

20th Annual

TOMATO DISEASE WORKSHOP

**The Ohio State University, OARDC
Arden Shisler Center, Wooster, OH**

October 20-21, 2005

PROCEEDINGS



Tomato Research Plots, OSU-OARDC North Central Experiment Station, Fremont, OH



20th Annual Tomato Disease Workshop
Wooster, Ohio October 20-21, 2005

TABLE OF CONTENTS

Table of contents.....i

Agenda.....iii

Participants.....vii

Sponsors.....xi

Genetic variation among *Beet curly top virus* isolates infecting weed and crop hosts in California. William M. Wintermantel 1

Progress on management and control of criniviruses in tomato. William M. Wintermantel..... 4

Field Assessment of Impact of Bacterial Speck Incidence on Yield of Processing Tomato, Woodland Area, California: A 2005 Progress Report. G. Miyao¹, R.M. Davis², D.J. Sweet¹, M.S. Kochi¹, F. Shulten-Baumer¹, N.D. Howell²,..... 8

Virulence in Bacterial Plant Pathogens: Significance in Diversity of Populations That Cause Bacterial Canker of Tomato. Anne Alvarez and Wendy Kaneshiro..... 11

The Effect of Spray Applications on *Clavibacter Michiganensis* subsp. *michiganensis* Populations. C. A. Laskey¹, M. K. Hausbeck¹, R. Hammerschmidt¹ 15

Efficacy of Fungicides/Bactericides in the Management of Foliar and Fruit Diseases of Tomato. Sally Miller, Melanie Lewis Ivey and Jhony Mera..... 18

Evaluation of Compost Tea for Managing Foliar Diseases in Tomato. M. T. McGrath ..22

New Products to Control Tomato Diseases. M.K. Hausbeck and B.D. Cortright, Michigan State University, East Lansing, MI..... 25

Shift in Performance of Fungicides for the Control of Tomato Early Blight. Thomas A. Zitter and Jessica L. Drennan..... 28

Effect of an Experimental Fungicide on Botrytis Gray Mold of Greenhouse Tomatoes. Melanie L. Lewis Ivey and Sally A. Miller. 31

Greenhouse Tomato Disease Research in Mississippi. David M. Ingram 34

Tomatoes for the Northeast Combining Early Blight and Late Blight Resistance. Martha Mutscher¹, Tom Zitter², Charles Bornt³37

Functional Analysis of the *Avr3a* gene family of *Phytophthora infestans*. Jorunn Bos, Thirumala Kanneganti, Carolyn Young and Sophien Kamoun 40

Role of *Phytophthora infestans* Protease Inhibitors and Their Target Tomato Proteases in Disease. Jing Song, Miaoying Tian, Joe Win, Nicolas Champouret, Hsin-Yen Liu and Sophien Kamoun..... 43

The IR-4 Pest Control Product Registration Process. Charles W. Meister, IR4 Field Research Coordinator, IFAS, University of Florida 45

Effect of Plant Growth Promoting Rhizobacteria on Tomato Diseases in an Integrated Management Program. C. Nava-Diaz³, M.D. Kleinhenz², D.J. Doohan², and S.A. Miller¹ 47

Use of Biorational Products to Manage Seedling Pre-and Post-Emergence Damping-Off. Fulya Baysal, Melanie Lewis Ivey, Jhony Mera and Sally Miller..... 50

20th Annual Tomato Disease Workshop

The Ohio State University-OARDC
Arden Shisler Center
Wooster, OH

October 20-21, 2005



AGENDA

Thursday October 20

- 7:30 a.m. Registration and Continental Breakfast
- 8:30 a.m. Ecology and Epidemiology of Beet Curly Top Virus in California and Impact on Tomato Production.
William Wintermantel, USDA ARS, Salinas, CA.
- 8:50 a.m. Control of Whitefly Transmitted Criniviruses in Tomato.
William Wintermantel, USDA ARS, Salinas, CA.
- 9:10 a.m. Field Assessment of Impact of Bacterial Speck Incidence on Yield of Processing Tomatoes.
Gene Miyao¹, M. Davis², D. Sweet², M. Kochi¹, F. Schulten-Baumer³, N. Howell². Univ. of California Cooperative Extension¹, University of California Davis², Univ. of Bonn³.
- 9:30 a.m. Significance of diversity in populations that cause bacterial canker of tomato.
Anne Alvarez, University of Hawaii at Manoa, Honolulu, HI
- 9:50 a.m. The Effect of Spray Applications on *Clavibacter michiganensis* subsp. *michiganensis* Populations.
Chandra Laskey, Mary Hausbeck, Ray Hammerschmidt, Michigan State University, East Lansing, MI.
- 10:10 a.m. Break

- 10:40 a.m. Efficacy of Fungicides/Bactericides in the Management of Tomato Early Blight and Bacterial Leaf Spot.
Sally Miller, Melanie Lewis Ivey and Jhony Mera, The Ohio State University-OARDC, Wooster, OH.
- 11:00 a.m. Evaluation of Compost Tea for Managing Foliar Diseases in Tomato.
Meg McGrath, Cornell University, Long Island, NY.
- 11:20 a.m. New Products to Control Tomato Diseases.
Mary Hausbeck, Brian Cartright, Michigan State University, East Lansing, MI.
- 11:40 a.m. Shift in Performance of Fungicides for the Control of Tomato Early Blight.
Thomas A. Zitter and Jessica L. Drennan, Cornell University, Ithaca, New York.
- 12:00 noon Lunch (provided)
- 1:15 p.m. Efficacy of Fungicides in Management of Botrytis in Greenhouse Tomatoes.
Melanie Lewis Ivey and Sally Miller, The Ohio State University-OARDC, Wooster, OH
- 1:35 p.m. Greenhouse Tomato Disease Research in Mississippi.
David M. Ingram, Mississippi State University, Raymond, MS
- 1:55 p.m. New Products for Tomato Disease Management– What’s New, What’s Coming.
Mike Bledsoe, Village Farms L. P, Heathrow, FL
- 2:15 p.m. Update on Breeding for Combined Early Blight and Late Blight Resistance.
Randy Gardner, North Carolina State University, Fletcher, NC.
- 2:35 p.m. Early blight resistance in freshmarket and processing tomato lines carrying late blight resistance.
Martha Mutschler, Tom Zitter and Charles Bornt, Cornell University, Ithaca, New York
- 2:55 p.m. Update on Breeding for Resistance in Tomato to *Clavibacter michiganensis* subsp. *michiganensis*.
David Francis, The Ohio State University-OARDC, Wooster, OH.
- 3:15 p.m. Break

- 3:45 p.m. Breeding for Resistance to Bacterial Spot in Tomato.
Wencai Yang and David Francis, The Ohio State University-OARDC, Wooster, OH.
- 4:05 p.m. Functional Analysis of the Avr3a Avirulence Gene of *Phytophthora infestans*
Jorunn Bos, Thirumala Kanneganti, Carolyn Young and Sophien Kamoun, The Ohio State University-OARDC, Wooster, OH
- 4:25 p.m. Role of *Phytophthora infestans* protease inhibitors and their target tomato proteases in disease.
Jing Song, Miaoying Tian, Joe Win, Nicolas Champouret, and Sophien Kamoun, The Ohio State University-OARDC, Wooster, OH
- 7:00 p.m. Wine and Cheese Social at the Stone House

Friday, October 21

- 7:30 a.m. Continental Breakfast
- 8:30 a.m. IR-4 Update: Product Solutions for Control of Tomato Diseases.
Charles Meister, Southeast Regional IR-4 Coordinator, University of Florida.
- 8:50 a.m. Effect of Plant Growth Promoting Rhizobacteria on Tomato Diseases in an Integrated Management Program.
Cristian Nava-Diaz, Matthew Kleinhenz, Douglas Doohan, and Sally Miller, The Ohio State University-OARDC, Wooster, OH.
- 9:10 a.m. Influence of Field Management on Rhizosphere Microbial Community Structure and Its Relation to Plant Health.
Fulya Baysal¹, Maria Soledad Benitez², Cristian Nava-Diaz², Sally Miller² and Brian McSpadden Gardener², Adana University¹, The Ohio State University – OARDC², Wooster, OH
- 9:30 a.m. Use of biorational products to manage seedling pre- and post- emergence damping-off.
Fulya Baysal¹, Melanie Lewis Ivey², Jhony Mera² and Sally Miller², Adana University¹ and The Ohio State University – OARDC², Wooster, OH
- 9:50 a.m. Tomato Disease Control From Programs Including Tanos and Curzate.
Marsha Martin, DuPont Crop Protection, Columbus, OH.
- 10:10 a.m. Break

- 10:30 a.m. Update on Performance of Biological Fungicides.
Robert Lund, Agraquest, Davis, CA
- 10:50 a.m. The Use of ProPhyt in Control of Tomato Diseases.
Vince Morton, Viva Inc, Greensboro, NC
- 11:10 a.m. Actinovate® SP Biological Fungicide For Foliar And Root Diseases.
Matt Kowalski, Natural Industries, Inc., Houston, TX
- 11:30 a.m. Meeting Wrap-up and Assignment of Next Workshop Place and Time
- 1:00 p.m. Optional Amish Farm Tour

PARTICIPANTS

Dawn Adams
Campbell Research
Davis, CA
dawn_adams@campbellsoup.com

Tito Alcantara
BHN Research
Immokalee, FL
tpa@bhnseed.com

Anne Alvarez
University of Hawaii
Honolulu, HI
alvarez@hawaii.edu

Fulya Baysal-Tustas
OSU/OARDC
Wooster, OH
baysal-tustas.1@osu.edu

Jim Beamesderfer
McConnell Agronomics Inc.
Denton, MD
mccagro@shore.intercom.net

Ron Becker
OSUE
Wooster, OH
becker.4@osu.edu

Mike Bledsoe
Village Farms
Heathrow, FL
mbledsoe@villagefarms.com

Hasan Bolkan
Campbell Research
Davis, CA
hasan_bolkan@campbellsoup.com

Jorunn Bos
OSU/OARDC
Wooster, OH
bos.4@osu.edu

George Bosveld
H.J.Heinz Company
Leamington, ON

Paul Bosveld
H.J.Heinz Company
Leamington, ON
paul.bosveld@ca.hjheinz.com

Gaby Briceno-Montero
OSU/OARDC
Wooster, OH
briceno-montero.1@osu.edu

Dain Bruns
Syngenta Crop Protection
Marysville, OH
dain.bruns@sygenta.com

Louise Carpenter
STA Laboratories
Gilroy, Ca
louise.carpenter@statlabs.com

Nancy Cohen
Campbell Research
Davis, CA
nancy_cohen@campbellsoup.com

George Collier
H.J.Heinz Company
Leamington, ON
george.collier@ca.hjheinz.com

Sylvaine Coulibaly
Unilever
Stockton, CA
sylvaine.coulibaly@unilever.com

Adam Dick
Tomato Solutions
Chatham, ON
adick@uoguelph.ca

Jim Dick
Tomato Solutions
Chatham, ON
jimdick@netrover.com

Dave Esplin
H.J.Heinz Company
Stockton, CA
david.esplin@us.hjheinz.com

David Francis
OSU/OARDC
Wooster, OH
francis.77@osu.edu

Randy Gardner
North Carolina State University
Fletcher, NC
randy_gardner@ncsu.edu

Janie Hansen
Unilever
Lodi, CA
janie.hansen@unilever.com

Adam Hartley
Red Gold LLC
Elwood, IN

Mary Hausbeck
Michigan State University
East Lansing, MI
hausbec1@msu.edu

Matthew Hofelich
OSU/OARDC
Fremont, OH
hofelich.4@osu.edu

Jim Holloway
Red Gold LLC
Elwood, IN

Jeff Huether
Cerexagri Inc.
Geneva, NY
Jeff.Huether@cerexagri.com

Kent Hughes
DuPont Ag. and Nutrition
Columbus, OH

David Ingram
Mississippi State University
Raymond, MS
davidi@ext.msstate.edu

Lewis W. Jett
University of Missouri
Columbia, MO
jettl@missouri.edu

Matt Kowalski
Natural Industries Inc.
Houston, TX
mattk@naturalindustries.com

John Lang
Craft Canada Inc.
Dresden, ON
john.lang@kraft.com

Chandra Laskey
Michigan State University
East Lansing, MI
laskeych@msu.edu

Janice LeBoeuf
Ontario Ministry of Agriculture and
Food
Ridgetown, ON
janice.leboeuf@omaf.gov.on.ca

Melanie Lewis Ivey
OSU/OARDC
Wooster, OH
ivey.14@osu.edu

Robert Lund
Agraquest
Davis, CA

Ken Martin
Furmano Foods
Northumberland, PA
ken.martin@furmanos.com

Marsha Martin
DuPont Ag. and Nutrition
Columbus, OH
marsha.j.martin@usa.dupont.com

Randy Martin
BioWorks, Inc.
Avon, IN
rmartin@bioworksinc.com

Brian McSpadden Gardener
OSU/OARDC
Wooster, OH
bbmg+@osu.edu

Charles Meister
University of Florida-IR4
Gainesville, FL
cmeister@ifas.ufl.edu

Sally Miller
OSU/OARDC
Wooster, OH
miller.769@osu.edu

Gene Miyao
University of California
Woodland, CA
emmiyao@ucdavis.edu

Vince Mortan
Viva Inc.
Greensboro, NC
mortv@aol.com

Martha Mutschler
Cornell University
Ithaca, NY
mam13@cornell.edu

Cristian Nava-Diaz
OSU/OARDC
Wooster, OH
nava-diaz.1@osu.edu

Martin Park
DuPont Crop Protection
Rensselaer, IN
martin.w.park@usa.dupont.com

Prakash Pradhanang
H.J.Heinz Company
Stockton, CA
prakash.pradhanang@us.hjheinz.com

Mark Ricker
Nunhems, USA
Acamp, CA
mark.ricker@nunhems.com

Charles Rivara
California Tomato Research Institute
Escalon, CA
chuck@tomatonet.org

Jing Song
OSU/OARDC
Wooster, OH
song.189@osu.edu

Ann Sundwall
Unilever
Lodi, CA
ann.sudwall@unilever.com

Nankui Tong
H.J.Heinz Company
Stockton, CA
nankui.tong@us.hjheinz.com

Margaret Tuttle McGrath
Cornell University
Riverhead, NY
mtm3@cornell.edu

Jeff Unverferth
Hirzer Canning Co.
Fort Jennings, OH
junverferth@hirzel.com

Curt Utterback
Red Gold LLC
Elwood, IN
cutterback@REDGOLD.com

Marleen Van der Torre
Koppert Biological Systems
Romulus, MI
marleen@koppertonline.com

Aaron Weaver
Weaver Truck Patch
Fredericksburg, OH

Aden Weaver
Fredericksburg, OH

Melvin Weaver
Fredericksburg, OH

William Wintermantel
USDA-ARS
Salinas, CA
wwintermantel@pw.ars.usda.gov

Ben Wright
Cerexagri Inc.
Brownsburg, IN
ben.wright@cerexagri.com

Wencai Yang
OSU/OARDC
Wooster, OH
yang.385@osu.edu

Raymond Yoder
Fredericksburg, OH

Tom Zitter
Cornell University
Ithaca, NY
taz1@cornell.edu

SPONSORS

20th Annual Tomato Disease Workshop

DuPont Crop Protection

Furmano Foods

H. J. Heinz Co. (Heinz Seed)

Red Gold

Widmer & Associates, Ltd.

Genetic variation among *Beet curly top virus* isolates infecting weed and crop hosts in California

William M. Wintermantel

USDA-ARS, 1636 E. Alisal Street, Salinas, CA 93905.

ABSTRACT

Curly top disease is caused by *Beet curly top virus* (BCTV) and related curtovirus species, and is transmitted by the beet leafhopper (*Circulifer tenellus*). The disease occurs in several large, but geographically separate regions of western North America. BCTV re-emerged in 2001 as a serious threat to agriculture in the San Joaquin Valley of California and has continued to exert pressure on agriculture in this region. BCTV infects a broad range of crop hosts including sugar beet, pepper, and tomato, as well as numerous native weeds. Prior molecular characterization of a limited number of curtoviruses from broad areas of the western United States suggested that two distinct curtovirus species, *Beet severe curly top virus* (BSCTV or CFH strain) and *Beet mild curly top virus* (BMCTV or Worland strain) were responsible for most crop disease, but little information existed on curtovirus species distribution among weed hosts or species prevalence in the California sugarbeet crop. The aim of this study was to clarify the genetic variability among curtovirus isolates in California, and to determine if specific weed hosts might be reservoirs for exceptionally severe virus species, such as BSCTV. Data collected over 2 years focused on molecular characterization of large numbers of BCTV isolates from weed and crop hosts of the beet leafhopper in the San Joaquin Valley. Total nucleic acid was isolated from individual plants, and both universal and specific primers were used to amplify viral DNA. PCR amplification coupled with sequence analysis identified the prevalence of both BSCTV and BMCTV as the predominant curtovirus species in California, infecting both weeds and crops. The Logan strain of BCTV, historically associated with California, was not identified among over 200 isolates characterized.

SUMMARY

Beet curly top virus (BCTV) and related viruses (collectively known as curtoviruses) transmitted by the beet leafhopper *Circulifer tenellus* (Baker) have caused significant problems to irrigated agriculture in the western US since 1899 (Carsner and Stahl, 1924). BCTV is known to infect a broad range of crop and weed hosts in many plant families (Bennett, 1971). Crop hosts for which natural BCTV infection has been reported include sugarbeet, tomato, pepper, bean, spinach, and cucurbits.

The leafhopper vector also feeds and breeds on an extensive range of plant hosts from different families (Cook, 1967). *C. tenellus* transmits curtoviruses most efficiently after a 48-hour acquisition-access feed on an infected source plant, but shorter feeding times (2-20 min.) also result in a low frequency of transmission. Curtovirus transmission by the vector requires a 4 hour latent period following ingestion, and leafhoppers can inoculate healthy plants by feeding for as little as a 1 min inoculation access period. Symptoms generally develop in plants within two weeks, depending on the host and age at infection. Leafhopper vectors retain the ability to transmit BCTV for days to weeks.

BCTV is a monopartite geminivirus and the type member of the genus *Curtovirus* within the family *Geminiviridae* (Fauquet et al., 2003). Viruses within this group are characterized by circular ssDNA genomes of approximately 3.0 kilobases encapsidated within twin spherical particles. Many strains (up to 14) of BCTV were initially distinguished on the basis of differential symptomatology in sugarbeet (reviewed in Klein, 1992).

The disease occurs in several large, but geographically separate regions of western North America. Curly top re-emerged in 2001 as a serious threat to agriculture in the San Joaquin Valley of California and has continued to exert pressure on agriculture in this region (Wintermantel and Kaffka, 2006). BCTV infects a broad range of crop hosts including sugarbeet, pepper, and tomato, as well as numerous native weeds. Prior molecular characterization of a limited number of curtoviruses from broad areas of the western United States suggested that two distinct curtovirus species, *Beet severe curly top virus* (BSCTV or CFH strain) and *Beet mild curly top virus* (BMCTV or Worland strain) were responsible for most crop disease (Stenger and McMahon, 1997), but little information existed on curtovirus species distribution among weed hosts or species prevalence in the California sugarbeet crop. The aim of this study was to clarify the genetic variability among curtovirus isolates in California, and to determine if specific weed hosts might be reservoirs for exceptionally severe virus species, such as BSCTV. Data collected over 2 years focused on molecular characterization of large numbers of curly top isolates from weed and crop hosts of the beet leafhopper in the San Joaquin Valley.

