“Understanding the ABCs (Awareness of Bacterial Challenges) with Antibiotics In Crops”

(Photos courtesy of Dr. Jim Graham, Dr. Jim Adaskaveg, Dr. Ronald French, and Dr. George Sundin)

Proceedings of the first Bacterial Challenges Mini-Summit

Hosted by: Interregional Research Project No. 4 (IR-4)

Atlanta, Georgia
September 11, 2014

New Jersey Agricultural Experiment Station Publication
No. A-27200-28-14, Supported by State, U.S. Hatch Act
and other U.S. Department of Agriculture funds.

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Thank You Speakers, Organizers and Participants of the First Bacterial Challenges Mini-Summit

In an effort to obtain a greater understanding about the bacterial disease issues that are being faced by growers, researchers and government, on September 11, 2014, following the IR-4 Food Use and Biopesticide Workshops in Atlanta, Georgia, IR-4 held its first Bacterial Challenges Mini-Summit entitled "Understanding the ABCs (Awareness of Bacterial Challenges) with Antibiotics in Crops". This Mini-Summit provided an opportunity for 125 attendees across the United States including growers, university personnel, industry, and government bodies to come together and discuss many of issues occurring with bacterial diseases on crops, to share research efforts conducted in this area, to discuss antibiotic review processes and provide decisions with regard to registering antibiotics for use in/on food crops, and to communicate the need for solutions. IR-4 was pleased to provide a forum for this mini-summit to help enhance the dialogue and communication around bacterial diseases and to encourage positive interactions among growers, researchers and government. This mini-summit provided a positive opportunity for communication and should pave the way for future summits and collaborations to help address disease issues affecting crops throughout the United States. As the Fungicide Coordinator of the IR-4 Project, I would like to personally thank all of you for your attendance and participation.

Regards,

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Abstract

Bacterial diseases continue to cause major damage and loss to US commodities. In citrus alone, growers are experiencing over a billion dollar loss due to HLB (Huanglongbing; also known as Citrus Greening Disease). This disease causes trees to lose leaves and late season fruit fall. Bacterial diseases affect many other high value specialty crops including Solanaceous crops such as tomato, pepper and potato, stone fruits such as cherry and peach, pome fruits including apples and pears and even tree nuts and olives. In addition to causing devastating losses, bacterial pathogens are difficult or impossible to control; there are not many tools to control these diseases and the registration process is more complicated because of the need to protect from bacterial resistance.

As a major resource for supplying pest management tools for specialty crop growers, the IR-4 Project understands the need for control of bacterial diseases on specialty crops and has been receiving numerous Project Clearance Requests (PCRs) for assistance. Currently, there are several issues impeding the progress toward bacterial disease solutions. Eradication programs have not contained the disease. Genetic technology, including the development of resistant or tolerant plants takes years to develop. Few conventional and biopesticide compounds are available for control and many of the compounds that are available for control can result in pathogen resistance if used improperly. In addition to these challenges, many of the potential conventional bacterial disease control products are antibiotics and require additional testing and review by the Environmental Protection Agency (EPA) in coordination with the Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) to ensure human safety by means of preventing resistance to human diseases before EPA tolerances are issued and products are labeled for use on food crops.

In an effort to obtain a greater understanding about the bacterial disease issues that are being faced by growers, researchers and government, IR-4 held its first Bacterial Challenges Mini-Summit entitled "Understanding the ABCs (Awareness of Bacterial Challenges) with Antibiotics in Crops" on September 11, 2014, following the IR-4 Food Use and Biopesticide Workshops in Atlanta, Georgia. The meeting started with a focus on the impact of bacterial diseases on various crops and the potential management strategies including but not limited to the use of antibiotics, genetic technology and biopesticides. Dr. Jim Dukowitz, the Commercial Products Manager of the Citrus Research and Development Foundation, Inc. (CRDF)[1], spoke about antimicrobial strategies for Florida Citrus including a discussion about the cause and symptoms of HLB, the economic impact of the disease (estimated annual losses have reached $1 billon), an overview of the mission and structure of CRDF, the research strategies that CRDF is focusing on and regulatory issues associated with this research. Dr. Jim Graham, a Soil Microbiologist at the

[1] Is a non-profit corporation to advance disease and production research and product development activities to ensure industry survival through innovation

University of Florida IFAS Citrus Research and Education Center focused on the epidemiology and impact of citrus canker on grapefruit and a rationale for the use of streptomycin on citrus. While Dr. Jim Adaskaveg, a Professor in the Department of Plant Pathology and Microbiology at the University of California, Riverside focused on bacterial diseases in a number of valuable tree crops grown in California including tree nuts, olive, stone fruits and pome fruits. Dr. Adaskaveg, like other researchers, stressed the issues associated with copper being the only registered compound for bacterial disease control and the need for alternate compounds with different modes of action to reduce the chance of resistance development and excess copper accumulation in the soil. Dr. Ronald D. French, an Extension Plant Pathologist and Diagnostician at the Department of Plant Pathology and Microbiology at Texas AgriLife Extension Service in Amarillo, Texas spoke about the cause of Zebra Chip of Potato and the research strategies of controlling this disease including an integrated approach of using antibiotics, insecticides, systemic resistance and nutrient supplements. Dr. Ken Johnson, a Professor of Plant Pathology at Oregon State University in Corvallis, Oregon spoke about the benefits of timing pesticide applications during different phases of growth and using an integrated control program with both conventional and biological compounds for Fire Blight control in pome fruit. Dr. George Sundin, a Professor, Tree Fruit Pathologist and Extension Specialist for the Department of Plant, Soil, and Microbial Sciences at Michigan State University focused his talk on the weather conditions in Michigan that favor bacterial diseases of stone and tree fruit including bacterial canker, bacterial spot and walnut blight. Dr. Sundin also evaluated both organic and conventional options for control, his positive experience with the use of kasugamycin in Michigan field trials and his studies on kasugamycin resistance.

Following these presentations discussions moved on to biotechnology efforts to develop citrus that are resistant to HLB. Dr. Ed Stover, a Horticulturist and Plant Breeder with the USDA Agricultural Research Service, focused on how transgenic citrus varieties are developed and why this technology is needed. Dr. Stover also stressed that host resistance or tolerance to HLB will offer the promise of a sustainable long-term solution to maintain citrus production. Dr. Manjul Dutt, at the Citrus Research and Education Center in Lake Alfred, Florida discussed his team's progress toward developing citrus varieties resistant to HLB and the use of RNAi technology to create trap plants that target the Asian Citrus psyllid vector. They both discussed the pros and cons of the transgenic plants being accepted by consumers.

The final session of the meeting focused on the processes and considerations undergone by EPA, CDC and FDA in addressing the use of antibiotics for various bacterial challenges as it relates to pesticidal efforts. Susan Jennings, the Public Health Coordinator of the Office of Pesticide Programs of the Environmental Protection Agency (EPA) spoke about the registration process and the added regulatory requirements when antibiotics are used on food crops including the data needs, interpretations and risk assessment. This includes studies on antimicrobial resistance and risk mitigation. EPA is required to consult with Food and Drug Administration (FDA) and CDC (Center for Disease Control and Prevention) when assessing the risks of antimicrobial resistance and protecting public health. Speakers were invited from these agencies to present their perspectives. Dr. Jean Patel D(ABMM), the Deputy Director of the Office of Antimicrobial Resistance in the Division of Healthcare Quality Promotion of CDC presented examples of the potential for development of antibiotic resistance in humans and the risks associated with pesticide antibiotic use. Dr. Heather Harbottle of the Microbial Food Safety Team (HFV-157), Office of New Animal Drug Evaluation, Center for Veterinary Medicine of FDA spoke about the microbial food safety risk assessment and the regulatory decision-making involved with the use of antibiotics in food-producing animals. Dr. Harbottle focused on the Guidance to Industry #152 document and its intent on preserving antibiotic tools that are important for treating human disease. She shared experiences with antimicrobial drug resistance risk assessment. The session concluded with a presentation by Dr. Shaunta Hill, Registration Division, Office of Pesticide Programs, EPA. Dr. Hill provided a detailed presentation on the registration process including the antibiotic considerations that were involved in the decision process to register kasugamycin bactericide (Kasumin® 2L) on Pome fruit Group 11-10.

This Mini-Summit provided an opportunity for attendees (125) including growers, university personnel, industry, and government bodies to come together and discuss many of issues that are occurring with bacterial diseases on crops and where they could share research efforts conducted thus far and the need for solutions. Speakers from EPA, FDA and CDC were also able to discuss antibiotic review processes and decisions that are involved when registering antibiotics for use in food crops. Hopefully it provided a better understanding of the information that is required for the registration of antibiotics on food crops. A question and answer session at the conclusion of the mini-summit also provided an opportunity for interaction between the speakers and audience. This mini-summit helped to enhance the dialogue and communication around bacterial diseases and encouraged positive interactions between growers, researchers and government that should pave the way for future summits and collaborations to help address disease issues affecting crops throughout the United States.

Structure and Overview of the Bacterial Disease Mini-Summit

"Understanding the ABCs (Awareness of Bacterial Challenges) with Antibiotics in Crops"

September 11, 2014
7:45 am to 1:00 pm
JW Marriott Atlanta Buckhead, 3300 Lenox Road NE, Atlanta, GA 30326-1333

Breakfast will be served starting at 6:30 AM for all participants

- **Introduction**
  - **7:45 am-8:00 am: IR-4 Welcome and Purpose of Summit-** Dr. Jerry Baron, Executive Director, IR-4; Moderator: Kathryn Homa, Fungicide Coordinator, IR-4

- **Grower Needs and Challenges / New and Existing Options for Control** (8:00 AM – 10:00 AM; 15 minute presentations plus time for questions)
  - **Affected Crops**
    - **Citrus**
      - **8:00 am-8:20 am: Experiences of setting up CRDF**
      - **Research efforts of control options for HLB –** Dr. Jim Dukowitz, Commercial Products Manager, Citrus Research and Development Foundation, Inc.
      - **8:20 am-8:40 am: Research efforts of control options for Citrus Canker**- Dr. Jim Graham, University of Florida
    - **Almond, olive, walnut**
      - **8:40 am-9:00 am: Research efforts of control options for bacterial diseases of almond, olive, walnut** - Dr. James Adaskaveg, University of California – Riverside, Dept. of Plant Pathology
    - **Potato**
      - **9:00 am-9:20 am: Zebra Chip –** Dr. Ronald D. French, Assistant Professor and Extension Plant Pathologist, Coordinator, Texas Plant Diagnostic Clinic, Department of Plant Pathology & Microbiology, Texas A&M AgriLife Extension Service
    - **Pome Fruit (Apples and Pears)**
      - **9:20 am-9:40 am: Fireblight-** Dr. Ken Johnson, Professor, Plant Pathology, Oregon State University

        - **Cherry and Other Stone Fruits**
            - **9:40 am-10:00 am: Research efforts of control options for bacterial diseases of stone fruit**
            - **Research and Experiences with Kasumin in Michigan-** Dr. George Sundin, Professor and Extension Specialist, Dept. Plant, Soil, and Microbial Sciences, Michigan State University

- **BREAK-** (10:00 AM – 10:20 AM)

- **Biotechnology Options for Control** (10:20 AM – 10:50 AM; 15 minute presentations including questions)
        - **10:20 am-10:35 am: Development of resistant citrus to Citrus Greening bacteria through genetic engineering-** Dr. Ed Stover, USDA/ARS, Research Horticulturalist/ Geneticist, U. S. Horticultural Research Laboratory, Fort Pierce, FL
        - **10:35 am-10:50 am: Development of HLB (greening disease) bacteria resistance through genetic engineering-** Dr. Manjul Dutt, Citrus Research and Education Center, University of Florida

- **Regulatory Review Processes and Perspectives** (10:50 AM – 11:50 AM; 20 minute presentations including questions)
    - **Processes / considerations undergone by EPA, CDC and FDA in addressing the use of antibiotics for various bacterial challenges as it relates to pesticidal efforts**
        - **10:50 am-11:10 am: EPA-** Susan Jennings, Public Health Coordinator, Office of Pesticide Programs, US Environmental Protection Agency
        - **11:10 am-11:30 am: CDC-** Dr. Jean B. Patel, D(ABMM), Deputy Director, Office of Antimicrobial Resistance Centers for Disease Control and Prevention
        - **11:30 am-11:50 am: FDA-** Dr. Heather C. Harbottle, Microbiologist, Division of Animal Food and Microbiology, Food and Drug Administration
            - **Discussion of the Guidance to Industry #152 document and its intent on preserving antibiotic tools that are important for treating human disease**
            - **Experiences with antimicrobial drug resistance risk assessment**

- **Recent Registration Example**

- **11:50 am–12:10 pm: Kasugamycin registration on Pome Fruits Crop Group 11-10-** Dr. Shaunta Hill, U.S. EPA: Office of Chemical Safety and Pollution Prevention, Registration Division/Fungicide Branch

- **Round Table Discussion-Break out groups (12:10 PM - 12:30 PM)**
  - **Organizing a commodity committee similar to CRDF to solve bacterial challenges**
  - **EPA/CDC/FDA registration questions/concerns**
  - **Sharing research and efficacy results/ideas**
    - **Citrus**
    - **Tree Fruit (other than citrus)**
    - **Miscellaneous crops**

- **Report / conclusions from each round table discussion (12:30 PM – 12:50 PM)- Reported by group leader**
- **Moving forward (12:50 – 1:00 PM) -** Jerry Baron, Executive Director, IR-4; Kathryn Homa, Fungicide Coordinator, IR-4
  - **Meeting feedback**
  - **What's next**
  - **Approaches and solutions**

Summary of Speaker Discussions

Dr. Jerry Baron, Executive Director of IR-4 provided a brief overview and purpose of the mini summit. He noted that IR-4 stakeholders asked for a Bacterial Disease Summit at our Food Use workshop last year (2013) and Atlanta seemed to be a good place to have the meeting since many of the participants involved only had to travel a short distance to this meeting location. Following this introduction, Kathryn Homa, Fungicide Coordinator IR-4, reviewed the agenda with attendees.

The first session of the meeting focused on grower needs and challenges in the field including new and existing options for control on various crops.

The first speaker, Dr. Jim Dukowitz, the Commercial Products Manager of Citrus Research and Development Foundation, Inc. (CRDF) began the meeting by communicating to the audience that Huanglongbing (HLB), also known as Citrus Greening Disease, is currently the most devastating citrus disease. It is a non-cultural bacterium and is vectored by the Asian citrus psylid. After the citrus tree becomes infected, no symptoms occur for over two years. Annual loss is now estimated at $1 billion per year. Short and long term strategies to combat HLB include conventional antibiotics that are already on the market including streptomycin, oxytetracycline, and kasuygamycin and agricultural antibiotics from other countries such as GRAS 25b like products including plant essential oils and biopesticides. There have been some good results, but suppliers and other challenges exist including commercialization requirements, effectiveness, and the ability to easily register the compounds. Regarding the regulatory issues, CRDF has held a number of meetings with regulatory agencies and CRDF is very thankful for the good advice and guidance that has been provided from these meetings. Currently, researchers are working with a pipeline of products and unique application methods including trunk injections and root applications. Bio Assays including graph-based assays (slower) and culture-based assays (faster) are also being used to test efficacy and phytotoxicity in citrus. Currently, field trials are the top research priority with oxytetracycline, streptomycin and GRAS-like products being tested in field trials this year. The research pipeline is now being enhanced with government funding that includes $21mm from MAC (the Multi-Agency Coordination group, composed of USDA representatives and stakeholders; USDA, APHIS) and $25mm from SCRI (Specialty Crop Research Initiative). Jim then spoke about CRDF including that it was organized in 2009 through the University of Florida, and that it consists of a 13 member board. Although it addresses a number of citrus diseases, 91% of the funds go to HLB solutions (approximately $40 million). Currently, this organization has 16 projects in their portfolio.

Dr. Jim Graham of the University of Florida spoke about his experiences with the use of Firewall™ 17 WP fungicide/bactericide (streptomycin) on grapefruit for control of citrus canker. Jim explained that the bacteria-causing canker is carried by water and when water hits the leaves at greater than 18 miles per hour, the bacteria is pushed in to the tree leaves via the stoma. The citrus canker eradication program ended in 2006 after a number of hurricanes hit the area.

Currently, copper is the only registered product for canker control and is used a lot. Jim stressed that applications of copper cannot protect against the bacteria that has entered the tree. In addition to this issue, copper resistance has occurred (although it has not yet been detected in Florida but has been detected in Argentina), copper entering the citrus agro-ecosystem is totaling more than 12 lb metallic copper/acre/season, and copper phytotoxicy is a problem. Jim stressed the importance of streptomycin and explained that applications of streptomycin are needed once the bacterium enters the citrus tree. Currently, there is a Section 18 for Firewall™ use during the 2013-2014 seasons on grapefruit. There are many benefits of streptomycin since it is locally systemic and has post penetration activity which copper does not have. It has also helped to manage copper burn, as it can be alternated with copper applications. Treatments are limited to two applications per year but that has reduced the copper toxicity. Streptomycin attributes for citrus canker currently outweigh the antibiotic risk in non-targets. In the meantime, streptomycin resistance will continue to be monitored.

Dr. Jim Adaskaveg from the University of California – Riverside, Dept. of Plant Pathology spoke about the large impact that bacterial diseases have on highly valued crops grown in California including almond, olive, walnut, stone fruit and pome fruit. Jim also explained how the rain that occurs (when it occurs) in California helps to provide a favorable environment for bacterial diseases. Jim then spoke about the disadvantages of copper and the use of alternatives to control bacterial diseases. Copper is one of the oldest pesticides available and is still being used a lot. Different plants have different sensitivities to copper phytotoxicity and bacterial pathogens are becoming less sensitive to copper use. Jim's research highlighted the dangers of using only one or two products with the increased sensitivity (tolerance) to copper and mancozeb. Although some of the newer products have fixed copper to help decrease the environmental load, it is important to continue to rotate chemistries to prevent resistance. The presentation presented the results from a number of conventionals, biopesticides and antibiotics when tested for control of bacterial diseases. He concluded his talk with a discussion of preventing/managing bacterial disease resistance.

Dr. Ronald D. French, Assistant Professor and Extension Plant Pathologist, Coordinator, Texas Plant Diagnostic Clinic, Department of Plant Pathology & Microbiology, Texas A&M AgriLife Extension Service talked about an emerging bacterial disease of potato called Zebra Chip. Ron explained that it can be confused with vine decline or blight. Tomatoes are also affected by this disease. In untreated areas of the field, as much as 70% incidence can occur. In the potato, sugar levels get altered. This causes a zebra pattern in the potato, which when fried will blacken chips and produce an off flavor. The only existing approach is to manage the Pysllid populations, which vectors the bacteria. Since there are a lot of insecticides being used in Texas to control the Pysllid, Ron and his colleagues are researching alternatives to insecticides including antibiotics, products that trigger plant defenses and nutrient alterations. Ron and his colleagues started trials in 2009 to screen a number of different products. In several trials, it was found that streptomycin was the only treatment that was better than the control. Ron also discovered that promising results occurred when streptomycin treatments were combined with other compounds and micronutrients including KPX (KeyPlex, a unique formulation of chelated micronutrients, enhanced alpha-keto acids, and humic acid).

Dr. Ken Johnson, Professor of Plant Pathology, Oregon State University started off his talk by providing a list of registered products and pending products for control of fire blight control on pome fruit. Ken stressed the need for applying products prior to bloom and that flower treatments are different than pre-bloom treatments. He explained that the sequence of applying materials for control is important and described using an integrated control approach that includes conventional and biopesticide products during different phases of growth. Ken provided a short overview of organic practices and then covered conventional control. He reported that kasugamycin is working well to control bacterial diseases in pome fruit and that it is a great new registered chemical for control of bacterial diseases. In concluding his talk, Ken mentioned that streptomycin resistance has actually decreased since 1988 from 80% to about 20% in 2010, because of this broader approach and better management of the orchards. However, there are still a variety of concerns about the materials for control including: cost, resistance, phytotoxicity, efficacy and compatibility of the materials when mixing.

Dr. George Sundin, Professor and Extension Specialist, Dept. Plant, Soil, and Microbial Sciences, Michigan State University spoke about his research experiences with bacterial diseases on cherry and other stone fruits in Michigan. He mentioned that bacterial infection can trigger frost disease, which can in turn cause more infection via open wounds on the plants. Canker is induced by freeze events. George mentioned that copper causes the most injury (phytotoxicity) to cherry trees after leaves are present, but he has not seen good efficacy using alternative treatments. In addition, research dollars are very low for this work in cherries. He concluded his presentation by describing his positive experience with Kasumin™ (kasugamycin) in Michigan since the Section 18 went into place. George is still determining if kasugamycin selects for resistance and is continuing with resistance monitoring in soil and plant surfaces. So far, no difference has been observed between kasugamycin-sprayed orchards and non-sprayed orchards. There is also no evidence of any effects of kasugamycin use on resistance to other antibiotics.

Grower presentations were followed by a discussion of biotechnology options for control. Dr. Ed Stover, USDA/ARS, Research Horticulturalist/ Geneticist, U. S. Horticultural Research Laboratory, Fort Pierce, FL discussed the development of resistant citrus to citrus greening bacteria through genetic engineering. Ed first explained that HLB is a serious problem that has led to the lowest citrus yields in 50 years. He then stated that host resistance is the best promise for the future of citrus in the US and that transgenics may provide the strongest resistance. Ed believes that transgenic crops will be more acceptable in the U.S. in the future. However, to date, there has been no release of transgenic crops for bacterial resistance and transgenics will be an ongoing effort even after positive candidates are identified. Ed focused on antimicrobial peptides, which are widely active to an array of micro-organisms. Ed concluded the presentation by stating that they have had some successes including transformations with modest resistances and others coming along that are even better. Currently, there is nothing ready for deregulation However, spinach “defensins” are showing promise in grapefruit and orange.

Dr. Manjul Dutt, Citrus Research and Education Center, University of Florida followed this talk with additional information about the development of HLB bacteria resistance through genetic engineering. Manjul discussed that the disease is usually fatal and that his lab has been working on the problem for 5 years. Two genes PR1 and PR2 have been identified as important for systemic acquired resistance genes. Currently, his group is looking for introgenic vectors for a robust transformation system. The Anthocyanin overexpressing RUBY gene obtained from the 'Moro' Blood Orange is being used in experiments and is only expressed in the somatic embryos using a citrus derived embryo specific promoter. Concluding the presentation, Manjul stated that citrus is difficult to grow and the growth of the trees takes a long time. However, his group have trees that are resistant and have made cuttings to use in further work.

The remaining discussions consisted of the regulatory requirements and review processes, perspectives and considerations undergone by the Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention (CDC) and Food and Drug Administration (FDA) in addressing the use of antibiotics for various bacterial challenges as it relates to pesticidal efforts.

