



South Atlantic U.S. Horticultural Rsch. Lab, Fort Pierce, FL Subtropical Insects Research

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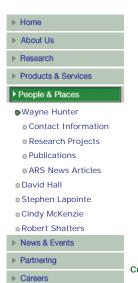


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Wayne B. Hunter wayne.hunter@ars.usda.gov Research Entomologist



Wayne Hunter, Ph.D. Research Entomologist

U. S. Horticultural Research Laboratory 2001 South Rock Road Fort Pierce, FL 34945 Telephone: (772) 462-5898 Fax: (772) 462-5986 Email: Wayne.Hunter@ars.usda.gov



Adult Glassy-winged Sharpshooter Leafhopper, GWSS, Homalodisca vitripennis Germar,. Vector of Pierce's disease of grapes, a xylemlimited bacterium, Xylella fastidiosa. Adults are about 1/2 inch, (12 mm) long.

Current Research Projects:

Dr. Hunter, Lead Scientist conducting research on the spread of plant diseases by insects. The main focus of research is on the glassywinged sharpshooter, GWSS, and other leafhoppers which spread the plant infecting bacteria, *Xylella fastidiosa*, at the U.S. Horticultural Research Lab, Fort Pierce, Florida.

Dr. Hunter works to provide solutions to insect problems under USDA-ARS National Program 304, Crop Protection and Quarantine.

Dr. Hunter's lab is elucidating GWSS biology using a genomics approach and has produced and published ~25,000 genetic sequences isolated from the GWSS.

These can be found at the National Center Biotechnology and Information, NCBI, expressed sequence tag, EST database for access by the research and education community.

Continuing examination of GWSS proteins have led to a deeper understanding of leafhopper feeding, development, and pathogen interactions.

He is the founder of the 'Southeastern Xylella Sharpshooter Initiative' aimed at increasing interactions among researchers on Pierce's disease and leafhoppers.

Dr. Hunter is also examining the use of insect viruses as a possible management tools to reduce insect populations, such as GWSS.

He has discovered several new insect viruses isolated from aphids, leafhoppers, psyllids, and fire ants.

Dr. Hunter also conducts research on exotic insect pests.

He has produced over 120,000 sequences from many different insect species:

Aphids, (Toxoptera citricida, Acyrthosiphon pisum, Aphis gossypii); Leafhoppers, (Homalodisca coagulata, Oncometopia nigricans); Asian Citrus Psyllid, (Diaphorina citri); Pink hibiscus mealybug, (Maconellicoccus hirsutus); citrus weevil, (Diaprepes abbreviatus); Asian longhorn beetle, (Anoplophora glabripennis); and parasitoids (Lysiphlebus testaceipes, Nasonia).

He was awarded 'Entomologist of the Year 2005' from the Florida Entomological Society for, "Internationally recognized contributions to insect genomics".

He is a member of the International Aphid Genomics working group, working with researchers from the USA, France, Spain, Japan, and Australia.

The aphid genome effort has resulted in the production of two pea aphid microarrays which are being used to increase our understanding of gene expression in aphid biology.

Dr. Hunter is also a member of the Genome Steering committee for the National Parasitoid Genome Project, Nasonia and the International Whitefly Genome Initiative.

He also organizes the 'International Insect Genomics Workshop at the International Plant & Animal Genome conference.

Dr. Hunter is a member of the 'International Grape Genome Program', and a co-developer of the 'North American Grape Genome Database', which is hosted at FloridaA&MUniversity.

North American grape varieties have greater resistance to grape diseases and insect pests.

Design of genetic markers linked to resistance traits are being used in a maker-assisted selection program to produce new grape varieties with resistance to important grape diseases, such as Pierce's Disease, and fungal pathogens.

Due to the economic importance of the Asian citrus psyllid, Diaphorina citri, as a vector of bacteria associated with the disease Huanglongbing, HLB in citrus, several new genomics efforts have been started to address many of the unknowns surrounding the cause and spread of HLB.