Using the extensive host range information available for curly top, reported weed and crop hosts of the virus were collected from throughout California. The majority of beet leafhopper flights are reported to be shorter than 100 miles, and the spring breeding grounds of the leafhopper, the foothills of western San Joaquin Valley, are well documented in California. Weed samples for this study were collected primarily from this area, with some samples originating from the southern portion of the Salinas Valley, as well. Collection locations were made using global positioning systems (GPS) in order to map the locations where curtoviruses were detected. Crop samples, consisting of sugarbeet, tomato, and pepper were also collected from the San Joaquin Valley. Sample collection was conducted from May through September over a three year period from 2002-2004. Samples were scored as positive or negative for curtoviruses using PCR-based virus detection methods described below. Based on this information, some areas were clearly “hot-spots” for the presence of curly top virus species, although no strain-specific hot-spots were identified.

Polymerase chain reaction (PCR)-based detection methods and DNA sequencing were used to confirm curtovirus infection and to identify different curtovirus species. This method involved using short strands of DNA (primers) that bind to complementary DNA sequences present in all curtovirus species (formerly known as different BCTV strains). After primer binding, an enzyme was used to extend the primers to make multiple copies of the original strand. The end result of this process is known as a PCR product. Samples that did not contain BCTV or related curtoviruses did not produce PCR products. The resulting PCR product was then directly sequenced. Sequencing results were compared with known sequences of curtovirus species to determine which species the isolate in question was most closely related to.

Results indicated that the highest incidence of infection was in sugarbeet (78%) and wild mustard (73%), with somewhat lower incidence in Russian thistle (57%), tomato (55%),

and London rocket (46%). Other weed and crop hosts had considerably lower incidence of curly top, as confirmed by detection of curtoviruses in plant tissue. Overall, 200 of 562 (36%) samples tested positive for BSCTV (formerly known as CFH strain) or BMCTV (formerly known as Worland strain). No traditional BCTV (formerly California/Logan strain) was found, although small pieces of DNA corresponding to the traditional BCTV (California/Logan) sequence were occasionally found interspersed among BSCTV or BMCTV sequences. Some recombinant curtoviruses were also identified. These involved sections of both BSCTV and BMCTV, suggesting recombination (exchange of viral genetic material) may readily occur between the different species within the region sequenced. The abundance of BSCTV and BMCTV, along with the lack of BCTV indicated a clear transition between curtovirus species prevalent in California during the mid 1900s and those present today, suggesting evolution and emergence of new curly top (curtovirus) species. Studies also addressed whether specific curtovirus species were associated with specific weed or crop hosts. Results demonstrated that all species were equally capable of infecting the different host species examined in this study, and that there appears to be little difference in host range between the different curtovirus species.

ACKNOWLEDGEMENT

Thanks to Rod Clark and Kelly Brannigan of the California Department of Food and Agriculture–Curly Top Virus Control Board for collection and GPS mapping of weed samples. This research was supported in part by funding provided by the California Beet Growers Association Industry Research Committee.

LITERATURE CITED

- Bennett, C. W. 1971. The curly top disease of sugarbeet and other plants. The Am. Phytopathol. Soc. Monogr. No. 7.
- Carsner, E. and Stahl, C. F. 1924. Studies on curly-top disease of the sugarbeet. J. Agr. Res. 28:297-320.
- Cook, W. C. 1967. Life history, host plants, and migrations of the beet leafhopper in the western United States. U.S.D.A. Tech. Bull. 1365. 122 p.
- Fauquet, C.M., Bisaro, D.M., Briddon, R.W, Brown, J.K., Harrison, B.D., Rybicki, E.P., Stenger, D.C. and Stanley, J. 2003. Revision of taxonomic criteria for species demarcation in the family *Geminiviridae*, and an updated list of begomovirus species. *Arch. Virol.* 148: 405-421.
- Klein, M. 1992. Role of *Circulifer / Neoliturus* in the transmission of plant pathogens. Pages 152-193 in: *Advances in Disease Vector Research*, Vol. 9. Springer-Verlag, New York, NY.
- Stenger, D. C. and McMahon, C. L. 1997. Genotypic diversity of beet curly top virus populations in the western United States. *Phytopathology* 87:737-744.
- Wintermantel, W.M. and Kaffka, S.R. 2006. Sugarbeet performance with curly top is related to virus accumulation and age at infection. *Plant Disease* 90: 000-000 (in press).

Progress on management and control of criniviruses in tomato

William M. Wintermantel

USDA-ARS, 1636 E. Alisal Street, Salinas, CA 93905.

ABSTRACT

Two crinivirus species infect Tomato (*Lycopersicon esculentum*): *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV). Recent studies demonstrated that transmission efficiency and persistence of ToCV in the vector varies significantly among the 4 vectors capable of transmitting ToCV. *Trialeurodes abutilonea* and *Bemisia tabaci* biotype B are highly efficient vectors of ToCV. *B. tabaci* biotype A and *T. vaporariorum* are less efficient vectors. The complete nucleotide sequence of the bipartite genome of ToCV was sequenced and compared with related crinivirus species. RNA 1 is organized into four open reading frames (ORFs), and encodes proteins involved in replication, and RNA 2 encodes nine ORFs including genes that encode a HSP70 homolog and two proteins involved in encapsidation of viral RNA. Two forms of resistance have been identified that reduce the impact of criniviruses on tomato. Acylsugar production on foliar trichomes reduces vector feeding and can slow the rate of TICV transmission under field conditions. Resistance to TICV infection was recently discovered in a wild species and studies are in progress to determine the efficacy of moving this resistance into cultivated tomato.

SUMMARY

The genus Crinivirus (family Closteroviridae) contains numerous new species identified within the past several years that are emerging as important pathogens throughout the world. Criniviruses often cause symptoms that are readily mistaken for physiological or nutritional disorders or pesticide phytotoxicity. These symptoms often include interveinal yellowing of leaves, an associated loss of photosynthetic capability, leaf brittleness, reduced plant vigor, yield reductions and early senescence, depending on the host plant affected. Symptoms are typically most apparent on middle to lower parts of plants, while new growth appears normal. Criniviruses remain confined to cells associated with host plant phloem, and symptoms are thought to result from plugging of the phloem with large viral inclusion bodies, which probably interfere with normal vascular transport in infected plants (Wisler and Duffus, 2001).

Two crinivirus species infecting tomato have been identified to date, *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV). TICV and ToCV cause identical symptoms on tomato, including interveinal chlorosis, leaf brittleness and limited necrotic flecking or leaf bronzing on tomato leaves. Both can cause a decline in plant vigor and reduce fruit yield (Wisler et al. 1998; Wintermantel and Wisler, 2006). Both tomato-infecting criniviruses are transmitted by the greenhouse whitefly, *Trialeurodes vaporariorum*. ToCV, however, is unique in that it is also transmitted by the banded wing whitefly (*T. abutilonea*) and *Bemisia tabaci* biotypes A and B (Duffus et al., 1996; Wisler et al., 1998). Both viruses have now been found in widespread areas of North America and Europe in both field and greenhouse environments, and are being increasingly identified in other subtropical as well as temperate areas of the world where

vectors are present. TICV occurs in tomato production fields along the west coast of North America, both in Mexico and California. ToCV is common in the southeastern United States and also has been found in Puerto Rico. Fields in southern California continue to experience high levels of TICV infection annually, and greenhouse producers have experienced significant economic losses from tomato criniviruses. Enclosed greenhouse production centers can lead to accumulation of high GHWF populations, which in turn facilitate viral spread throughout the facility. This is particularly a problem with organic or reduced chemical production operations, where insecticide use is either decreased or not possible.

Host range and transmission of TICV and ToCV

Recent studies demonstrated that transmission efficiency and persistence of ToCV varies significantly among the different whitefly vectors. *T. abutilonea* and *B. tabaci* biotype B are highly efficient vectors of ToCV. *B. tabaci* biotype A and *T. vaporariorum* are less efficient vectors, but are fully capable of transmission. ToCV persists for up to 5 days in *T. abutilonea*, 2 days in *B. tabaci* biotype B, and only 1 day in *B. tabaci* biotype A and *T. vaporariorum* (Wintermantel and Wisler, 2006). ToCV has a moderately wide host range, infecting 24 host plant species in 7 families (Wintermantel and Wisler, 2006). TICV is known to infect 25 host plant species in 8 families (Duffus et al., 1996).

ToCV genome and relationships

Recent studies determined the complete nucleotide sequence of the bipartite genome of ToCV and compared ToCV with related crinivirus species. RNA 1 is organized into four open reading frames (ORFs), and encodes proteins involved in replication, based on homology to other viral replication factors. RNA 2 is composed of nine ORFs including genes that encode a HSP70 homolog and two proteins involved in encapsidation of viral RNA, referred to as the coat protein and minor coat protein (Wintermantel et al., 2005). These proteins are characteristic of the genus crinivirus. The function of many crinivirus proteins remains to be determined, and ToCV encodes two putative proteins that appear to be unique among known criniviruses (Wintermantel et al., 2005). Analysis of genetic differences with respect to biological function may provide insight into the role crinivirus proteins play in virus infection and transmission.

Control of Criniviruses:

One important control measure to limit spread of criniviruses may be the identification of these viruses in nursery stock and ornamental plants. ToCV and TICV have moderate host ranges, affecting a number of host plants in several different families, many of which include weed species common near tomato fields, as well as ornamentals (Duffus et al., 1996; Wintermantel and Wisler, 2006). Viruses can be introduced to new areas through the distribution of susceptible ornamentals and nursery crops not suspected of harboring viruses. Due to the extensive host ranges of some of these viruses, it would be appropriate to consider testing plant material for crinivirus infection as a means of reducing accidental movement of virus. Once introduced, the viruses may become established in weed populations and moved by vectors. Application of virus testing of nursery material, coupled with effective vector control will reduce spread of these viruses, as well as limit damage to susceptible crops both in greenhouse and field environments.

Currently, the only method available to reduce losses from criniviruses in tomato is insecticide-based control. Imidocloprid based products are most frequently used for whitefly control, and can be applied as a foliar spray, a seed treatment or through drip application. While insecticides effectively reduce whitefly populations, such control methods are inefficient for control of viruses, since whiteflies can transmit a virus before being killed by an insecticide. In addition, most GHWF-transmitted criniviruses do not produce symptoms until 3 to 4 weeks after infection occurs. Therefore infection can be widespread by the time symptoms are observed and control measures are implemented.

The most effective form of control for any plant virus, if available, is strong stable resistance. Two types of resistance have been identified that reduce the impact of criniviruses on tomato production; resistance to the virus and resistance to the whitefly vector. To date there are no tomato cultivars known to have resistance against TICV or ToCV. In an effort to identify sources of resistance to tomato-infecting criniviruses, we began testing *Lycopersicon* and *Solanum* accessions from the C.M. Rick Tomato Germplasm Center at the University of California-Davis in attempts to identify resistance to TICV. Early in the project we identified apparent resistance in *Lycopersicon pennellii* and *L. hirsutum* that prevented infection by TICV in tests in which whiteflies were given a choice of which plants to feed on in mass feeding experiments. Ultimately it was determined that these plants could be infected by TICV and that resistance was based on a reduced desire of *T. vaporariorum* to feed on these species. Previous research demonstrated that *L. pennellii* and *L. hirsutum* exhibited resistance to insect feeding through production of acylsugars on foliar trichomes (Liedl et al, 1995). Preliminary field trials demonstrated a significant delay in TICV infection in acylsugar producing tomato lines compared with non-acylsugar lines over two years of field testing in southern California coastal production areas. Additional research on the benefits of insect based control as a component of tomato IPM is continuing.

Additional studies examined wild germplasm for true resistance to TICV infection, and recently identified what appears to be true resistance to TICV infection in a wild *Solanum* species. Efforts are in progress to determine the genetics of this resistance and if this resistance can be introgressed into cultivated tomato.

LITERATURE CITED

1. Duffus, J.E., Liu, H.-Y., and Wisler, G.C. 1996. Tomato infectious chlorosis virus—a new clostero-like virus transmitted by *Trialeurodes vaporariorum*. *Eur. J. Plant Pathology* 102: 219-226.
2. Liedl, B. E., D. M. Lawson, K. K. White, J. A. Shapiro, D. E. Cohen, W. G. Carson, J. T. Trumble, and M.A. Mutschler. 1995. Acylglucoses of the wild tomato *Lycopersicon pennellii* (Corr.) D'Arcy alters settling and reduces oviposition of *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 88:742-748.
3. Wintermantel, W.M. and Wisler, G.C. 2006. Vector Specificity, Host Range and Genetic Diversity of *Tomato Chlorosis Virus*. *Plant Disease* 90: 000-000 (in press).
4. Wintermantel, W.M., Wisler, G.C., Anchieta, A.G., Liu, H.-Y., Karasev, A.V., and Tzanetakis, I.E. 2005. The complete nucleotide sequence and genome organization of *Tomato chlorosis virus*. *Arch. Virol.* 150: 2287-2298.
5. Wisler, G.C., Li, R.H., Liu, H.-Y., Lowry, D.S., and Duffus, J.E. 1998. Tomato chlorosis virus: a new whitefly-transmitted, phloem limited, bipartite clostero-virus of tomato. *Phytopathology* 88: 402-409.

6. Wisler, G.C. and Duffus, J.E. 2001. Transmission properties of whitefly-borne criniviruses and their impact on virus epidemiology. In *Virus-Insect-Plant Interactions*. K.F. Harris, O.P. Smith, and J.E. Duffus, eds. Academic Press. San Diego, CA. pp. 293-308.

Field Assessment of Impact of Bacterial Speck Incidence on Yield of Processing Tomato, Woodland Area, California: A 2005 Progress Report

G. Miyao¹, R.M. Davis², D.J. Sweet¹, M.S. Kochi¹, F. Shulten-Baumer¹, N.D. Howell²,

University of California Cooperative Extension, 70 Cottonwood Street, Woodland, CA, 95695 and ²Department of Plant Pathology, UC Davis, 1 Shields Ave, Davis, CA 95616

ABSTRACT

Two field tests initiated in 2005 indicated that bacterial speck incidence reduces processing tomato yield at relatively low disease levels and continues at a linear rate. Disease incidence was measured by visually assessing both percent leaf area diseased and percent leaves diseased at multiple periods during the season.

INTRODUCTION

Bacterial speck, caused by *Pseudomonas syringae* p.v. *tomato*, is the most important springtime disease of California tomatoes. When rainy conditions persist, crop losses as high as 25% have been documented (G. Miyao, 1995, unpublished). In areas with high disease pressure, multiple applications of copper and other chemicals are commonly applied.

Copper products have limited success in keeping the disease under control. Copper resistance is reported worldwide (1,2). Various control strategies have been effective in reducing disease incidence and impact on yield (3).

MATERIALS AND METHODS

Early bacterial speck incidence was identified in 2 commercial tomato fields near Woodland, California in the southern Sacramento Valley in the spring of 2005. Both fields were flagged to allow repeat visits to the same plant location areas to follow disease progression especially during the spring when rainy weather conditions persisted. In one of the fields, forty 5' x 5' square plots were established in a direct seeded field with double seed lines per 5' centered bed. In a second field established with transplants, 150 individual plants were flagged. In both fields, the intent was to monitor a broad range of disease levels to assess impact on crop yield. The visual rating scale was pre-transformed with ratings of 0, 2.5, 10, 21, 35, 50, 65, 79, 90, 97.5 or 100%. One measure of disease level was on a percent of leaf area diseased. Another measure was on percent diseased leaves (with any disease present).

At harvest, fruit were hand picked and sorted into categories of marketable red, pink, green, sun-damaged and rots on a weight basis and converted to percent. Correlations were used to compare disease level with yield outcome.

RESULTS AND DISCUSSION

The best fit was measured between leaf area diseased at an early plant growth stage and marketable yield (figure 1). The disease reduced marketable yield linearly. As a conversion from pounds per plot to tons per acre basis, marketable yields were reduced 0.66 tons per acre for each 10% increase in leaf area diseased. If price was \$50 per ton,

the calculated cost of a 10% leaf area diseased level would be a gross revenue decline of \$33 per acre. Total fruit biomass was not reduced.

Both of the field harvest results were similar although yield levels were different between the two fields.

While the results of the tests are preliminary, it appears that relatively low disease levels reduce yields when they occur at an early plant growth stage prior to early flowering. The impact on yield appears to be from sun damaged fruit and culls rather than a reduction in total fruit produced. The results suggest that low disease levels can impact crop yield suggesting that control programs are needed at first sign of the disease. Further tests are needed to confirm the year 2005 results.

LITERATURE CITED

1. Bender C.L, and Cooksey, D.A. 1986. Indigenous plasmids in *Pseudomonas syringae* pv. *tomato*: conjugative transfer and role in copper resistance. *J Bacteriol.* 165(2):534–541.
2. Cooksey, D. A. 1987. Characterization of a copper resistance plasmid conserved in copper-resistant strains of *Pseudomonas syringae* pv. *tomato*. *Appl Environ Microbiol.* 1987 Feb;53(2):454–456
3. Graves, A. S., and Alexander, S. A. 2002. Managing bacterial speck and spot of tomato with acibenzolar-S-methyl in Virginia. Online. *Plant Health Progress* doi:10.1094/PHP-2002-0220-01-RS.

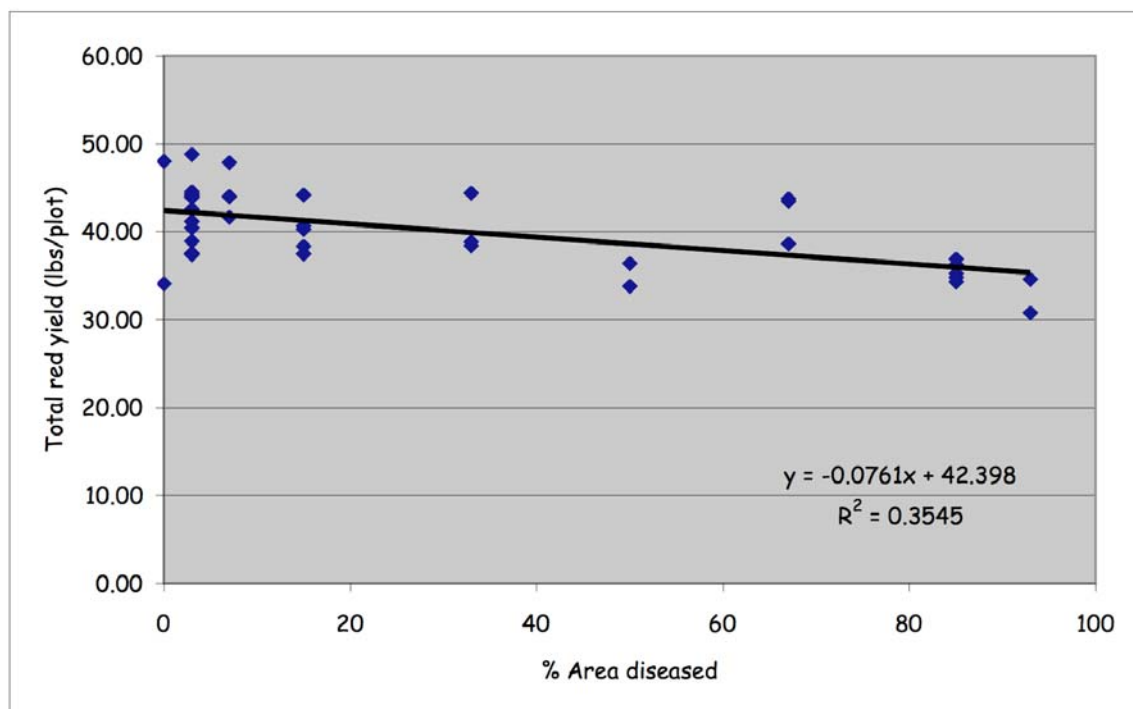


Figure 1. Impact of percent leaf area diseased on marketable fruit yield from 19 May 2005 rating (early bloom stage), Field 2, Woodland, CA.