The presentations began with Susan Jennings, Public Health Coordinator, Office of Pesticide Programs, US EPA. Susan set the stage for how EPA deals with antibiotics. She began the presentation by stressing the need to make the best possible regulatory decisions to protect public health, non-target species, and the environment. She then explained the registration process for an antibiotic pesticide and explained the need for an extensive data set and risk assessment including resistance management and the FDA #152 document (Guidance for Industry #152 – Analysis of risk of development of antimicrobial resistance among bacteria of human health concern in/on treated crops), as a qualitative assessment. Antibiotic registration reviews, in addition to conventional pesticide requirements, must also focus the most on antibiotic resistance and this requirement started in 2005. Susan stated that the EPA can accept data from other sources but this has to be approved/agreed/discussed with EPA before submission. Susan also clarified that during risk assessment, EPA consults with CDC and FDA on potential for such a use to cause resistance and be a public health concern. However, EPA ultimately makes the decision to register the product.

Dr. Jean B. Patel, D(ABMM), Deputy Director, Office of Antimicrobial Resistance Centers for Disease Control and Prevention spoke about the role of CDC in the registration process of antibiotics on food crops. Jean explained that CDC provides advice but is not a regulatory agency. She explained that antimicrobial resistance is one of the most important public health issues at this moment and that anywhere antibiotics are used can be a possible source for resistance. As an example, she stated that resistant bacteria (KPC producers (CRE Colonization)) are now endemic in New York City and have been identified in a number of other states. Dr. Patel presented a hypothetical scenario for carbapenem-resistant Enterobacteriaceae amplification from the misuse of pesticides. She then concluded that the potential misuse of antibiotics in agriculture could contribute to antibiotic resistance in humans.

Dr. Heather C. Harbottle, Microbial Food Safety Team (HFV-157), Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration started off the presentation by discussing the uses of antimicrobials in food-producing animals. She then went into further detail about the purpose of the Guidance to Industry #152 document and its intent on preserving antibiotic tools that are important for treating human disease. Release, hazard identification and qualitative risk assessment were explained in detail. Then she provided an example of the process of reviewing the 152 assessment.

The final presenter at the bacterial disease mini-summit was Dr. Shaunta Hill, U.S. EPA: Office of Chemical Safety and Pollution Prevention, Registration Division/Fungicide Branch. She began the presentation by discussing the general pesticide registration process, the public comment period and a detailed discussion regarding the recent kasugamycin registration decision on Pome Fruits Crop Group 11-10. She provided details about the chemical compound, as an example of what type of data were reviewed and the antibiotic concerns including resistance concerns. After discussing the details about the regulatory decision, she explained the risk reduction measures that are taking place as indicated on label wording including monitoring for resistance.

The summit closed with a short question and answer session with the various speakers. In closing, the summit helped to enhance the dialogue and communication around bacterial diseases and encouraged positive interactions between growers, researchers and government. It is hoped that these discussions and collaborations will continue in an effort to address these disease issues affecting so many crop growers in the United States.

Speaker Presentations

Antimicrobial Strategies for Florida Citrus

IR-4 Bacterial Challenge Mini-Summit

September 11, 2014

Agenda

- HLB Challenge
- CRDF Overview
- Antimicrobial strategies
- Regulatory Issues

What is HLB?

- The most devastating disease known to citrus
- Pathogen: Non-culturable bacterium, Candidatus Liberibacter asiaticus (C Las)
- Vector: Asian Citrus Psyllid (ACP, Diaphorina citri)
- Disease progression:
  - Asymptomatic for one to two years
  - Progressive loss of leaves, decline in root mass and overall plant vigor
  - Lower productivity and declining fruit quality
  - Symptomatic fruit are extreme in size (unusually small or large), atypical in color, asymmetric in shape, and yield juice of reduced quality

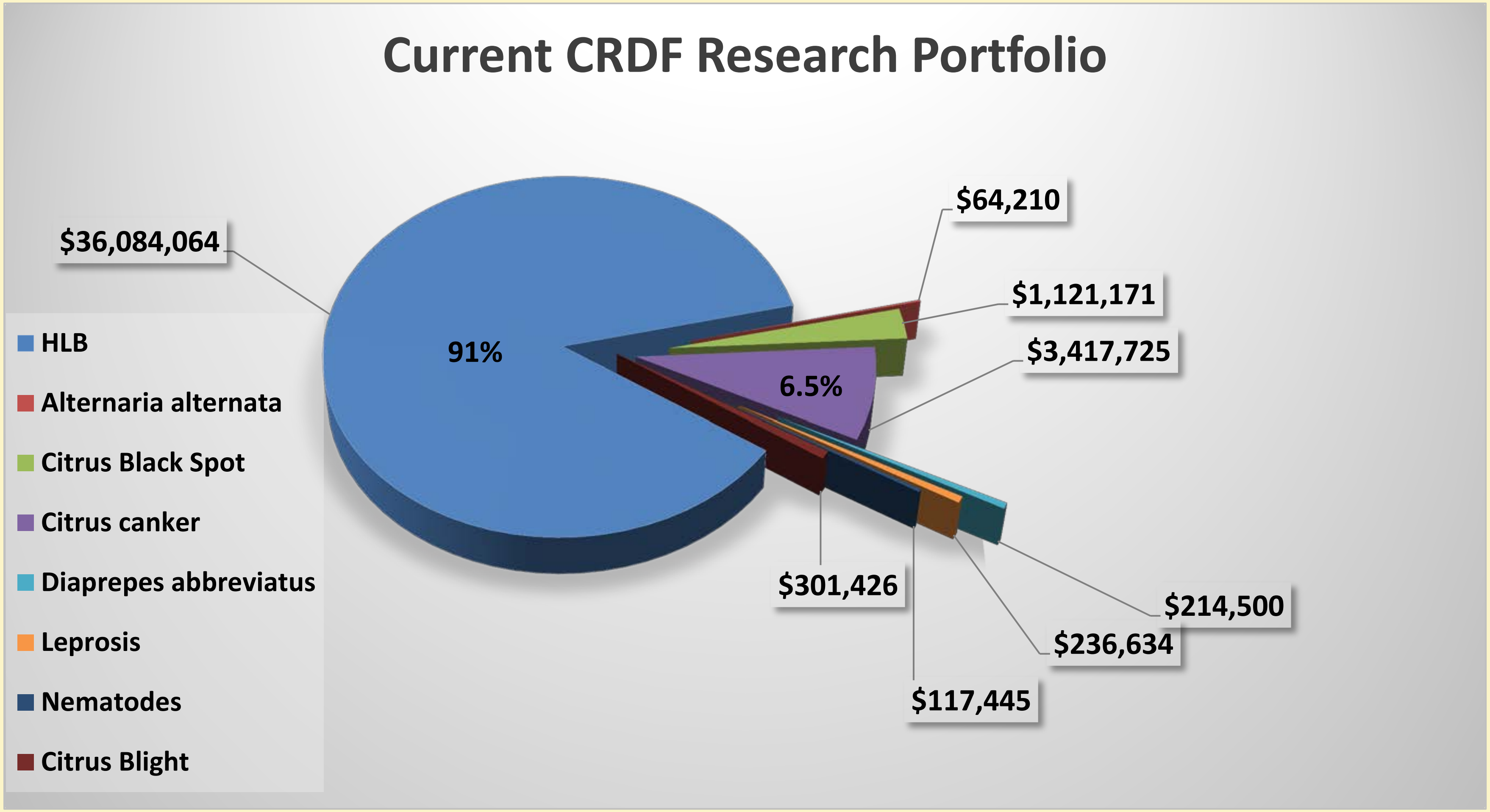
Economic Impact of HLB

- HLB spread from two counties in Florida in 2005 to 32 by 2008. Today all 34 citrus producing counties in Florida are infected with HLB.
- Since the 2007-2008 season, annual orange production in Florida has declined from 170 million boxes to 104 million boxes. Most recent estimates for the coming year are 89 to 95 million boxes, much attributed to HLB. (Lowest in 50 years.)
- Since the 2006-2007 growing season, average annual loss is approximately $1 billion per year.

CRDF Mission and Structure

- Created in 2009 as a non-profit corporation organized under Florida State laws as a Direct Service Organization of the University of Florida
- Mission to advance disease and production research and product development activities to ensure industry survival through innovation.
- The organization is headed by a 13-member Board of Directors that includes individuals from industry, academia and government.
- The COO handles the day-to-day management of business affairs, and Program Managers oversee the research and commercial product delivery portfolios.

CRDF Emphasis on HLB Solutions



2014-15 CRDF Delivery Project Topics

Therapy for Existing Trees
Antimicrobial Strategies
Naturally Occurring Microbial Products
Thermal Therapy to reduce *CLas* titer
Plant Growth Regulator Interactions with HLB
Strategic Inoculum Removal to Manage HLB in Florida
Case Analysis of Success in Responding to HLB
New Plantings
Asian Citrus Psyllid Management
Tolerant Rootstock Plantings
Psyllid Shield – Delivering RNAi with CTV Vector
Integrating HLB Management Tools into Model New Groves
Candidate HLB Tolerant Scion Evaluation in Field Trials
Genetic Technology (MCTF): Deploying Canker-Resistance Genes
Diaprepes Root Weevil Pheromone
Citrus Leafminer Area-Wide Mating Disruption
CTV Vector as a Tool to Deliver Solutions
HLB Escapes

Antimicrobial Strategies

- Conventional Antibiotics
  - Used for human or animal health.
  - Streptomycin, oxytetracycline focus due to precedence for their use on food crops. Kasugamycin?
  - Others, e.g. Penicillin, lack required data sets for agriculture use.
- GRAS-like
  - Simple plant essential oils. Sponsored research is underway to formulate and deliver compounds that qualify for GRAS status (or even better, 25(b) status)
  - Potential for more rapid deployment through a reduced commercialization and regulatory pathway

Antimicrobial Strategy (2)

- Biopesticides
  - Looking at a class of compounds used in agriculture but not formulated for vascular disease of trees.
  - In discussion with dominant industry patent holder on their proprietary compounds.
  - Opportunity to repurpose products. Potentially shorter regulatory path.
- Agricultural antibiotics
  - Used on food crops in other countries
  - Pose unique challenges, e.g. reliable suppliers, IP issues
- New molecular entities
  - Specificity and potency customized to treat HLB and not used for human or animal health. Referred to as non-antibiotics.

CRDF

Near Term Commercialization Requirements

- Effective against HLB
- Phloem-mobile antimicrobials
- Non-phytotoxic
- Already registered with EPA for use in plant agriculture – ideally long history of safe use in tree fruit crop agriculture
- Formulation and delivery system
- Commercial viability
  - Cost to develop and evaluate active ingredients and products
  - Time and cost to market
  - Return on investment
- Essential to have reliable, well-resourced commercial partners to successfully commercialize these technologies

Biological Assays

- Graft-based assay
  - Infected scion soaked in test solution and grafted onto uninfected rootstock, follow by PCR
  - Slow, low-throughput
  - Evidence of efficacy *in planta* and first look at phyto-toxicity
  - Open contest with InnoCentive™ promotion
- *Liberibacter crescens* culture-based assay
  - Culturable cousin of C Las
  - Much faster, higher-throughput followed by *in planta* confirmation on *CLas*

CRDF

Prior Results

- Compounds screened
  - ~100+ by graft assay and
  - ~400+ by culture assay
- Wide variety of categories of chemicals
  - Antibiotics and agricultural antibiotics
  - Polycation polymers
  - Biopesticides, plant essential oils, terpenoids
  - New actives and non-antibiotic derivatives
  - Host immune modulators

CRDF

Creating a Pipeline for Screening

- Looking inside the box…
  - Companies, facilitate turn-key screening
- Looking outside the box…
  - Repurpose products with regulatory advantage
  - Failed antibiotics with good safety profiles
  - 25(b) minimum risk pesticides
  - All commercial compounds fitting our chemical profile and having existing tolerances on other food crops

Current Testing Resources

Liquid Culture Assay on *L. crescens* ➪ MIC90

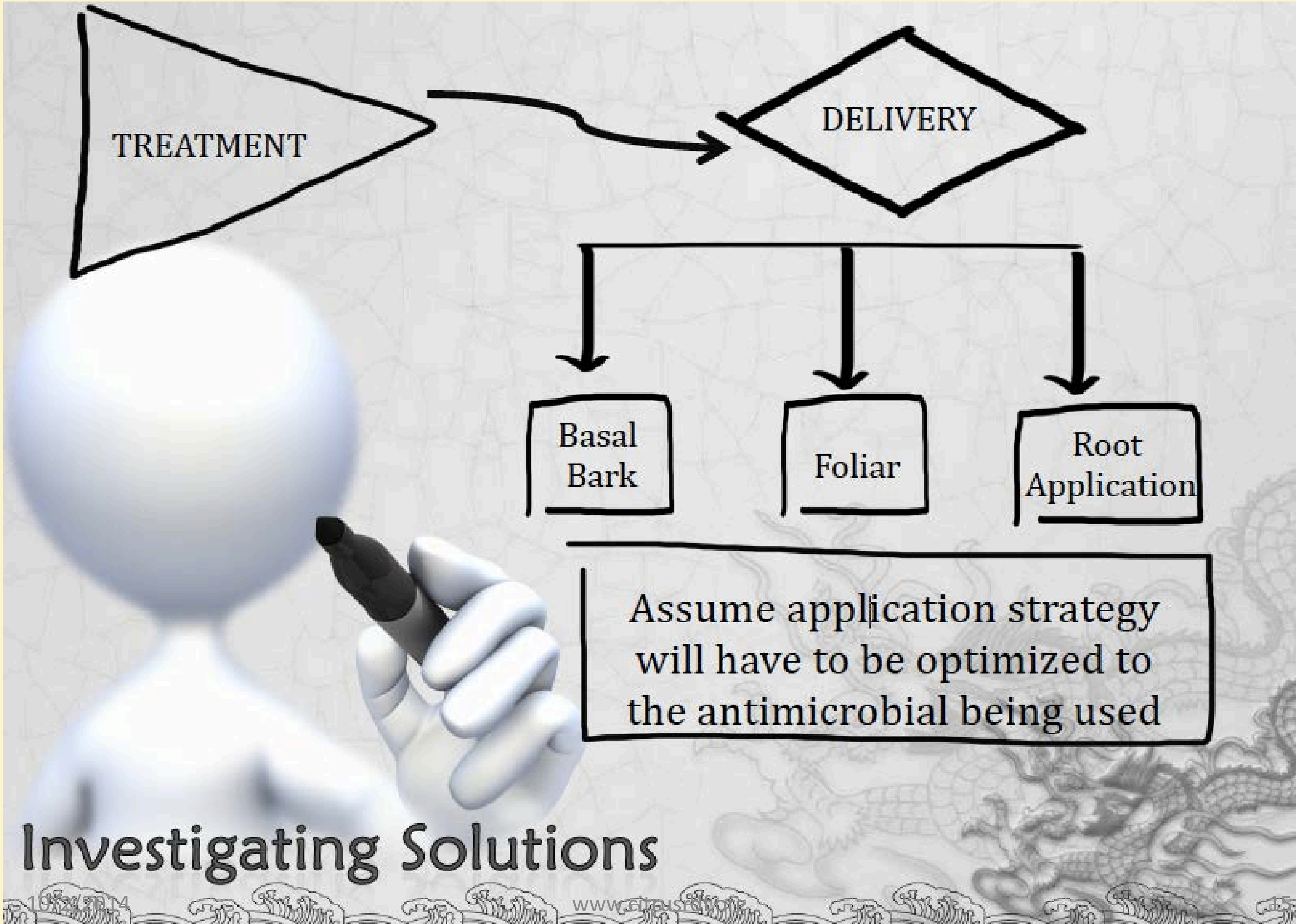
(Dr. Erik Triplett Laboratory)

Citrus Flush Assay on *C. asiaticus* ➪ Confirmation

(Dr. Claudio Gonzalez)

Trunk Injection ➪ Dose

(Dr. Nian Wang)


TREATMENT
DELIVERY
Basal Bark
Foliar
Root Application
Assume application strategy will have to be optimized to the antimicrobial being used
Investigating Solutions

Risk Remaining at this Stage

- Technical
  - Commercial scale delivery, efficacy, phytotoxicity
- Cost
- Regulatory
- Commercial Partner(s)
- Market acceptance

Build on Success–Expanding Teams

- Pivot our focus – connecting corporations
- Sponsored research base from CRDF and others, researchers, reviewers, public solutions
- Improved communication; knowledge and data sharing between and within grower, researcher, government, corporate sectors
- CRDF Board and Committees; RMC, CPDC, IRCC
- Research pipeline now enhanced with government funding \$21mm MAC, \$25mm SCRI

What comes next?

1. Field trials with top priority

Category of Compounds	Description	Partner
Antibiotics	Oxytetracycline Streptomycin (Kasugamycin?)	Commercial
GRAS-like	Plant Essential Oils Natural Products – cymene, carvacrol, etc.	CRDF-sponsored formulation research

2. Field trials with major challenges

Category of Compounds	Description	Partner
Agricultural Antibiotics	Zhongshengmycin Validoxylamine A	Source? - Research

3. Field trials with commercial partners

Category of Compounds	Description	Partner
Biopesticides	Surfactin (lipopetides)	Company C
Host Immune Modifiers	One marketed compound One pipeline compound	Company C

4. Development pipeline

Category of Compounds	Description	Partner
Polycation polymers	Proprietary biodegradable polymers	Company D
Bacterial protein target LdtR (Gonzalez)	Lead refinement - Highly active, no human homolog, transcription regulator, essential role in cell wall remodeling	Company D
Bacterial protein target SecA (Wang)	Lead refinement - Highly active, no human homolog, protein secretion, essential role in effector secretion	Company D
Non-antibiotic derivatives	Tetracycline derivatives	Company E

Regulatory Issues

1. Identification of specific issues of the health regulatory agencies around the universe of compounds that we may include in the testing protocol
2. For the antimicrobial agents screened by CRDF (or others)
    a. Are there differences in concerns related to cross over issues with human health use?
    b. Are there potential labelling issues associated with allergenicity of specific antibiotics?
    c. From a process component standpoint are there any concerns over byproduct use in animal feed, flavoring or fragrances?
3. To expedite data collection to allow use, can a regional registration be pursued with geographically limited residue information?
4. How to best coordinate and communicate with the regulatory community as preliminary information starts to become available from the ongoing research program

Rationale for use of Firewall™ (streptomycin) for citrus canker control in Florida grapefruit

Rationale for use of Firewall™ (streptomycin) for citrus canker control in Florida grapefruit*

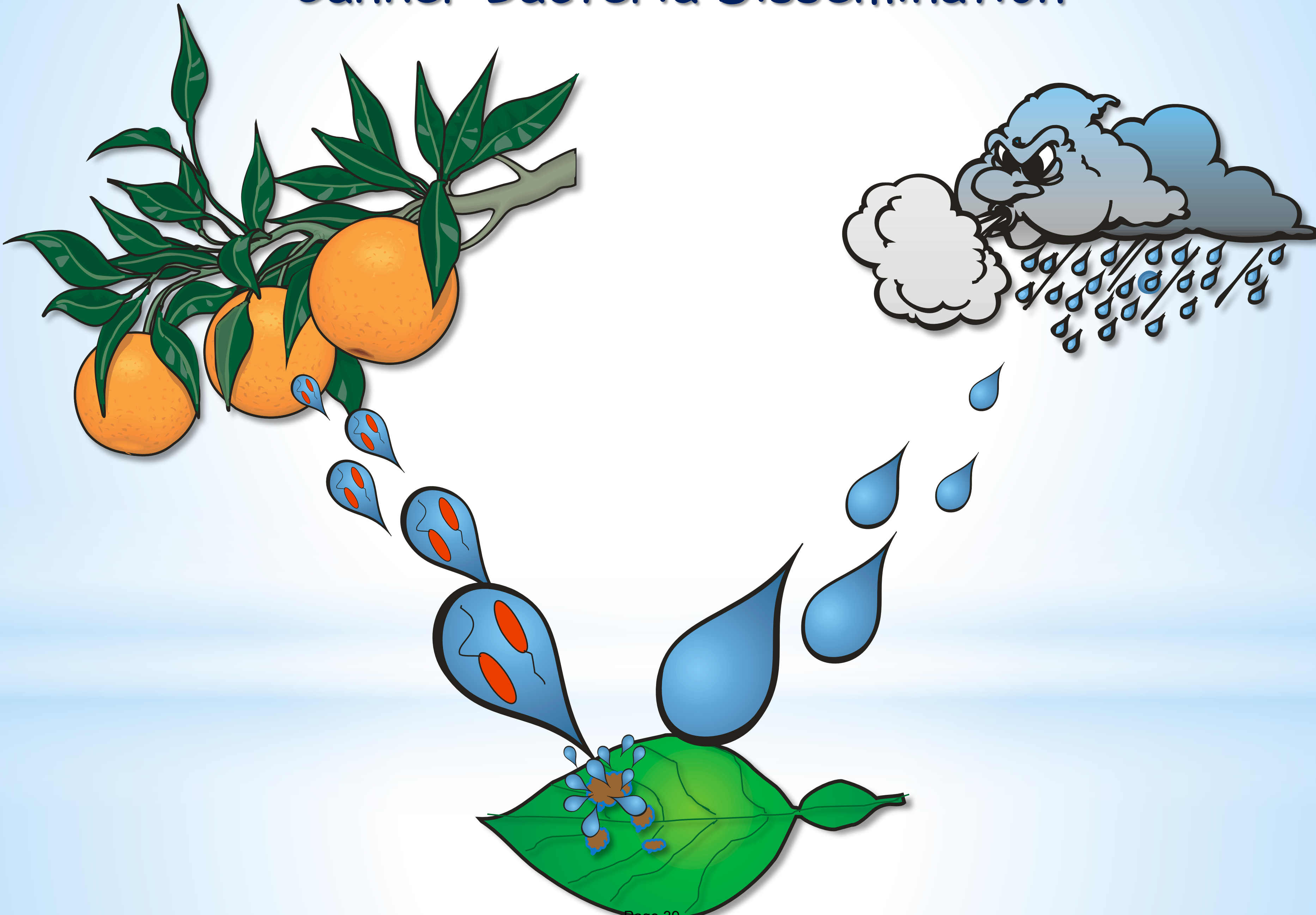
Jim Graham
Soil Microbiologist

Sept 11, 2014
Atlanta, GA

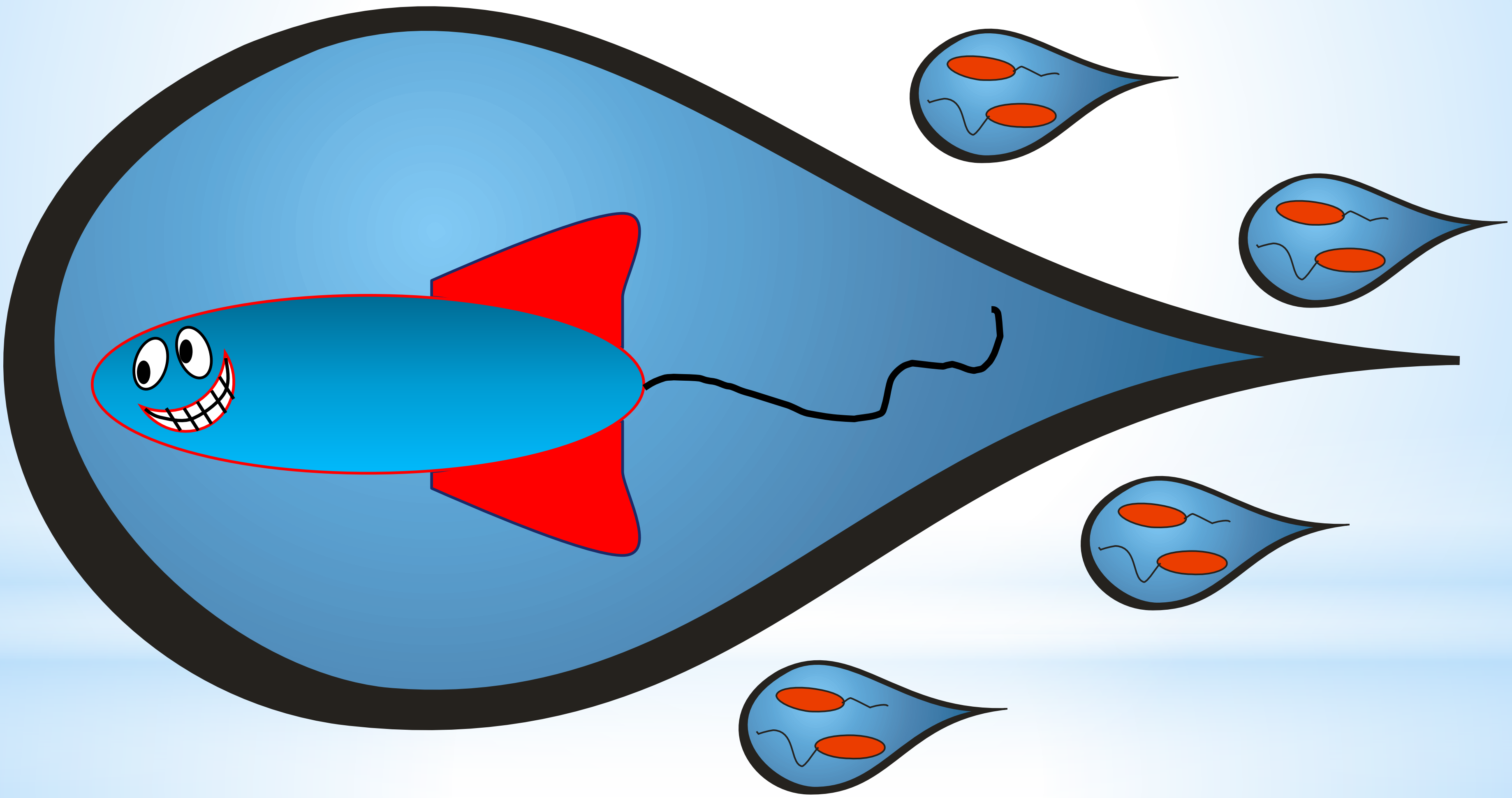
UF UNIVERSITY of FLORIDA
IFAS
Citrus Research and Education Center

*Research supported by CRDF Citrus Research and Development Foundation, Inc.