International Asian Citrus Psyllid Genome Consortium

Hunter, Wayne B. (Lead) USA

National Program Staff:

Dr. Kevin Hackett, NPL, ARS, Beltsville, MD (Insects)

Dr. Gail Wisler, NPL, ARS, Beltsville, MD (Plant Disease)

Steering Committee:

Dr. Susan Brown, KSU, Arthropod Genomics Consortium, KS

Dr. Stephen Richardson, Human Genome Sequencing Center, Baylor Univ., TX

Dr. Jay Evans, USDA, ARS, Honey bee genomics, Beltsville, MD

Working Group Members:contact Wayne.Hunter@ars.usda.gov

Project ID:	29473
Project Status:	Accepted
Project Type:	Single Species Project
Lead Contact's Name:	Wayne Hunter, Wayne.hunter@ars.usda.gov
Submitting Organization:	USDA, ARS, U.S.Hort. Res. Lab, Ft. Pierce, FL
Contact's Name:	Robert Shatters, Jr., Robert.shatters@ars.usda.gov
Contact's Name:	David Hall, <u>David.hall@ars.usda.gov</u>
Submitting Organization:	USDA, ARS, U.S.Hort. Res. Lab, Ft. Pierce, FL
Locus Tag Prefix:	DIACI
Source of DNA:	W. Hunter, Colonies, U.S.Horticultural Res. Lab
	2001 South Rock Road, Ft. Pierce, FL, USA

The actual causes of Huanglongbing, HLB, (known as 'Citrus Yellow Dragon Disease' or 'Citrus Greening') in citrus is currently unknown. While the bacteria, Candidatus Liberibacter asiaticus, CLa, has been shown to be associated with the occurrence of HLB in citrus, there is a lack of data on all the microorganisms associated with the 'cause' of HLB. There is growing agreement within the scientific community that the cause may be due to the interaction of several different pathogens.

To resolve this issue a genomics approach was proposed and developed, incorporating a Metagenomics approach to identify all possible micro organisms associated with HLB. By using high-resolution community-wide genomic information, we can describe the composition, function and emergent properties of the integrated microbial community within the citrus tree and within the psyllid vector more accurately.

While a genomics effort is being applied to the Asian citrus psyllid, AsCP, to provide insight into psyllid biology, the Metagenomic project provides a deeper understanding of the micro-world within the psyllid. The DNA from psyllids or from the phloem of citrus trees contains a community of organisms that may include a wide range of organisms from bacterial to viral species at different levels of diversity and abundance. Since many microbes resist being cultured, culture-independent methods such as genomics is often the only way for identifying and enumerating microbes in samples like citrus phloem and the psyllid.>

Therefore, an International Asian Citrus Psyllid Genomics consortium was organized, bringing together researchers working on insects, bacteria, psyllid vectors, plant disease, Genomics, Bioinformatics, Pathology, Microbiology and others. The main objectives will be to identify the causal agents of HLB, and then to apply these answers to the development of innovative management strategies against this devastating citrus disease.

International Diaphorina citri Wolbachia Genome Consortium

David Hall, RL, USHRL, ARS, Ft. Pierce, FL Yong Ping Duan, USHRL, ARS, Ft. Pierce, FL Wayne Hunter, USHRL, ARS, Ft. Pierce, FL Robert Shatters, USHRL, ARS, Ft. Pierce, FL Dave Schneider, ARS, Cornell Univ., NY Working Group forming:

Project ID:	29451
Project Status:	Accepted
Project Type:	Single Species Project
Contact's Name:	Wayne Hunter, Wayne.hunter@ars.usda.gov
Contact's Name:	Yong Ping Duan, YongPing.Duan@ars.usda.gov
Contact's Name:	Robert Shatters, Jr., Robert.shatters@ars.usda.gov
Contact's Name:	David Hall, <u>David.hall@ars.usda.gov</u>
Submitting Organization	USDA, ARS, U.S.Hort. Res. Lab, Ft. Pierce, FL
URL:	http://www.ars.usda.gov/main/site_main.htm?modecode=66-18
Consortium Name:	International Diaphorina citri Wolbachia Genome Consortium
Organism Name:	Wolbachia endosymbiont of Diaphorina citri
Strain/isolate/breed:	FL
Locus Tag Prefix:	WDIAC
Source of DNA:	Yong Ping Duan, USDA, ARS, U.S.Hort. Res. Lab,
	2001 South Rock Road, Ft. Pierce, FL, USA

General Information on GWSS:





Scientific name of the glassy-winged sharpshooter, GWSS: Homalodisca vitripennis Germar, is the main vector of the plant infecting pathogenic bacterium, *Xylella fastidiosa*.

Xylellabacterium, causes Pierce's Disease of grapes, and many other 'Scorch-like' plant diseases.