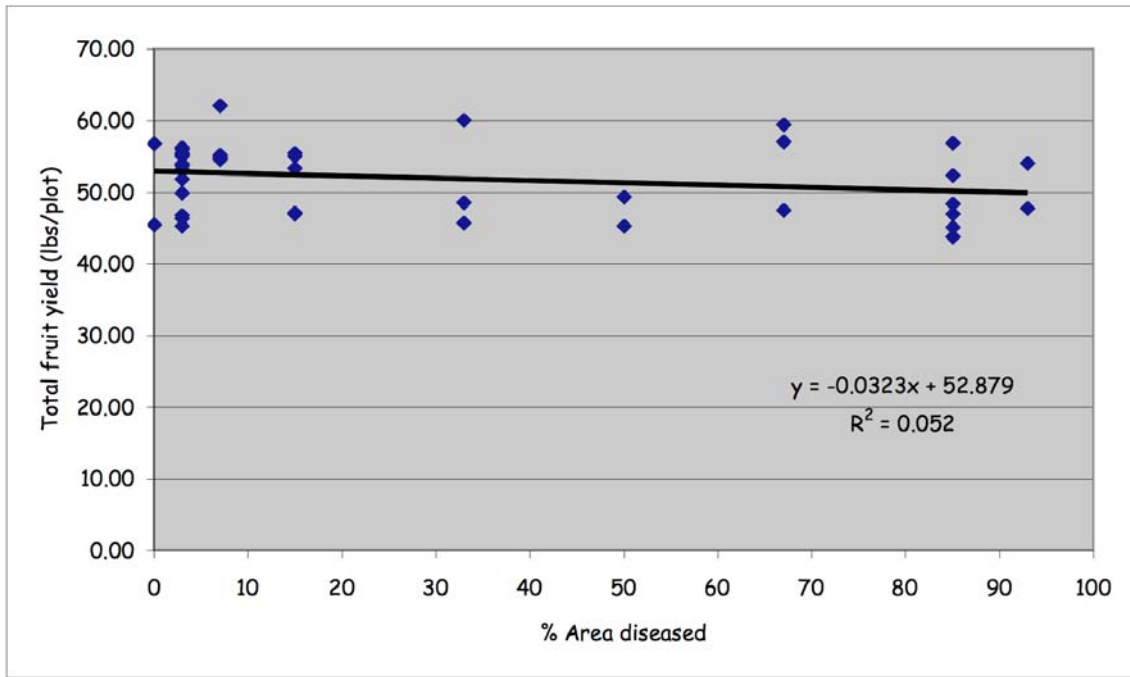


Figure 2. Impact of percent leaf area diseased on total fruit biomass yield from 19 May, 2005 rating (early bloom stage), Field 2, Woodland, CA

Virulence in Bacterial Plant Pathogens: Significance in Diversity of Populations That Cause Bacterial Canker of Tomato

Anne Alvarez and Wendy Kaneshiro

Department of Plant and Environmental Protection Sciences
University of Hawaii, 3190 Maile Way, St. John 307, Honolulu, HI 96822

ABSTRACT

Methods to differentiate yellow seed saprophytes from *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) were assessed, and a potential etiological role for hypovirulent and nonvirulent *Cmm* was studied. Half of the *Cmm* strains isolated from seed were hypovirulent or nonvirulent, but ELISA using MAb Cmm1, MicroLog™ and rep-PCR still consistently differentiated these *Cmm* subpopulations from saprophytes. Co-inoculation of virulent and nonvirulent *Cmm* strains did not alter the capacity of either strain to colonize tomato stems, however, further studies are necessary to confirm these observations. Until it is established that hypovirulent and nonvirulent *Cmm* strains pose no threat to seed health, they cannot be ignored when developing new detection assays.

INTRODUCTION

Strains of the bacterial canker pathogen, *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), exhibit marked differences in virulence, regardless of geographic origin (5). Twenty-six percent of 236 *Cmm* strains isolated from around the world were hypovirulent or nonvirulent, implying that these subpopulations hold some significance. Nonvirulent strains of a related pathogen, which alone cause no symptoms, caused disease when co-inoculated into plants (7), and the epidemiological potential of such strains remains largely unknown. Current tomato seed assays require presumptive *Cmm* be confirmed with plant bioassays, ensuring that some hypovirulent and all nonvirulent strains are misidentified, with unknown consequences. The purpose of this study was to determine the prevalence of hypovirulent and nonvirulent strains from tomato seed, to find methods of separating *Cmm* subpopulations from yellow saprophytes, and to determine whether co-inoculation of strains of varying virulence affects colonization.

MATERIALS AND METHODS

Seed assays. Seed samples (10 – 25 g) from seven seed lots were soaked for 4 hr at room temperature in 30 – 75 ml 0.01 M phosphate buffered saline (PBS, pH 7.4) amended with 100 mg/L cycloheximide and 0.02% Tween 20, then agitated for 15 minutes in a laboratory blender (Stomacher 400, Seward, Inc.). Seeds from two highly contaminated lots were individually macerated in PBS. Sub-samples of extract (0.1 ml) were plated onto CMM semiselective medium (1). Plates were incubated at 28°C for 7 d then *Cmm*-like colonies (yellow to orange, semi-fluidal, convex, with entire margins) were spotted onto YSC medium (containing 10 g yeast extract, 20 g sucrose, 20 g calcium carbonate, and 17 g agar per liter) and tested by ELISA using MAb Cmm1 (clone 103-142)(2).

Characterization of bacterial strains. Virulence was determined on four-week-old tomato seedlings (cultivar ‘Kewalo’) by wound-inoculation of a 72-hour culture into the stem between the cotyledons. Plants were observed for 21 d and rated on a scale of 0 - 7

(4). Endocellulase production was detected by the procedure of Nissinen et al. (7). PCR was done using Cm₃/Cm₄ (8) and CMM-5/CMM-6 (3), with conditions similar to those reported. Rep-PCR was performed using the BOXA1R primer (BOX-PCR), as described in Louws and Cuppels (6). Species identifications were obtained with MicroLog³TM (release 4.2.04, Biolog, Inc.) using the Gram positive database (release 6.11).

PCR for 16S rDNA sequence analysis was done using primers 264F (5'-GAT GAT CAG CCA CAT TGG GAC -3') and 1078R (5'-CCC AAC ATC TCA CGA CAC GAG -3'), with 27 µl Platinum PCR SuperMix high fidelity (Invitrogen), 1 µl each primer (10 µM), and 1 µl genomic DNA (50 ng). PCR conditions were: 5 min at 94 °C, followed by 35 cycles of 45 sec at 94 °C, 45 sec at 55 °C, and 45 sec at 72 °C, and ending with 5 min at 72 °C. Most significant alignments were obtained through BLASTN search of the NCBI database (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Coinoculation of virulent and nonvirulent strains. Two strains were selected based on differences in colony morphology, virulence, and reactivity with MAb Cmm1. Plants were inoculated with individual cultures or were co-inoculated by placing equal amounts of inoculum (10⁶ CFU per strain) at the wound site. After 30 d, ten 1-cm stem sections taken from the inoculation point towards the apical meristem were individually ground in PBS buffer and dilution plated to CMM medium. Colonies recovered from plants inoculated with individual strains were enumerated after 5 d. Colonies of the MAb Cmm1-negative virulent strain from the co-inoculated plants were enumerated in this manner, and then plates were blotted with nitrocellulose membranes and run using an immunoblot (4) to enumerate colonies of the MAb Cmm1-positive nonvirulent strain.

RESULTS AND DISCUSSION

Over 27,000 bacterial colonies were isolated from the nine seed lots. After transfer to YSC plates and ELISA using MAb Cmm1, 60 ELISA-positive strains were identified. Presumptive *Cmm* were identified as *C. michiganensis* by 16S rDNA sequence analysis, and 32 were characterized further (Table 1). Additionally, 12 yellow seed saprophytes identified by 16S rDNA as non-*Clavibacter* species were tested to compare reactivities of the various methods with non-target strains. Sixteen of the 32 *Cmm* strains (52%) were either nonvirulent or hypovirulent, and reactivity with PCR and the endocellulase assay was variable. Virulent strains gave more uniform reactions with these methods. None of the saprophytes reacted with either primer set, but seven produced endocellulase. All 32 *Cmm* strains were identified as *C. michiganensis* using MicroLog³TM, whereas the saprophytes were not. BOX-PCR also differentiated the 32 strains from saprophytes based on three bands (296, ~500 and ~700 bp) present in *Cmm* fingerprints but absent from non-*Cmm* fingerprints. ELISA using MAb Cmm1, MicroLog³TM and BOX-PCR were the most reliable of the methods tested for differentiating hypovirulent and nonvirulent *Cmm* from similar-appearing seed saprophytes.

Both virulent and nonvirulent *Cmm* strains colonized tomato stems when inoculated alone, with the nonvirulent strain doing so to a lesser extent (7 cm, compared to 10 cm for the virulent strain). Co-inoculation had no apparent effect on colonization by either the virulent or the nonvirulent strain, as colony counts remained unchanged between individually inoculated and co-inoculated plants. As these conclusions are based on limited studies of a few strains, further studies using new MAbs and strain-specific primers must be undertaken to understand the nature of the interactions between different bacterial populations. Additional studies to assess the ability of hypovirulent and

nonvirulent *Cmm* to infest and survive on seed will also give insight into the potential role these *Cmm* subpopulations play in bacterial canker epidemiology.

Table 1. Characterization of 32 presumptive *Cmm* strains isolated from tomato seed using selected phenotypic and genotypic tests.

Virulence	Virulence	# of strains	Ab Cmm1	PCR reactivity		Endo cellulase production	MicroLog™ (Cm)	ep-PCR
				m ₃ /m ₄	MM-5/ MM-6			
V	N	6	.		-	-	+	
V	N	1	.		-	+	+	
V	N	8	.		+	+	+	
H		1	.		-	+	+	
H		1	.		-	+	+	
V		1	.		-	+	+	
V		1	.		+	+	+	
		4						

LITERATURE CITED

1. Alvarez, A.M., Kaneshiro, W.S. and Vine, B.G. (2005) Diversity of *Clavibacter michiganensis* subsp. *michiganensis* populations in tomato seed: What is the significance? *Acta Horticulturae* (ISHS) 695:205-214.
2. Alvarez, A.M., Derie, M., Benedict, A., and Gabrielson, R. 1993. Characteristics of a monoclonal antibody to *Clavibacter michiganensis* subsp. *michiganensis*. *Phytopathology* 83:1405.
3. Dreier, J., Bermphohl, A., and Eichenlaub, R. 1995. Southern hybridization and PCR for specific detection of phytopathogenic *Clavibacter michiganensis* subsp. *michiganensis*. *Phytopathology* 85:462- 468.
4. Kaneshiro, W.S. 2003. Detection and characterization of virulent, hypovirulent, and nonvirulent *Clavibacter michiganensis* subsp. *michiganensis*. M.S. Thesis, University of Hawaii.
5. Kaneshiro, W.S. and Alvarez, A.M. 2001. Specificity of PCR and ELISA assays for hypovirulent and avirulent *Clavibacter michiganensis* subsp. *michiganensis*. *Phytopathology* 91:S46. Publication no. P-2001-0330-AMA.

6. Louws, F.J. and Cuppels, D.A. 2001. Molecular Techniques. Pages 321-333 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd Edition, N.W. Schaad, J.B. Jones and W. Chun, eds. American Phytopathology Society Press, St. Paul, MN.
7. Nissinen, R., Kassuwi, S., Peltola, R., and Metzler, M.C. 2001. *In planta*-complementation of *Clavibacter michiganensis* subsp. *sepedonicus* strains deficient in cellulase production or HR induction restores virulence. Eur. J. Plant Pathol. 107:175-182.
8. Sousa Santos, M., Cruz, L., Norskov, P., and Rasmussen, O.F. 1997. A rapid and sensitive detection of *Clavibacter michiganensis* subsp. *michiganensis* in tomato seeds by polymerase chain reaction. Seed Sci. Technol. 25:581-584.

The Effect of Spray Applications on *Clavibacter Michiganensis* subsp. *michiganensis* Populations

(Research in Progress)

C. A. Laskey¹, M. K. Hausbeck¹, R. Hammerschmidt¹

¹Department of Plant Pathology, Michigan State University, East Lansing, MI 48824

ABSTRACT

The effect of bactericidal spray applications on *Clavibacter Michiganensis* subsp. *michiganensis* (*Cmm*) populations was assessed throughout the 2005 growing season. *Cmm* was allowed to spread from infected tomato plants to healthy plants. Epiphytic population levels were monitored on 22 July, 12 August, and 2 September, 2005. Untreated plants had the highest epiphytic population of *Cmm* while Actigard and Tanos treated tomatoes tended to have lower epiphytic populations. However, differences may not be significant. Future studies will focus on *Cmm* population studies in the greenhouse in response to bactericides as well as repetition in the field next season.

INTRODUCTION

Bacterial canker, caused by *Cmm* is a seed borne disease that has caused great economic losses for the Michigan tomato industry [1]. In the field, disease symptoms can include marginal leaf necrosis, fruit lesions, wilting, stunting, and plant death. Transplants that have a higher than 10^8 CFU of *Cmm* /g of tissue are severely reduced in their ability to survive and produce in the field [1]. Infected transplants have been shown by Ricker et. al have shown that secondary spread of *Cmm* in the field from infected transplants can result in yield loss and cause systemic infections in surrounding plants [2]. The objective of this experiment was to evaluate different bactericidal products on *Cmm* population growth and spread through the growing season.

MATERIALS AND METHODS

Experimental Plots

On 10 June 2005, tomato (*L. esculentum* cv. Mountain Spring) transplants grown in a commercial like setting were hand transplanted 18 inches apart into raised beds covered with black plastic in muck soil in Laingsburg, MI. The plot followed a fresh market planting design and drip irrigation was provided. The beds were 2' wide, 6" high and 50' long with rows spaced at 5.5' apart. Each row comprised of two treatments, 22.5' long with a five-foot section in the middle, reserved for inoculated plants. Treatments were arranged in a complete randomized block design.

Treatments

Treatments consisted of Kocide (copper hydroxide) at 1.5 lbs/acre, Oxidate (hydrogen dioxide) at 4 pints/acre alternated with Kocide at 1.5lbs/acre, Tanos (famoxadone and cymoxanil) at .5lbs/acre alternated with Kocide at 1.5 lbs/acre, Actigard (acibenzolar-S-methyl) at rates of 0.02, 0.03, and 0.05 lbs/acre with remaining applications at 0.05lbs/acre alternated with Kocide at 1.5 lbs/acre and an untreated control. On 27 June 2005, treatments were applied preventively on a 5-day interval ending 31 August 2005. All treatments received alternating Bravo WS and Manzate applications to prevent fungal infections and were applied at the same time as the treatments.

Inoculation

On 24 June 2005, transplants were clip inoculated with a spontaneous rifampicin resistant *Cmm* mutant at 10^8 CFU/ml and incubated on a raised bench in a glass greenhouse. On 29 June 2005, 5 days after inoculation, transplants were hand transplanted in the center 5' of every row between two randomized treatments.

Sampling

On 22 July, 12 August, and 2 September 2005, three asymptomatic leaflets of approximately the same age and size were randomly selected from the lower 2/3 of the tomato plant at each sample location. Treatments were sampled from sites located at 0', 9', 18' from the inoculated plants. Each sample was placed in a sterile plastic bag and transported on ice to the laboratory for immediate processing. Each leaflet was weighed, placed in 20 ml of 0.01 M sodium phosphate buffer (pH 7.0 with 0.1% peptone) in a 125 mL flask, and shaken on a rotary shaker at 140 rpm for 1 hour at room temperature[3]. The buffer from each of the three leaflets was combined and a ten-fold dilution series was plated on NBY amended with 75 μ g/ml Rifampicin and 100 μ g/ml Cycloheximide to select for *Cmm* rifampicin resistant mutant and inhibit contaminant growth. *Cmm* colonies were counted after 5 days incubation at room temperature.

RESULTS AND DISCUSSION

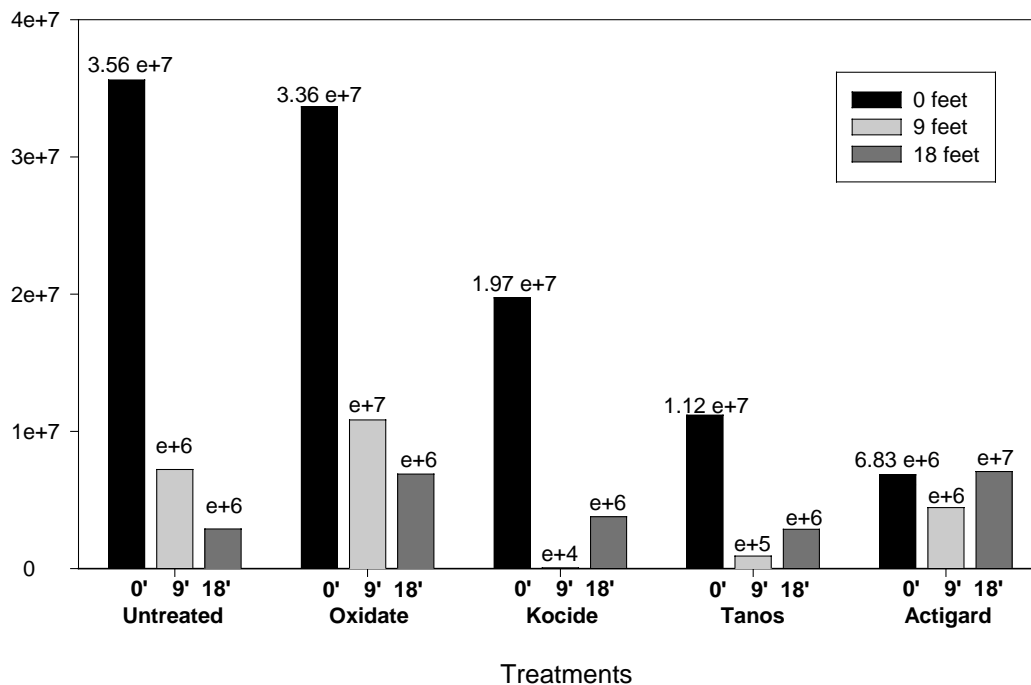


Figure 1. *Cmm* populations on tomato foliage located 0', 9', and 18' from inoculated plants when treated with various products.

Under our field experimental conditions, *Cmm* spread 9' from the point of infection within 3 weeks and to 18' in 6 weeks (Figure 1). Oxidate-treated plants had higher epiphytic populations of *Cmm* at the beginning and the end of season than the control. Tanos and Actigard consistently had lower *Cmm* populations at the end of the season than other treatments (graphs not pictured). However, difference observed between treatments may not be significant.

Due to the variability of population levels on any given date, *Cmm* populations need to be monitored over the course of the season to assess the ability of treatments to limit pathogen spread. Future studies will be needed to further define the relationship between epiphytic *Cmm* population levels, disease development, and treatment efficacy.

LITERATURE CITED

1. Hausbeck, M.K., et al., *Effect of bactericides on population sizes and spread of Clavibacter michiganensis subsp michiganensis on tomatoes in the greenhouse and on disease development and crop yield in the field*. Phytopathology, 2000. **90**(1): p. 38-44.
2. Ricker, M.D. and R.M. Riedel, *Effect Of Secondary Spread Of Clavibacter-Michiganensis Subsp Michiganensis On Yield Of Northern Processing Tomatoes*. Plant Disease, 1993. **77**(4): p. 364-366.
3. Chang, R.J., S.M. Ries, and J.K. Pataky, *Dissemination Of Clavibacter-Michiganensis Subsp Michiganensis By Practices Used To Produce Tomato Transplants*. Phytopathology, 1991. **81**(10): p. 1276-1281.