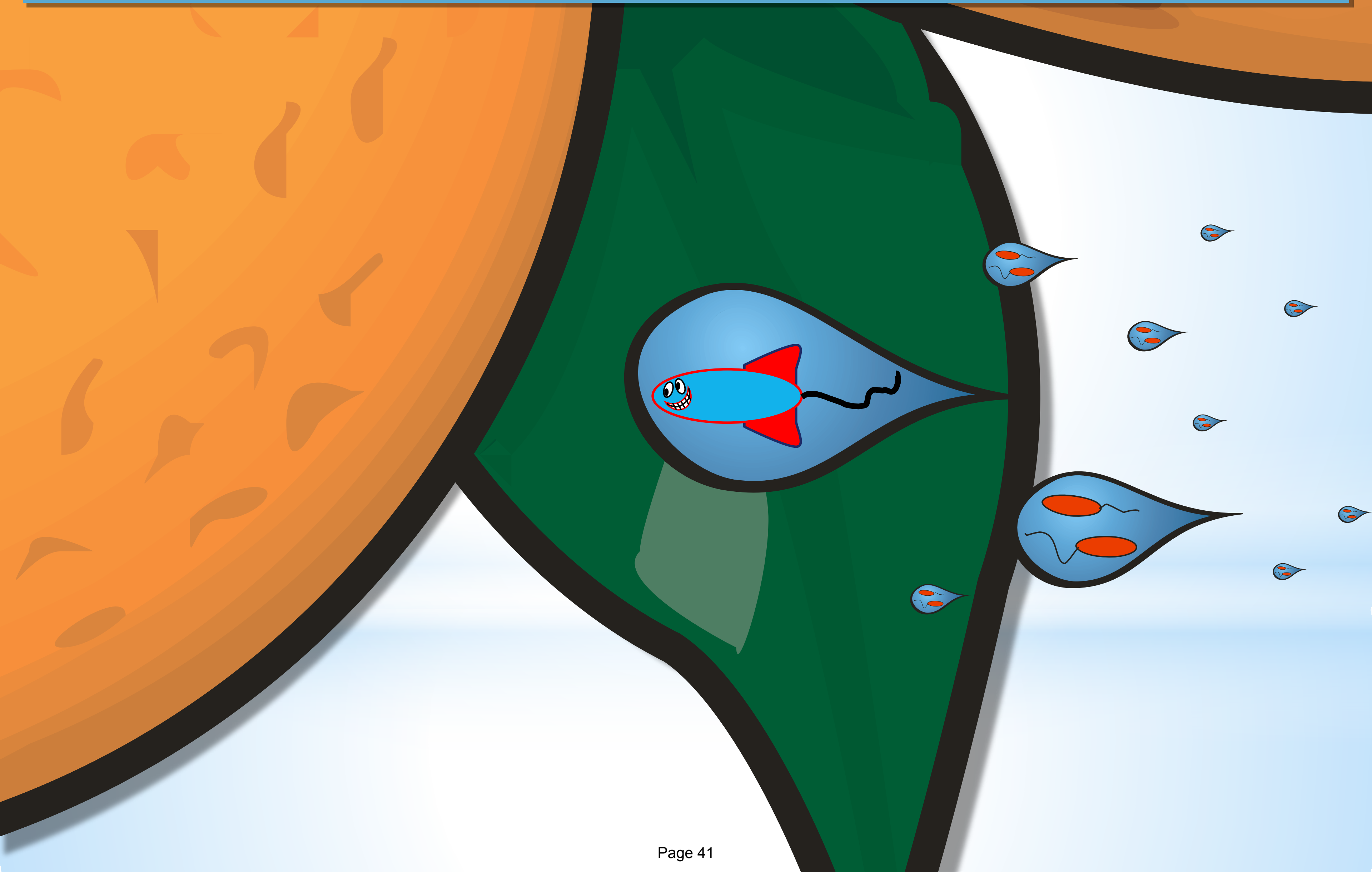
Canker Bacteria Dissemination

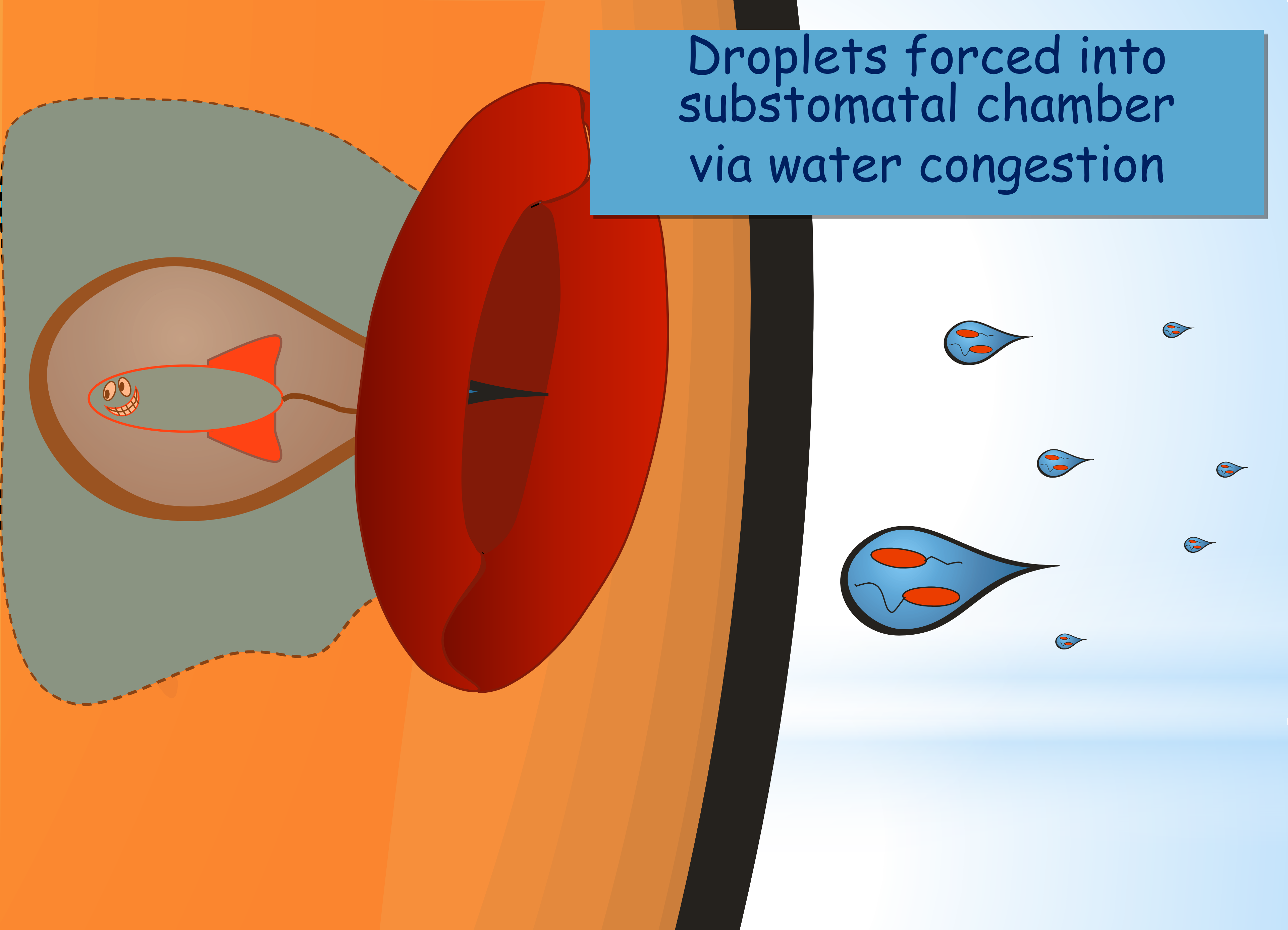


Squadron of bacterial laden droplets caught up in winds takes flight

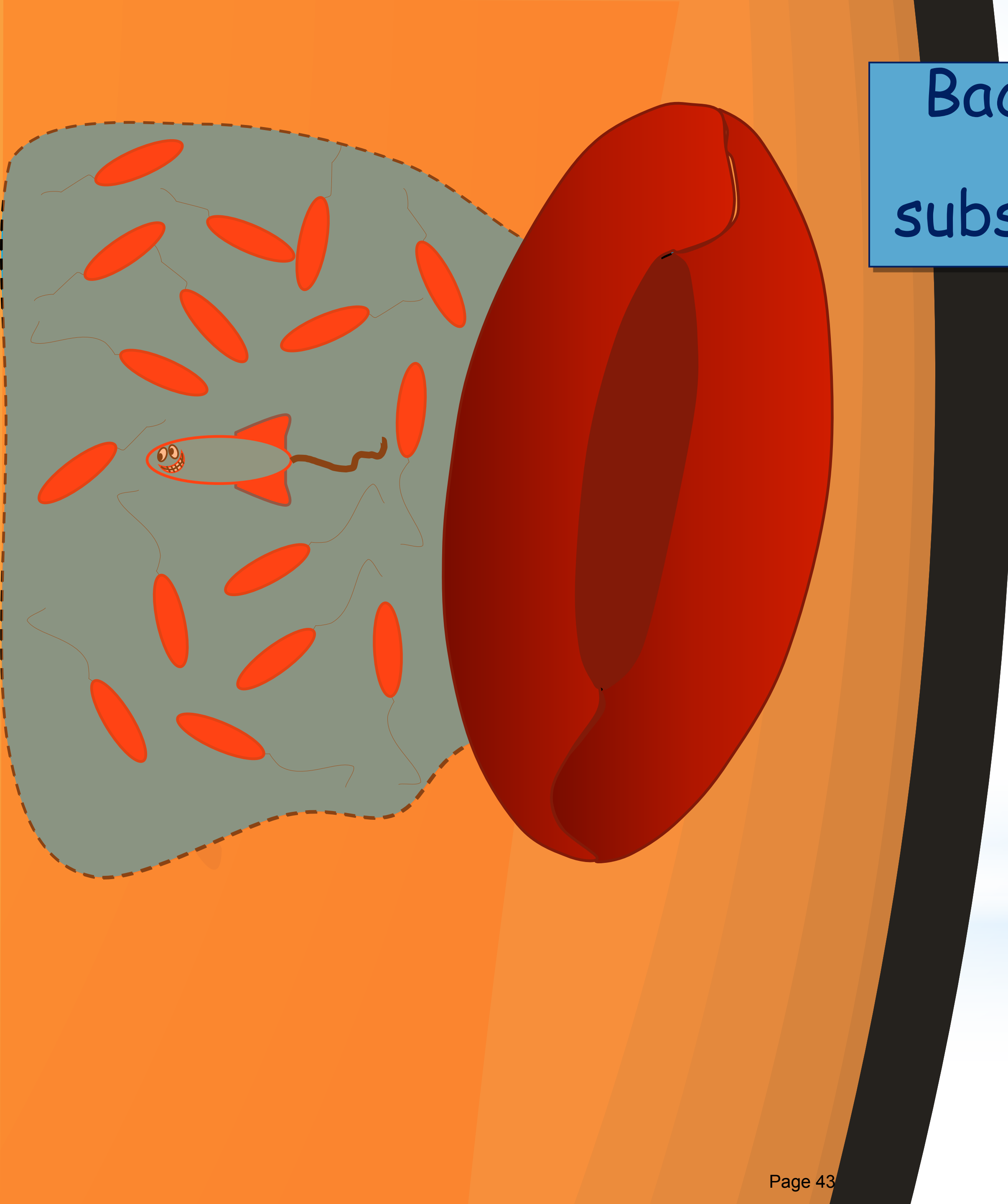


Droplets impact citrus leaves and fruit at wind speeds > 18mph

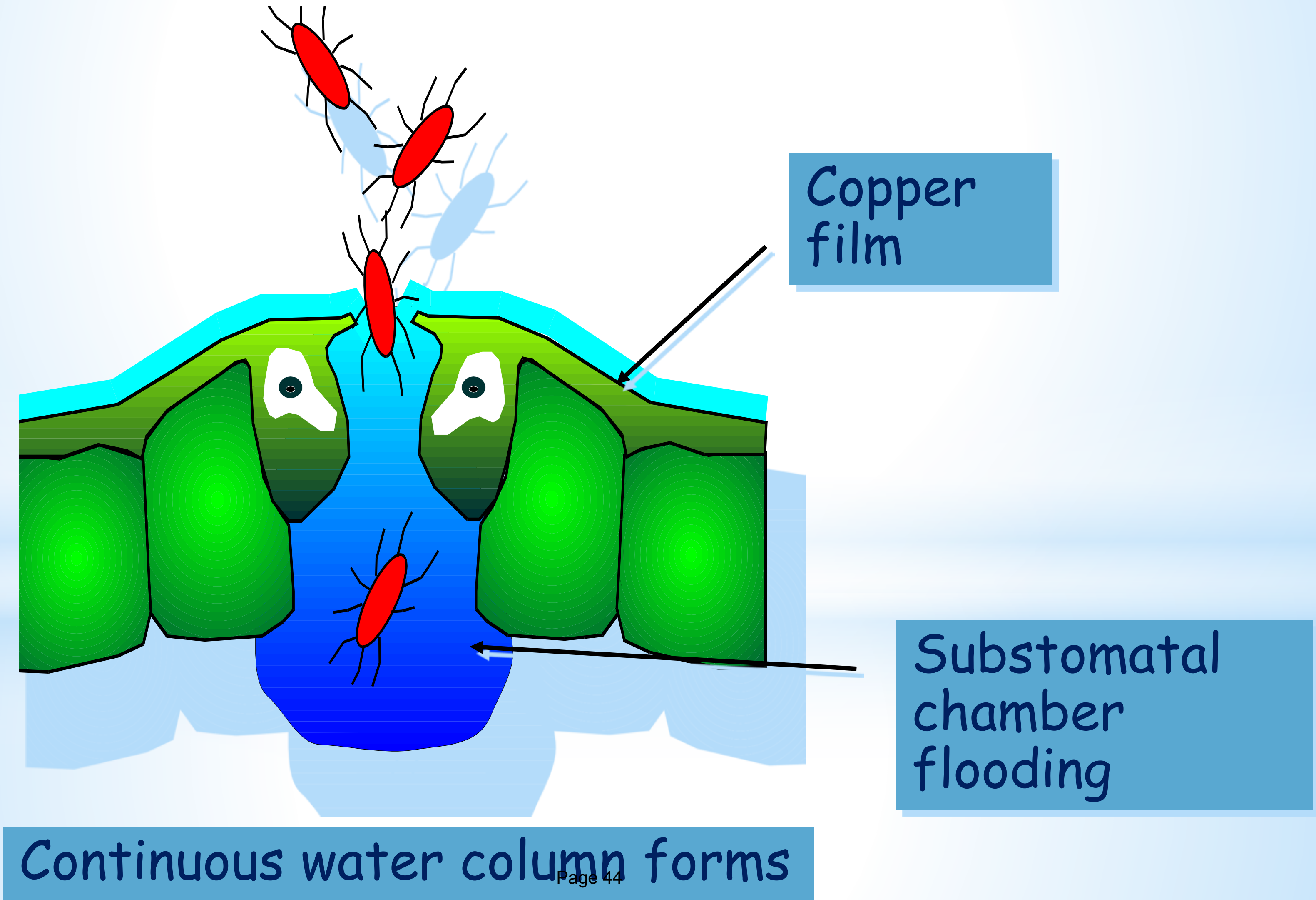



Droplets forced into
substomatal chamber
via water congestion

Bacteria reproduce rapidly in substomatal chamber



Copper film cannot protect entry points when the droplets exceed 18 mph



Stomatal infections on copper-treated grapefruit leaves and fruit after tropical storm in 2008

Florida situation after canker eradication ended in 2006

* Citrus canker caused by *Xanthomonas citri* subsp. *citri* (Xcc) is a serious bacterial disease of all citrus cultivars, grapefruit is most susceptible
* Severe infections cause defoliation, blemished fruit, premature fruit drop, twig dieback, and general tree decline
* Grapefruit is the most important fresh citrus variety with a value of $135 mil in 2011-12 compared to $174 mil in 2005-06
* 22% decrease in crop value due to loss of eligibility for export markets that do not have canker (e.g. EU) and diversion of fruit to juice processing
* Copper is the only bactericide registered for control of canker on Florida citrus
* Copper sprays are recommended season long (March to October) at 21 day intervals to protect the fruit (10-11 sprays)

Leaf, stem and fruit lesions on grapefruit

Concerns for use of Copper as the only registered bactericide

* Copper is the only effective bactericide, no new products have been registered for canker control
* <u>Disadvantages</u>: **induction of copper resistance (CuR) in Xcc populations** and accumulation in citrus soils with potential phytotoxic and adverse environmental effects
* Target for Cu entering citrus agro-ecosystems is 12.5 lb metallic/acre/season
* Season long protection of grapefruit increases risk for Cu burn that may render fruit unsuitable for fresh market
* Florida grapefruit industry has been in a **non-routine emergency situation** since eradication ended in 2006
* Section 18 for FireWall™ was approved for the 2013-14 and 2014-15 crop seasons

Advantages of FireWall™ 50 WP in Florida grapefruit

* Streptomycin is locally systemic in leaves and fruit and has post penetration activity that Cu does not
* 4 years of streptomycin resistance (SmR) risk assessment in a Florida grapefruit grove detected <u>No SmR</u>
* Recommended for management of CuR and Cu burn:

  Mix FireWall™ with a reduced rate of Cu in 2 sprays per season
* Less Cu in sprays reduces the yearly input

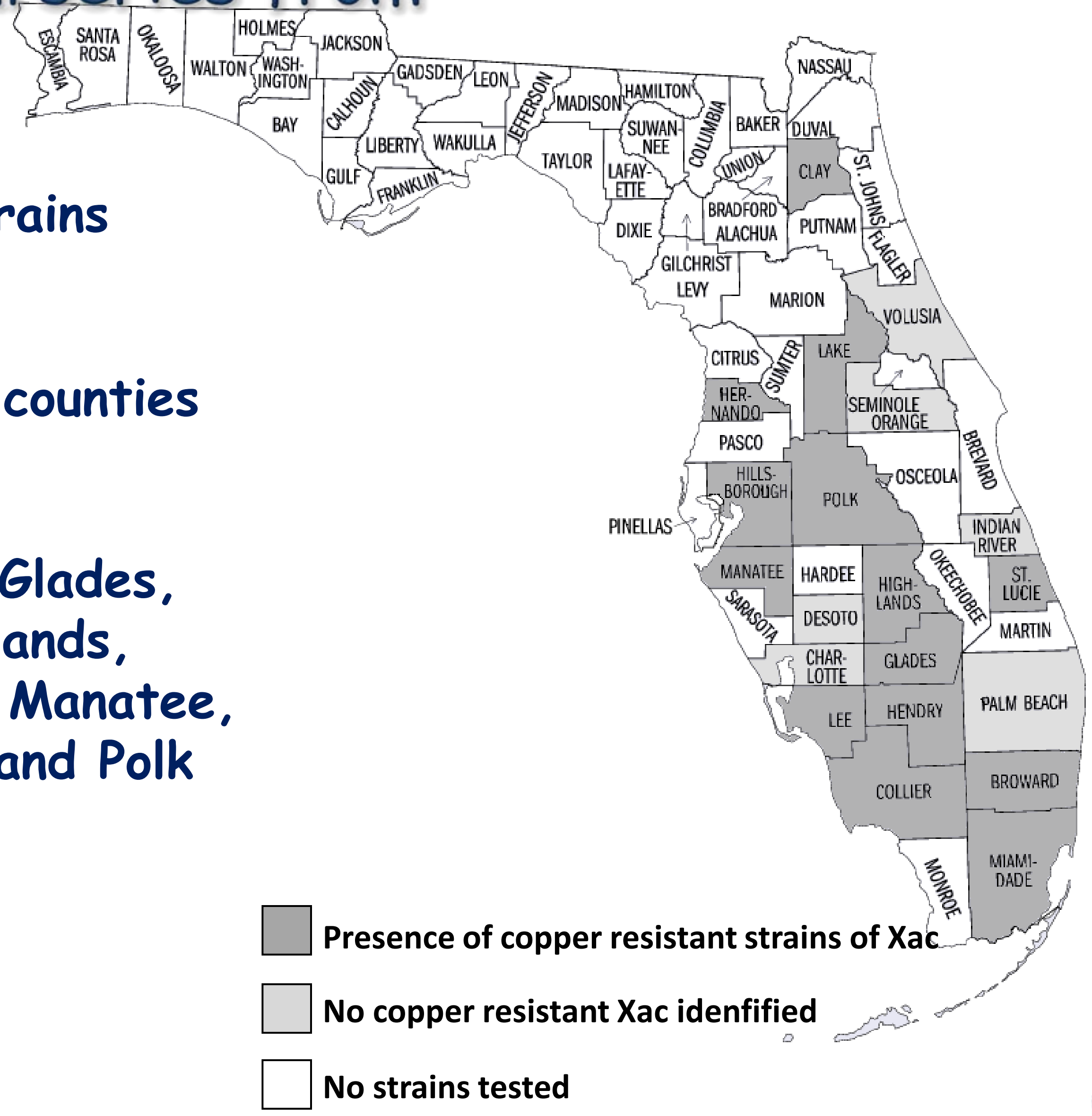
One advantage of tank mixing FireWall™ is to lower metallic Cu in sprays later in the season

4 year assessment of CuR and SmR risk in Florida and Brazil

* Plasmid-borne CuR genes in Xcc have been identified in several sites in Argentina where long-term Cu use in citrus
* CuR genes in Xcc are highly related to those in *Xanthomonas* strains from other crops (eg. tomato): implicates horizontal transfer of plasmids as source of risk
* Survey for CuR strains of Xcc in Florida and Brazil and *Xanthomonas alfalfae* subsp. *citrumelonis* (Xac), the cause of citrus bacterial spot (CBS) in Florida
* Xcc: No CuR detected in Florida and Brazil
* Xac: CuR strains widespread in Florida citrus nurseries
* SmR in Florida: none detected in Xcc or phyllosphere bacteria isolated after 11 sprays per season for 3 yr

Survey for CuR strains of Xac in Florida citrus nurseries from 1999-2009

- ✓ 31 of 54 (57%) Xac strains screened were Cu^R
- ✓Present in 14 of the 20 counties surveyed
- ✓ Broward, Clay, Collier, Glades, Hendry, Hernando, Highlands, Hillsborough, Lake, Lee, Manatee, Miami-Dade, St. Lucie, and Polk



FireWall™ attributes for canker outweigh concern for antibiotic risk in non-targets

* <u>Stewardship</u>: Mixing of FireWall™ with Cu increases canker control and may reduce risk of Xcc resistance to each MOA
* <u>Environmental concerns</u>: Reduces total Cu input to avoid exceeding metallic Cu limit per season (12.5 lbs)
* <u>Crop value</u>: Lower metallic in July-August sprays reduces risk of Cu burn that affects marketability


Thanks for your attention!