Since the GWSS can fly long distances, and feeds from a wide range of plants it can spread these bacteria far and wide through many different crops. Grapes, almonds, peaches and other tree crops especially are heavy hit by these pathogens.

Examination of the developmental genes within the eggs, embryos, and nymphs is aiding efforts to develop environmentally safe and efficient long-term management strategies to disrupt insect development and reduce the spread of *Xylella*caused diseases.

How damage is caused:

GWSS is a piercing-sucking feeder that extracts fluid from the xylem tissues of plants. This feeding spreads the bacterial diseases to other host plants. The pest is highly mobile, flying long distances, and feeds on many different plants, thus spreading the disease to many plants in a single day. The GWSS has over 100 different host plants on which it feeds. The bacterium Xylella fastidiosa is spread during GWSS feeding. The interactions between bacterium and plant induces the symptoms similar to drought, wherin the leaves and stems dry out, thus infected plants lose their vigor and gradually die.

Other diseases vectored by GWSS (strains of Xylella fastidiosa):

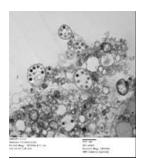
Pierce's disease (grapes) Leaf scorch (almonds) Alfalfa dwarf (alfalfa) Oleander scorch (oleander) Phony peach disease (peaches) Plum leaf scald (plums) Citrus variegated chlorosis (citrus)



Many other leafhopper species, such as the black-winged sharpshooter, *Oncometopia nigricans*can also transmit and spread *Xylella*caused plant diseases.

Intensive comparisons of the genetics between different leafhopper species is providing insights into how the pathogen interacts with the insect vectors, and adding to our understanding of pathogen acquisition and transmission.

Discovering Insect Viral Pathogens:



Newly discovered insect viruses, specific to sharpshooters, such as HoCV-1, which reduces GWSS survival, along with discovery of viruses in other insects, like fire-ants, SINV-1, and in many other insect pests demonstrates that there are a lot of unexploited insect pathogens yet to be discovered which may serve to reduce insect pests.

Iridovirus infection in GWSS fatbody tissues (pictured above) within the head of adults that were injected with virus into the lower abdomen.

Examination of virus infection shows that IIV6 infects fatbody, muscle, and nerve tissues of GWSS.

Mortality ranged from 20-30%, and egg production was reduced.

Insect infecting viruses may provide a means to reduce insect pests in an environmentally friendly manner.

The main obstacle is the development of a method for mass production of virus in a cost efficient manner.

Modern technological breakthroughs may soon make this approach a feasible, cost effective means of insect management.



New discoveries of insect infecting viruses, which are highly specific to the insects which they infect may provide new means to manage insect pests.

In the Diaprepes root weevil, DRW, we have shown that infected males can transfer virus to non-infected females, which subsequently become infected and can then transfer the virus back into males during mating.

In insects that mate multiple times using a virus suppression holds great promise as more individuals become infected and either die or become sterilized.

Exotic Insect Pests:



TOXOPTERA citricida, the Brown Citrus Aphid, BrCA, is the main vector of Citrus Tristeza virus, CTV, in citrus growing regions.

The aphids produce two morphologically distinct adults one and one with wings (Alate), and without wings (Apterous).

In the winged stage the aphids disperse to nearby host trees and begin to feed thus transferring the virus and infecting more citrus trees.

Genomic analyses is permitting a closer examination of the genes and proteins playing critical roles in virus acquisition and transmission, as well as exposing the intimate interactions between insect and host plants.

Dr. Hunter's Most Recent Publication:

D. C. S. G. Oliveira, W. B. Hunter, J. Ng,C. A. Desjardins, P. M. Dang, and J. H. Werren. <u>Data mining cDNAs reveals three new single stranded RNA viruses in *Nasonia* (Hymenoptera: Pteromalidae). Insect Molecular Biology, 2010, 19 (Suppl 1), 99-107.</u>

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W.B. Hunter, S.E. Dowd, D.S. Katsar, R.G. Shatters Jr., C.L. McKenzie and D.G. Hall. Psyllid Biology: <u>Expressed Genes in Adult Asian Citrus Psyllids, Diaphorina citri Kuwayama.</u>. The Open Entomology Journal, 2009, 3, 18-29.