Efficacy of Fungicides/Bactericides in the Management of Foliar and Fruit Diseases of Tomato

Sally Miller, Melanie Lewis Ivey and Jhony Mera

Department of Plant Pathology, The Ohio State University
Ohio Agricultural Research and Development Center (OARDC), Wooster, OH 44691

ABSTRACT

A number of products and combinations were tested for their potential utility in management of bacterial and fungal diseases of processing tomatoes. Several product combinations significantly reduced bacterial leaf spot severity. Cuprofix, Actigard, and Tanos + Manzate + Kocide alternated with Manzate + Kocide were the most effective. The combination of Tanos + Manzate + Kocide alternated with Manzate + Kocide was also active against bacterial canker. Amistar or Reason alternated with Bravo Weather Stik performed best in reducing early blight severity.

INTRODUCTION

Foliar and fruit diseases consistently cause economic loss in processing and fresh market tomato production. Early blight (*Alternaria solani*) and septoria leaf spot (*Septoria lycopersici*) are the two most common fungal foliar diseases in Ohio and other areas where high humidity and rainstorms occur during the growing season. Anthracnose (*Colletotrichum* spp.) is the most common fungal disease of tomato fruit. Bacterial spot (*Xanthomonas* spp.), bacterial speck (*Pseudomonas syringae* pv. *tomato*) and bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) are also common, and are very difficult to manage once they are established in the field. Bacterial stem rot, pith necrosis, botrytis grey mold, late blight, buckeye rot and sclerotinia white mold occur relatively sporadically. New fungicides and bactericides are being evaluated for efficacy in controlling these diseases in tomatoes. The purpose of this study was to evaluate plant protection products for management of early blight, anthracnose and bacterial diseases of tomato.

MATERIALS AND METHODS

Field trials were conducted at the Ohio Agricultural Research and Development Center North Central Experiment Station in Fremont, OH in 2004 and 2005. 'Peto 696' tomato seeds were hot water-treated and sown into 288-cell plug trays containing Metromix 360 seedling mix. Seedlings were transplanted 1 ft apart into single rows 25 ft long on the beds in late May. Starter fertilizer (N-P-K 10-34-0; 0.7 qt/50 gal water) was applied in the transplant water. Treatments were arranged in a randomized complete block design with four replications. Treatment rows were alternated with untreated border rows. Plants were overhead irrigated as needed. The bacterial spot trial was inoculated both years with *Xanthomonas campestris euvesicatoria* 767 (T1P3) at 10⁷ CFU/ml (copper sensitive). Bacterial canker appeared naturally in the bacterial spot trial in 2005. Treatments were applied using a tractor-mounted CO₂-pressurized sprayer (60 psi, 44.31 gal/A, 3 MPH) on a 7-10 day schedule. Disease severity was evaluated using a modified Horsfall-Barratt rating scale. Fruit were harvested from all plants in each plot and weights were recorded. Weights for marketable fruit, green fruit, and fruit with

anthracnose, bacterial disease, blossom end rot and other rots were determined from a 14-24 kg sub-sample from each plot. The area under the disease progress curve (AUDPC) was calculated using all ratings. Data were analyzed by ANOVA using SAS software and means were separated using Fisher's least significant difference test.

RESULTS

All of the treatments evaluated in 2004 significantly reduced bacterial spot symptoms in processing tomatoes compared to the untreated control (Table 1). Cuprofix and Actigard (applied with Amistar alternated with Bravo for fungal disease control) and Tanos + Kocide 2000 + Manzate alternated with Kocide 2000 + Manzate were the most effective in reducing bacterial spot severity, and were significantly better than the Kocide 2000 + mancozeb standard treatment. All of the treatments except Serenade + Kocide 2000, Serenade + Kocide 2000 alternated with Kocide 2000 + Manzate, and Kocide 2000 + Manzate) significantly reduced the tonnage of tomato fruit with anthracnose symptoms (Table 1). Total marketable yield varied from 31.5 tons/A (Tanos (12 oz) + Kocide 2000 + Manzate alternated with Kocide 2000 + Manzate) to 24.3 tons/A (untreated control) but did not differ significantly among treatments and the control. The proportion of marketable fruit was significantly higher than the control for all treatments except Serenade + Kocide 2000 alternated with Kocide 2000 + Manzate (data not shown).

Table 1. Effect of various treatments on severity of bacterial spot and anthracnose in processing tomatoes, 2004.

Treatment and rate/A	Foliar bacterial spot		Anthracnose
	% disease	AUDPC	(ton/A)
Cuprofix 40DF 2 lb + (Amistar alt. Bravo)	7.3 f	407.8 cde	0.8 fg
Actigard 50WG ¹ (6) + (Amistar alt. Bravo)	7.3 f	626.3 c-f	0.6 g
Tanos 50DF 12 oz + Kocide 2000 2 lb + Manzate 75DF 2 lb <i>alt. Manzate 75DF 2 lb + Kocide 2000 2 lb</i>	10.8 ef	252.5 f	1.4 fg
Airone SC 1.3 pt + Manzate 75DF 2 lb	14.3 de	336.4 def	
Amistar 1.8 oz <i>alt. Manzate 75DF 2 lb + Kocide 2000 2 lb</i>	14.3 de	503.9 c-f	1.6 c-f
ManKocide 61WDG 3 lb	14.3 de	659.0 bcd	1.6 c-f
Tanos 50DF 10 oz + Kocide 2000 2 lb + Manzate 75DF 2 lb <i>alt. Manzate 75DF 2 lb + Kocide 2000 2 lb</i>	14.3 de	309.6 ef	2.0 c-f
Tanos 50WG 8 oz + Kocide 2000 2 lb + Manzate 75DF 2 lb <i>alt. Manzate 75DF 2 lb + Kocide 2000 2 lb</i>	17.9 de	249.6 ef	1.9 c-f
Serenade Max WPB 1 lb + Kocide 2000 2 lb <i>alt. Kocide 2000 2 lb + Manzate 75DF 2 lb</i>	19.0 cd	804.3 b	5.4 ab
Airone SC 2.5 pt + Manzate 75DF 2 lb	19.0 cd	801.6 bcd	1.7 efg
Kocide 2000 2 lb + Manzate 75DF 2 lb	21.4 cd	439.9 c-f	5.6 ab
Airone SC 1.9 pt + Manzate 75DF 2 lb	28.5 bc	471.3 cde	2.7 b-e
Cuprofix 40DF 2 lb	28.5 bc	701.4 bc	3.3 be
Serenade Max WPB 1 lb + Kocide 2000 2 lb	28.5 bc	716.0 bc	4.6 abc
Tanos 50DF 10 oz + ManKocide 2 lb <i>alt. ManKocide 61WDG 3 lb</i>	44.3 b	769.3 bc	3.0 b-e
Untreated control	67.6 a	1780.3 a	12.1 a

Several product combinations were also tested for bacterial leaf spot management in 2005. In that year, bacterial canker also appeared naturally in the plots late in the season.

Bacterial spot was severe in this trial, with 96% of the foliage affected in the untreated control (Table 2). The 8 oz rate of Tanos combined with Manzate plus Kocide 2000 at 2lb/A or Manzate plus GFJ52 at 1 lb/A and alternated with Manzate plus Kocide 2000 or GFJ52, Manzate plus Kocide 2000 alone, and JE874-SE 7.58 fl oz/a plus Manzate plus Kocide alternated with Manzate plus Kocide significantly reduced bacterial leaf spot severity compared to the untreated control. Bacterial canker severity was low-moderate (19% severity in the untreated control), and all of the treatments except the Serenade Max + Kocide + Biotune significantly reduced its severity in the trial. Treatments containing the 3.2 and 7.58 fl oz/A rates of JE874-S, and Tanos plus GFJ 52 significantly reduced anthracnose incidence in fruit compared to the untreated control. There were no significant differences among treatments and the control in marketable yield (data not shown).

Table 2. Effect of various treatments on severity of bacterial spot and anthracnose in processing tomatoes, 2005.

Treatment and rate/A	% foliar bacterial spot	% bacterial canker	% anthracnose
Tanos 8oz+Manzate 2 lb+ Kocide2000 2 lb alt. Manzate 2 lb+ Kocide2000 2 lb	72.3 d	2.8 d	12.0 bc
Tanos 8oz+Manzate 2 lb+ GFJ52 1 lb alt. Manzate 2 lb+ GFJ52 1 lb	76.9 cd	9.5 bc	10.5 c
Manzate 2b + Kocide2000 2 lb	80.5 bcd	7.3 cd	14.1 abc
JE874-SE 7.58fl oz+Manzate 2 lb+Kocide2000 2 lb alt. Manzate 2 lb+ Kocide2000 2 lb	80.5 bcd	9.6 bc	10.2 c
JE874-SE 3.2 fl oz+Manzate 2 lb+Kocide2000 2 lb alt. Manzate 2 lb+ Kocide2000 2 lb	84.1 a-d	7.3 cd	10.4 c
JE874-SE 19fl oz+Manzate 2 lb+Kocide2000 2 lb alt. Manzate 2 lb+ Kocide2000 2 lb	89.9 abc	9.6 bc	20.1 a
Tanos 8oz+Manzate 2 lb+ Kocide2000 1 lb alt. Manzate 2 lb+ Kocide2000 1 lb	91.1 abc	6.1 cd	12.1 abc
Serenade Max 1 lb + Kocide2000 1 lb + Biotune .25%	94.1 ab	14.3 ab	16.8 abc
Untreated control	96.0 a	19.0 a	19.4 ab

Additional products were tested in a separate trial for utility in the management of early blight in 2005 (Table 3). Amistar or Reason (5.5 oz/A rate) alternated with Bravo Weather Stik effectively reduced the severity of early blight. Bravo Ultrex alone, the 8.2 oz/A rate of Reason alternated with Bravo Weather Stick and several rates of an experimental product did not reduce early blight disease severity compared to the untreated control. There were no significant differences in marketable yield (data not shown).

Table 3. Effect of various treatments on severity early blight in processing tomatoes, 2005.

Treatment and rate/A	% early blight*	AUDPC
Amistar 2 oz alt. Bravo Weather Stik 2.75 pt	13.1 c	172.9 c
Reason 5.5 oz + Bond .1% alt. Bravo Weather Stik 2.75 pt	16.6 bc	133.5 c
Bravo Ultrex 1.5 lb	26.5 abc	279.0 abc
Reason 8.2 oz + Bond .1% alt. Bravo Weather Stik 2.75 pt	31.3 abc	243.0 abc
IR5885 CTL WP 1.35 lb	35.3 abc	214.5 abc
IR5885 CTL WP 1.1 lb	41.5 ab	254.5 abc
IR5885 CTL WP 1.6 lb	46.3 a	332.3 ab
Untreated control	43.4 a	356.3 a

* 23 Aug 2005 – 4th rating

CONCLUSION

Several products or combinations significantly reduced bacterial leaf spot severity. Cuprofix, Actigard, and Tanos + Manzate + Kocide alternated with Manzate + Kocide were the most effective. Products containing copper are expected have some activity in these trials, since the strain of the bacterial spot pathogen used to inoculate plants was copper sensitive. Copper-insensitive strains are widespread throughout Ohio and other tomato-producing regions, and in cases where they are predominant, the copper-containing products are likely to have significantly reduced efficacy. A treatment containing Tanos plus Manzate plus Kocide alternated with Manzate plus Kocide was also active against bacterial canker. Amistar or Reason alternated with Bravo Weather Stik performed best in reducing early blight severity.

Evaluation of Compost Tea for Managing Foliar Diseases in Tomato

M. T. McGrath

Dept of Plant Pathology, Cornell University, LIHREC,
3059 Sound Avenue, Riverhead, NY 11901

ABSTRACT

A bacterial-dominant compost tea applied at least weekly to foliage was evaluated for control of naturally-occurring diseases in tomato grown in a research field dedicated to organic production. Tea was tested alone or with other products in 2003 and 2004. Tea was applied starting 32 and 27 days after transplanting, respectively, for a total of 14 and 10 applications. Tomatoes were transplanted no-till into a hairy vetch-rye mulch. An additional treatment was a side-dressing of nitrogen applied as a band of peanut meal to some plots. Disease incidence and severity were not significantly reduced by compost tea, the biofungicide Sonata, the two used together, or compost tea followed by rescue treatments of Champion and JMS Stylet-oil made after disease detection. Diseases were powdery mildew, Septoria leaf spot, and bacterial speck. Possible reasons compost tea was ineffective include lack of appropriate organisms in compost, unsuitable recipe or brewing conditions, applications made at the wrong time of day, and treatment needed earlier in crop development, perhaps including a seed treatment.

INTRODUCTION

Compost teas have been used for disease control for years with limited research. Interest in compost tea has increased with increase in organic crop production. Evaluations are needed for specific diseases using teas made from defined recipes.

MATERIALS AND METHODS

'Paragon' or 'Red Sun' tomato seed were hot water treated (25 min at 122 F) to control seed-borne bacterial pathogens, then placed in trays with an organic soil-less mix on 2 May 03 or 5 May 04, respectively. The hairy vetch-rye cover crop was flail chopped on 16 Jun 03 and 1 Jun 04 to form a mulch. Seedlings were no-till transplanted 26 Jun 03 and 11 Jun 04 with fish hydrolysate fertilizer (approx. 2.8 ml/plant of Organic Gem Liquid Fish 3-3-3 diluted in water 1:64). A tractor equipped with a fluted coulter and an S-tine was used to cut 4-in. deep strips through the field. Seedlings were placed in these holes by hand. There were 10 plants spaced 2-ft apart in each single-row plot. Drip irrigation tube was laid on the soil surface next to the plants. Plants were watered as needed. Plants were pruned then trellised to maintain up-right growth habit. Weeds were managed by mowing between plots and hand-weeding in the planted rows. Straw was placed around the base of plants (1/2 bale/plot) because the straw from the vetch cover crop was not sufficient to suppress weeds.

Treatments. Peanut meal was applied at 625 lb/A (equivalent to 50 lb/A of N) to the high nitrogen treatment plots on 17 Jul 2003 and 23 Jun 2004 to supplement the nitrogen provided by the vetch mulch and starter fertilizer. Foliar disease management treatments were applied using a CO₂-pressurized backpack sprayer with a single nozzle boom. Each plot side was treated with the boom held sideways to obtain thorough coverage. Compost tea was applied separately from the other treatments. Fungal-based compost (4 lb dairy manure-based vermicompost and 4 lb grape pumice compost in 2003 or 4 lb leaf-based compost in 2004) was brewed aerobically with 12 oz Fertrell Liquid Kelp, fish hydrolysate (7 oz Fertrell Omega Grow in 2003 and 4 oz Organic Gem Liquid Fish 3-3-3 or Neptune's Harvest Benefits of Fish 2-4-1 in 2004), and humic acid (7 oz Humate International Humate in 2003 and 16 oz Fertrell Bio-Hume in 2004) for about 24 hour in a 60-gal Sotillo brewer. Compost tea was filtered and applied at full strength (no dilution) with Nu-Film-P (6 oz/A) to foliage on 18 and 30 Jul; 1, 5, 12, 19, 22, and 28 Aug; and 5,

9, 12, 17, and 24 Sep in 2003. Additionally, tea was applied as a soil drench using a watering can on 23 Jul due to wet conditions. The tea used on 5 Sep was brewed for 48 hr because rainy weather on 4 Sep prevented application. To minimize potential damage to organisms in the compost tea, it was applied at low pressure (40 psi) using a nozzle with a large orifice that causes little resistance (FloodJet). Most applications were made before 10 am. In 2004, fish hydrolysate (1 oz/30 gal) was also added before the tea was applied on 8, 16, 23, and 29 Jul; 4, 11, 17, and 25 Aug; and 1 and 8 Sep.

Sonata (2 qt/A) was applied on 12, 19, and 26 Aug; and 6, 15, 22, and 26 Sep with a TwinJet (TJ110-8003) nozzle at 50 psi and 50 gpa. In 2004, Sonata was applied on 11, 17, and 25 Aug; and 1, 8 and 17 Sep immediately after compost tea. An additional treatment in 2004 was compost tea applied preventively followed by rescue treatments applied after disease detection, which were JMS Stylet oil applied for powdery mildew on 25 Aug and 1 Sep and the copper fungicide Champion applied on 3, 12 and 17 Sep.

Samples of tea, compost, and leaves were submitted to the Soil FoodWeb Laboratory in Port Jefferson, NY, for analysis of the organismal content. Samples of tea were collected on 5 Aug 03 and 8 Sep 04 from the brewer and from the spray nozzle. The two samples were collected to determine if the spray nozzle had a detrimental impact on the microbes. In 2004, compost samples were submitted on 28 Apr and 8 Sep. Leaves were collected before and after tea was applied on 8 Sep to assess the delivery and deposition of microbes.

Disease assessment. Incidence, severity and defoliation for the major diseases occurring were rated as percent leaves and leaf area affected on 22 Sep, 29 Sep and 6 Oct 2003 and 1, 9, 16, and 24 Sep and 1 Oct 2004.

Harvest. Red fruit and those turning red were harvested weekly from the center 8 of 10 plants in each plot from 27 Aug through 15 Oct 2003 and from 26 Aug through 7 Oct 2004. Fruit were graded by size, counted, and weighed.

Statistical analysis. Means were separated using Fisher's protected least significant difference test ($P \leq 0.05$).

Results and discussion

Powdery mildew, bacterial speck and Septoria leaf spot developed naturally. In 2003, symptoms of powdery mildew were first observed in one plot on 3 Sep. Septoria leaf spot was seen on 8 Sep. These diseases became widespread in the field by 22 Sep causing severely affected leaves to die. A few spots due to early blight and leaf mold were seen; incidence was too low to rate. In 2004, powdery mildew was first observed on 20 Aug. Septoria leaf spot and bacterial speck were seen on 1 Sep.

No significant differences ($P \leq 0.05$) were detected among treatments in disease incidence, severity or defoliation (Tables 1 and 2). However, in 2003 disease severity and defoliation were numerically lowest where Sonata and compost tea were applied. Based on the organismal analysis, the compost tea used in these two experiments was primarily bacterial, although the ingredients used were anticipated to produce a fungal-dominant tea and the recipe was adjusted in an effort to obtain more fungi. Active and total bacterial biomass for tea from brewer were rated by the Soil FoodWeb Laboratory as good and excellent in 2003 (33.8 and 576 ppm, respectively) and good in 2004 (16 and 288 ppm, respectively). Values were higher for compost tea sample from the nozzle, likely reflecting that Nu-Film-P can be a food source for bacteria. Active and total fungal biomass was rated low, being only 1.28 and 1.73 ppm, respectively, in 2003 and 0.91 and 2.78 ppm, respectively, in 2004. These teas were described as bacterial with good fungal biomass. The leaf-based compost used in 2004 had very high fungal activity before the experiment was started (99.8 ppm on 28 April); it was only 36.8 ppm on 8 Sep. Active bacterial biomass increased from 47.8 ppm in Apr to 69.4 ppm in Sep. The leaf organism assay revealed that leaf coverage with bacteria and fungi was inadequate on leaves

collected immediately before tea application. Leaves collected about 1 hour after tea was applied were 76% covered by bacteria and 17% covered by fungi, which is considered very good. There are several possible reasons the compost tea was ineffective, including lack of appropriate organisms in compost, unsuitable recipe or brewing conditions, applications made at the wrong time of day (perhaps late in the day better than morning), and treatment needed earlier in crop development, perhaps including a seed treatment.