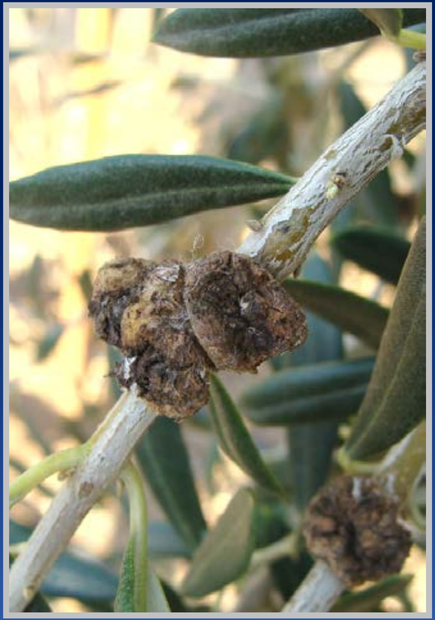
Bacterial diseases of tree crops in California and the need for copper alternatives for their management

Bacterial diseases of tree crops in California and the need for copper alternatives for their management
- Almond, olive, walnut, pome, and others -

James E. Adaskaveg, Professor
Dept. of Plant Pathology and Microbiology
University of California, Riverside

Major foliar bacterial diseases of tree crops in California

Walnut blight - *Xanthomonas arboricola* pv. *juglandis*



Olive knot
Pseudomonas savastanoi pv. *savastanoi*

Fire blight - *Erwinia amylovora*

Cherry blossom and leaf blast, bacterial canker – *Pseudomonas syringae* pv. *syringae*

Bacterial spot of almond
Xanthomonas arboricola pv. *pruni*
(a newly emerging disease)

2010-2011 Agricultural statistics for walnuts, olives, and almonds in California

Crop	Quantity harvested	Total value ($)
Almonds	1 million tons	3.9 billion
Walnuts	461,000 tons	1.3 billion
Sweet cherries	85,000 tons	220 million
Pome fruits	400,000 tons	160 million
Olives	71,200 -206,000 tons*	53 -140 million

Values for olives are for 2010 and 2011. Environmental factors account for variability in olive yield.

Almonds are 3rd among top commodities in CA (after dairy and grapes).

Walnuts are 9th among top commodities in CA.

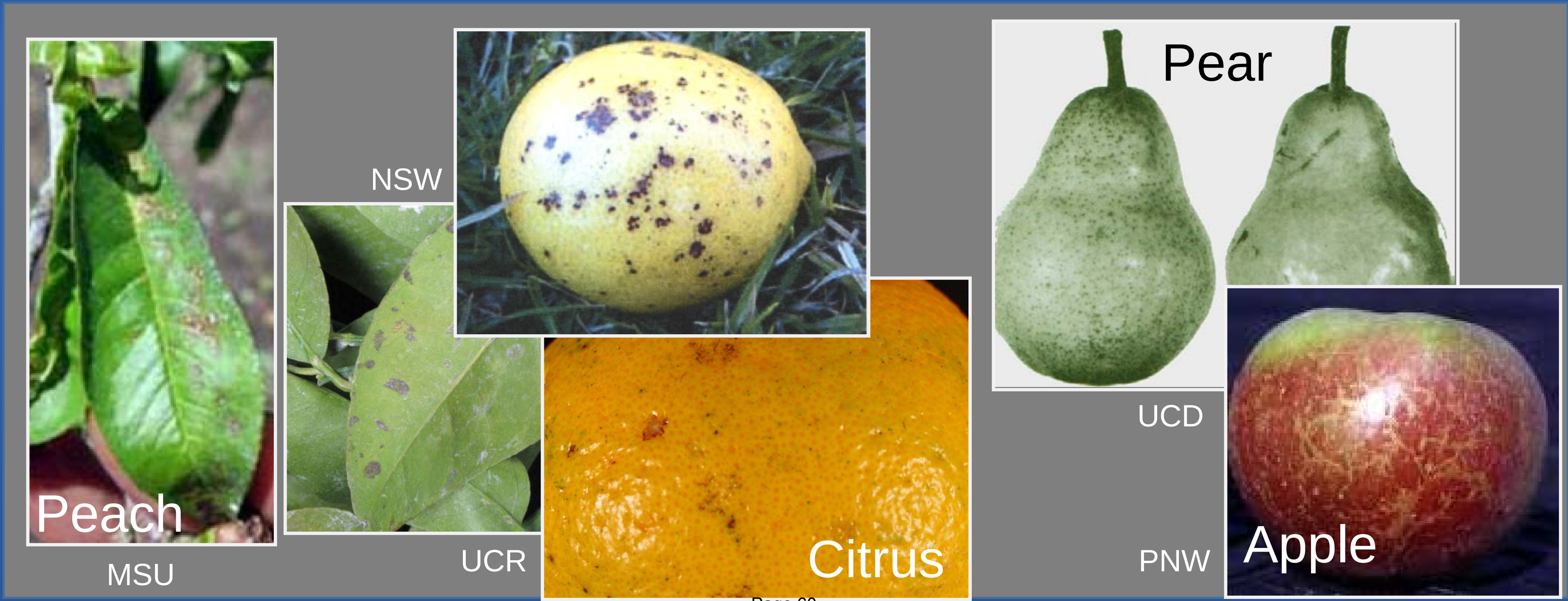
California produces >99% of walnuts, olives, and almonds in the US.

Copper use in agriculture

- Copper products are among the oldest pesticides used in agriculture against fungi *and* bacteria – first used in the 1880s for grape downy mildew control.
- Evolved from soluble forms to insoluble, “fixed” compounds, to new formulations with polymers have resulted in reduced rates.
- Copper is used as a protectant, there is no post-infection activity (must be applied prior to disease).
- Persistence provides residual effect.
- Generally safe to plants, essential micronutrient, compatible with other chemicals, and economical.

Disadvantages of copper use

- Repeated applications with copper may cause phytotoxicity.
  - Leaves: spots, irregular interveinal necrosis
  - Fruit: spots; russeting of pears and apples
- Does not degrade – accumulation in the environment
- Resistance in many bacterial pathogens, new resistance likely to occur – no effective alternatives.



Copper sensitivity levels in bacterial pathogens of tree crops in California

Surveys 2009-2013

Disease	Pathogen (Strains from California)	Crop	Copper Sensitivity (ppm)*	Years of Cu Usage
Bacterial spot**	*Xanthomonas arboricola* pv. *pruni*	Almond	<10 ppm	0
Blast/Canker	*Pseudomonas syringae* pv. *syringae*	Cherry	30-40 ppm	50
Olive knot	*Pseudomonas savastanoi* pv. *savastanoi*	Olive	10-15 ppm (50 ppm)	40
Fire blight	*Erwinia amylovora*	Apple/Pear	<10 ppm	<5
Walnut blight	*Xanthomonas arboricola* pv. *juglandis*	Walnut	150-200 ppm	50

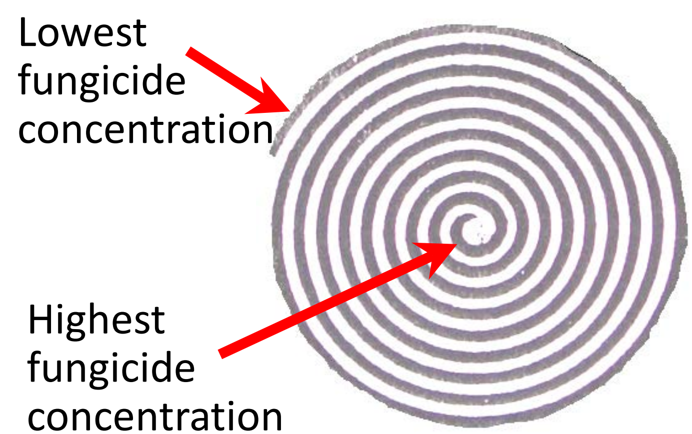
*- Growth at <10 ppm is considered Cu-sensitive.

** - Newly introduced into California.

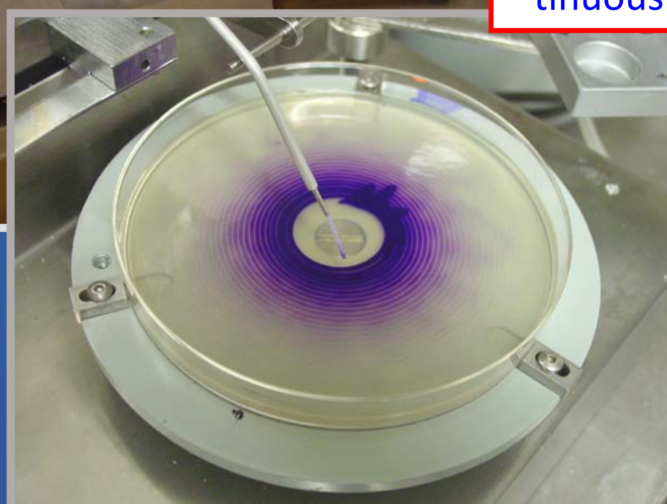
Spiral Gradient Dilution Assay

Growth response of fungal and bacterial plant pathogens to concentration gradients of toxicants

Spiral plater



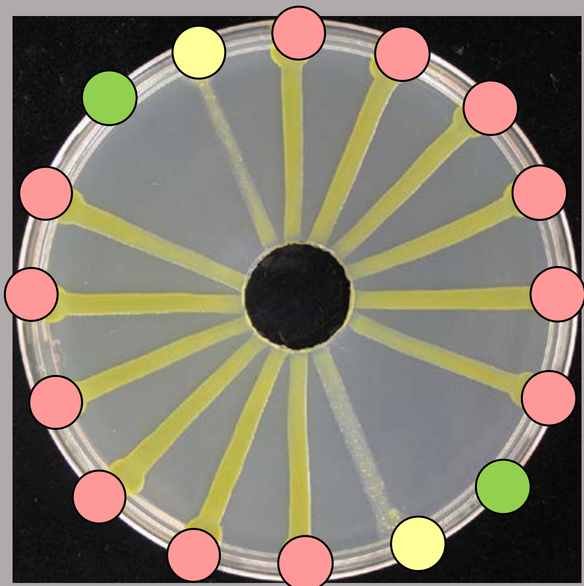
After a 2-4 h incubation period a continuous exponential gradient is formed.



Visualization of concentration gradient using dye

Sensitivity tests with Cu, mancozeb, and Cu-mancozeb for strains of *X. arboricola pv. juglandis*

Order of isolates is the same for the three plates.

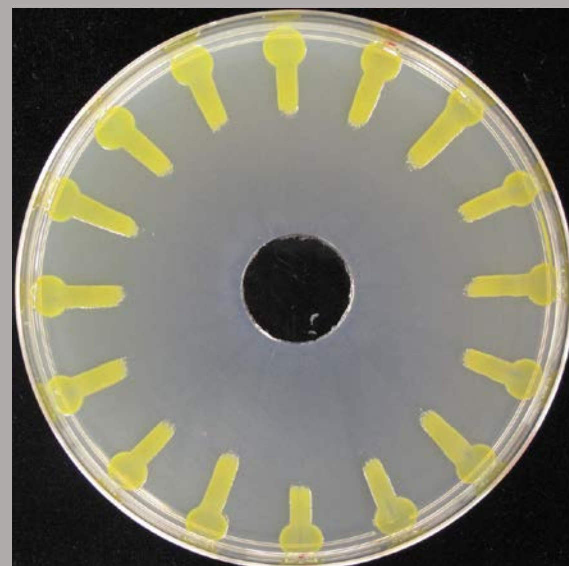


50 ppm copper

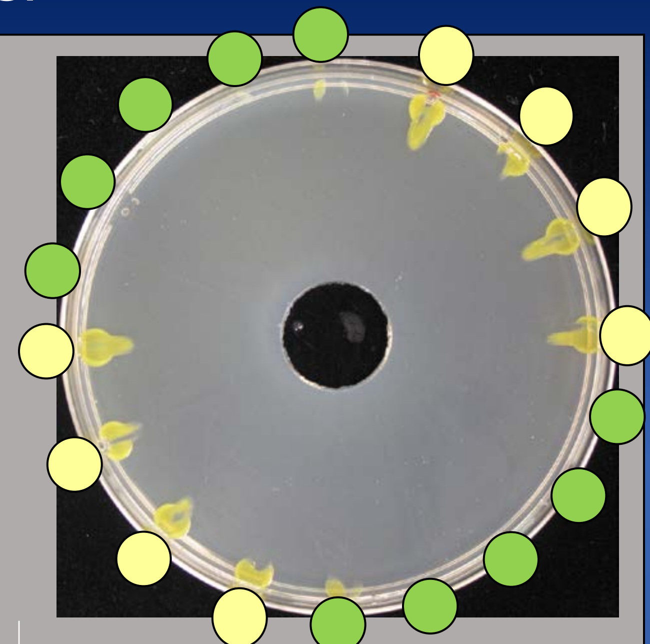
Cu-resistant isolates

Cu moderate-resistant

Cu-sensitive



Mancozeb gradient 0.4 - 40 ppm



Cu 50 ppm + mancozeb gradient 0.04 - 4 ppm

Cu-Mze less sensitive

Cu-Mze sensitive

Cu-resistant isolates are not inhibited by 50 ppm copper.

Mancozeb has some antibacterial activity (MIC 0.5-5 ppm).

Some Cu-resistant isolates are inhibited less by Cu-mancozeb.

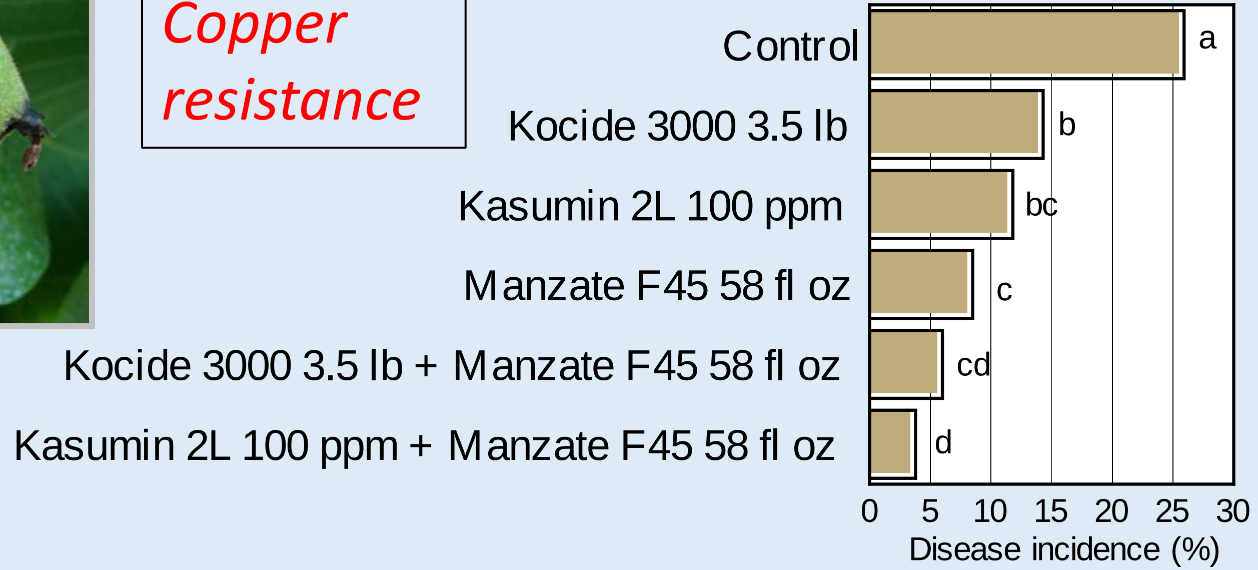
Pesticide Resistance Management

The most effective way to combat pesticide resistance is to mix or alternate compounds with different modes of action. If possible, at least one rotational mix partner should be a multi-site material (such as Cu).

- Avoid repeated application of the same MOA
- Rotation or mixtures of different MOA
- Reduced exposure period during active growth of the pathogen
- Integration of chemical and non-chemical approaches
- Maintain labeled dose rates

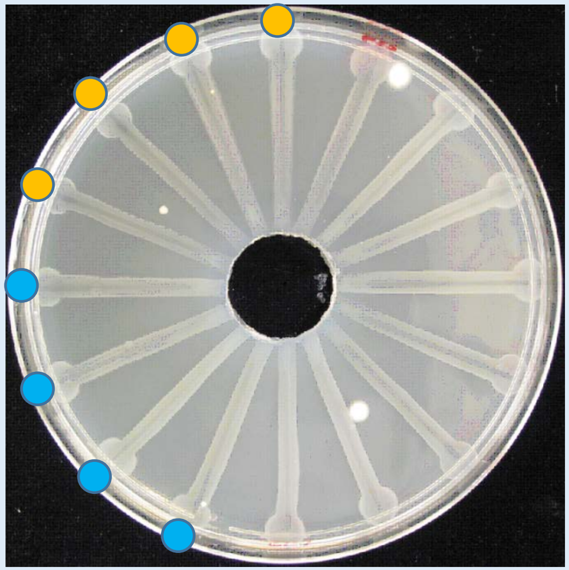
Efficacy of selected treatments for walnut blight on cv. Vina walnut under natural rainfall conditions - Yuba-Sutter Co. CA -

Copper resistance

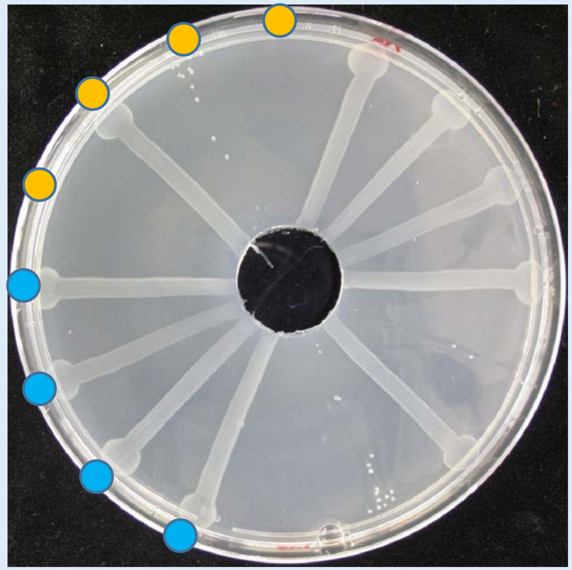


Air-blast spray applications were done on 3-24 and 3-29 (catkin emergence), 4-6, 4-15, 4-22, 4-29, and 5-11-10. Incidence of disease is based on 100 fruit evaluated for each 4 single-tree replications.

