptibility of source plants to Sugarcane Fiji disease virus influences the acquisition and transmission of the virus by the planthopper vector *Perkinsiella saccharicida*. J Appl Entomol 130: 67-71. doi:10.1111/j.1439-0418.2005.01023.x.

Dhileepan K, Greet A, Ridley A, Croft BJ & Smith GR (2003) Fiji disease resistance in sugarcane: Relationship to cultivar preference in field populations of the planthopper vector *Perkinsiella saccharicida*. An Appl Biol 143: 375-379. doi:10.1111/j.1744-7348.2003.tb00307.x.

Dintinger J, Boissot N, Chiroleu F, Hamon P & Reynaud B (2005) Evaluation of maize inbreds for Maize stripe virus and Maize mosaic virus resistance: Disease progress in relation to time and the cumulative number of planthoppers. Phytopathology 95: 600-607.

Fisk J (1980) Effects of HCN, phenolic acids and related compounds in Sorghum bicolor on the feeding behaviour of the planthopper Peregrinus maidis. Entomol Exp Appl 27: 211-222. doi:10.1007/bf00333831.

Herold R (1972) Maize mosaic virus. CMI/AAB Description of Plant Viruses #94.

Hu L-X, Chi H, Zhang J, Zhou Q & Zhang R-J (2010) Life-table analysis of the performance of *Nilaparvata lugens* (Hemiptera: Delphacidae) on two wild rice species. J Econ Entomol 103: 1628-1635. doi:10.1603/ec10058.

Jin H, Domier LL, Kolb FL & Brown CM (1998) Identification of quantitative loci for tolerance to Barley yellow dwarf virus in oat. Phytopathology 88: 410-415.

Kang B-C, Yeam I & Jahn MM (2005) Genetics of plant virus resistance. Ann Rev Phytopathol 43: 581-621. doi:doi:10.1146/annurev.phyto.43.011205.141140.

Kennedy GG (1976) Host Plant Resistance and the Spread of Plant Viruses. Environ Entomol 5: 827-832.

Kennedy GG & Schaefers GA (1974) Evidence for nonpreference and antibiosis in aphid-resistant red raspberry cultivars. Environ Entomol 3: 773-777.

Lapidot M, Friedmann M, Pilowsky M, Ben-Joseph R & Cohen S (2001) Effect of host plant resistance to Tomato yellow leaf curl virus (TYLCV) on virus acquisition and transmission by its whitefly vector. Phytopathology 91: 1209-1213. doi:10.1094/phyto.2001.91.12.1209.

Maris PC, Joosten NN, Peters D & Goldbach RW (2003) Thrips resistance in pepper and its consequences for the acquisition and inoculation of Tomato spotted wilt virus by the Western flower thrips. Phytopathology 93: 96-101. doi:10.1094/phyto.2003.93.1.96.

Martin DP, Willment JA & Rybicki EP (1999) Evaluation of maize streak virus pathogenicity in differentially resistant *Zea mays* genotypes. Phytopathology 89: 659-700.

Metcalf ZP (1943) General catalogue of the Hemiptera, fasc. IV. Fulgoroidea, part 3, Araeopidae (Delphacidae). Smith College, Northhamptom, Mass, pp. 552

Ming R, Brewbaker JL, Pratt RC, Musket TA & McMullen MD (1997) Molecular mapping of a major gene conferring resistance to maize mosaic virus. Theor Appl Genetics 95: 271-275. doi:10.1007/s001220050559.

Namba R & Higa SY (1971) Host plant studies of the corn planthopper, *Peregrinus maidis* (Ashmead), in Hawaii. Proc Hawaiian Entomol Soc 21: 105-108.

Napompeth B (1973) Ecology and population dynamics of the corn planthopper, *Peregrinus maidis* (Ashmead) (Homoptera: Delphacidae) in Hawaii. Ph. D Dissertation, University of Hawaii, Honolulu, Hawaii.

National Agriculture Statistics Service. 2011. Statistics of Hawaii Agriculture: Seed Crops. U.S. Department of Agriculture, Honolulu, HI.

Ndjiondjop MN, Albar L, Fargette D, Fauquet C & Ghesquière A (1999) The genetic basis of high resistance to Rice yellow mottle virus (RY*MV*) in cultivars of two cultivated rice species. Plant Disease 83: 931-935.

Parejarearn A, Lapis DB & Hibino H (1984) Reaction of selected rice varieties to rice ragged stunt virus and its vector *Nilaparvata lugens*. Philippine Phytopathol 20: 15-16.

Redinbaugh MG, Jones MW & E. GR (2004) The genetics of virus resistance in maize (*Zea Mays L.*). Maydica: 183-190.

Ridley AW, Dhileepan K, Walter GH, Johnson KN & Croft BJ (2008) Variation in acquisition of Fiji disease virus by *Perkinsiella saccharicida* (Hemiptera: Delphacidae). J Econ Entomol 101: 17-22. doi:10.1603/0022-0493(2008)101[17:viaofd]2.0.co;2.

Sharp GL, Martin JM, Lanning SP, Blake NK, Brey CW, Sivamani E, Qu R & Talbert LE (2002) Field evaluation of transgenic and classical sources of Wheat streak mosaic virus resistance. Crop Sci 42: 105-110.

Singh BU & Rana BS (1992) Stability of resistance to corn planthopper, *Peregrinus-maidis* (Ashmead) in sorghum germplasm. Insect Sci Appl 13: 251-263.

Singh BU & Seetharama N (2008) Host plant interactions of the corn planthopper, *Peregrinus maidis* Ashm. (Homoptera: Delphacidae) in maize and sorghum agroecosystems. Arthropod-Plant Intera 2: 163-196. doi:10.1007/s11829-007-9026-z.

Takara J & Nishida T (1983) Spatial distribution of the migrants of the corn delphacid, *Peregrinus maidis* (Ashmead) (Homoptera:Delphacidae) in cornfields. Proc Hawaiian Entomol Soc 24: 7.

Tsai JH (1996) Development and oviposition of *Peregrinus maidis* (Homoptera: Delphacidae) on various host plants. Florida Entomol 79: 19-26.

Vega FE, Barbosa P, Kuosell HL, Fisher DB & Nelsen TC (1995) Effects of feeding on healthy and diseased corn plants on a vector and non-vector insect. Experientia 51: 293-299.

Zera AJ & Denno RF (1997) Physiology and ecology of dispersal polymorphism in insects. Ann Rev Entomol 42: 207-230. doi:doi:10.1146/annurev.ento.42.1.207.