Neither compost tea nor the peanut meal nitrogen treatment significantly affected yield. High nitrogen plants, those that had received peanut meal, were observed to be larger and greener than those that had not (low nitrogen treatment) in 2003 but not in 2004. Compost tea applications, which can be a source of nutrients, did not have a detectable impact on plant appearance. High nitrogen plants not receiving a foliar treatment produced numerically more marketable fruit (at least 2.5 inch in diameter) than low nitrogen nontreated plants (16 vs 10 per plant in 2003 and 21 vs 19 in 2004), but these differences were not statistically significant.

Table 1. Effect of compost tea, biofungicide Sonata, and nitrogen fertilization on disease severity and subsequent defoliation due to disease in 2003.

Treatment	Disease severity (% leaf area affected)				Defoliation(%)
	Powdery mildew		Septoria leaf spot		
	29 Sep	6 Oct	22 Sep	6 Oct	6 Oct
Low Nitrogen, Nontreated	7.1	13.0	6.8	20.0	17.3
Low Nitrogen, Compost tea	5.4	18.0	8.0	21.3	22.5
High Nitrogen, Nontreated	5.5	17.6	5.5	20.0	18.8
High Nitrogen, Compost tea	2.4	10.8	5.5	18.8	12.3
High Nitrogen, Compost tea + Sonata AS 2 qt/A	0.9	5.6	3.8	18.8	11.3
<i>P</i> value	0.81	0.78	0.83	0.99	0.88

Table 2. Effect of compost tea, OMRI-approved fungicides, and nitrogen fertilization on disease severity and subsequent defoliation due to disease in 2004.

Treatment	Disease severity or incidence (%) *					
	Powdery mildew		Septoria leaf spot		Bacterial speck	
	1 Sep	24 Sep	24 Sep	1 Oct	24 Sep	1 Oct
Low Nitrogen, Nontreated	11	53	30	41	28	44
Low Nitrogen, Compost tea	3	35	35	51	36	46
High Nitrogen, Nontreated	11	43	28	34	35	44
High Nitrogen, Compost tea	10	46	56	53	18	43
High Nitrogen, Sonata AS 2 qt/A	9	62	36	48	33	44
High Nitrogen, Compost tea + Sonata	8	40	45	48	38	55
High Nitrogen, Compost tea + JMS Stylet-oil 5 qt/100 gal + Champion WP 4 lb/A	9	40	39	41	38	48
<i>P</i> value	0.82	0.61	0.29	0.74	0.72	0.97

* Powdery mildew was assessed as proportion of leaf tissue affected. Septoria leaf spot and bacterial speck were assessed as proportion of leaflets affected.

New Products to Control Tomato Diseases

Dr. M.K. Hausbeck and B.D. Cortright, Michigan State University, East Lansing, MI

Studies in 2004 and 2005 evaluated new products and industry standards for efficacy against bacterial canker, early and late blights of tomato (Table 1).

Table 1. Products used in tomato trials.

Product	Active ingredient	Labeled
Acrobat 50WP	dimethomorph	yes
Actigard 50WG	acibenzolar-S-methyl	no
Amistar 80WG	azoxystrobin	yes
Bravo Weather Stik 6SC, Bravo Ultrex 82.5WDG.....	chlorothalonil	yes
Cabrio 20WG	pyraclostrobin	yes
Champ 57.6DF, Kocide 2000 54DF, Kocide 61.4DF	copper hydroxide	yes
Dithane 75DF, Manzate 75DF	mancozeb	yes
Endorse 11.3 DF.....	polyoxin D zinc salt	no
Ranman 3.3SC.....	cyazofamid	yes
Ridomil Gold Bravo 76.5WP.....	mefenoxam + chlorothalonil	yes
Serenade Max 20WP	<i>Bacillus subtilis</i>	no
Tanos 50WG	famoxadone + cyazofamid	yes
BAS 55007F 4.17SC, CTL 75WP, TM 459 1.67SC, TM 465, TM 473 4FL.....	--	no

New Product Tests for Bacterial Canker

A study of bacterial diseases on tomato was conducted at Michigan State University using raised beds spaced 8 ft apart. Planting holes were punched in the tops of the beds using a water wheel with a hole spacing of 18 in. on 8 June and one 'Mt Spring' transplant from a 72-cell plug tray was planted in each hole. Treatment plots consisted of 20 ft of row with a 2 ft buffer space between treatments within the row, and one buffer row between each treatment row. Treatments were replicated four times in a randomized complete block design. The plot received applications of 20-20-20 (5 lb/A) weekly through the drip tube. Fungicide sprays were applied using a CO₂ backpack sprayer with three 8003XR nozzles spaced 19 in apart. The sprayer was operated at 50 psi and was calibrated to deliver 50 gal/A. Ten sprays were made in 2004 to the 7-day treatments on 26 June; 2, 9, 16, 23, and 31 July; 7, 14, 21, and 25 August. Plots were evaluated for foliar bacterial infection (based on a rating of 0 to 100%) on 2 and 14 September 2004. Yields were taken from the inner five ft of plants on 7 and 15 September 2004. Ten sprays were made in 2005 to the 7-day treatments on 29 June; 6, 13, 20, and 27 July; 3, 10, 17, and 26 August; and 2 September. Plots were evaluated for foliar bacterial and fungal infection and bacterial defoliation (based on a rating of 0 to 100%) on 30 August and 15 September 2005. Yields were taken from the inner five feet of plants on 2 and 13 September 2005.

Defoliation in 2004 was caused by both bacterial canker and bacterial speck. Canker symptoms caused a blighting and yellowing that resulted in death of entire

petioles on one side of the stem. All treatments had less bacterial canker and bacterial speck than the untreated control for the last foliar rating (Fig. 1A). Bacterial pressure in the 2005 trial was moderate with 33.8% bacterial defoliation in the untreated sections by the final rating date. All treatments in 2005, with the exception of Serenade Max + Biotune, had less canker than the untreated control (Fig. 1B).

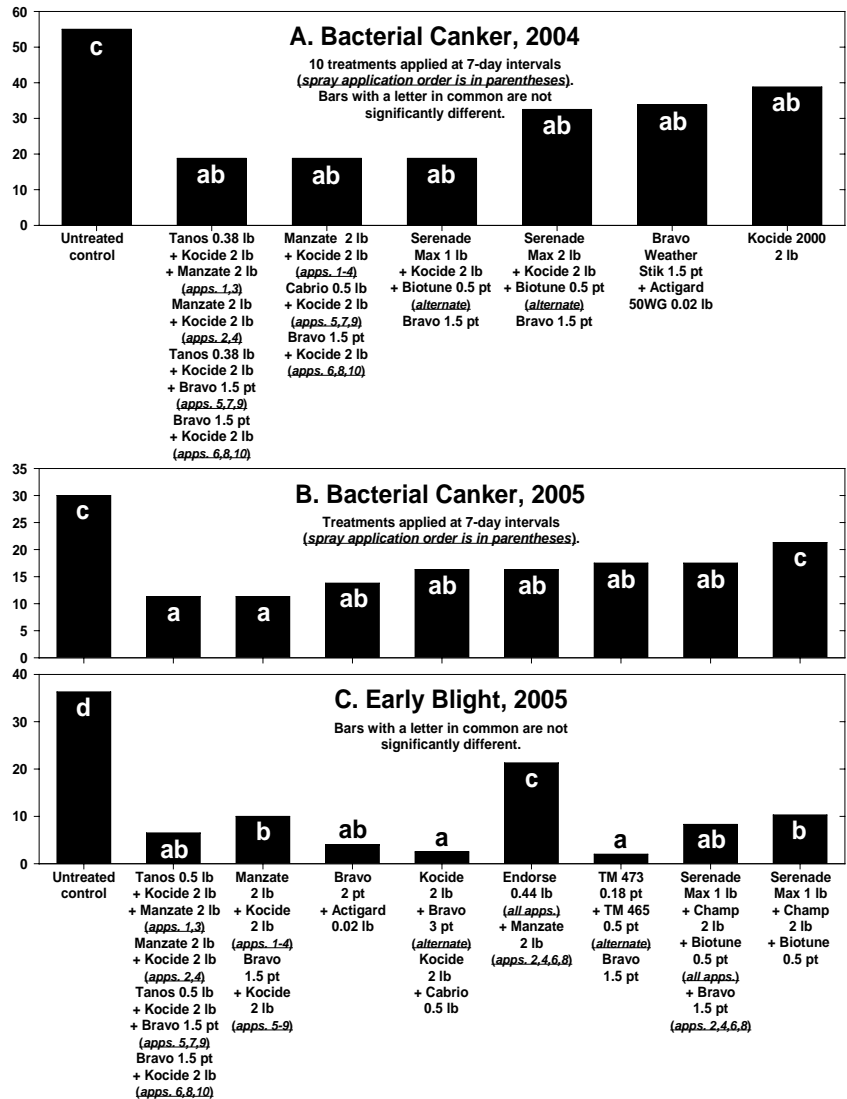
New Product Test for Early Blight

The same treatments evaluated in 2005 for bacterial canker were also rated for early blight. All treatments significantly limited early blight compared to the untreated control, although plants treated with Endorse + Manzate had more disease than all other fungicide treatments (Fig. 1C).

New Product Test for Late Blight

A tomato late blight study was conducted at a grower cooperator's farm in Calhoun County, MI, on a sandy clay loam soil that was previously planted to winter wheat. Plots consisted of one 20-ft row spaced 8 ft apart, with 18 in. between plants. On 9 June, 72-cell 'Mt. Spring' tomato plugs were transplanted into the field. Eighteen treatments were replicated four times in a randomized complete block design. A spore suspension (1×10^5 spores/ml) was prepared from 11-day old cultures of *Phytophthora infestans* (US-6, A1) grown on rye agar and applied to plants in the field on 31 July, 7, 14, and 21 August. Tomato early blight (*Alternaria solani*) infection was suppressed by applications of Endura 70WG (4 oz/A) on 17 July and again on 5 August. Fungicide sprays were applied using a CO₂ backpack boom sprayer equipped with three 8003XR nozzles spaced 19 in. apart, operated at 50 psi to deliver 50 gal/A. Sprays were applied on a 7-10 day schedule on 9, 20, and 25 July; 5, 11, 19, and 27 August; and 4 September. Plants were visually assessed for foliar disease on 26 August and 9 September. Yields were taken by stripping the fruit from the three center plants of each

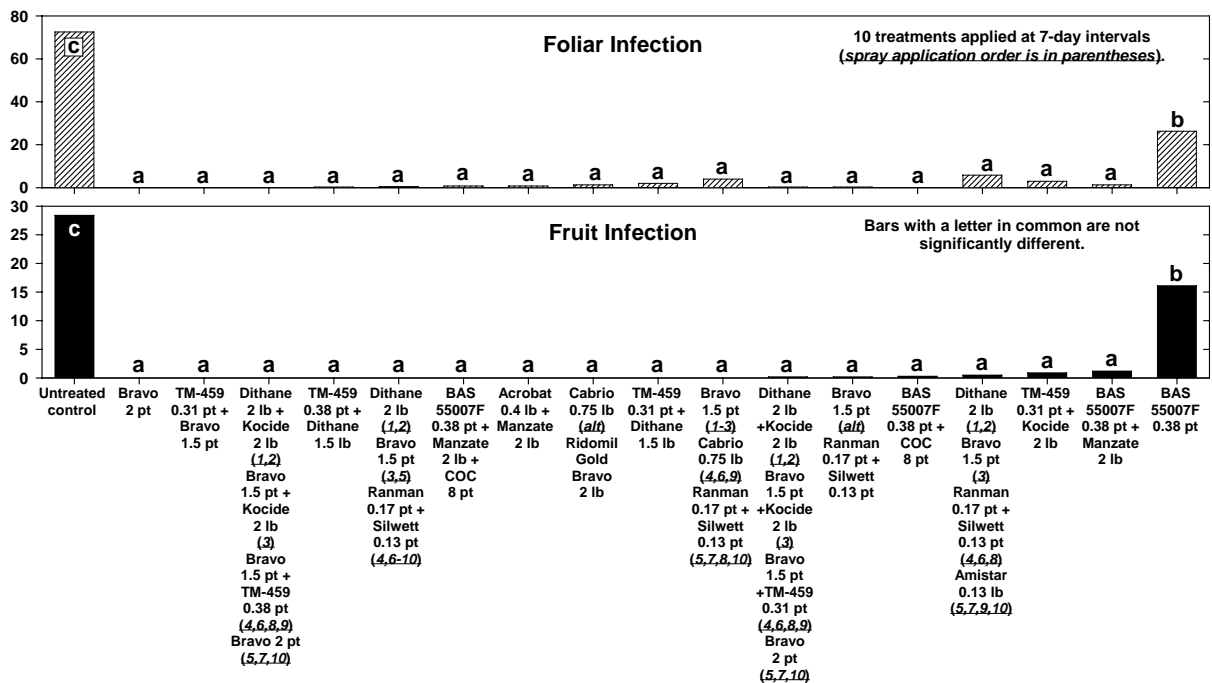
Fig. 1. New product tests for bacterial canker in 2004 (A) and 2005 (B), and early blight in 2005 (C).



treatment plot and the fruit was sorted by disease on 13 September.

Disease developed 7 days after the first inoculation and spread across the plot by the last evaluation date. All treatments provided significant disease control compared to the untreated on the first evaluation date (Fig. 2). All treatments provided significantly better foliar blight control than the untreated on the last observation date. BAS 55007F 4.17SC applied alone had a significantly higher level of disease than the other treatments. All other treatments were very effective in controlling foliar late blight symptoms. As with the foliar ratings, all treatments had fewer infected fruit than the untreated. The treatment of BAS 55007F 4.17SC applied alone had a higher level of infected fruit than the other chemical treatments. The remaining treatments were very effective in controlling late blight fruit infections. There were no significant differences in total yield among treatments and the untreated.

Fig. 2. New product study for late blight, 2004.



Shift in Performance of Fungicides for the Control of Tomato Early Blight

Thomas A. Zitter and Jessica L. Drennan
Department of Plant Pathology, Cornell University, Ithaca, NY 14853

ABSTRACT

Most tomato cultivars (*Solanum esculentum*) are very susceptible to foliar infection with early blight (EB) caused by *Alternaria tomatophila*. Fungicide sprays consisting of protectants (mancozeb and chlorothalonil), strobilurins (i.e. azoxystrobin and pyraclostrobin), and more recently boscalid, are commonly used for control. Concern for fungicide resistance has required that more attention be paid to the selection and scheduling of all products. Use of *A. tomatophila* in our spray trials has resulted in high infection levels and a clearer separation for efficacy among protectant and newer fungicides. Mancozeb was shown to be equal or better than chlorothalonil in providing control, and can be an effective tool in managing resistance concerns among fungicides of differing chemistry.

INTRODUCTION

Early blight (EB) is an extremely common disease of potato (*Solanum tuberosum*) and tomato (*Solanum esculentum*) and has been traditionally thought to be caused by a single fungal species, *Alternaria solani* Sorauer. Simmons (5) reported that two morphologically and culturally distinct species are responsible for the disease, and assigned *A. tomatophila* Simmons as the cause of tomato EB. The key differences described are in length, widths, and branching patterns of the beaks as well as cultural differences. Frazer (2) confirmed these morphological differences and showed that *A. tomatophila* is more virulent than *A. solani* on tomato leaves, petioles, and stems, and that isolates of *A. tomatophila* can exist as either light or dark phenotypes. Since this report, the light phenotype of *A. tomatophila* has been used in our subsequent field trials to provide better distinction and separation for efficacy among the products tested (6, 7).

Early blight infections in upstate New York typically begin the middle of July and can increase dramatically during the August. Weather conditions are generally favorable for disease development during this time, resulting in plant defoliation in excess of 60% in the unsprayed control plots (6, 7).

Fungicides are commonly used to control early blight and consist of protectant products like mancozeb (Dithane) and chlorothalonil (Bravo), or systemic fungicides belonging to the strobilurin class (1). Strobilurin compounds are site specific fungicides, and although very effective initially, resistance has been identified for a number of fungi including EB (1, 4).

MATERIALS AND METHODS

Fungal isolate and inoculations. The light phenotype of *A. tomatophila* was grown on V-8 with a 12-hour photoperiod and at a temperature of 27° C. This isolate was used to inoculate the disease spreader rows in the field plots (approx. 20,000 spores/ml applied in mid-July).

Field establishment and spray treatments for fungicide spray plots. Supersonic tomato seedlings were transplanted in early June in field plots located at Freeville, NY.

Treatments were arranged in a randomized complete block design with three (2004) or four (2005) replications. Each spray plot consisted of eight plants spaced 22 in. apart within a 15 ft row with 10 ft between each block and 14 ft between each row. Inoculated spreader rows provided a constant disease source, which ran the length of the plot, and were equidistant from each treatment row. Fungicides were applied with a CO₂ pressurized boom sprayer at 60 psi, delivering 23 gal/A, through four TeeJet XR11003 flat fan nozzles spaced 20 in. apart.

Data collection. Defoliation was assessed using the Horsfall-Barratt rating scale on a weekly basis where 0 = no defoliation and 11 = dead plants (3). Foliar data were converted using the area under the disease progress curve (AUDPC) model to account for foliar disease, which progressed over time. The data were analyzed using one-way ANOVA at $P=0.05$ and significant differences between means were separated using Tukey's Studentized Range Test at $\alpha=0.05$.

RESULTS AND DISCUSSION

In both 2004 and 2005, fungicide treatments provided significantly better control of early blight defoliation when compared with the unsprayed control (Table 1). Chlorothalonil (Bravo) has long been considered the standard fungicide for comparison sake, and in our trials it has performed well, and did so in the 2004-2005 trials. However when compared with mancozeb (trialed as Dithane) in a head-to-head comparison, mancozeb was equivalent or even slightly better than chlorothalonil. This enhanced performance was apparent in a reduction in the percent defoliation and in the reduced number of EB-infected fruit. Mancozeb also proved to be a good mixing partner when it was included with sprays like Phostrol (phosphorous acid) and Forum (dimethomorph) that were intended for late blight control. This is important since many fungicides need to be tank-mix with a protectant like mancozeb to control the full range of diseases affecting tomato. Boscalid (Endura) provided the best control of EB both seasons, although in both trials the product was used on a weekly basis and not on an alternating basis as prescribed on the label. The added value of using mancozeb with boscalid was observed in the 2004 trial, when with the addition of mancozeb to the Endura and Forum sprays, significantly better disease control was achieved.

Despite the occurrence of reduced sensitivity of *A. solani* (and probably *A. tomatophila*) to strobilurins (4), this resistance is found exclusively in states that relied heavily and without rotation of these materials for potato production. In our trials, Quadris Opti (azoxystrobin + chlorothalonil) and Amistar (azoxystrobin) performed well when alternated with chlorothalonil and mancozeb in 2004 and 2005, respectively, and continue to have a place for tomato disease control. Boscalid is an additional fungicide with a different mode of action that will fit nicely into rotational programs.

Previous work in potato has shown that repeated use of the same protectant fungicides like chlorothalonil and triphenyltin hydroxide can result in a shift of pathogens (*A. solani* and *A. alternata*) and an overall reduction in efficacy. We have also demonstrated that rotation of multi-site protectant fungicides can be as important as the rotation of fungicides with more specific modes of action.

Table 1. Performance of fungicides for the control of early blight (*A. tomatophila* - light phenotype) during trials in 2004 and 2005 at Freeville, NY.