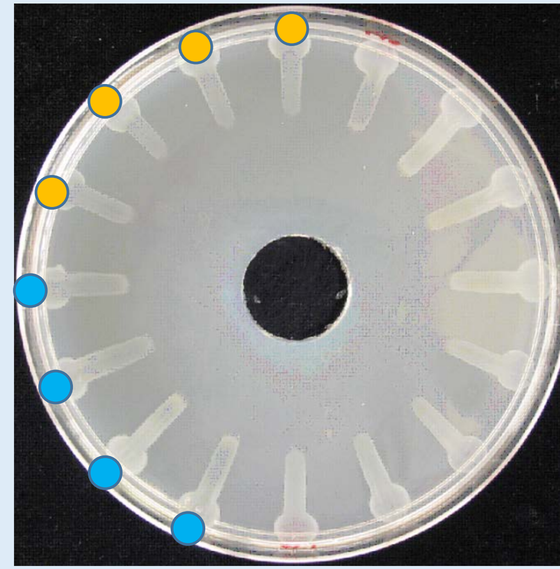
Copper and kasugamycin sensitivity of *Pseudomonas* spp. in California



Control



Cu 50 ppm MCE



Gradient of kasugamycin (8 – 80 ppm)

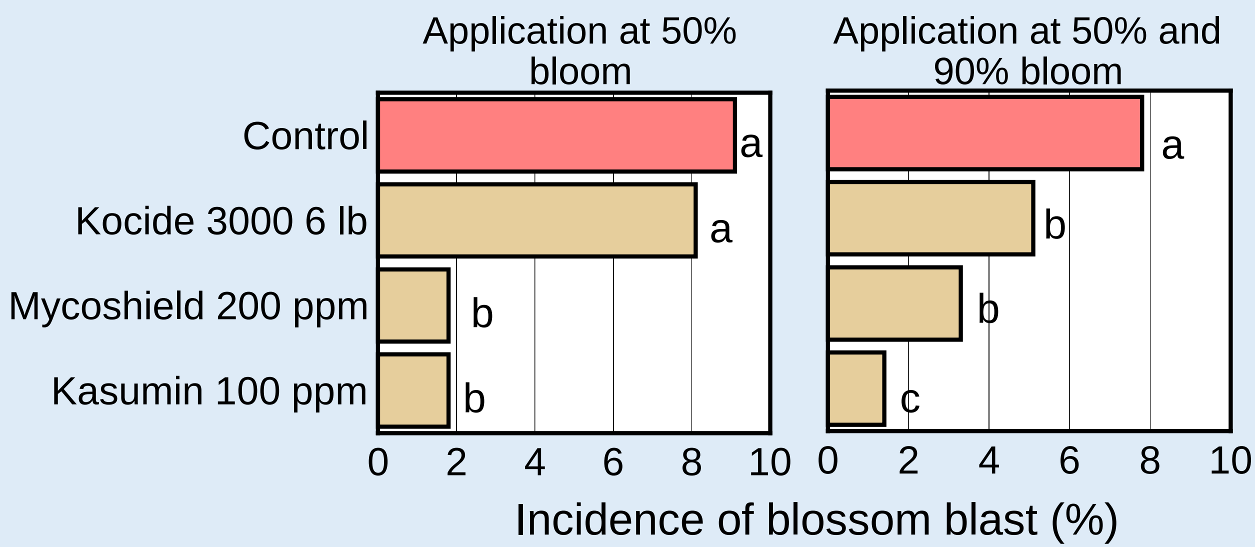
● ***P. savastanoi*** **pv.** ***savastanoi*** **- olive**

● ***P. syringae*** **pv.** ***syringae*** **- cherry**

- Some strains of *P. savastanoi* pv. *savastanoi* have reduced sensitivity to copper – field applications are effective.
- Most strains of *P. syringae* pv. *syringae* are resistant to copper – field applications not effective.

New treatments for the management of bacterial blast - Air-blast spray applications of non-inoculated trees -

Natural incidence studies



Applications were made using an airblast sprayer at 100 gal/A on 3-9 and 3-16-11. Blossoms were evaluated on 3-30-11.

Control

Kasugamycin

Selected bacterial diseases of tree crops in California
- Available treatments for integration into management programs -

Treatment	Walnut blight	Olive knot	Almond bacterial spot
Copper products	Effective but widespread resistance	Effective, but resistance locally	Effective, no resistance to date
Copper-Mancozeb	Reduced sensitivity at some locations	---	Not registered for this disease
Gallex	---	Labor intensive	---
Biologicals (Actinovate, Regalia, etc.)	Effective only under low disease pressure, inconsistent		
Phosphonates	Efficacy low, inconsistent	---	Efficacy low, inconsistent
Antibiotics (streptomycin, oxytetracycline, kasugamycin)	**Effective but not registered**		

--- = not registered

In screening of many compounds, no highly effective alternatives to copper except antibiotics have been identified.

Selected bacterial diseases of tree crops in California
- Available treatments for integration into management programs -

Treatment	Fire blight	BB/BC
Copper products	Effective but potential phytotoxicity	Effective, but resistance locally
Copper-Mancozeb	Effective, but potential phytotoxicity & label restrictions	---
Biologicals (Actinovate, Regalia, Blossom Protect, etc.)	Effective only under low disease pressure, inconsistent	
Phosphonates	Efficacy low, inconsistent	---
Antibiotics (streptomycin, oxytetracycline, kasugamycin)	**Effective but streptomycin resistance, need kasugamycin, and other alternatives (antimicrobial peptides?)**	**Effective but not registered**

BB/BC – Stone fruit bacterial blast and canker. --- = not registered.

- ***Mechanisms of streptomycin resistance in CA (plasmid or chromosomal) appear to confer fitness penalties in the pathogen. Thus, strep can still be a component in a rotation program.***
- ***In screening of many compounds, no highly effective alternatives to streptomycin except other antibiotics have been identified.***

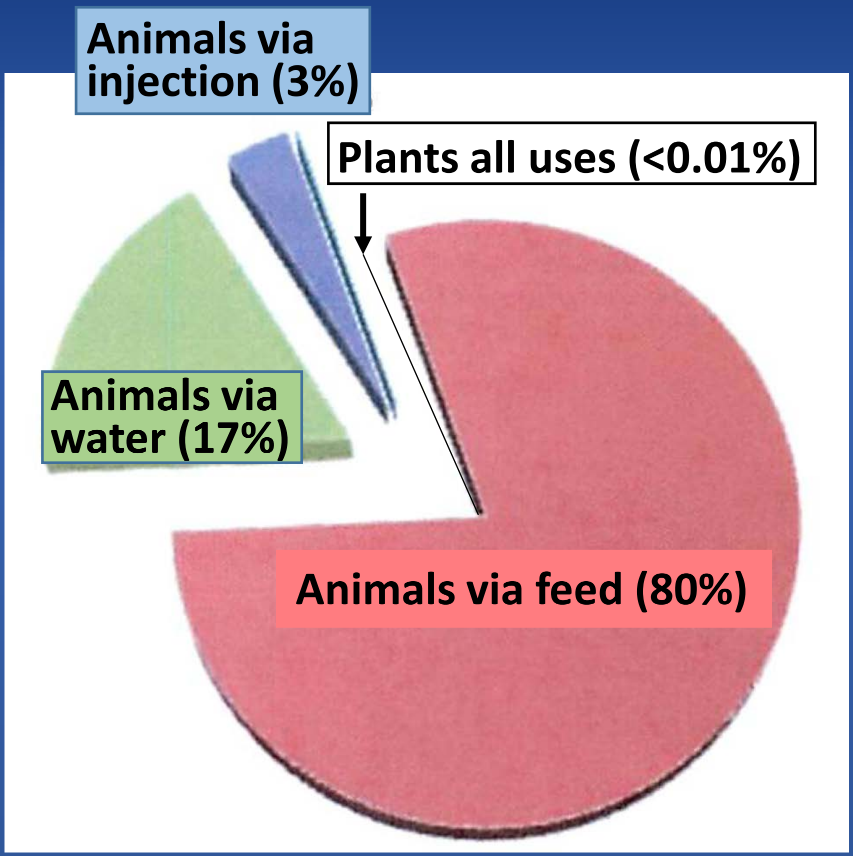
Kasugamycin as a copper alternative in managing bacterial diseases of tree crops in California

- Kasugamycin has high activity against diseases caused by *Erwinia* and *Pseudomonas* species and moderate activity against *Xanthomonas* species.
- Usage patterns have been developed – mixtures, rotations, limits on the number of applications, total product/season, PHI, MRLs, baseline sensitivities.
- Kasugamycin is not used in animal and human medicine and unlikely will be (low activity against mammalian pathogenic bacteria was shown).

Use of antibiotics in plant agriculture

V.O. Stockwell & B. Duffy Rev. sci. tech. Off. int. Epiz., 2012, 31 (1), 199-210

- Antibiotic use in agriculture in the United States 2009:
  - Animal agriculture: 13,100,000 **tonnes** a.i.
  - Plant agriculture: 16,465 **kg** a.i. (<0.01%)
- Regulations reduce direct human exposure to antibiotics used in plant agriculture.
  - Examples - re-entry times, preharvest intervals, MRLs, etc.
- Antibiotics are non-persistent on plant surfaces and break down rapidly.
- A direct link between antibiotic sprays on plants and antibiotic resistance in clinical bacteria has not been demonstrated.



Antibiotic use in plant vs. animal agriculture

- With limited total use of antibiotics in plant agriculture, regulations need to be separated from animal agriculture
  - Residue limits and risk – Toxicity and resistance development is dependent on dosage (*levels below functional thresholds are irrelevant*).
  - Registration times of 8-10 years are not reasonable
  - Older antibiotics (streptomycin and oxytetracycline) could have new uses on various crops (e.g., almonds, cherry, walnut).
- Human pathogens are not common phyllosphere colonizers, the probability of acquisition of antibiotic resistance genes from resident bacteria on plants is minimal.
- Development and registration of new products such as antimicrobial peptides need to be stream-lined under biopesticides in an effort to provide rotational products with different MOAs.

Conclusion: Antibiotic use in plant agriculture

- There is a great need for managing bacterial diseases in ***plant*** agriculture.
- Find solutions for new integrated management programs that reduce risks of any individual component.

Host

Genetically modified plants

Can be developed and Integrated, but they are not the final answer and have their own issues (e.g., consumer acceptance)

New modes of copper activation

Mancozeb is effective, but when used exclusively shifts in sensitivity are occurring with eventual resistance

Management

New and old antibiotics

Streptomycin, oxytetracycline, & kasugamycin have different modes of action and should be available on new crops and used in rotations.

Pathogen

New products

Antimicrobial peptides, biologicals, and others need to be explored, developed and registered as an integral component

Environment

Thank you

Management of Zebra Chip of Potato with Alternative Chemistries

Understanding the ABC's with Antibiotics in Crops

Management of Zebra Chip of Potato with Alternative Chemistries

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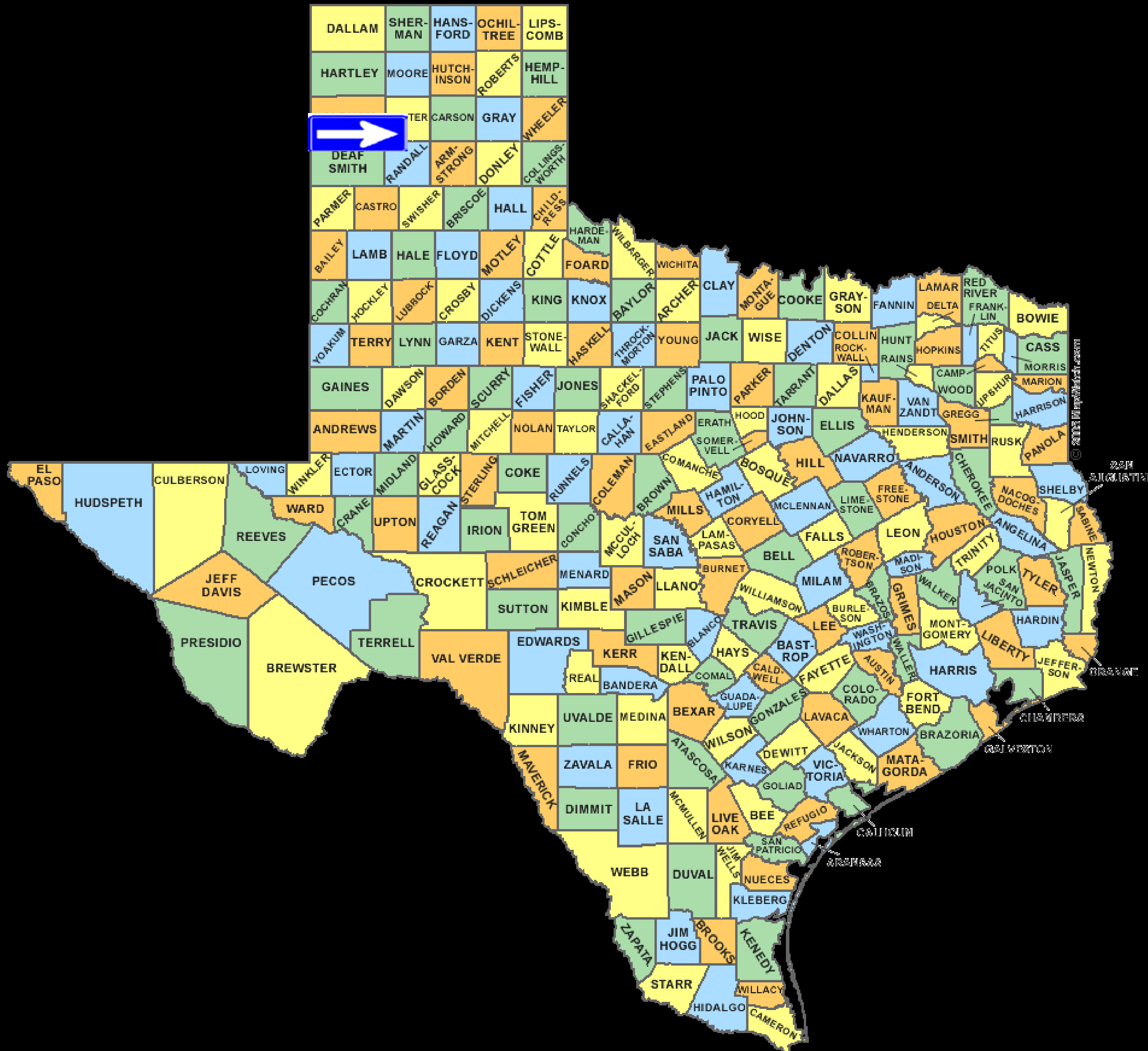
September 11, 2014



DALLAM
SHER-MAN
HANS-FORD
OCHIL-TREE
LIPS-COMB
HARTLEY
MOORE
HUTCH-INSON
ROBERTS
HEMP-HILL
CARSON
GRAY
WHEELER
DEAF SMITH
RANDALL
ARM-STRONG
DONLEY
COLLINGS-WORTH
PARMER
CASTRO
SWISHER
BRISCOE
HALL
CHILD-RESS
HARDE-MAN
WILBARGER
WICHITA
CLAY
MONTA-GUE
COOKE
GRAY-SON
FANNIN
LAMAR
RED RIVER
BOWIE
BAILEY
LAMB
HALE
FLOYD
MOTLEY
COTTLE
FOARD
COCHRAN
HOCKLEY
LUBBOCK
CROSBY
DICKENS
KING
KNOX
BAYLOR
ARCHER
DELTA
FRANK-LIN
CASS
YOAKUM
TERRY
LYNN
GARZA
KENT
STONE-WALL
HASKELL
THROCK-MORTON
YOUNG
JACK
WISE
DENTON
COLLIN
ROCK-WALL
HUNT
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DAWSON
BORDEN
SCURRY
FISHER
JONES
SHACKEL-FORD
STEPHENS
PALO PINTO
PARKER
TARRANT
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KAUF-MAN
VAN ZANDT
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MITCHELL
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TAYLOR
CALLA-HAN
EASTLAND
ERATH
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SOMER-VELL
JOHN-SON
ELLIS
HENDERSON
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RUSK
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EL PASO
HUDSPETH
CULBERSON
LOVING
WINKLER
ECTOR
MIDLAND
GLASS-COCK
STERLING
COKE
RUNNELS
COLEMAN
BROWN
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ANDERSON
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SHELBY
SAN AUGUSTINE
REEVES
WARD
CRANE
UPTON
REAGAN
IRION
TOM GREEN
CONCHO
MCCUL-LOCH
MILLS
HAMIL-TON
CORYELL
MCLENNAN
LIME-STONE
FREE-STONE
NACOG-DOCHES
SABINE
JEFF DAVIS
PECOS
CROCKETT
SCHLEICHER
MENARD
SAN SABA
LAM-PASAS
BELL
FALLS
LEON
HOUSTON
TRINITY
ANGELINA
NEWTON
JASPER
ROBER-TSON
MADI-SON
POLK
TYLER
MASON
LLANO
BURNET
WILLIAMSON
MILAM
BRAZOS
GRIMES
WALKER
SAN JACINTO
SUTTON
KIMBLE
GILLESPIE
BLANCO
TRAVIS
LEE
BURLE-SON
MONT-GOMERY
LIBERTY
HARDIN
PRESIDIO
TERRELL
VAL VERDE
EDWARDS
KERR
KEN-DALL
HAYS
BAST-ROP
WASH-INGTON
WALLER
HARRIS
JEFFER-SON
ORANGE
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CALD-WELL
FAYETTE
AUSTIN
COLO-RADO
FORT BEND
CHAMBERS
KINNEY
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BEXAR
GUADA-LUPE
GONZALES
LAVACA
WHARTON
BRAZORIA
GALVESTON
WILSON
DEWITT
JACKSON
MATA-GORDA
ZAVALA
FRIO
ATASCOSA
KARNES
VIC-TORIA
GOLIAD
MAVERICK
DIMMIT
LA SALLE
MCMULLEN
LIVE OAK
BEE
REFUGIO
CALHOUN
SAN PATRICIO
ARANSAS
WEBB
DUVAL
JIM WELLS
NUECES
KLEBERG
ZAPATA
JIM HOGG
BROOKS
KENEDY
STARR
HIDALGO
WILLACY
CAMERON

Publications

January 2009, Volume 93, Number 1
Page 108
DOI: 10.1094/PDIS-93-1-0108C

Disease Notes

First Report of the Detection of '*Candidatus* Liberibacter' Species in Zebra Chip Disease-Infected Potato Plants in the United States

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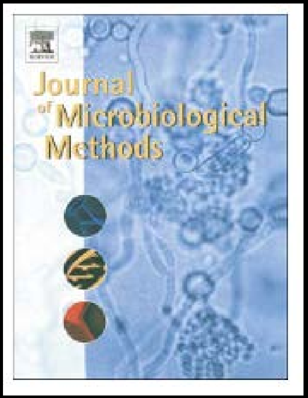
January 2009

Publications

Multiplex real-time PCR for detection, identification and quantification of '*Candidatus* Liberibacter solanacearum' in potato plants with zebra chip

Wenbin Li, Jorge A. Abad, Ronald D. French-Monar, John Rascoe, Aimin Wen, Neil C. Gudmestad, Gary A. Secor, Ing-Ming Lee, Yongping Duan, Laurene Levy



May 2009

April 2010, Volume 94, Number 4
Page 481
DOI: 10.1094/PDIS-94-4-0481A

Disease Notes

First Report of "*Candidatus* Liberibacter solanacearum" on Field Tomatoes in the United States

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April 2010

Zebra Chip on potato (2007)

Zebra Chip on potato (2007)

In 2013, untreated plots in South Texas used for potato psyllid monitoring had 70% incidence of ZC. Commercial potato fields had up to 5% incidence of ZC.

August 15, 2008

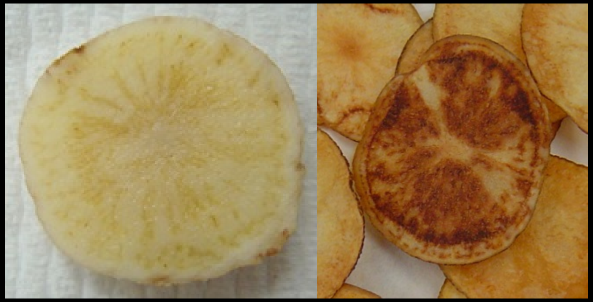
August 15, 2008 (Tomato)

Zebra Chip (ZC)

- Caused by *Candidatus* liberibacter solanacearum.
- Phloem-limited bacterium.
- The bacterium has been determined to be transmitted primarily by the potato psyllid, *Bactericera cockerelli.*

- Sugar levels in the potato tubers are altered which results in caramelization when sliced and fried.
- Potato chip processors reject infected potatoes due to the unacceptable dark stripes and the off-taste of the fried chips.

Zebra Chip on Potato



Managing Zebra Chip

- Currently, the only effective approach to managing ZC is to target the potato psyllid (using insecticides.

Can we target the pathogen, symptom expression, or plant health?

- By suppressing pathogen development, establishment, delaying the onset of symptoms, or promote increased tuber production, the potential for reduced losses in potato tuber production and symptoms could complement strategies such as insect/vector management

Some insectcides used in Texas in commercial potato production (2009)

- Admire Pro (imidacloprid)
- Platinum (thiometoxam)
- Movento (spirotetramat)
- Agri-Mek (abamectin)
- Fulfill (pymetrozine)
- Knack (IGR)
- Venom (dinotefuran)
- Radiant (spinetoram)
- Oberon (LBI)

Thimet (phorate)
Beleaf (flonicamid)
Baythroid (cyfluthrin)
Leverage
Thiodan
Asana (esfenvalerate)
Rimon (IGR)

Visit: https://www.fritolayag.com/public/zc/Goolsby_John_ZC2009.pdf

Benevia, Verimark, y Torac (2013 and/or 2014)

Alternatives to insecticides?

- 1)Target the pathogen with an **antibiotic/bactericide**.
- 2)Trigger a plant defense response similar to a systemic acquired resistance (SAR) or induced systemic resistance (ISR).
- 3)Utilize nutrient supplements to offset the effects of ZC by supplying key nutrients (micro and macro) lost or unavailable to the plant due to insect or pathogen related stress.
- 4)Use bactericides, systemic resistance, nutrients in an integrated approach with/without insecticides.

Some antibiotics for beef cattle (2008)

- Amoxicillin, Ampicillin, Ceftiofur sodium, Ceftiofur hydrochloride, Enrofloxacin, Erythromycin, Florfenicol, Oxytetracyclin, Penicillin, Sulfamethazine calf, Sulfamethazine cow, Sulfadimethoxine, Tilmicosin, andTylosin. http://pubs.ext.vt.edu/400/400-008/Table_1.html

Some antibiotic drug classes used in cattle for treating pneumonia or bovine respiratory disease (2008)

- Aminoglycoside, Cephalosporin, Floroquinolone, Macrolide, Phenicol, Tetracycline

California Cattlemen's Magazine (September 2008)
http://www.vetmed.ucdavis.edu/vetext/local-assets/pdfs/pdfs_beef/cca0809-pneumonia-treat.pdf

Some antibiotics for plant use?

- 1) Oxytetracycline hydrochloride for bacterial diseases of non-crop bearing trees: oak, elm, sycamore, palm. (Injection only)
- 2) Oxytetracycline hydrochloride for crop bearing trees: apples and pears (fire blight, *Erwinia*), peaches and nectarines (bacterial spot, *Xanthomonas*).
- 3) Oxytetracycline calcium for crop bearing trees: apples and pears (fire blight), peaches and nectarines (bacterial spot, *Xanthomonas*).

Some antibiotics for plant use?

- 4)Streptomycin sulfate: Apples and Pears (Fire Blight), celery (blight, FL), Chrysanthemums and Hydrangea (wilt), Dieffenbachia (stem rot), Ornamentals (*Pseudomonas*, *Xanthomonas*), Tomatoes and Peppers (spot and/or speck), Roses and Lonicera (crown gall), *Tobacco (Blue Mold),* and Potato (seed treatment, *Erwinia,* soft rot/blackleg)

Springlake, TX 2009 Trial

Field Trial (Treatments)

1) Untreated
2) Actigard
3) Streptomycin sulfate (Agri-Mycin 17)
4) P-016B
5) K-Phite (Low)
6) K-Phite (High)
7) Actinovate
8) Heads-Up
9) KPX-B1 (Low)
10) KPX-B1 (High)
11) KPX-B2 (Low)
12) PPX-B2 (High)
13) Oxytetracycline calcium (Mycoshield)
14) Phostrol
15) Phostrol + Streptomycin sulfate

Methodology

- Experiments arranged in a Randomized Complete Block Design (RCBD).
- For each treatment, 4 replications of 20 plants each, for a total of 80 plants per treatment.
- Plots located in ~Springlake, TX
- Planted: April 14, 2009.