Dr. Hunter's Publication List (2002-2009)

Dr. Hunter's Publications (1995 -2002):

Hunter, W.B., Sinisterra, X.H., McKenzie, C.L., and Shatters, Jr., R.G. 2002. Iridovirus infection and vertical transmission in citrus aphids. Proceedings of the Florida Horticultural Society 114:70-72.

McKenzie, CL., Lapointe, SL., **Hunter, WB.**, and GJ. Puterka. 2002. Efficacy of Surround for control of Asian citrus psyllid on citrus. Arthropod Management Tests 27: (Report).

McKenzie, C.L., W.B. Hunter, S.L. Lapointe, and P.M. Dang. 2002. Iridovirus: potential entomopathogenic biocontrol agent for whitefly. Silverleaf Whitefly National Research, Action, and Technology Transfer Plan: Fourth Annual Review of the Second 5-Year Plan and Final Report for 1992-2002. USDA-ARS 2002-01: 162.

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Funk, C J., **Hunter, W.B.**, and Achor, DS. 2001. <u>Replication of insect iridescent virus 6 in a whitefly cell line</u>. Journal of Invertebrate Pathology 77: 144-146.

Mayer, Richard T., **Wayne B. Hunter**, Stephen L. Lapointe, C. L. McKenzie, R. Shatters, J. Shapiro, Hamed Doostdar and A. A. Weathersbee III. 2001. Diaprepes Root Weevil: Recent Advances at the U.S. Horticultural Research Laboratory, Fort Pierce. Citrus & Vegetable Magazine, Part 2, Vol. 65, No. 12, August 2001, pp. 8-13.

Mayer, Richard T., **Wayne B. Hunter**, Stephen L. Lapointe, C. L. McKenzie, R. Shatters, J. Shapiro, Hamed Doostdar and A. A. Weathersbee III. 2001. Diaprepes Root Weevil: Recent Advances at the U.S. Horticultural Research Laboratory, Fort Pierce. Citrus & Vegetable Magazine. Part 1, Vol. 65, No. 11, July 2001, pp. 9-12.

Hunter, W.B., C.P. Patte, X.H. Sinisterra, D.S. Achor, C.J. Funk and J.E. Polston. 2001. <u>Discovering new insect viruses: Whitefly iridovirus (Homoptera: Aleyrodidae: Bemisia tabaci)</u>. Journal of Invertebrate Pathology 78: 220-225.

Lapointe, S. L., W. B. Hunter, and C. J. Funk(2000). <u>Infection of the Diaprepes root weevil, Diaprepes abbreviatus (Coleoptera: Curculionidae)</u>, by an Iridovirus.

Hunter, W.B. and H.T. Hsu. 1999. Synthetic diets and piercing-sucking insect research. Intl. J. Ecol. Environ. Science 25: 259-266.

Tang, Y.Q., S.L. Lapointe, L.C. Brown, and **W. B. Hunter**. 1999. Effects of host plant and temperature on the biology of Toxoptera citricida (Homoptera: Aphididae). Environ. Entomol. 28(5): 895-900.

Hunter, W. B., E. Hiebert, S. E. Webb, J. H. Tsai & J. E. Polston. 1997. <u>Location of geminivirus in the whitefly, Bemisia tabaci (Homoptera: Aleyrodidae)</u>. Plant Dis. 82: 1147-1151.

Hunter, W. B., E. Hiebert, S. Webb, J. E. Polston, and J. H. Tsai. 1996 Precibarial and Cibarial Chemosensilla in the Whitefly, Bemisia Tabaci(Gennadius), (Homoptera: Aleyrodidae) Int. J Insect Morphol. and Embryol. 25(3): 295-304.

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Hunter, W. B.& H. T. Hsu. 1996. Inoculation of thrips cell mono-layers with a tospovirus. pp. 359-363. In International Symposium on Tospoviruses and Thrips of Floral and Vegetable Crops. George Kuo (ed). Acta Horticulturae, No. 431. ISHS. Asian Vegetable Research & Development Center, AVRDC.

Hunter, W. B.& H. T. Hsu. 1995. Establishing Thrips Cell Cultures to Study Tospoviruses.pp. 163-166. In Thrips Biology and Management, Bruce L. Parker, Margaret Skinner, and Trevor Lewis (eds). Plenum Press, New York.

Hunter, W. B., H. T. Hsu & R. Lawson. 1995. A novel method for acquisition of Tospoviruses by thrips. Phytopathology 85(4): 480-483. (Public Database).