2004 Season cool, wet - Treatments (spray schedule) ^a	% defol.	EB inf. fruit	2005 Season hot, dry – Treatments (spray schedule) ^a	% defol.	EB inf. fruit
Control (A-H)	199 f	31 f	Control (A-H)	150 c	60
Bravo (AB/CDEF/GH)	160 e	23 ef	Bravo (AB/CDEF/GH)	111 b	54
Dithane alone (not tested)	--	--	Dithane (AB/CDEF/GH)	89 ab	54
Dithane (AB/CDEF/GH) with Phostrol ^b (--/-DEF/GH)	128 abcd	11 abcde	Dithane (AB/CDEF/GH) with Phostrol (--/--EF/GH)	92 b	48
Bravo (AB/C-E-/G-) with Quadris Opti --/-D-F/-H	133 bcde	16 bcde	Dithane (AB/CD-F/-H) with Actigard (AB/----/--) and Amistar (--/--E-/G-)	86 ab	59
Endura (AB/CDEF/GH) with Dithane + Forum ^b (--/-DEF/GH)	98 a	2 ab	Endura (AB/CDEF/GH) Dithane + Forum (--/--EF/GH)	56 a	38
Endura (AB/CDEF/GH) with Forum (--/-DEF/GH)	139 cde	5 abcd	Endura alone (not tested)	--	--

^a Weekly spray schedule with sprays A and B applied in Jul, CDEF applied in Aug, and GH applied in Sep.

^b Phostrol and Forum sprays were included for anticipated occurrence of late blight in the plots.

LITERATURE CITED

1. Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. *Pest Manag. Sci.* 58:649-662.
2. Frazer, J. T. 2002. Two species of *Alternaria* cause early blight of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*). Cornell University Master's Thesis 72pp.
3. Horsfall, J.G., and Barratt, R.W. 1945. An improved grading system for measuring plant diseases. *Phytopathology* 35:655.
4. Pasche, J. S., Wharam, C. M., Gudmestad, N. C., 2004. Shift in sensitivity of *Alternaria solani* in response to QoI fungicides. *Plant Dis.* 88:181-187.
5. Simmons, E.G. 2000. *Alternaria* themes and variations (244-286): species on Solanaceae. *Mycotaxon* 75:1-115.
6. Zitter, T. A., and Drennan, J. L. 2005. Comparing fungicides for controlling the light phenotype *Alternaria tomatophila* in tomato, 2004. *Fungic. Nematic. Tests.* 60:V072.
7. Zitter, T.A., and Drennan, J. L. 2006. Comparing fungicides for early blight control in tomato, 2005. *Fungic. Nematic. Tests* 61: (in press).

Effect of an Experimental Fungicide on Botrytis Gray Mold of Greenhouse Tomatoes.

Melanie L. Lewis Ivey and Sally A. Miller

The Ohio State University, Ohio Agricultural Research and Development Center,
Wooster, OH 44691 (ivey.14@osu.edu)

ABSTRACT

The experimental fungicide V-10135 was evaluated for management of Botrytis gray mold of tomatoes grown under greenhouse conditions. Two rates of fungicide were compared with an untreated control and Botran (standard). The high rate of V-10135 and Botran significantly reduced the number of petioles infected with *Botrytis* compared to the untreated control. However, neither rate of V-10135 nor the Botran treatment effectively reduced foliar symptoms compared to the control. Total yield was significantly higher in plants treated with Botran than in untreated plants or those treated with V-10135, but fruit in the Botran treatment had significantly more ghost spot than fruit from plants treated with V-10135 or not treated.

INTRODUCTION

Botrytis gray mold, caused by *Botrytis cinerea*, is a widespread problem of greenhouse grown vegetables including tomatoes, lettuce, and cucumbers. On greenhouse tomatoes, the pathogen can become established on dying tissue at leaf scars, fruit stems and wounds causing stem cankers. It also can infect the petioles, flowers and green or ripening fruit of the plants.

To control Botrytis gray mold an integrated approach should be taken. Effective control can be achieved by maintaining low relative humidity and warm temperatures, by promoting ventilation with adequate plant spacing and pruning and by plant sanitation. Biopesticides such as Rootshield, Prestop and *Rhodosporidium diobovatum* strain S33 may prevent the formation of stem cankers when applied preventatively. However, if an outbreak of Botrytis gray mold occurs in the greenhouse a fungicide program may be needed. Fungicides allowed for use on greenhouse tomatoes in Ohio include Manex, 2,6-dichloro-4-nitroaniline (Botran), pyrimethanil (Scala) and fenhexamid (Decree).

The objective of this study was to evaluate the effect of V-10135 (Valent Inc.), an experimental fungicide, for the control of Botrytis gray mold on greenhouse tomatoes.

MATERIALS AND METHODS

Botrytis cinerea L. was isolated from naturally infected begonia flowers and maintained at 20 °C on potato-dextrose agar (PDA). Inoculation was carried out with conidia from 8 day-old PDA cultures. Conidia were collected from PDA plates by flooding the plates with sterile water and dislodging the conidia with a sterile glass hockey stick. The conidial suspension was filtered through two layers of sterile cheesecloth and the spore concentration was adjusted to 3×10^4 conidia/ml. Tomato seeds (cv. Big Beef) were hot water-treated prior to seeding. Three week-old tomato seedlings were transplanted into 6-inch pots containing Wooster silt loam and arranged in

a randomized complete block design with four replications of ten plants each per treatment. Seven week-old tomato plants were spray inoculated (25 ml/plant) 7 days after the first fungicide application. Prior to inoculation the plants were artificially wounded by pruning the bottom two leaves and suckering the plants. High humidity was maintained by pre-misting the seedlings for 24 hr prior to inoculation and misting four times per day following inoculation. Greenhouse temperatures were maintained between 65 and 75 °F. V-10135 (0.25 and 0.50 lb ai/200 gal), Botran 75W (1 lb/A) and water were sprayed onto the tomato plants on a 7-day schedule for a total of four applications. Percent foliar disease was recorded 7 days after the second application and 4 days after the fourth application using the modified Horsfall-Barrett rating scale. The number of petioles and fruit infected and the weight of infected and healthy fruit were determined 4 days after the last fungicide application was applied. Data were analyzed by ANOVA using SAS software and means were separated using Fisher's protected least significant difference test. The experiment was done once.

RESULTS

Disease pressure was low and no stem cankers were observed. Foliar symptoms included chlorosis and water-soaking at the tips of leaflets and on the petioles (Figure 1a). Ghost spots were observed on green fruit (Figure 1b). Late stage symptoms included dead leaves and sporulation on senesced petioles.

Both the low (0.25 lb ai/200 gal) and high (0.50 lb ai/200 gal) rate of V-10135 reduced foliar symptoms compared to the untreated control and Botran, however; the reduction was only significant when compared to Botran (Table 1). Both rates of V-10135 reduced the number of petioles infected compared to the untreated control, however; only the high rate was significant (Table 1). Compared to the untreated control, Botran significantly reduce the number of petioles infected but did not reduce percent foliar disease. Plants treated with Botran produced significantly more fruit than those treated with V-10135 or not treated, however a larger proportion of this fruit was infected with Botrytis gray mold (Table 2). There was no significant difference in percent healthy fruit among treatments (data not shown). Both rates of V-10135 significantly reduced the proportion of fruit with ghost spots compared to Botran but not compared to the untreated control (Table 2).

DISCUSSION

V-10135 is an experimental fungicide that is being evaluated for its ability to control Botrytis gray mold on greenhouse grown tomatoes. In this study, both rates of V-10135 reduced the percent foliar disease, the number of petioles infected and the percent diseased fruit. However, the reduction was not always significant. Plants treated with V-10135 produced a higher yield of green fruit compared to the untreated control but the increase was not significant. Although Botran significantly reduced the number of infected petioles the treatment had the highest percent foliar disease suggesting that the petioles had died and fallen off the plant before being counted. Initial results suggest that V-10135 (both rates) can reduce the number of petioles and fruit infected with Botrytis gray mold, however more trials are needed to confirm its efficacy.



A



B

Figure 1. Symptoms of *Botrytis cinerea* on ‘Big Beef’ greenhouse tomatoes. (A) water-soaking and sporulation on petioles, (B) ghost spots on green fruit.

Table 1. Effect of various treatments on percent foliar disease and the number of petioles infected when challenged with *Botrytis cinerea*.

Treatment	Percent (%) Foliar (4 Aug)	Percent (%) Foliar (15 Aug)	Number of Petioles Infected
Botran 75W 1 Lb/A	7.7 a	30.9 a	1.8 b
V-10135 0.25 Lb/200 gal	1.9 b	20.3 a	2.6 ab
V-10135 0.50 Lb/200 gal	1.8 b	18.8 a	2.4 b
Untreated	3.1 b	25.8 a	3.7 a

¹Values in a column followed by the same letter are not significantly different at $P \leq 0.05$. Means were separated using Fisher’s protected least significant difference test.

Table 2. Total fruit yield and percent diseased fruit of cv. “Big Beef” tomato treated with V-10135 and Botran.

Treatment	Total Yield (g)	Percent (%) Diseased Fruit
Botran 75W 1 Lb/A	169.6 a	37.2 a
V-10135 0.25 Lb/200 gal	40.4 b	9.9 b
V-10135 0.50 Lb/200 gal	50.1 b	4.2 b
Untreated	38.9 b	18.6 ab

¹Values in a column followed by the same letter are not significantly different at $P \leq 0.05$. Means were separated using Fisher’s protected least significant difference test.

Greenhouse Tomato Disease Research in Mississippi

David M. Ingram

Central Mississippi Research & Extension Center
1320 Seven Springs Road, Raymond, MS 39154

ABSTRACT

During the past 3-4 years, research has been conducted on three diseases causing economic losses to greenhouse tomatoes in Mississippi and the United States. Botrytis gray mold, Pythium root rot and bacterial canker research has focused on finding new and alternative products with high food safety and short pre-harvest intervals for managing these diseases in the greenhouse environment. Emphasis has been placed on identifying bio-pesticides with suitable efficacy against the pathogens that cause these diseases. Several bio-pesticides have been identified that have shown effectiveness in managing the major greenhouse tomato diseases. Further research is needed to determine long term efficacy of these products and to continue to test new products as they are brought onto the market.

SUMMARY

There are approximately 850 acres of greenhouse tomatoes (*Lycopersicon esculentum* Mill.) grown in the United States (2). Major producing states include Texas, Colorado, Arizona, Virginia, and Pennsylvania. One of the greatest economic concerns in greenhouse tomato production is that of diseases. Fungi, bacteria, and viruses are responsible for most infectious diseases found in the greenhouse situation. Environmental conditions are also frequently the cause of abiotic problems.

Most growers begin transplanting tomato seedlings into greenhouses about mid-October (6). Harvest begins about January 1 and plants may continue to produce fruit through the end of June, depending on temperature. During November through March, temperatures in Mississippi are cool enough to require supplemental heating. In this situation, greenhouses are closed and little venting to bring in outside air with lower humidity is practiced. Humidity levels are commonly 90% or greater for most of the day. Freestanding water is also common in many operations. These environmental conditions are ideal for the initiation and proliferation of gray mold (3). Gray mold caused by *Botrytis cinerea* Pers.: Fr., is probably the most ubiquitous disease of tomato. In addition, many days during this period are cloudy and rainy, resulting in an inability to maintain dry canopy conditions. Tomatoes also tend to grow rapidly, producing great amounts of lush, succulent tissue, which shades the lower plant canopy resulting in highly susceptible plant tissues to gray mold infection.

Gray mold affects all aboveground plant parts. The most characteristic symptom is the fuzzy, gray-brown mold growth on plant parts. Conidiophores and conidia are easily observed with a hand lens and spores can be dislodged by shaking, creating a cloudy mass. Gray mold causes a soft rot of fruit with visible whitish mycelium produced on the fruit surface. Another interesting aspect of gray mold is the occurrence of "ghost spots" on fruit. The spores germinate and the germ tube penetrates the fruit surface, however, the mycelium aborts, leaving a necrotic fleck with a whitish halo around it. Ghost spot symptoms are most apparent on mature green fruit.

Research on gray mold has been directed at finding bio-pesticides that are efficacious either alone or in combination with traditional fungicide chemistries. Three years of research indicate that the bio-fungicides Milsana and Serenade are effective in reducing disease severity of gray mold on tomato foliage. Marketable and total yield have been increased by 10-18% (expressed in pounds per plot), but these yield increases have not been shown to be statistically significant. Milsana and Serenade have also been shown to be similar in effectiveness as the standard, Decree. Having the ability to alternate these products will assist growers with resistance management issues associated with this pathogen.

Pythium root rot has been a continuous problem in the ornamental (5) and greenhouse tomato industry. Aboveground symptoms in the plant manifest as chlorotic, generally unthrifty plants that may wilt during the heat of the day. These plants can be easily visibly discerned from healthy plants by the general lack of vigor. A survey of Pythium species occurring on greenhouse tomatoes in Mississippi was conducted during 2003-2004. Eleven locations were surveyed. Growing media consisted of pine bark fines (ground pine bark) or perlite. Ten random samples were collected from each location (non-destructive grab samples from three plants constituted each sample). Twenty root pieces (1.0 cm in length) were cut at random from each sample and plated onto water agar. Pythium colonies growing from the root pieces were individually cataloged and placed in storage for identification. One hundred percent of greenhouses were infected with Pythium. Incidence ranged from 3.5% to 36.5% and was generally dependent on the age of the growing medium. The older the growing medium was, the greater the incidence of Pythium. Three species were isolated; *Pythium myriotylum* (79.4%), *Pythium ultimum* var. *ultimum* (14.9%), and *Pythium spinosum* (5.6%). In the laboratory, fungicide sensitivity tests revealed that *P. myriotylum* was not able to grow on either of two fungicides, even at 0.01 ppm after 48 hours. *Pythium ultimum* and *Pythium spinosum* were able to grow at a concentration of 0.01 ppm after 48 hours, suggesting the possibility of fungicide resistance in some of the population. Products such as etridiazole have been labeled for Pythium root rot in greenhouse tomatoes as a result of this work.

Objectives of Pythium research over the last three years have been identical to that of gray mold; searching for bio-fungicides to use either alone or in combination with traditional fungicide chemistry for the control of root rot in tomatoes. The bio-fungicides Mycstop and Companion have proven to be effective in reducing disease severity of Pythium root rot, with respect to root symptoms observed and visual assessments of plant vigor. However, there are the same problems with showing significant yield benefits from using these products. The standard fungicide, Terramaster, performs consistently well when used alone or in combination with these bio-fungicides. Having the ability to alternate these products provides growers the ability to assist with resistance management issues associated with this pathogen.

Bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al, is a destructive disease of tomato, both in field and greenhouse production systems (4). Symptoms of bacterial canker generally include a systemic wilting of the entire plant, marginal necrosis of leaflets and the upward curling of leaf edges. The disease usually develops on the lower part of the plant, however if there is a wound in the upper part of the plant then the disease can develop there and rapidly move downward resulting in plant death. Stems may display external discoloration but often cankers are not formed. The vascular tissue of infected plants turns a reddish-brown color, which is

especially prominent at the nodes. The bacterium also may affect fruit resulting in a symptom called bird's-eye spot, which gives the fruit a rather scabby appearance from lesions produced on the surface. The lesions on fruit have brown centers and are surrounded by a white halo.

Copper bactericides are labeled for bacterial canker on other crops but efficacy on bacterial canker in tomato is uncertain. Recently, a company in Utah has begun to develop specific bacteriophages (AgriPhage®, OmniLytics, Inc., Salt Lake City, UT) for the control of bacterial spot and speck (1) in field grown tomato. A bacteriophage is basically a virus that is pathogenic to the bacterium and results in lysis of the bacterial cells. The grower sends a diseased plant sample to the company who then finds a bacteriophage that is highly pathogenic on that isolate of bacteria. The product (AgriPhage) is formulated and shipped to the grower, who then applies the product.

With one year of experiments completed, the best inoculation procedure has been identified; wounding plant tissue and introducing the bacterial inoculum into the wound. Agriphage and ProPhyt products show promise in reducing leaf area necrosis caused by the bacterium, however, more research needs to be conducted in this area.

LITERATURE CITED

1. Balogh, B., Jones, J.B., Momol, M.T., Olson, S.M., Obradovic, A., King, P., and Jackson, L.E. 2003. Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. *Plant Dis.* 87:949-954.
2. Cook, R. and Calvin, L. 2005. Greenhouse tomatoes change the dynamics of the North American fresh tomato industry. *USDA/Economic Research Report Number 2.* April 2005. 81 p.
3. Jarvis, W.R. 1992. *Managing diseases in greenhouse crops.* American Phytopathological Society Press, St. Paul, MN.
4. Jones, J.B., Jones, J.P., Stall, R.E. and Zitter, T.A., eds. 1991. *Compendium of tomato diseases.* American Phytopathological Society Press, St. Paul, MN.
5. Moorman, G.W., Kang, S., Geiser, D.M., and Kim, S.H. 2002. Identification and characterization of *Pythium* species associated with floral crops in Pennsylvania. *Plant Dis.* 86:1227-1231.
6. Snyder, R.G. 1993. *Greenhouse tomato handbook.* Mississippi State University Extension Service Publication 1828. 26 p.

Tomatoes for the Northeast Combining Early Blight and Late Blight Resistance

Martha Mutscher¹, Tom Zitter², Charles Bornt³
Depts. of Plant Breeding and Genetics¹, Dept. of Plant Pathology², CCE Capital District
Vegetable Program³,
Cornell University, Ithaca, NY 14853

ABSTRACT

Early blight and late blight have consistently been identified as the main tomato diseases in the northeast US. Growers have traditionally relied on calendar- or weather-driven fungicide sprays in order to control both diseases. Plant breeding efforts to identify and incorporate resistance for both diseases have progressed to the point that resistant lines with good horticultural characteristics are close to being released. This paper describes the progress made during 2005 in trials conducted in two regions of New York.

INTRODUCTION

Late blight (LB) (caused by *Phytophthora infestans*) is an increasingly significant problem in processing and fresh market tomato production. *P. infestans* is a pathogen with a variety of isolates. The original resistance genes, *Ph-1* and *Ph-2*, were both quite race specific, and so were not widely used in most breeding lines. Additional late blight resistance from *L. pimpinellifolium* L3708 was found by Black and Hanson and was transferred to elite breeding lines. A series of seven late blight-resistant lines was released that approach the type required of processing tomato. The late blight resistance in the lines involves the *Ph-3* gene plus added hypostatic genes that, together, allow the resistance to control more pathogen isolates. The first commercial processing tomato hybrids containing this resistance may be on the market for the 2006 growing season. Several breeding programs are transferring this resistance to fresh market tomato as well.

Resistance to early blight (EB) of tomato has previously been reported (2, 4). The pathogens causing EB exist as two different species, *Alternaria solani* which causes the traditional early blight of potato and *Alternaria tomatophila* which causes much of the disease on tomato (5). *A. tomatophila* has two phenotypes, appearing as light or dark in color in culture, with the light phenotype being significantly more pathogenic on tomato (1).

Tomato cultivars for the NE require earlier season and modified characteristics to adapt to the cooler late season and the diseases prevalent in the area. One aspect of our breeding program has been combining resistance to late blight and early blight in lines suited to the conditions of the NE. The expectation is that use of both resistances in a well designed IPM program should reduce fungicide use, risk of crop loss and costs of production. LB and EB resistance is being combined into fresh market lines developed at Cornell University. Dr. Randy Gardner of NCSU has been following a similar strategy to combine late blight and early blight resistance in fresh market tomato.