Methodology

- Treatments were sprayed weekly for a total of 8 spray schedules.
- Sprays began with plant establishment (two weeks after planting)
- Sprays ended June 24, 2009.
- Vines Killed: August 11, 2009 (117 DAP)
- Harvest: August 17, 2009 (123 DAP)

Total yield, total yield of U.S. No. 1, under 4 oz. and Culls/No.2 potatoes (Springlake, TX)

Treatment	Total Yield Cwt/A	U.S. No. 1 Cwt. Per Acre						
		Total Yield	4-6 oz	6-10 oz	10-18 oz	Over 18 oz	Under 4 oz.	Culls/ No.2
1-Untreated	311.5	235.2	108.7	76.7	49.9	0.0	68.5	7.7
2-Actigard	384.1	285.6	118.3	90.8	76.5	12.6	73.8	12.1
3-Agri-Mycin	398.1	309.8	123.4	103.6	82.8	0.0	69.7	18.6
4-P-016B	323.1	256.5	116.6	81.3	58.6	0.0	55.9	10.6
5-K-Phite(Low)	315.1	239.1	102.4	73.8	62.9	0.0	67.3	8.7
6-K-Phite(High)	353.1	261.4	114.2	83.7	63.4	0.0	83.0	8.7
7-Actinovate	310.5	251.0	104.1	79.9	67.0	0.0	51.8	7.7
8-Heads-Up	285.6	206.4	95.1	56.6	54.7	0.0	74.1	5.1
9-KPX-B1(Low)	341.7	268.9	111.8	91.2	65.8	0.0	66.1	6.8
10-KPX-B1(High)	217.6	166.3	73.6	50.1	42.6	0.0	40.9	10.4
11-KPX-B2(Low)	308.8	235.2	99.7	73.3	62.2	0.0	58.1	15.5
12-KPX-B2(High)	275.6	195.8	82.5	77.0	36.3	0.0	68.5	11.4
13-Mycoshield	289.9	212.7	86.4	78.4	47.9	0.0	62.2	15.0
14-Phostrol	325.5	232.3	119.1	63.4	49.9	0.0	77.2	16.0
15-Phost+AgriM	330.1	251.4	109.4	82.5	59.5	0.0	62.7	16.0
Average	318.0	240.5	104.4	77.5	58.7	0.8	65.3	11.4
L.S.D. (.05)	ns	73.7	ns	ns	ns	6.5	ns	ns

Total yield, total yield of U.S. No. 1 (Springlake, TX)

Treatment	Total Yield Cwt/A	U.S. No. 1 Cwt. Per Acre			
		Total Yield	4-6 oz	6-10 oz	10-18 oz
1-Untreated	311.5	235.2	108.7	76.7	49.9
2-Actigard	384.1	285.6	118.3	90.8	76.5
3-Agri-Mycin	398.1	309.8	123.4	103.6	82.8
4-P-016B	323.1	256.5	116.6	81.3	58.6
5-K-Phite(Low)	315.1	239.1	102.4	73.8	62.9
6-K-Phite(High)	353.1	261.4	114.2	83.7	63.4
7-Actinovate	310.5	251.0	104.1	79.9	67.0
8-Heads-Up	285.6	206.4	95.1	56.6	54.7
9-KPX-B1(Low)	341.7	268.9	111.8	91.2	65.8
10-KPX-B1(High)	217.6	166.3	73.6	50.1	42.6
11-KPX-B2(Low)	308.8	235.2	99.7	73.3	62.2
12-KPX-B2(High)	275.6	195.8	82.5	77.0	36.3
13-Mycoshield	289.9	212.7	86.4	78.4	47.9
14-Phostrol	325.5	232.3	119.1	63.4	49.9
15-Phost+AgriM	330.1	251.4	109.4	82.5	59.5
Average	318.0	240.5	104.4	77.5	58.7
L.S.D. (.05)	ns	73.7	ns	ns	ns

Conclusion

- The only treatment that was statistically higher than the untreated control (grower practice only) was Streptomycin sulfate (Agri-Mycin)

Springlake, TX **2010** Trial

Methodology

- Experiments arranged in a Randomized Complete Block Design (RCBD).
- For each treatment, **4 replications of 18 plants** each, for a total of 72 plants per treatment. (Note: one replication discarded)
- Plots located in ~Springlake, TX
- Seed Source: Russet Norkotah 223

Methodology (cont)

- Size of plots: 10’ 5”
- Spacing between hills: 9”
- Spacing between rows: 36”
- Number of rows per plot: 2
- Potatoes Planted March 30, 2010
- Potatoes Vines Killed August 16, 2010
- Harvested August 24, 2010

Methodology (cont)

- Treatments were sprayed at a 2-week interval starting May 28 for a total of **5 sprays.**
- Sprays ended July 15, 2010.

Field Trial (Treatments)

1) Untreated
2) Firewall (Streptomycin Sulfate)
3) Prophyt (Potassium Phosphite)
4) K-Phite (Salts of Phosphorous acid)
5) Keyplex (Yeast Extract)
6) Phostrol (Phosphite) + Fwal
7) Actigard (Acibenzolar S-methyl)
8) Fwal + Saver (Salicylic acid)
9) Firewall + SAver
10) K-Phite + Actigard
11) K-Phite + Firewall
12) K-Phite + SAver
13) Keyplex + SAver
14) Keyplex + Actigard
15) Renew (RenCs)

Springlake
Table 1a.

Total yield, total yield of U.S. No.1, under 4 ounce and culls/No.2 potatoes and general rating of 15 entries in the Chemical Treatment Trial grown near Springlake, Texas-2010.

Variety or Selection	Total Yield Cwt/A	U.S. No. 1 Cwt. Per Acre				Over 18 oz	Under 4 oz.	Culls/ No.2
		Total Yield	4-6 oz	6-10 oz	10-18 oz			
1-Untreated	143.2	103.5	78.7	22.8	2.1	0.0	39.7	0.0
2-Fwal	168.4	131.3	85.6	38.7	6.9	0.0	37.2	0.0
3-Proph	146.7	96.4	84.7	7.0	4.7	0.0	50.3	0.0
4-KPh	172.0	117.3	98.1	19.2	0.0	0.0	54.7	0.0
5-KPX	164.7	114.8	88.1	21.0	5.7	0.0	49.9	0.0
6-Phost + Fwal	144.7	101.8	90.2	7.3	4.4	0.0	42.9	0.0
7-Actgd	174.2	130.0	95.3	23.4	11.4	0.0	44.2	0.0
8-Fwall + SAv	143.9	108.0	75.7	32.3	0.0	0.0	36.0	0.0
9-Fwall + Acgd	168.7	141.2	97.7	41.5	2.0	0.0	27.5	0.0
10-KPh + Acgd	150.4	118.5	103.9	5.5	9.2	0.0	31.9	0.0
11-KPh + Fwal	120.5	94.3	69.7	22.9	1.8	0.0	26.2	0.0
12-KPh + SAv	114.6	90.8	86.3	4.5	0.0	0.0	23.7	0.0
13-KPX + Sav	82.5	59.2	42.7	11.8	4.7	0.0	23.4	0.0
14-KPX + Acgd	145.0	98.6	74.1	20.1	4.5	0.0	46.4	0.0
15-RenCs	128.3	98.4	69.5	25.9	3.0	0.0	29.9	0.0
Average	144.5	106.9	82.7	20.3	4.0	0.0	37.6	0.0
L.S.D. (.05)	ns	ns	ns	ns	ns	ns	ns	ns

[1] 1=very poor to 5= excellent

Total Yield (all tubers), Springlake, TX (2010)

Treatment	Yield (Cwt/A)	Significant
Firewall	**224.6***	***Significantly better than untreated**
Untreated	186.7	--------------------
Phostrol + Firewall	149.8**	**Significantly worse than untreated
K-Phite + Actigard	148.8**	**Significantly worse than untreated
K-Phite + Firewall	140.5**	**Significantly worse than untreated
K-Phite + SAver	138.3**	**Significantly worse than untreated
Keyplex + SAver	120.3**	**Significantly worse than untreated

L.S.D. (.05)= 32.6

Total Yield of US No.1 (4-18oz tubers), Springlake, TX 2010

Treatment	Yield (Cwt/A)	Significant
Firewall	**182.7***	***Significantly better than untreated**
Firewall + Actigard	**179.2***	***Significantly better than untreated**
Untreated	140.7	--------------------
Prophyt	103.5**	**Significantly worse than untreated
Keyplex + SAver	90.0**	**Significantly worse than untreated

L.S.D. (.05)= 30.2

Conclusions

- Streptomycin sulfate treatment was the only treatment that had significant higher total yields.
- Streptomycin sulfate and Streptomycin sulfate+Actigard were the only treatments that had significant higher total yields for U.S. No. 1 tubers

Springlake, TX **2011** Trial

Field Trial (Treatments)

1) Control (grower practice)
2) Firewall (Streptomycin Sulfate)
3) Prophyt (Potassium Phosphite)
4) K-Phite (Salts of Phosphorous acid)
5) Keyplex (Yeast Extract)
6) Phostrol (Phosphite) + Fwal
7) Actigard (Acibenzolar S-methyl)
8) Fwal + Saver (Nitrogen, Potassium, others)
9) Firewall + SAver
10) K-Phite + Actigard
11) K-Phite + Firewall
12) K-Phite + SAver
13) Keyplex + SAver
14) Keyplex + Actigard
15) Renew (K20, P205, Salicylic acid, nutrients)

Methodology

- Experiments arranged in a Randomized Complete Block Design (RCBD).
- For each treatment, 4 replications of 30 plants each, for a total of 120 plants per treatment.
- Plots located in Springlake Potatoes (Barrett's) (Highway 385 S Springlake, TX)
- Seed Source: Russet Norkotah 223

Methodology (cont)

- Spacing between hills: 9”
- Spacing between rows: 36”
- Number of rows per plot: 2
- Potatoes Planted March 24, 2011
- Potatoes Vines Killed August 15, 2011
- Harvested August 22, 2011

Methodology (cont)

- Treatments were sprayed at a 2-week intervals starting May 10 for a total of 6 sprays. (back-pack)
- Sprays ended July 22, 2011.
- Every spray date, ten leaves from each treatment were sampled for molecular diagnosis (PCR) of Ca. Liberibacter solanacearum.

Springlake
Table 1a.

Total yield, total yield of U.S. No.1, under 4 ounce and culls/No.2 potatoes and general rating of 15 entries in the Western Regional Russet Trial grown near Springlake, Texas-2011.

Variety or Selection	Total Yield Cwt/A	U.S. No. 1 Cwt. Per Acre						
		Total Yield	4-6 oz	6-10 oz	10-18 oz	Over 18 oz	Under 4 oz.	Culls/ No.2
1-Untreated	77.3	20.3	16.8	3.5	0.0	0.0	23.6	33.4
2-Fwal	119.1	36.3	26.8	8.6	1.0	0.0	39.5	43.2
3-Proph	138.7	40.3	29.4	11.0	0.0	0.0	44.2	54.2
4-KPh	156.7	37.3	17.3	18.2	1.8	0.0	50.7	68.7
5-KPX	86.8	20.0	16.0	4.0	0.0	0.0	31.9	34.8
6-Phost + Fwal	162.3	45.5	33.4	11.6	0.5	0.0	50.7	66.1
7-Actgd	146.7	35.2	22.6	9.2	3.4	0.0	48.1	63.4
8-Fwall + SAv	143.3	52.8	40.0	12.7	0.0	0.0	43.2	47.3
9-Fwall + Acgd	51.9	19.4	13.6	5.8	0.0	0.0	16.3	16.3
10-KPh + Acgd	132.3	28.2	21.8	6.5	0.0	0.0	37.1	67.0
11-KPh + Fwal	122.8	25.3	16.9	8.4	0.0	0.0	47.6	49.9
12-KPh + SAv	125.2	35.3	27.3	6.0	2.1	0.0	37.6	52.3
13-KPX + Sav	101.2	31.8	23.1	8.7	0.0	0.0	28.2	41.1
14-KPX + Acgd	123.6	33.2	27.3	6.0	0.0	0.0	40.5	49.9
15-RenCs	53.7	10.0	6.6	3.4	0.0	0.0	19.2	24.5
Average	116.1	31.4	22.6	8.2	0.6	0.0	37.2	47.5
L.S.D. (.05)	51.8	20.0	17.3	8.2	ns		20.5	28.5

Total Yield (all tubers)-statistically better than untreated

Treatment	Yield (Cwt/A)
1-Control (grower practice only)	77.3
6-Phostrol + Firewall	**162.3**
4-K-Phite	**156.7**
7-Actgard	**146.7**
8-Firewall + SAver	**143.3**
3-Prophyt	**138.7**
10-K-Phite + Actigard	**132.3**

L.S.D. (.05)= 51.8

Note: Firewall=119.1

Total Yield of US No.1 (4-18oz tubers)- statistically better than untreated

Treatment	Yield (Cwt/A)
8-Firewall + SAver	52.8
6-Phostrol + Firewall	45.5
1-Control (grower practice only)	20.3

L.S.D. (.05)= 20.0

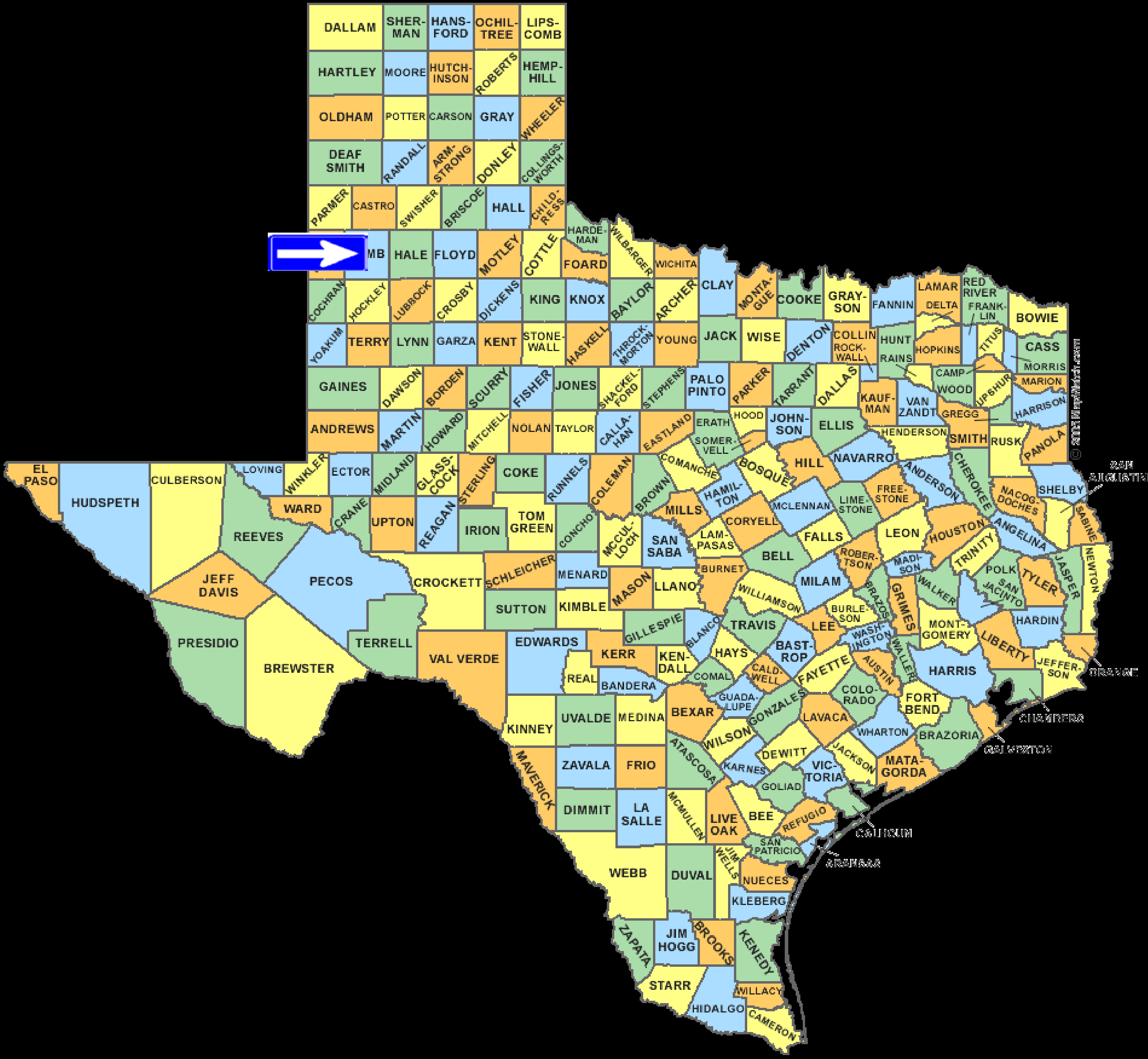
Note: Firewall=36.3

Conclusion

- Several treatments had higher total yields, but that also included small tubers and culls.
- Firewall + Phostrol and Firewall+SAver were the only treatments that had significant higher total yields for U.S. No. 1 tubers (4-18 oz.)
- Although not statistically higher, Firewall alone had numerically higher yields for Total yield and for U.S. No. 1 tubers

2012 Field Trials (Treatments): Springlake

1) Control (Grower Application of Chemicals)
2) Streptomycin Sulfate (FireWall)
3) Salts of Phosphorous Acid
4) Actigard (Acibenzolar S-methyl)
5) SAver □(Nitrogen, Potassium, others)
6) KPX-B1 (Micronutrients)
7) Renew (K_20, P_205, Salicylic acid, nutrients)
8) Actigard + SAver
9) Streptomycin Sulfate + SAver
10) KPX-B1 + Renew



DALLAM
SHER-MAN
HANS-FORD
OCHIL-TREE
LIPS-COMB
HARTLEY
MOORE
HUTCH-INSON
ROBERTS
HEMP-HILL
OLDHAM
POTTER
CARSON
GRAY
WHEELER
DEAF SMITH
RANDALL
ARM-STRONG
DONLEY
COLLINGS-WORTH
PARMER
CASTRO
SWISHER
BRISCOE
HALL
CHILD-RESS
MB
HALE
FLOYD
MOTLEY
COTTLE
HARDE-MAN
WILBARGER
FOARD
WICHITA
CLAY
MONTA-GUE
COOKE
GRAY-SON
FANNIN
LAMAR
RED RIVER
BOWIE
COCHRAN
HOCKLEY
LUBBOCK
CROSBY
DICKENS
KING
KNOX
BAYLOR
ARCHER
DELTA
FRANK-LIN
CASS
YOAKUM
TERRY
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GARZA
KENT
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HASKELL
THROCK-MORTON
YOUNG
JACK
WISE
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HOPKINS
TITUS
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GAINES
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BORDEN
SCURRY
FISHER
JONES
SHACKEL-FORD
STEPHENS
PALO PINTO
PARKER
TARRANT
DALLAS
KAUF-MAN
VAN ZANDT
WOOD
UPSHUR
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EASTLAND
ERATH
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JOHN-SON
ELLIS
HENDERSON
SMITH
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SOMER-VELL
EL PASO
HUDSPETH
CULBERSON
LOVING
WINKLER
ECTOR
MIDLAND
GLASS-COCK
STERLING
COKE
RUNNELS
COLEMAN
BROWN
COMANCHE
BOSQUE
HILL
NAVARRO
ANDERSON
CHEROKEE
NACOG-DOCHES
SHELBY
SAN AUGUSTINE
HAMIL-TON
FREE-STONE
LIME-STONE
MCLENNAN
WARD
CRANE
UPTON
REAGAN
IRION
TOM GREEN
CONCHO
MCCUL-LOCH
MILLS
LAM-PASAS
CORYELL
SAN SABA
FALLS
LEON
HOUSTON
TRINITY
ANGELINA
SABINE
REEVES
PECOS
JEFF DAVIS
CROCKETT
SCHLEICHER
MENARD
BURNET
BELL
ROBER-TSON
MADI-SON
WALKER
POLK
SAN JACINTO
TYLER
JASPER
NEWTON
MASON
LLANO
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KIMBLE
GILLESPIE
BLANCO
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LIBERTY
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TERRELL
VAL VERDE
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BAST-ROP
FAYETTE
AUSTIN
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JEFFER-SON
ORANGE
BREWSTER
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COLO-RADO
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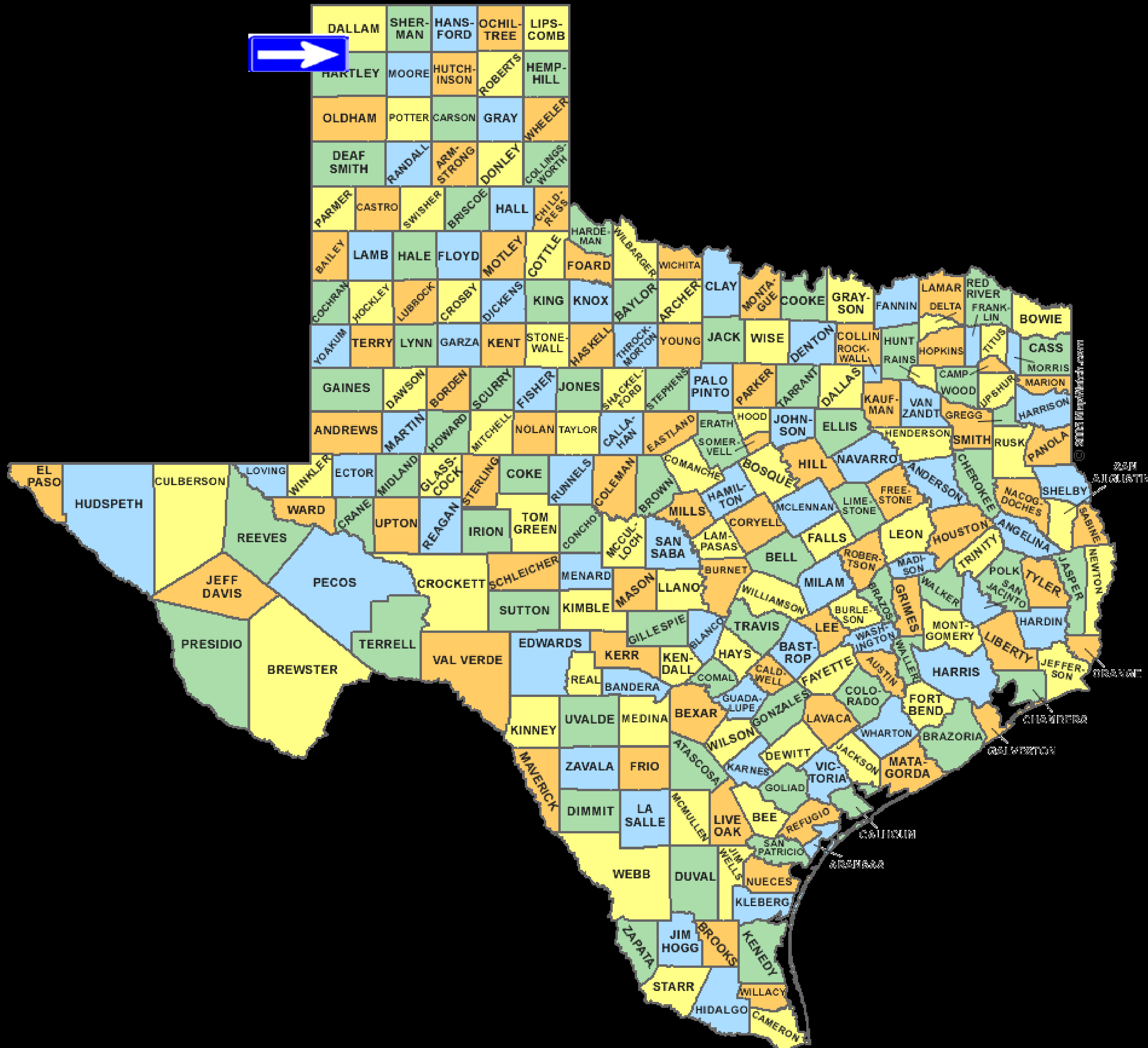
ZAVALA
FRIO
ATASCOSA
KARNES
VIC-TORIA
GOLIAD
DIMMIT
LA SALLE
MCMULLEN
LIVE OAK
BEE
REFUGIO
CALHOUN
SAN PATRICIO
ARANSAS
WEBB
DUVAL
JIM WELLS
NUECES
KLEBERG
ZAPATA
JIM HOGG
BROOKS
KENEDY
STARR
HIDALGO
WILLACY
CAMERON