Dr. Hunter's Project List

Published EST Gene Sequences:

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Hunter, W.B., Hunnicutt, L.E., Wistrom, C.M., Purcell, A.H. 2007. Gene expression in adult blue-green sharpshooters, *Graphocephala atropunctata*(Signoret) (Hemiptera: Cicadellidae)6,481 mRNA sequences. Acc. nos.: EH655849-EH662332. National Center for Biotechnology Information, NCBI. (Public Database).

Mozoruk, J, Hunnicutt, LE, Cave, RD, <u>Hunter, W.B.</u>, Bausher, MG. 2006. Profiling transcriptional changes in *Citrus sinensis*(L.) Osbeck challenged by herbivory from the xylem-feeding leafhopper *Homalodisca coagulata*(Say) by cDNA macroarray analysis. Plant Science 170:1068-1080.

Hunter, W.B., Hunnicutt, L.E., Hall, D.G. 2006. Ribosomal proteins of the Asian citrus psyllid, *Diaphorina citri*. 88 Proteins, Acc. nos: DQ765535-DQ673441. National Center for Biotechnology Information, NCBI. http://www4.ncbi.nlm.nih.gov/. (Public Database).

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Lysiphlebus testaceipes

(Hymenoptera: Braconidae), Acc. Nos.- DQ062217- DQ062237 and AY863030-AY961584. Set of 123 putative full cDNA sequences with identities.

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Hunter W.B., Dang PM, Lee L. 2005. Adult alate Aphis gossypii, (Hemiptera: Aphididae), Acc. No.-DR389179-DR397419.

8,241 mRNA sequences. NCBI.

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Hunter W.B., Smith M. 2005. 5th-instar *Anoplophora glabripennis*larva (Coleoptera: Cerambicidae) cDNA clones, Acc. Nos.-DR108748-DR109303, Set of 556 mRNA sequences. <u>Plus two full-length</u> cDNA, DQ067276 & DQ067275. NCBI. http://www.ncbi.nlm.nih.gov/

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, Dang, PM, McKenzie, CL. 2005 *Diaphorina citri*Kuwayama (Hemiptera: Psyllidae) *Diaphorina citri*, Acc. nos.-DN465721-DN470410, 4,686 mRNA sequences

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Hunter W.B., McKenzie CL, Shatters RG. Weathersbee AA. Hall DG. Gene expression in field collected Asian citrus psylla, *Diaphorina citri*Kuwayama (Hemiptera: Psyllidae) 1,220 EST's, Acc. Nos. DN202325- DN201110. NCBI. http://www.ncbi.nlm.nih.gov/

Werren, J, **Hunter, W**., Dang, P. 2005. *Nasonia*, Produced cDNA expression libraries, (Sequencing done by TIGR, 17,000 sequences, TIGR database).

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U.S.Horticultural Research Laboratory, 2001 South Rock Rd., Fort Pierce, FL34945, USA.

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U.S.Horticultural Research Laboratory, 2001 South Rock Rd., Fort Pierce, FL34945, USA.

Accession no. CF587442-CF588411.

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Bausher, M., McKendree, W., Dang, P., Chaparro, J., Shatters, R., **Hunter, W.B.**, Niedz, R 2002. Published 2,306 electronic pages of "EST high quality sequences from a *Citrus sinensis*(sweet orange) whole seedling cDNA library" to NCBI EST database.

<u>http://www.ncbi.nlm.nih.gov/</u>
(Public Database).

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Hunter, W.B., Backus, E.A., Hunnicutt, L.E., Habibi, J., Dang, P.M. 2006. Salivary gland gene expression in glassy-winged sharpshooter, Homalodisca vitripennis (syn. H. coagulata) (Hemiptera: Cicadellidae), Published 3,933 sequences. Accession nos.: EG372472-EG368539. National Center for Biotechnology Information, NCBI. (Public Database).

Hunter, W.B., Backus, E.A., Hunnicutt, L.E., Habibi, J., Dang, P.M. 2006. Midgut gene expression in glassy-winged sharpshooter, Homalodisca vitripennis (syn. H. coagulata) (Hemiptera: Cicadellidae), Published 3,933 sequences. Accession # EG368538-EG365118. National Center for Biotechnology Information, NCBI. (Public Database).

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Sequencing Links:

Asian Citrus Psyllid ESTs http://www.uttyler.edu/biology/faculty/bextine/psyllid consortium/links.php

Last Modified: 12/21/2010

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