MATERIALS AND METHODS

Freeville conventional trial. Twenty two lines fixed for EB and LB resistance were established in plots consisting of 10 plants each with two replications. The standard

varieties Mountain Fresh and Supersonic along with line NC 96LB were used as checks. All plants were inoculated with the light phenotype of *A. tomatophila*. Plants were rated on three dates (19 Aug, 6 Sep, 19 Sep) for % defoliation using the Horsfall Barrett 0 to 11 rating scale (3), and for stem lesions using a 0 to 5 scale (0 = clean, 5 = stems cankered).

Kinderhook organic trial. This trial was conducted at the Roxbury Farm in Kinderhook, NY and consisted of a subset of 19 Cornell lines, three NC lines, and the standard varieties Sunchief and Mountain Fresh. The plots consisted of 8 plants with three reps. Plants were not inoculated in this trial.

RESULTS AND DISCUSSION

A three year cooperative project is focusing on testing the developing lines to determine their utility, any characteristics needed, and the proper use of dual resistant lines or hybrids. In this first year of the project, self progeny of the Cornell lines were screened in a severe early blight trial in Freeville NY during the summer of 2005. These lines and dual blight resistant lines developed by Dr. Randy Gardner were also included in a production trial in a commercial organic farm in Kinderhook, NY.

The results of the Early Blight trial confirmed that the Cornell lines were fixed for the early blight resistance (Table 1). The results of this early blight trial also demonstrated the difference in the degree of disease control the resistance provides on stems vs. on the foliage. The stem ratings on all of the resistant lines are uniformly very low (from 0.0 to 0.2), in contrast to the ratings of the susceptible controls (>4.0). However foliar symptoms, as measured by the % defoliation on the last reading date (19 Sep) or by area under the disease progress curve (AUDPC), were not as well controlled as the stem symptoms. There was also considerable variation among the resistant lines for foliar disease development. This could have been due to differences among these lines for maturity and fruit load/development, rather than any true difference for resistance.

Table 1. Early blight defoliation or stem ratings for inoculated trial, Freeville, NY

Entry	% Defoliation 9/19	AUDPC 8/19 to 9/19	Stem rating 9/19	Entry	% Defoliation 9/19	AUDPC 8/19 to 9/19	Stem rating 9/19
Supersonic	73.5	136.9	4.6	048143-03	70.3	87.4 *	0.0 *
Mountain Fresh	80.3	134.6	4.1 *	048143-05	73.9	84.6 *	0.0 *
NC 96LB	48.9	60.1 *	0.2 *	048143-07	76.9	106.0	0.0 *
048111-06	47.0 *	51.6 *	0.0 *	048144-01	70.7	102.2	0.0 *
048111-09	45.4 *	53.5 *	0.0 *	048144-04	68.8	103.7	0.0 *
048112-08	46.8	52.9 *	0.0 *	048144-07	63.7	106.0	0.0 *
048142-05	60.4	69.8 *	0.0 *	048144-09	78.3	95.9 *	0.1 *
048142-06	60.7	78.8 *	0.0 *	048117-02	68.7	78.8 *	0.0 *
048143-01	63.8	74.7 *	0.2 *	048117-04	67.6	82.3 *	0.0 *
048143-02	52.8	65.5 *	0.0 *	037104-08	77.4	93.8	0.0 *
				037104-08	86.7	98.2	0.2 *

Entries are significantly different from the Supersonic control by Dunnett's Test; Family error rate = 0.0500, Individual error rate = 0.00426

The late blight/early blight resistant lines being developed Cornell and similar lines being developed by Randy Gardner of NCSU were also included in production trial in a commercial organic farm in Kinderhook NY (Table 2) to evaluate horticultural characteristics of the resistant lines and hybrids. This trial was not inoculated, there was no appreciable early blight development and late blight did not occur.

The results of the trials in Kinderhook and Freeville allowed the selection of lines with the best horticultural characteristics in addition to disease resistance. The NC experimental hybrid NC0571 was close in % of #1 fruit to the control varieties Mt Fresh and Sunchief, but lower in total production. NC0576 had greater total production, but a slightly lower % of #1 fruit. The better inbreds in terms of production, fruit size, as well as fruit characters such as shape, smoothness, blossom end scar (data not shown) are included in Table 2. Production levels of these inbreds were high, through fruit size was smaller than that of the hybrids. In the coming year we will continue regional testing on the smaller set of selected dual resistant lines as well as hybrids created using the selected inbred lines. We will also test these lines and hybrids in disease trials with modest input of chemical controls, in addition to the resistance, to extend control of foliar symptoms of early blight.

Table 2. Production on controls and selected fresh market tomato lines fixed for early blight and late blight. Kinderhook, NY

Variety	Type	Total # marketable	Total wt marketable	#1 as % total fruit no.	#2 as % total fruit no.
Mt. Fresh	hybrid	461	303.6	86.8	13.2
Sunchief	hybrid	429	248.0	73.0	27.0
NC0571	hybrid	367	214.2	77.1	22.9
NC0576	hybrid	453	221.4	60.7	39.3
048143-07	inbred	537	246.0	59.8	40.2
NC 96LB	inbred	430	192.6	57.0	43.0
048111-06	inbred	386	169.6	53.6	46.4
048143-01	inbred	587	241.8	48.9	51.1
048142-06	inbred	591	223.2	48.1	51.9
048144-07	inbred	798	260.8	28.8	71.2

LITERATURE CITED

1. Frazer, J. T. 2002. Two species of *Alternaria* cause early blight of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*). Cornell University Master's Thesis. 72pp.
2. Gardner, R. G. 1988. NC EBR-1 and NC EBR-2 early blight resistant tomato breeding lines. *HortScience* 23:779-781.
3. Horsfall, J.G., and Barratt, R.W. 1945. An improved grading system for measuring plant diseases. *Phytopathology* 35:655.
4. Nash, A. F., and Gardner, R. G. 1988. Tomato early blight resistance in a breeding line derived from *Lycopersicon hirsutum* PI 126445. *Plant Dis.* 72:206-209.
5. Simmons, E.G. 2000. *Alternaria* themes and variations (244-286): species on Solanaceae. *Mycotaxon* 75:1-115.

Functional Analysis of the *Avr3a* gene family of *Phytophthora infestans*

Jorunn Bos, Thirumala Kanneganti, Carolyn Young and Sophien Kamoun.

Dept of Plant Pathology, The Ohio State University, OARDC, 1680 Madison Ave.,
Wooster, OH 44691

ABSTRACT

Plant pathogens secrete molecules that promote the infection process and disease. Plants may defend themselves against attack by recognizing these pathogen molecules, resulting in resistance. The plant pathogen *Phytophthora infestans* was responsible for the Irish potato famine in the nineteenth century. This plant pathogen causes late blight on potato and tomato, a devastating disease that leads to billions of dollars in economic losses each year. To understand how this pathogen is able to cause disease on host tomato and potato plants, we aim to identify *P. infestans* molecules that are secreted during infection, and investigate their functions. The *Avr3a* gene of *P. infestans* encodes a small secreted protein that is recognized by R3a in *Solanum demissum* (Armstrong *et al.*, 2005; Huang *et al.*, 2005). The *Avr3a* gene family is represented by at least two polymorphic members, *Avr3a_S*^{19E⁸⁰M¹⁰³} and *Avr3a_C*^{19K⁸⁰I¹⁰³} that confer a virulent and avirulent phenotype on R3a potato plants, respectively. We performed functional characterization studies aimed at identifying the virulence function of AVR3a to gain insights in the mechanisms by which this protein contributes to plant susceptibility. Eventually, understanding of the mechanisms by which *P. infestans* causes disease will help us to generate more resistant crops and reduce yield loss.

SUMMARY

In plant-microbe interactions recognition of pathogen molecules by plants results in the induction of plant defense responses and resistance. Plant pathogen proteins, encoded by avirulence (*Avr*) genes are recognized by products of plant resistance (*R*) genes. This interaction typically follows the gene-for-gene model, which postulates that the concurrent expression of matching pairs of pathogen *Avr* genes and plant *R* genes results in resistance (Staskawicz *et al.*, 1995; Dangl and Jones, 2001). In the simplest illustration of this model the AVR and R proteins interact directly. However, recent studies implicate the involvement of a third component, the virulence target (Dangl and Jones, 2001; Martin *et al.*, 2003). According to the “Guard hypothesis” the AVR protein interacts with this virulence target in both resistant and susceptible plants (Van der Biezen and Jones, 1998). However, in resistant plants the virulence target is “guarded” by the R protein, which monitors alterations in the target and promotes defense signaling.

Oomycetes, such as *Phytophthora*, downy-mildews, and *Pythium*, form a unique branch of eukaryotic plant pathogens with an independent evolutionary history (Kamoun, 2003). Among the oomycetes, *Phytophthora* species cause some of the most destructive plant diseases in the world. The most notable and best-studied oomycete is *Phytophthora infestans*, the Irish famine pathogen. *P. infestans* causes late blight, a devastating and re-emerging disease of potato and tomato (Birch and Whisson, 2001; Kamoun and Smart,

2005). In our working model, *P. infestans* delivers proteins to different cellular compartments of the host where they interact with plant targets to reprogram host defenses and promote susceptibility. To understand the mechanisms underlying disease and susceptibility, we aim to identify and characterize these *P. infestans* proteins that are secreted during infection.

The *Avr3a* gene family encodes two polymorphic secreted proteins that are the main focus of our studies (Armstrong *et al.*, 2005). *P. infestans* isolates that are avirulent on *R3a* potato carry the avirulence gene *Avr3a*, which encodes the mature protein AVR3a^{KI} (containing amino acids K⁸⁰ and I¹⁰³), whereas virulent isolates carry only the virulence allele *avr3a*, encoding the mature protein AVR3a^{EM} (containing amino acids E⁸⁰, and M¹⁰³) (Armstrong *et al.*, 2005). Thus, *P. infestans* isolates that secrete AVR3a^{KI} are recognized by host potato plants that express the R3a resistance protein, which leads to plant resistance. However, in susceptible plants there is no recognition of AVR3a due to the absence of the matching R protein and therefore no induction of defense responses. We aim to find out how AVR3a contributes to this establishment of disease in these susceptible plants. The main questions are: what are the molecular mechanisms by which AVR3a alters host defense responses? What protein features of AVR3a are important for its functions in avirulence and virulence? What plant protein(s) does AVR3a target in its host? Ultimately, knowledge about the molecular basis of plant susceptibility to *P. infestans* will help generate more resistant crops and improved disease management strategies.

LITERATURE CITED

Armstrong, M.R., Whisson, S.C., Pritchard, L., Bos, J.I.B., Venter, E., Avrova, A.O., Rehmany, A.P., Böhme, U., Brooks, K., Cherevach, I., Hamlin, N., White, B., Fraser, A., Lord, A., Quail, M.A., Churcher, C., Hall, N., Berriman, M., Huang, S., Kamoun, S., Beynon, J.L. and Birch, P.R.J. (2005) An ancestral oomycete locus contains late blight avirulence gene *Avr3a*, encoding a protein that is recognized in the host cytoplasm. *Proc. Natl Acad. Sci. USA*, 102, 7766-7771.

Birch, P.R.J. and Whisson, S. (2001) *Phytophthora infestans* enters the genomics era. *Mol. Plant Pathol.* 2, 257–263.

Dangl, J.L. and Jones, J.D. (2001) Plant pathogens and integrated defence responses to infection. *Nature*, 411, 826–833.

Huang, S., van der Vossen, E.A.G., Kuang, H., Vleeshouwers, V.G.A.A., Zhang, N., Borm, T.J.A., van Eck, H.J., Baker, B., Jacobsen, E. and Visser, R.G.F. (2005) Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *Plant J.* 42, 251-261.

Kamoun, S., Hamada, W. and Huitema, E. (2003) Agrosuppression: A bioassay for the hypersensitive response suited to high-throughput screening. *Mol. Plant Microbe Interact.* 16, 7-13.

Kamoun, S., and Smart, C.D. (2005) Late blight of potato and tomato in the genomics era. *Plant Disease*, 89, 692-699.

Martin, G.B., Bogdanove, A.J. and Sessa, G. (2003) Understanding the functions of plant disease resistance proteins. *Annu. Rev. Plant Biol.* 54, 23-61.

Staskawicz, B.J., Ausubel, F.M., Baker, B.J., Ellis, J.G. and Jones, J.D.G. (1995)

Molecular genetics of plant disease resistance. *Science*, 268, 661–667.

Van der biezen, E.A. and Jones, J.D. (1998) Plant disease-resistance proteins and the gene-for-gene concept. *Trends Biochem. Sci.* 23, 454–456.

Role of *Phytophthora infestans* Protease Inhibitors and Their Target Tomato Proteases in Disease

Jing Song, Miaoying Tian, Joe Win, Nicolas Champouret, Hsin-Yen Liu
and Sophien Kamoun

Department of Plant Pathology, The Ohio State University, OARDC,
1680 Madison Ave., Wooster, OH 44691

ABSTRACT

The oomycete *Phytophthora infestans* causes late blight, a reemerging and ravaging disease of potato and tomato. *P. infestans* has evolved gene families of Kazal-like extracellular serine protease inhibitors and cystatin-like cysteine protease inhibitors. Some of these protease inhibitors show inhibition activity towards tomato proteases. The study of the interaction between *P. infestans* protease inhibitors and tomato proteases will unravel the role of these molecules in the disease progress and provide possible ways to engineer plants for resistance against this pathogen.

SUMMARY

Protein-protein interactions play an important role in the battle between plants and pathogens. Among these proteins, proteases and protease inhibitors are molecules that are frequently involved in such molecular process. Proteases are enzymes that cleave peptide bonds linking amino acids in protein molecules. In plants, proteases perform their biological functions by processing proteins to generate bioactive mature forms or by degrading endogenous or exogenous proteins. P69 family from tomato, for example, contains six members, two of which are pathogenesis-related (PR) proteins involved in defense against pathogens' attack (Jorda et al., 2000). Protease inhibitors (PIs) are molecules that inhibit the function of proteases. Many naturally occurring protease inhibitors are proteins. Protease inhibitors interact with their target proteases to perform their biological functions, such as metabolism regulation; thereby blocking the proteases enzymatic activity. Based on their active sites, PIs are classified as serine, cysteine, or other protease inhibitors.

Data mining of genomic and cDNA sequences revealed that *P. infestans* evolved 18 extracellular protease inhibitor genes belonging to two major structural classes: (i) Kazal-like serine protease inhibitors (EPI1-14) and (ii) cystatin-like cysteine protease inhibitors (EPIC1-4) (Tian et al., 2004). We hypothesize that *P. infestans* secretes proteins that inhibit host proteases and facilitate infection by protecting secreted *P. infestans* proteins from proteolytic degradation and/or by perturbing host defense signaling cascades that include proteolytic steps.

Among the 14 Kazal-like serine protease inhibitor genes, *epi1*, *epi10* and *epi12* are up-regulated during infection of tomato by *P. infestans*, which suggests that they may play a role during the interaction. Previous biochemical studies showed that both recombinant EPI1 and EPI10 specifically inhibit subtilisin A among major serine proteases, and inhibit and interact with the pathogenesis-related (PR) P69B subtilisin-like serine protease of tomato. We extended our biochemical analyses to EPIC1 and EPIC2, secreted *P. infestans* proteins with similarity to cystatin-like protease inhibitor domains. The *epiC1* and *epiC2* genes are strongly up-regulated during infection of tomato by *P.*

infestans. EPIC1 and EPIC2B were unstable in tomato apoplastic fluids and were degraded by tomato P69B but EPI1 protected both proteins from degradation. Affinity purified P69B was sufficient to degrade EPIC1 and EPIC2B but not EPI1a, suggesting selectivity in degradation by P69B. Coimmunoprecipitation experiments revealed that EPIC2 interacts with a novel papain-like extracellular cysteine protease, termed Phytophthora Interacting Protein 1 (PIP1). The interaction was further confirmed by coimmunoprecipitation using in planta expressed PIP1 protein. Characterization of PIP1 revealed that it is a PR protein closely related to Rcr3, a tomato apoplastic cysteine protease that functions in fungal resistance. Altogether, our findings suggest that a cascade of inhibition of host proteases initiated by EPI1 occurs in the tomato apoplast during infection by *P. infestans*. We will expand our study on molecular characterization of these *P. infestans* protease inhibitors and the interacting tomato proteases to further investigate their roles in disease progression. The study of this proteases and protease inhibitor interaction is providing insights into key molecular processes regulating susceptibility to an economically important pathogen as well as providing hints for engineering plants to be more resistant to pathogens.

LITERATURE CITED

- Erwin, D.C. and Ribeiro, O.K. 1996. *Phytophthora* Diseases Worldwide. St. Paul, Minnesota, APS Press
- Kamoun, S. 2003. Molecular genetics of pathogenic oomycetes. *Eukaryot. Cell* 2: 191-199.
- Jorda, L., Coego, A., Conejero, V. and Vera, P. 1999. A genomic cluster containing four differentially regulated subtilisin-like processing protease genes is in tomato plants. *J. Biol. Chem.* 274: 2360-2365.
- Jordá L. and Vera. P. 2000. Local and Systemic Induction of Two Defense-Related Subtilisin-Like Protease Promoters in Transgenic Arabidopsis Plants. Luciferin Induction of PR Gene Expression. *Plant Physio.* 124(3): 1049 - 1058.
- Tian M., Benedetti B. and Kamoun S. 2005. A Second Kazal-Like Protease Inhibitor from *Phytophthora infestans* Inhibits and Interacts with the Apoplastic Pathogenesis-Related Protease P69B of Tomato. *Plant Physiology*, July, Vol. 138, pp. 1785-1793.
- Tian M., Huitema E., da Cunha L., Torto-Alalibo T. and Kamoun S. 2004. A Kazal-like extracellular serine protease inhibitor from *Phytophthora infestans* targets the tomato pathogenesis-related protease P69B. *J. Biol. Chem.* 279: 26370-26377.
- Tornero, P., Conejero, V. and Vera, P. 1997. Identification of a new pathogen-induced member of the subtilisin-like processing protease family from plants. *J. Biol. Chem.* 272: 14412-14419.

THE IR-4 PEST CONTROL PRODUCT REGISTRATION PROCESS

Charles W. Meister, IR4 Field Research Coordinator, IFAS, University of Florida

ABSTRACT

The National IR4 Project assists pest control product registrants develop new and expanded registrations for minor and specialty crop uses by undertaking a series of activities that are renewed every year. The IR4 process is described with an update on current projects being researched for tomato disease control.

DISCUSSION

The National IR4 Project is headquartered at Rutgers University with regional offices at The University of Florida, University of California at Davis, Michigan State University and Cornell University at Geneva. It operates in every state with the assistance of IR4 state liaison representatives usually associated with state universities. The USDA/ARS also participates in each of the four IR4 regions. The IR4 partnership encompasses a network of scientists and consultants in every state, the agriculture chemistry industry, the EPA and specialty growers and grower groups.

The IR4 registration process begins with the submission of a Project Clearance Request (PCR) form. This request may be submitted as a hard copy or electronically and provides the basis for all future activities. It documents the pest control product, specialty crop, pest problem and proposed use pattern. All requests should be accompanied with efficacy/crop safety data and can be submitted by anyone except the product registrant/owner.

The PCR is reviewed at national HQ to be sure that it is valid and given a number which is used to track it through the process. There are over 9,000 requests in the system. Each PCR is sent to the registrant for comments on its future as a possible IR4 project. Many requests are handled by the registrant and given a category 05. Some are not acceptable (cat. 11) while others may need more information such as efficacy/crop safety data before they are approved (cat. 06). Most, however, are found to be acceptable (cat. 03) and registrants encourage IR4 to proceed with research.