2012 Field Trials in Dalhart, TX: Treatments

1) Control
2) Streptomycin Sulfate (FireWall)
3) Salts of Phosphorous Acid
4) Actigard (Acibenzolar S-methyl)
5) SAver (Nitrogen, Potassium, others)
6) <u>KPX 1000DPX –low </u>(Experimental Micronutrients)
7) Renew (K20, P205, Salicylic acid, nutrients)
8) Actigard + Salicylic acid
9) Streptomycin Sulfate + Salicyclic Acid
10) <u>KPX 1000DPX –high </u>(Experimental Micronutrients)

All treatments are in addition to grower application of chemicals



DALLAM
SHER-MAN
HANS-FORD
OCHIL-TREE
LIPS-COMB
HARTLEY
MOORE
HUTCH-INSON
ROBERTS
HEMP-HILL
OLDHAM
POTTER
CARSON
GRAY
WHEELER
DEAF SMITH
RANDALL
ARM-STRONG
DONLEY
COLLINGS-WORTH
PARMER
CASTRO
SWISHER
BRISCOE
HALL
CHILD-RESS
HARDE-MAN
WILBARGER
BAILEY
LAMB
HALE
FLOYD
MOTLEY
COTTLE
FOARD
WICHITA
CLAY
MONTA-GUE
COOKE
GRAY-SON
FANNIN
LAMAR
RED RIVER
COCHRAN
HOCKLEY
LUBBOCK
CROSBY
DICKENS
KING
KNOX
BAYLOR
ARCHER
DELTA
FRANK-LIN
BOWIE
YOAKUM
TERRY
LYNN
GARZA
KENT
STONE-WALL
HASKELL
THROCK-MORTON
YOUNG
JACK
WISE
DENTON
COLLIN
ROCK-WALL
HUNT
RAINS
HOPKINS
TITUS
CASS
MORRIS
GAINES
DAWSON
BORDEN
SCURRY
FISHER
JONES
SHACKEL-FORD
STEPHENS
PALO PINTO
PARKER
TARRANT
DALLAS
KAUF-MAN
VAN ZANDT
CAMP
WOOD
UPSHUR
MARION
HARRISON
GREGG
ANDREWS
MARTIN
HOWARD
MITCHELL
NOLAN
TAYLOR
CALLA-HAN
EASTLAND
ERATH
HOOD
SOMER-VELL
JOHN-SON
ELLIS
HENDERSON
SMITH
RUSK
PANOLA
EL PASO
HUDSPETH
CULBERSON
LOVING
WINKLER
ECTOR
MIDLAND
GLASS-COCK
STERLING
COKE
RUNNELS
COLEMAN
BROWN
COMANCHE
BOSQUE
HILL
NAVARRO
ANDERSON
CHEROKEE
SHELBY
SAN AUGUSTINE
WARD
CRANE
UPTON
REAGAN
IRION
TOM GREEN
CONCHO
MCCUL-LOCH
MILLS
HAMIL-TON
CORYELL
MCLENNAN
LIME-STONE
FREE-STONE
NACOG-DOCHES
SABINE
REEVES
PECOS
SAN SABA
LAM-PASAS
BELL
FALLS
LEON
HOUSTON
TRINITY
ANGELINA
JEFF DAVIS
CROCKETT
SCHLEICHER
MENARD
MASON
LLANO
BURNET
ROBER-TSON
MADI-SON
POLK
TYLER
JASPER
NEWTON
SUTTON
KIMBLE
WILLIAMSON
MILAM
BRAZOS
GRIMES
WALKER
SAN JACINTO
PRESIDIO
TERRELL
GILLESPIE
BLANCO
TRAVIS
LEE
BURLE-SON
MONT-GOMERY
LIBERTY
HARDIN
BREWSTER
VAL VERDE
EDWARDS
KERR
KEN-DALL
HAYS
BAST-ROP
WASH-INGTON
WALLER
HARRIS
JEFFER-SON
ORANGE
REAL
BANDERA
COMAL
CALD-WELL
FAYETTE
AUSTIN
COLO-RADO
FORT BEND
CHAMBERS
KINNEY
UVALDE
MEDINA
BEXAR
GUADA-LUPE
GONZALES
LAVACA
WHARTON
BRAZORIA
GALVESTON
WILSON
DEWITT
JACKSON
MATA-GORDA
MAVERICK
ZAVALA
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JIM WELLS
NUECES
KLEBERG
ZAPATA
JIM HOGG
BROOKS
KENEDY
STARR
HIDALGO
WILLACY
CAMERON

Methodology

- Experiments arranged in a Randomized Complete Block Design (RCBD).
- For each treatment, <u>6 replications of 30 plants</u> each, for a total of 180 plants per treatment.
- Total of 10 treatments
- Plots located in Springlake, TX (Springlake Potatoes Inc) and Dalhart, Texas (CSS Farms)
- Seed Source: Russet Norkotah 278

Methodology (Springlake)

- Planted March 29, 2012
- Plots were sprayed at approximately 2-week intervals.
- Springlake: June 5, June 19, July 3, July 18 (**4 sprays**)
- Vines killed July 23, 2012

Springlake Plots (June 20, 2012)

Methodology (Dalhart)

- Planted May 7, 2012
- Plots were sprayed at approximately 2-week intervals.
- Dalhart: June 29, July 13, July 25, August 13, August 27 (**5 sprays**)
- Vines killed September 4, 2012

Dalhart Plots (July 25, 2012)

SPRINGLAKE: Total Yield (All tubers incl. >18oz, <4oz, culls)

Treatment	Yield (Cwt/A)
KPX-B1 + Renew	299.9
Streptomycin Sulfate	298.0
Streptomycin Sulfate + SAver	293.2
SAver	286.6
Salts of Phosphorous Acid	277.6
Actigard	270.2
KPX-B1	265.8
Actigard + SAver	261.5
Control	243.2
Renew	240.2

L.S.D. (.05)= 63.6; Average=273.6; Difference high yield –control yield=56.7

SPRINGLAKE: Total Yield of US No.1 Tubers (4-18 oz)

Treatment	Yield (Cwt/A)
KPX-B1 + Renew	**202.6**
Streptomycin Sulfate	188.1
Streptomycin Sulfate + SAver	187.3
Salts of Phosphorous Acid	179.8
SAver	164.2
KPX-B1	150.9
Renew	149.7
Actigard	149.6
Actigard + SAver	146.4
Control	**141.5**

L.S.D. (.05)= 54.0; Average=166.1; Difference high yield –control yield=61.1

DALHART: Total Yield (All tubers incl. >18oz, <4oz, culls)

Treatment	Yield (Cwt/A)
KPX 1000DPX-high	278.2
Streptomycin Sulfate + SAver	276.5
Actigard	251.8
KPX 1000DPX-low	247.5
SAver	240.2
Actigard + SAver	234.1
Streptomycin Sulfate	233.7
Renew	232.6
Control	229.3
Salts of Phosphorous Acid	216.4

L.S.D. (.05)= 61.9; Average=195.7; Difference high yield –control yield=48.9

DALHART: Total Yield of US No.1 Tubers (4-18 oz)

Treatment	Yield (Cwt/A)
KPX 1000DPX-high	217.2
Streptomycin Sulfate + SAver	214.5
Actigard	209.9
KPX 1000DPX-low	206.9
Renew	191.8
SAver	190.4
Actigard + SAver	186.6
Streptomycin Sulfate	183.4
Control	182.1
Salts of Phosphorous Acid	174.5

L.S.D. (.05)= 61.9; Average=195.7; Difference high yield –control yield=35.1

Treatment Ranking for US No. 1

Treatment (Springlake)	Treatment (Dalhart)
KPX-B1 + Renew	**KPX 1000DPX-high**
Streptomycin Sulfate	**Streptomycin Sulfate + SAver**
Streptomycin Sulfate + SAver	Actigard
Salts of Phosphorous Acid	KPX 1000DPX-low
SAver	*Renew*
KPX-B1	SAver
Renew	Actigard + SAver
Actigard	*Streptomycin Sulfate*
Actigard + SAver	Control
Control	Salts of Phosphorous Acid

Zebra Chip presence (tuber chip frying, % ZC in tubers)

Treatment (Springlake)	Treatment (Dalhart)
KPX-B1 + Renew (3%)	KPX 1000DPX-high
Streptomycin Sulfate (3%)	Streptomycin Sulfate + SAver
Streptomycin Sulfate+SAver (2%)	Actigard (2%)
Salts of Phosphorous Acid	KPX 1000DPX-low
SAver(2%)	Renew
KPX-B1	Salicylic Acid (3%)
Renew	Actigard + SAver
Actigard	Streptomycin Sulfate
Actigard + SAver (2%)	Control (7%)
Control (2%)	Salts of Phosphorous Acid

Conclusions

- In Springlake, KPX-B1 (micronutrients) plus Renew (supplements and micronutrients) had significantly better yields than the control for U.S. no. 1 tubers.

- In Dalhart, although no treatment was significantly better than the control, a micronutrient treatment, KPX 1000DPX-high dose, was the highest yielding for U.S. no. 1 tubers.

Conclusions (cont.)

- The treatment Streptomycin Sulfate + SAver was the second highest yielding in Springlake and third in Dalhart.
- Streptomycin sulfate was the second highest yielding treatment in Springlake.
- Recent observations/preliminary studies would indicate that ZC levels suffer a reduction north of Springlake (Would explain why streptomycin sulfate alone may not have worked in Dalhart as well as in Springlake).

Overall Conclusions (2009-2012)

- Based on data, the potential exists for Streptomycin sulfate to be incorporated in an Integrated Disease Management strategy. More data would fine-tune number of applications and rates.

- Oxytetracycline calcium was only tested once and was statistically lower yielding than Streptomycin sulfate and not different to the untreated control.

Overall Conclusions

- The nutrient approach also has the potential to be an alternative or complementary management strategy.
- If the use of Streptomycin sulfate with or without nutrients can decrease insecticide use, it could fit into a sound IPM or BMP approach.

Overall Conclusions

- The judicious use of insecticides in combination with a plant nutritional approach, the use of streptomycin sulfate or other bactericide, plant resistance, and other factors could prove valuable in an integrated disease management approach for potatoes and ZC disease management in the future.

Acknowledgements

- USDA Specialty Crops Research Initiative
- Texas Department of Agriculture
- Albert Patton, former Extension Assistant
- Luz Serrato-Diaz, former Extension Assistant
- Dr. Creighton Miller, Jeff Koym, and Doug Scheuring (Potato Breeding)

Thank you

Visit: http://zebrachip.tamu.edu

Grower Needs and Challenges / New and Existing Options for Fire Blight Control

Grower Needs and Challenges / New and Existing Options for Fire Blight Control

Dormant

- Fixed coppers – CuOH, CuOCl, Cu_2O, …
- Lime sulfur – lime sulfur (apple bloom thinning)
- Biologicals – Blossom Protect, BlightBan A506, Bloomtime Biological

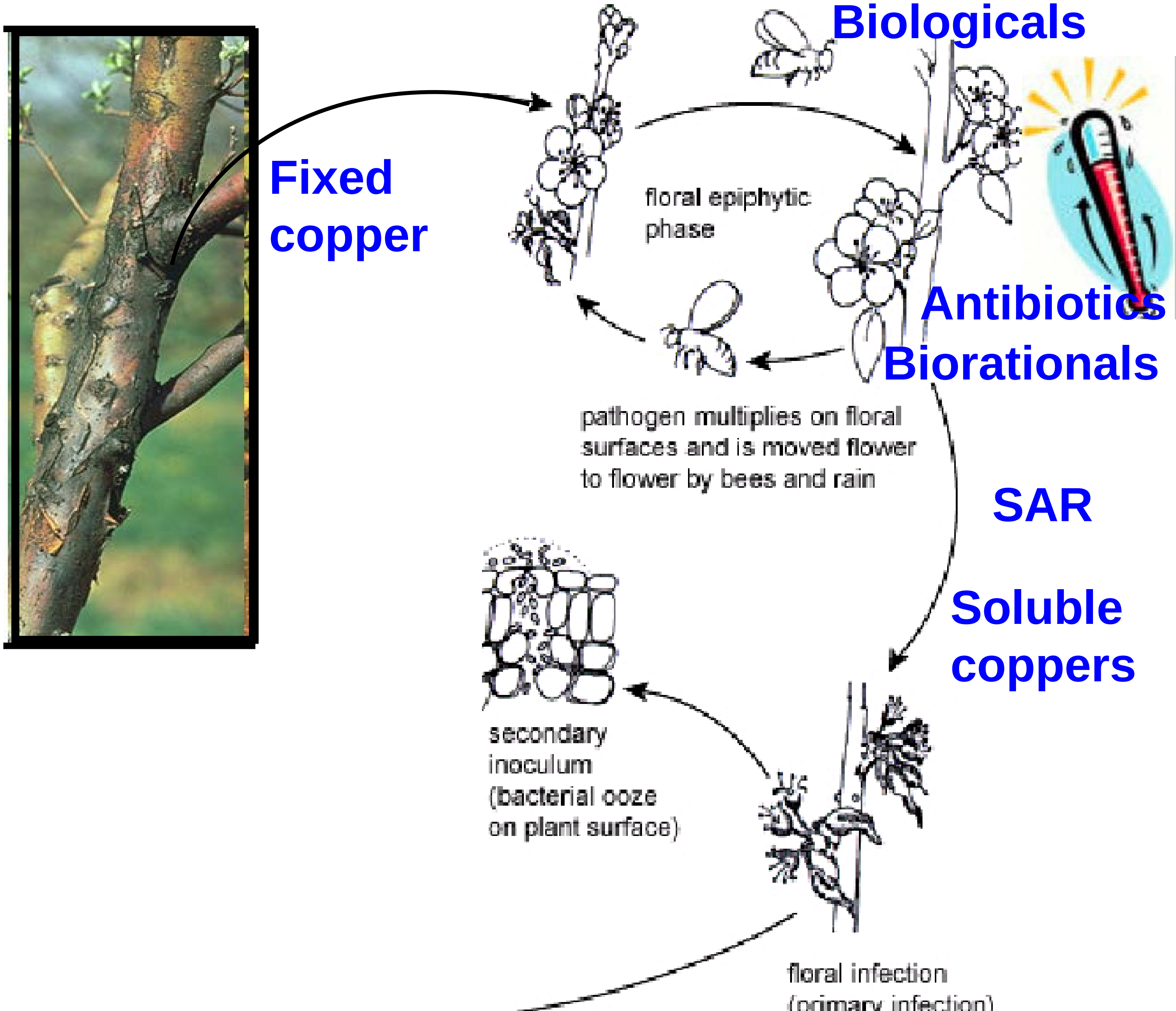
Early bloom

- Antibiotics – streptomycin, oxytetracycline, kasugamycin (pending)
- Biorationals – Serenade, Double Nickel

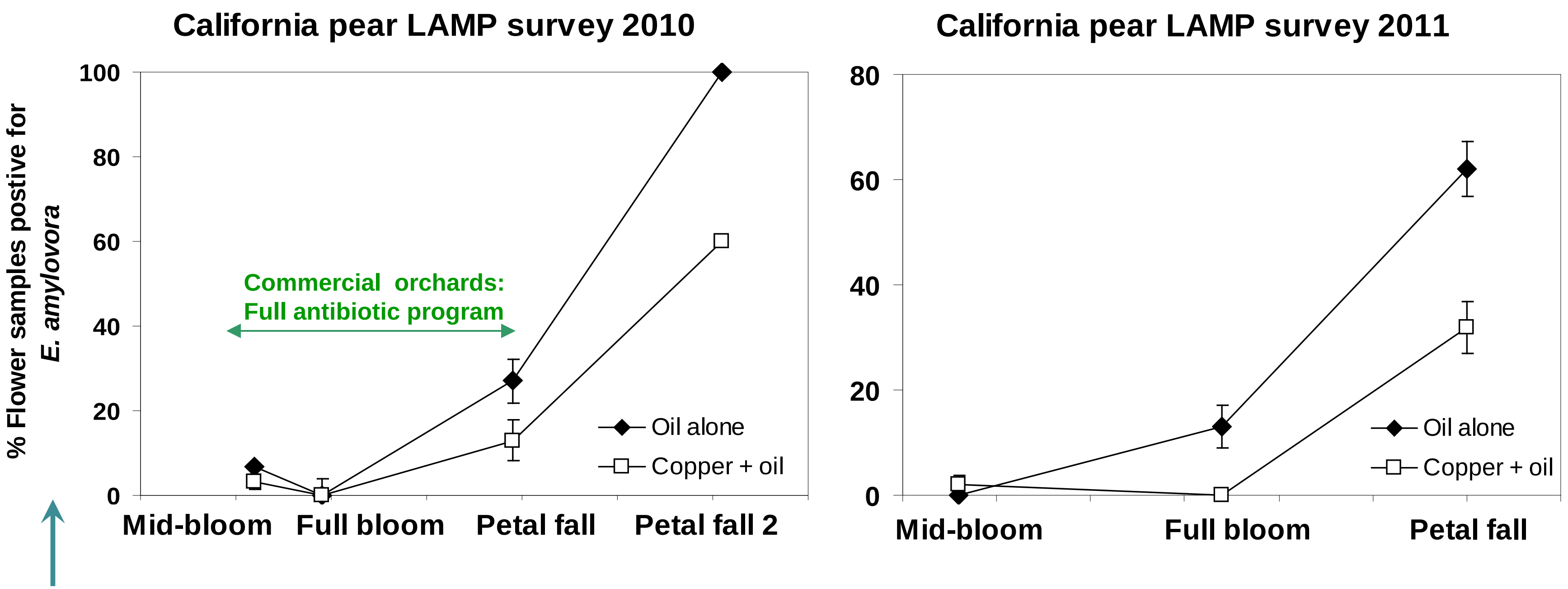
Late bloom

- Soluble Coppers – Cueva, Previsto (pending)
- SAR inducer – Actigard (pending)
- Growth regulator – Apogee (apples)

Ken Johnson
Oregon State U.


Biologicals
Fixed copper
floral epiphytic phase
Antibiotics
Biorationals
pathogen multiplies on floral surfaces and is moved flower to flower by bees and rain
SAR
Soluble coppers
secondary inoculum (bacterial ooze on plant surface)
floral infection (primary infection)

Fixed coppers:



Delayed dormant copper application

Fixed copper applied before bloom that delays the build-up of pathogen populations in flowers.

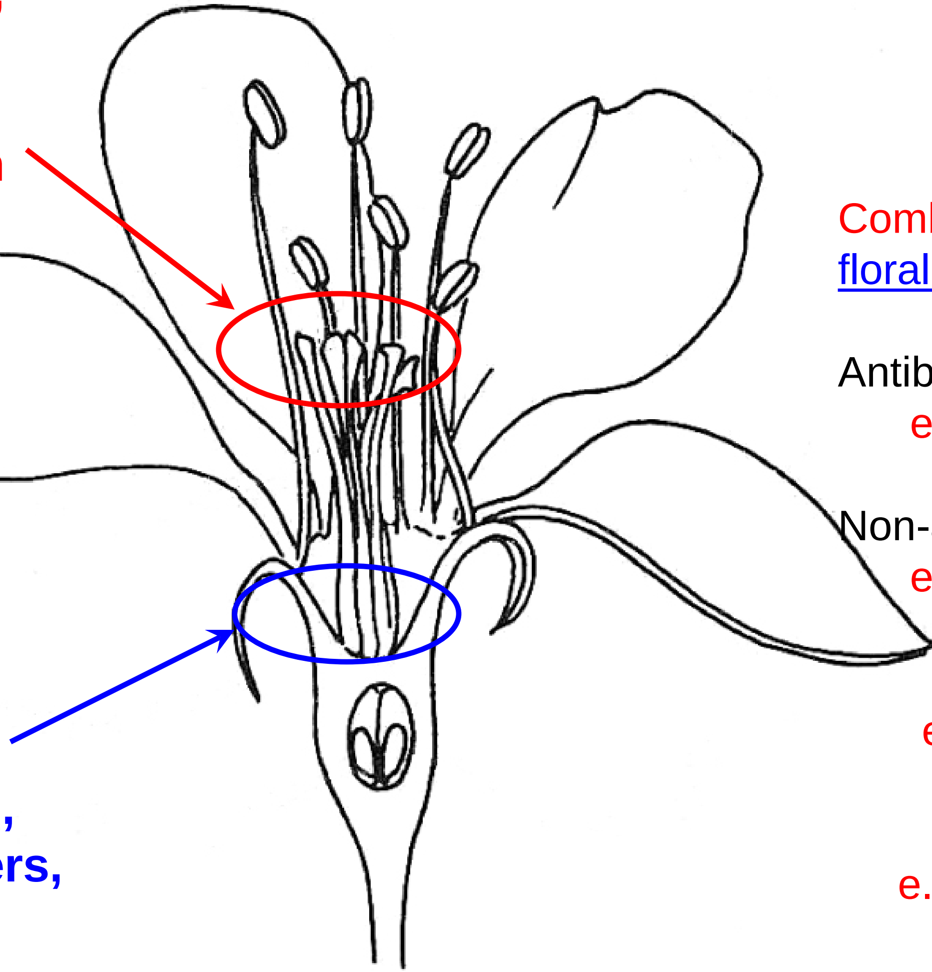
It is used in orchards with a recent history of fire blight.