Every year IR4 holds a three day food use workshop to identify PCR's that will be researched the following year. Over 100 scientists from across the country meet each day to voice support for their particular project need. Each pest management discipline (weeds, diseases, insects/mite) meet on separate days to identify about 15 priority A projects that will be funded for future research. Priority B projects are also identified for possible upgrades later in the year. Additional Projects are considered when proposals are submitted after the workshop. Every year, 2 or 3 tomato projects are identified for research. A tomato disease control project is identified for research every other year, on average.

A month later IR4 Coordinators and Study Directors meet at National Headquarters to develop a work plan for the following year. More than 100 Projects previously identified are addressed at this National Research Planning Meeting. Field Research sites are assigned according to the EPA regional requirements. Laboratory assignments are made so that one site will analyze all samples from a given project.

One Study Director is assigned to each project and a research protocol is prepared and reviewed by the specified Field and Laboratory Research Director. Protocols are signed January-March and test material is ordered so work can start as soon as possible. Each Project may require data from 3 to 15 sites according to the nature of the new use.

Field and Laboratory data are compiled according to good laboratory practices and submitted to the Study Director. All data is assembled into a petition package and reviewed by the registrant before submission to EPA for a pest control product/crop residue tolerance. The IR4 Project tries to complete research from protocol signature date until EPA submission in 30 months. See Figure 1.

The IR4 Project also supports research with Biopesticides to secure new and expanded registrations for minor and specialty uses. These Projects come to IR4 each year in requests that are part of a grant proposal to research Biopesticides for efficacy, crop safety or limited environmental data for EPA. Product residue data is not required. This year 113 proposals were submitted to IR4 for funding. Nine proposals requested funds to research Biopesticides for control of tomato diseases and nematodes.

There are at least 20 tomato disease project requests listed in the IR4 data base at www.ir4.rutgers.edu. The current status of several project requests are presented in Table 1. IR4 developed residue data that allowed the expansion of the Decree label to greenhouse tomato and Amistar, Cabrio, Endura to field tomato. Projects that will lead to Switch-greenhouse and Captan- greenhouse transplant and Topsin-M-field tomato labels are complete and will be submitted to EPA. More efficacy and crop safety data need to be collected on specific uses for traditional materials: V-10135, V-10161, Tanos, Quintec, Serenade, Captan and the Biopesticides: Agriphage, Endorse, Kasugamycin, Phosphonic acid, Vacciplant before they can be considered for registration.

Table 1. IR-4 PRODUCT REQUESTS FOR CONTROL OF TOMATO DISEASES

<i>Product</i>	<i>Disease</i>	<i>Status</i>
AGRIPHAGE	Bacterial canker	need greenhouse efficacy
AMISTAR	Foliar diseases	Labeled for field use
CABRIO	Foliar diseases	Labeled for field use
CAPTAN	Rhizoctonia, Pythium	GH transplant complete
CAPTAN	P.capsici	need efficacy
DECREE	Botrytis	Labeled for greenhouse
ENDURA	Botrytis, early blight	Labeled for field
ENDORSE	Botrytis, powdery mildew	need greenhouse efficacy
KASUGAMYCIN	Bacterial diseases	need efficacy
MILSANA	Botrytis, powdery mildew	Labeled
PHOSPHONIC ACID	Bacterial diseases	need efficacy
QUINTEC	Powdery mildew	need crop safety
SERENADE	Bacterial diseases	need efficacy
SWITCH	Botrytis, powdery mildew	Greenhouse use complete
TANOS	Bacterial canker	need efficacy
TOPSIN-M	Foliar diseases	GH and field use complete
V-10135	Botrytis, Sclerotinia	need efficacy
V-10161	P.capsici	need efficacy
VACCIPLANT	Bacterial diseases	need efficacy

Effect of Plant Growth Promoting Rhizobacteria on Tomato Diseases in an Integrated Management Program

C. Nava-Diaz³, M.D. Kleinhenz², D.J. Doohan², and S.A. Miller¹

Departments of ¹Plant Pathology and ²Horticulture and Crop Science. Ohio Agricultural Research and Development Center, The Ohio State University, Wooster Ohio. 44691.

ABSTRACT

Three rifampicin-resistant strains of *Bacillus* spp. (*B. subtilis* MI600, *B. subtilis* GBO3 and *B. amyloliquefaciens* IN937) were integrated in an intensive tomato management program that included mulch, drip irrigation and a forecasted fungicide spray program to improve fresh market tomato growth and productivity and reduce the intensity of diseases caused by *Alternaria solani*, *Septoria lycopersici* and *Pseudomonas cichorii*. *Bacillus* spp. population densities were 10^4 - 10^6 CFU g⁻¹ root during the seedling stage and dropped to less than 10^3 CFU g⁻¹ root during the flowering and fruiting stages when fungal and bacterial diseases were observed. Significant increases in plant height were observed in all *Bacillus*-inoculated tomato plants, however, foliar diseases incited by *A. solani* and *S. lycopersici* were not reduced. GBO3+IN937-inoculated plants were more susceptible to bacterial stem rot caused by *P. cichorii* than those inoculated with MBI600 or non-inoculated control plants. Plants grown on rye mulch had lower severity of foliar diseases and stem rot than those grown on plastic mulch. No significant increase in yield was induced by *Bacillus* spp. In a dry year, marketable yields were 64 and 32 ton/ha for plants grown under plastic and plant residue mulches, respectively. In a wet year, marketable yields were 10 and 23 ton/ha for plants grown under plastic and plant residue mulches, respectively.

SUMMARY

The intensive management of tomato includes water management, use of black plastic mulch, and use of fungicides. Water management not only affects uptake and utilization of nutrients, but also the development of certain diseases, herbicide activation and quality and quantity of tomato production. Drip irrigation is one of the most common delivery systems, in which water under pressure flows through a pipe. Water deficiency during vegetative, flowering or fruiting stages results in yield reductions of 25, 52 and 43 % respectively (Rutledge *et al.* 1999). The use of black plastic mulch is widely accepted since plastic mulch controls weeds and reduces certain diseases, conserves moisture and increases quality and quantity of marketable fruit. The main disadvantage of the use of plastic is the expense associated with installation, removal and disposal of the black plastic (Rutledge *et al.* 1999). Mulching fresh market tomato with residue from the previous winter annual crop may be an alternative to plastic mulch to control weeds and reduce production costs and impact on the environment. The fact that the severity of foliar diseases in fresh market tomato is lower in plants grown under plant residue mulch compared with those grown on plastic mulch is also attractive (Mills *et al.* 2002). The most important fungal foliar diseases in Ohio are early blight (*Alternaria solani*) and septoria leaf spot (*Septoria lycopersici*). Management of foliar diseases of tomato is achieved by crop rotation, balanced nutrition, destruction of infected leaves, eradication of weed or volunteer plants (Jones, 1997; Pitblado, 1994) and the use of fungicides.

Amistar 80WG, Bravo Weather Stik, Dithane M45, Tanos 50DF, Kocide 2000, Manzate75DF, and Cabrio 20 WG are among the fungicides applied to manage foliar and fruit diseases, particularly early blight (*Alternaria solani*), septoria leaf spot (*Septoria lycopersici*), and anthracnose (*Colletotrichum gloeosporioides*, *C coccodes*) on tomato.

The use of plant growth-promoting rhizobacteria (PGPR) represents a potentially attractive alternative disease management approach since PGPR have been reported to increase yield and protect crops simultaneously (Ramamoorthy *et al.* 2002; Raupach, 1998). PGPR have been reported to stimulate plant growth and improve stand under stress conditions (van Loon *et al.* 1998). Three strains of PGPR, *Bacillus subtilis* MBI600 (Microbio, Bolder, CO) and GBO3 (Gustafson, Inc., Plano, TX), and *Bacillus amyloliquefaciens* IN937 (Auburn University, Auburn, AL), have been reported to act as biological control agents against various plant pathogens in numerous field and vegetable crops (Martinez-Ochoa, 2000; Zehnder *et al.* 2000; Raupach, 1998; Ryu *et al.* 2000; EPA, 2004a,b). Plant disease control using PGPR has been variable across locations and crops; several factors may influence the ability of PGPR to affect plant growth parameters and disease suppression such as colonization (Bloemberg and Lugtenberg 2001; Benizri *et al.* 2001), soil moisture (Meikle *et al.* 1995), competition (Young *et al.* 1995), nutrients, inorganic compounds and plant-derived factors (Milner *et al.* 1995; Rodriguez and Pfender, 1997). These observations may help explain the variable effectiveness of PGPR in numerous studies. The goal of this study was to evaluate the effect of soil moisture and mulch type on PGPR colonization of tomato rhizospheres and subsequent effect on plant height, foliar diseases and yield of tomato. Our observations indicated that *Bacillus* spp. colonize tomato roots and act as PGPR, but under our conditions do not reduce the severity of foliar diseases. In fact, bacterial stem rot was higher in plants colonized by two of the *Bacillus* spp. strains than in the control or in plants inoculated by a third strain. The effect of mulch type on tomato yield was linked to weather conditions during this study; in a dry year, yields were higher for plants grown with plastic mulch than with rye residue mulch, while the opposite was true in a wet year.

Literature Cited

- Benizri, E., Baudoin, E., and Guckert, A. 2001. Root colonization by inoculated plant growth promoting rhizobacteria. *Biocontrol Sci. Technol.* 11:557-574.
- Bloemberg, G. V., and Lugtenberg, B. J. J. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology* 4: 343-350.
- EPA. 2004a. *Bacillus subtilis* MBI600 129082. http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_129082.htm
- EPA. 2004b. *Bacillus subtilis* GBO3 (129068). http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_129068.htm.
- Jones, J.P. 1997. Early blight. In Jones J.B., Stall, R.E. and Zitter, T.A. 1997. *Compendium of Tomato Diseases*. APS press. Minnesota. USA: 13 pp.
- Martinez-Ochoa, N. 2000. Biological control of the root-knot nematode with rhizobacteria and organic amendments. Ph.D. dissertation. Auburn University. Alabama: 120 p.
- Meikle, A., Amin-Hanjani, S., Glover, L.N., Killham, K., and Prosser, J. 1995. Matric potential and the survival and activity of a *Pseudomonas fluorescens* in soil. *Soil Biology and Biochemistry* 27(7): 881-892.

- Mills, D.J., Coffman, C.B., Teasdale, J.R., Everts, K.L., Abdul-Baki, A.A. Lydon, J., and Anderson, J.D. 2002. Foliar disease in fresh-market tomato grown in different bed strategies and fungicide spray programs. *Plant Disease* 86: 955 – 959.
- Milner, J.L., Raffel, S.J., Lethbridge, B.J., and Handelsman, J. 1995. Culture and conditions that influence accumulation of zwittermycin A by *Bacillus cereus*. *Appl Microbiol Biotechnol* 43: 685-691
- Pitblado, R.E. 1994. Septoria leaf spot. In Howard, R.J., Garland, J.A. and Seaman, W.L. 1994. Diseases and pest of vegetable crops in Canada. The Canadian Phytopathological Society. Entomological Society of Canada. Canada: 275 pp.
- Ramamoorthy, V., Raguchander, T., and Samiyappan, R. 2002. Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads. *European Journal of Plant Pathology* 108: 429-441.
- Raupach, G.S. 1998. Induced systemic resistance as the principal component of integrated pest management of *Cucumis sativus* L. by PGPR. Ph.D. dissertation. Auburn University. Alabama. USA: 120 p.
- Rodriguez, F., and Pfender, W.F. 1997. Antibiosis and antagonism of *Sclerotinia homeocarpa* and *Drechslera poae* by *Pseudomonas fluorescens* PF-5 in vitro and in planta. *Phytopathology* 87: 614-621
- Rutledge, A.D., Wills, J.B., and Bost, S. 1999. Commercial tomato production. Agricultural Extension Service. The University of Tennessee. PB 737: 31 p.
- Ryu, C.M. Reddy, M.S., Zhang S., Murphy, J.F. and Kloepper, J.W. 2000. Plant growth promotion of tomato by biological preparation (LS213) and evaluation for protection against cucumber mosaic virus. <http://www.ag.auburn.edu/~mreddy/>.
- Van Loon, L.C., Bakker, P.A.H.M., and Pieterse, C.M.J. 1998. Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* 36: 453-483.
- Young, C.S., Lethbridge G., Shaw, L.J., and Burns, R.G. 1995. Survival of inoculated *Bacillus cereus* spores and vegetative cells in non-planted rhizosphere soil. *Soil Biology and Biochemistry* 27 (8): 1017-1026.
- Zehnder, G.W., Yao, C., Murphy, J.F., Sikora, E.R. and Kloepper, J.W. 2000. Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. *Biocontrol* 45: 127-137.

USE OF BIORATIONAL PRODUCTS TO MANAGE SEEDLING PRE- AND POST- EMERGENCE DAMPING-OFF

Fulya Baysal, Melanie Lewis Ivey, Jhony Mera and Sally Miller

Department of Plant Pathology, The Ohio State University
Ohio Agricultural Research and Development Center (OARDC), Wooster, OH 44691

ABSTRACT

Composted cow manure, *T. hamatum* 382, Omega grow plus, Moncut and Endorse significantly reduced damping-off of pepper caused by *Rhizoctonia solani*. Seedlings treated with compost, Omega Grow or Serenade were taller than those in the inoculated control, but none were taller than the non- inoculated control seedlings. Post- emergence damping-off was low in this study, at 3.1- 3.7%. Results are primarily based pre-emergence damping-off. Damping- off was significantly lower in the 3.5 g/L rate of Muscodor than in the untreated, inoculated control. Based on the results of this study, there are several biorational alternatives to traditional fungicides that are suitable for consideration in transplant and micro-vegetable production.

INTRODUCTION

In greenhouse environments, damping-off caused by *Rhizoctonia solani* and *Pythium* spp. can cause significant losses in transplant and micro-vegetable production. Damping-off is associated with plant crowding, variable temperatures, over-watering, high humidity and no fungicides highly effective against damping-off pathogens. The goal of this research was to develop tactics to reduce the incidence of *Rhizoctonia* damping-off in greenhouse vegetables. We focused on pepper, which is particularly susceptible to damping-off.

MATERIALS AND METHODS

Nine treatments were evaluated for efficacy against *Rhizoctonia* damping-off in pepper (cv. California wonder- organic seed) seedlings: Muscodor (biofumigant *Muscodor albus*), composted cow manure, Omega Grow (fish proteins) and Omega Grow Plus (fish proteins and oil), *Trichoderma hamatum* 382, Endorse, Serenade (Rhapsody) ASO (*Bacillus subtilis* QST 713), Thiram and Moncut. *Trichoderma hamatum* 382 was evaluated in Fafard Superfine Germinating mix and peat potting mix (peat: perlite 70:30 (v:v); amended with 3.8g/L dolomitic lime, 1.3g/L Mississippi lime, 0.8 g/L potassium nitrate, 0.8g/L triple super phosphate and 0.8g/L gypsum). All treatments except Muscodor were applied at the time of sowing pepper seeds. Muscodor (7.5 or 3.5 g) was mixed with *Rhizoctonia solani* strain122 (5 g dried inoculum prepared in chopped potato/soil medium), Fafard's superfine germinating mix (1 L) and sterile water (100 ml) 7 days prior to sowing pepper seeds. A Muscodor-only control and a soil-only control were prepared in the same way, omitting *R. solani* 122 inoculum or both inoculum and Muscodor, respectively. For the remaining treatments, on the day of sowing, the center 48 cells of 288-cell trays were filled with *R. solani* 122-infested potting mix (0.5 g inoculum/100 ml Fafard's Superfine Germinating mix or peat potting mix) and the remaining cells of each flat were filled with non-inoculated potting mix. Treatments were applied to the entire flat, with the exception of the Muscodor, *T. hamatum* 382 and Thiram treatments, which were only applied to the center 48 cells of each flat. The center

48 cells of each flat were seeded with pepper seeds. All flats were placed in the greenhouse in a randomized complete block design with four replications. Greenhouse temperatures were set to 80 °F day-time and 70 °F night-time. Automatic overhead watering was set up for one pass at 4ft/min., three times daily.

RESULTS

Pre-emergence damping-off, post-emergence damping-off and healthy plant number was assessed 4, 7 and 10 days after emergence and top growth of seedlings assessed 4 weeks after planting to estimate effects on root growth and development plant height and fresh weight (Tables 1, 2 and 3).

Table 1. Efficacy of treatments against *Rhizoctonia* damping-off of pepper (cv. California Wonder)

Treatment	<i>R. solani</i> 122	Percent Healthy	Percent Damping Off	Plant Height (cm)	Fresh weight (g)
Omega grow	+	30.2 i*	69.8 a	2.9 ab	2.4 f
Omega grow plus	+	82.3 cd	17.7 hi	1.8 g	2.3 f
<i>Trichoderma hamatum</i> 382	+	89.6 abc	10.4 ijk	2.5 b-f	3.9 bcd
Serenade ASO 0.5%	+	68.2 efg	31.8 def	2.5 b-f	2.8 def
Serenade ASO 1%	+	41.1 h	58.9 b	2.8 abc	2.5 ef
Serenade ASO 2%	+	25.5 i	74.5 a	2.7 a-d	2.5 f
Moncut	+	78.12 de	21.88 ghi	2.1 fg	2.8 def
Endorse 1.6 lb /100 gal	+	71.3 ef	28.7 efg	2.5 b-f	2.9 def
Endorse 2 lb /100 gal	+	74.5 de	25.5 fgh	2.3 c-f	2.7def
Thiram	+	88.5abc	11.5 ijk	2.3 def	3.8 cde
Untreated control	+	64.1 fg	35.9 cde	2.1 fg	3.5 def
Untreated control	-	98.4 a	1.6 k	2.6 b-e	4.8 bc

Table 2. Efficacy of composted cow manure treatment against *Rhizoctonia* damping-off of pepper (cv. California Wonder)

Treatment	<i>R. solani</i> 122	Percent Healthy	Percent Damping Off	Plant Height (cm)	Fresh weight (g)
Composted cow manure	+	95.3 a*	4.7 jk	2.7 a-d	5.2 ab
Untreated control	-	93.7 ab	6.3 jk	3.1 a	6.2 a
Untreated control	+	61.1 fg	38.9 cde	2.3 fg	3.6 def

Table 3. Efficacy of Muscodor treatment against *Rhizoctonia* damping-off of pepper (cv. California Wonder)

Treatment	<i>R. solani</i> 122	Percent Healthy	Percent Damping Off	Plant Height (cm)	Fresh weight (g)
Muscodor 7.5 g/L planting mix	+	58.3 g*	41.7 c	2.5 b-f	3.1 def
Muscodor 7.5 g/L planting mix	-	59.4 g	40.6 c	2.3 c-f	2.7 ef
Muscodor 3.5 g/L planting mix	+	76.1 de	23.9 fgh	2.4 c-f	3.5 c-f
Muscodor 3.5 g/L planting mix	-	84.4 bcd	15.6 hij	2.3 def	3.1 def
Untreated control	+	64.1 fg	35.9 cde	2.1 fg	3.4 def
Untreated control	-	97.9 a	2.1 k	2.2 efg	3.4 def

*Values are the means of four replicate flats; means followed by the same letter are not significantly different at $P \leq 0.05$

CONCLUSIONS

Composted cow manure, *T. hamatum* 382, Omega grow plus, Moncut and Endorse significantly reduced damping-off of pepper caused by *Rhizoctonia solani*. Seedlings treated with compost, Omega Grow or Serenade were taller than those in the inoculated control, but none were taller than the non- inoculated control seedlings. Post- emergence damping-off was low in this study, at 3.1- 3.7%. Results are primarily based pre-emergence damping-off. Damping- off was significantly lower in the 3.5 g/L rate of Muscodor than in the untreated, inoculated control. Based on the results of this study, there are several biorational alternatives to traditional fungicides that are suitable for consideration in transplant and micro-vegetable production.