‘Integrated control programs’

the sequence of materials is important

Lime sulfur, Biologicals

early bloom 30 & 70%



Antibiotics, Biorationals, Soluble coppers,

full bloom to petal Fall

Combining a stigma product with a floral cup product improves control

Antibiotic approach:

e.g., Bloomtime Biological then Oxytetracycline

Non-antibiotic approach:

e.g., Bloomtime Biological then Serenade Opti

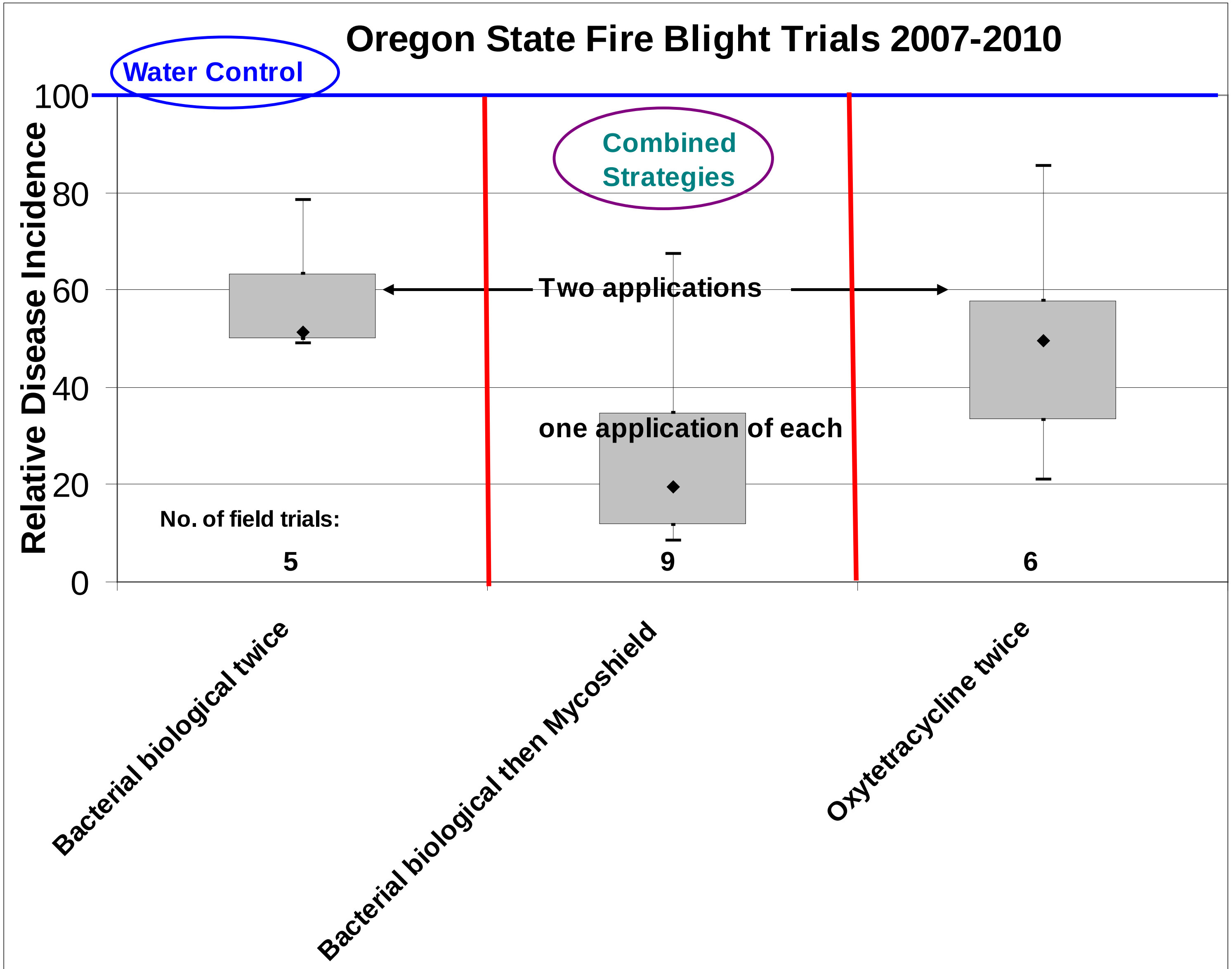
e.g., Lime sulfur & fish oil then Blossom protect

e.g., Blossom Protect then Previsto

e.g., Blossom Protect then Serenade Opti

very good to excellent control

Integrated control:

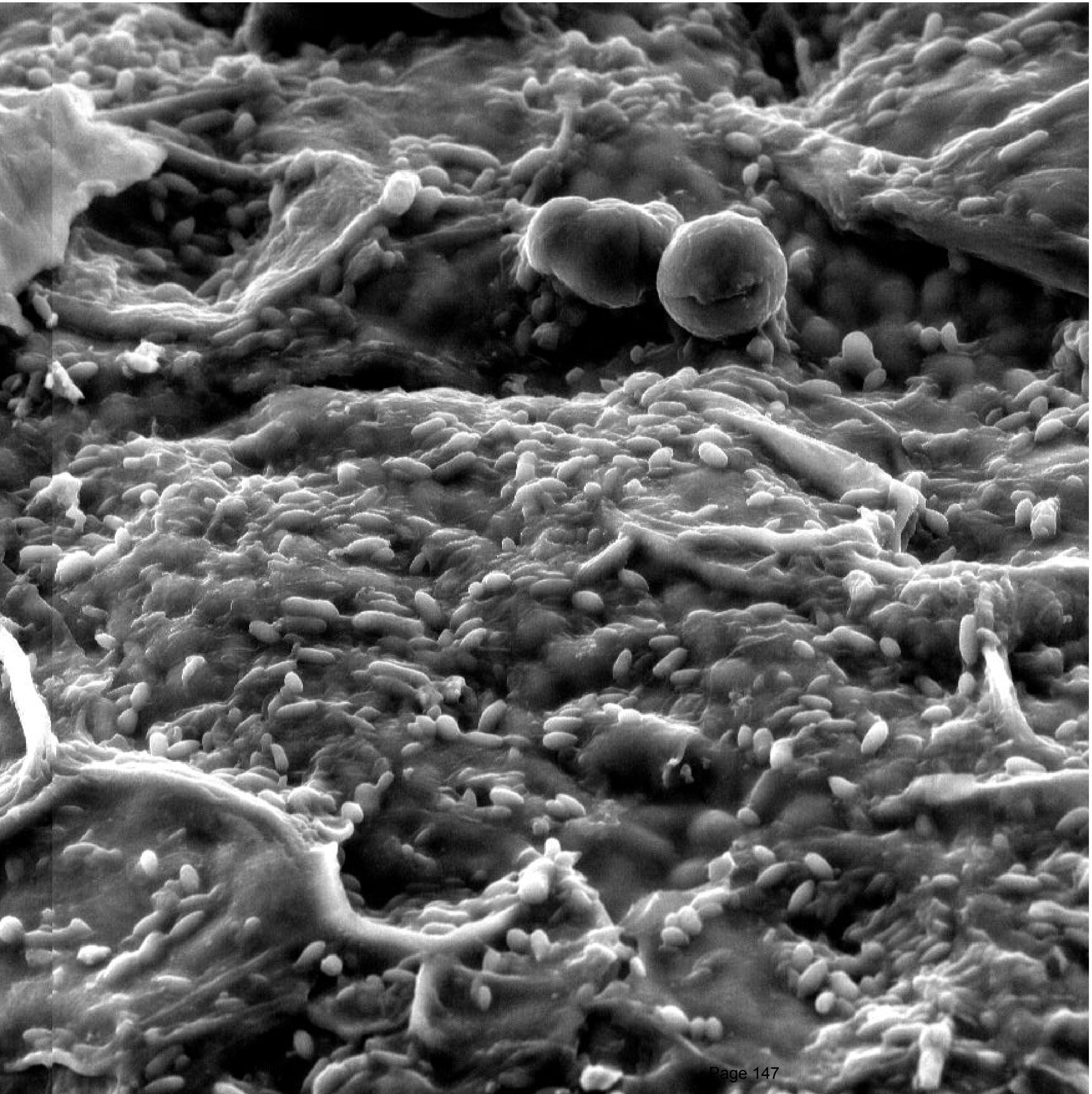


Based on integrated control concept, biological materials have different timings

- Bacterial Stigma Colonizers
  - (BlightBan A506) early bloom
  - (Bloomtime Biological)

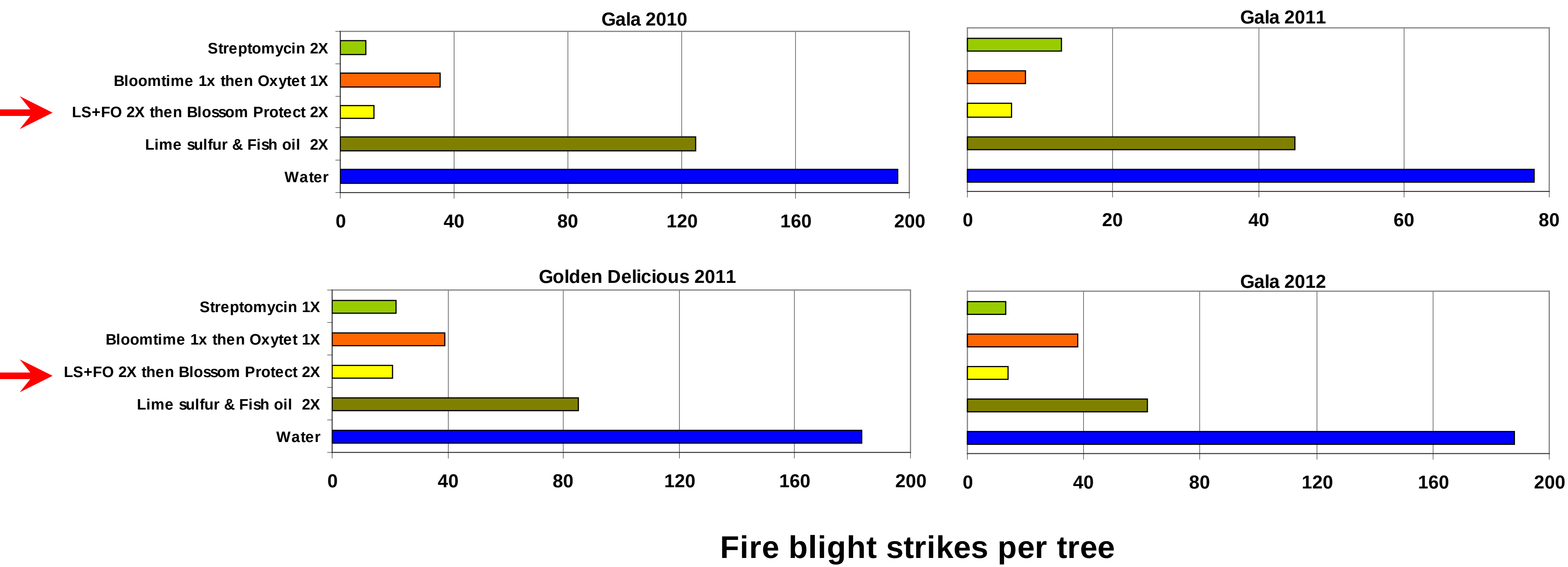
- Yeast floral cup colonizer
  - Blossom Protect early to mid-bloom

- Biorationals based on Bacillus spp.
  - Serenade Opt or ASO full bloom to petal fall
  - (Double Nickel)
  - (Taegro - not registered)



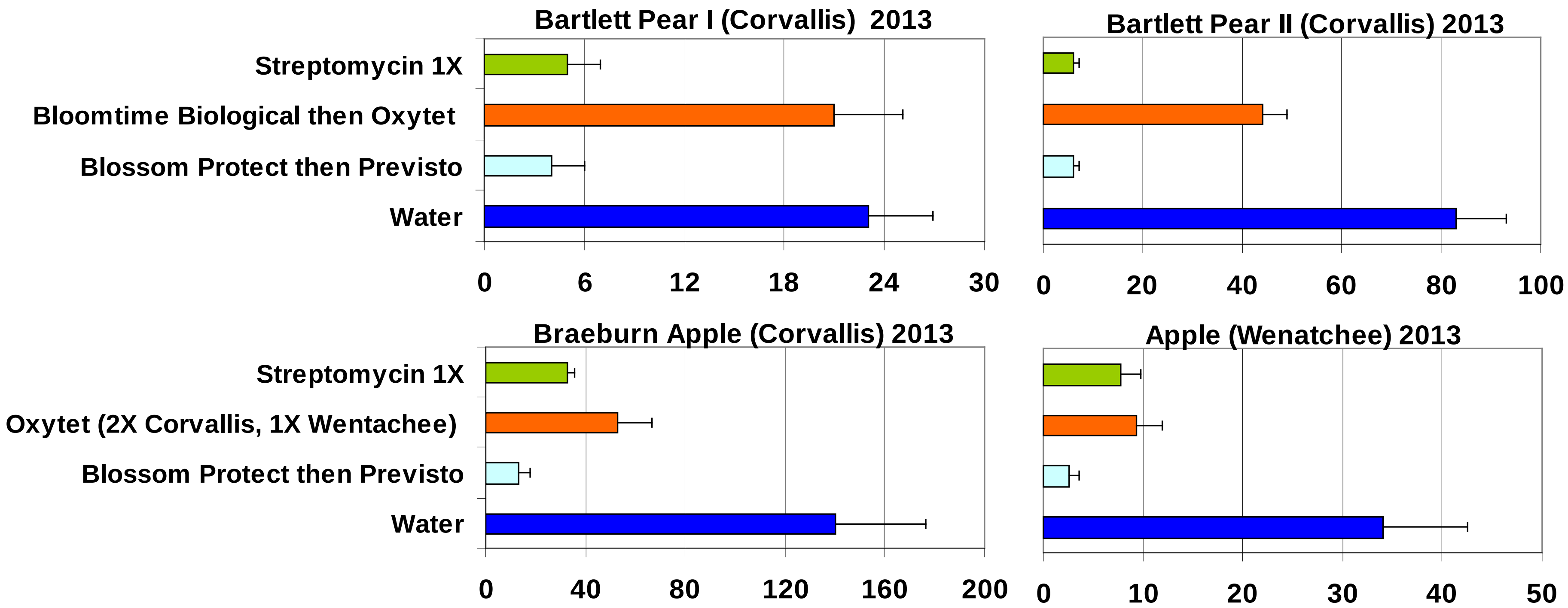
Blossom Protect yeast cells on nectary of pear flower sampled near petal fall

Blossom Protect (yeast)



Integrated control ✓
Blossom Protect ✓
Followed by new Previsto copper ✓

Replicated, inoculated orchard trials:



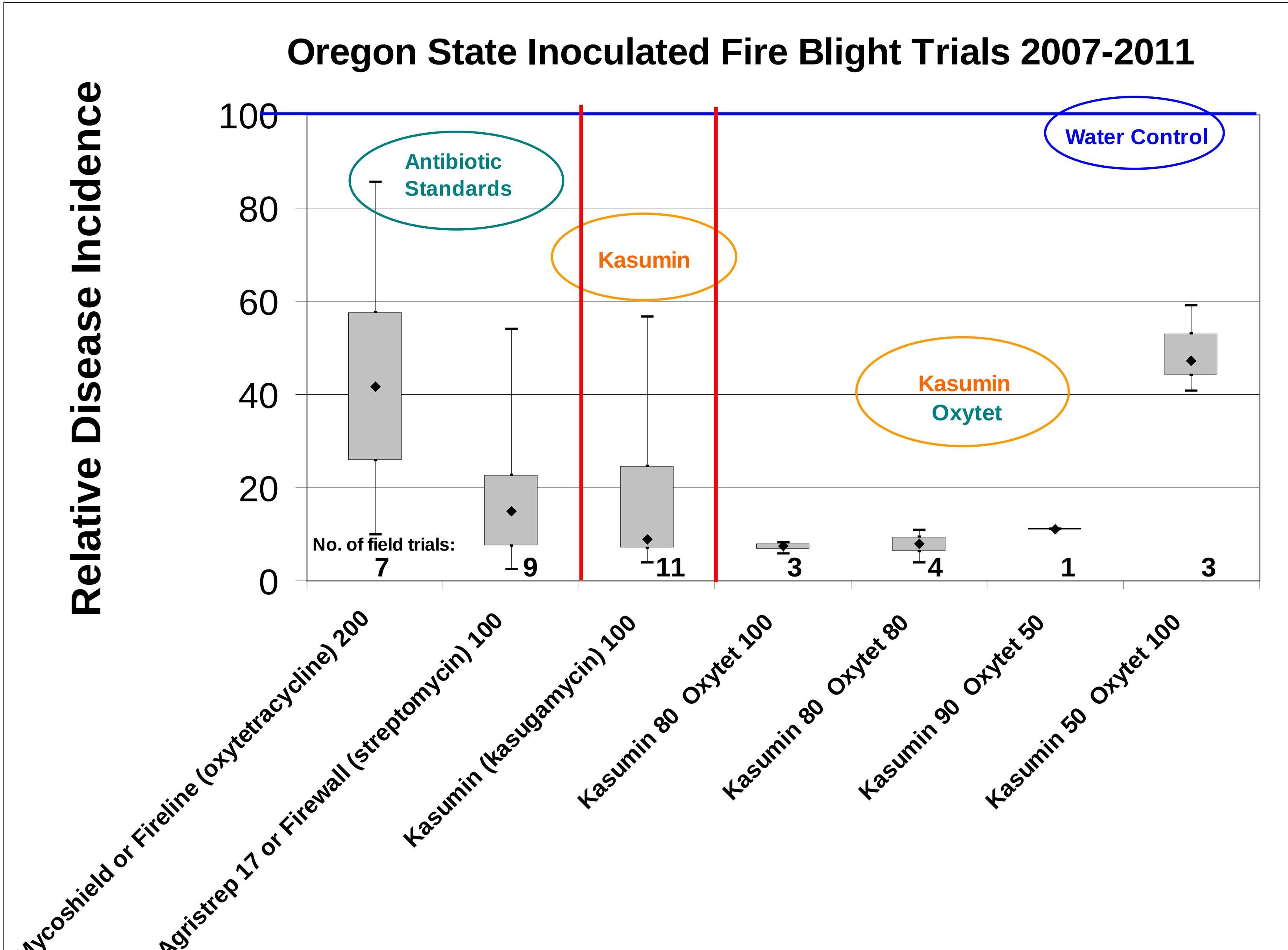
Antibiotics full bloom to petal-fall

- Oxytetracycline
  - Mycoshield
  - FireLine

- Streptomycin
  - Agrimycin 17
  - FireWall

- Kasugamycin
  - Kasumin (pending)

Antibiotic performance:



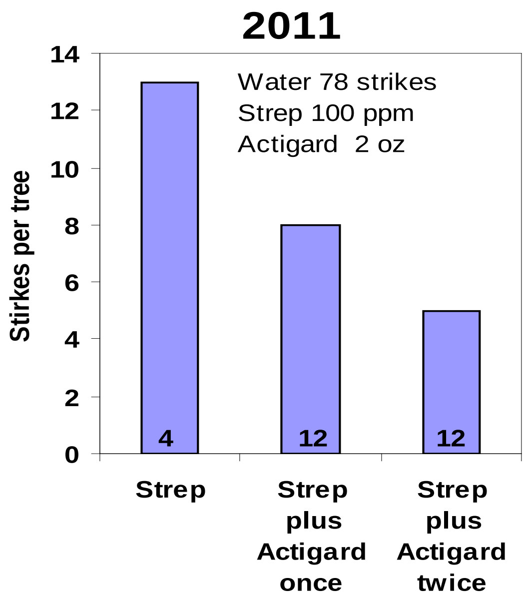
Streptomycin resistance:

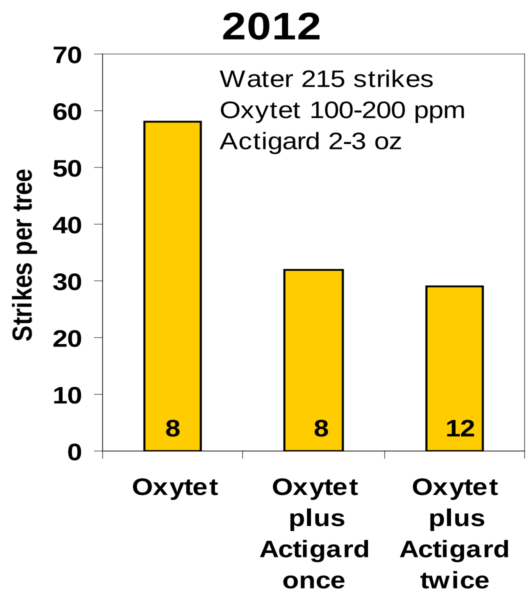
	% *E. amylovora* strep-resistant	
Washington	1988: (Loper et al.)	2010:
Isolates:	76 of 95 **(80%)**	5 of 23 **(22%)**
Orchards:	29 of 32 **(90%)**	2 of 8 **(25%)**
Oregon & CA	1992-1998:	2009:
Isolates:	-	6 of 19 **(31%)**
Orchards:	52 of 125 **(41%)**	5 of 13 **(38%)**

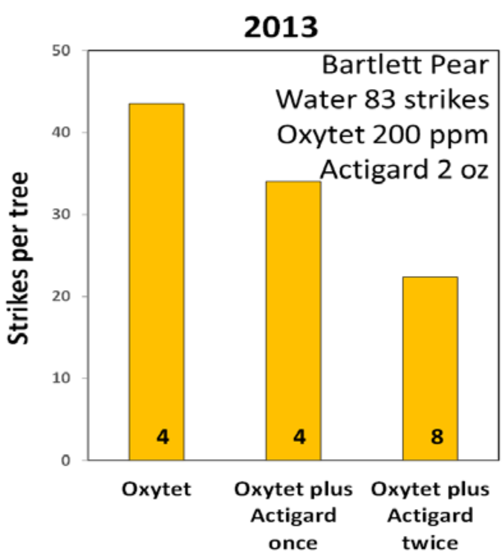
Based on the data, our recommendation has shifted from
“Don’t use strep” to
“Strep once per season, but only mixed with full rate of oxytet”

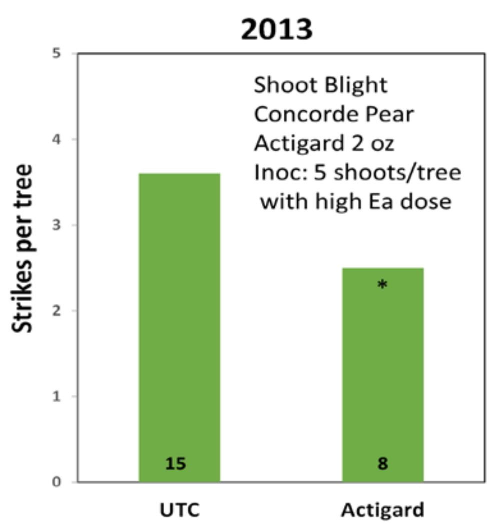
Actigard in combo with antibiotics

Inoculated orchard trials:



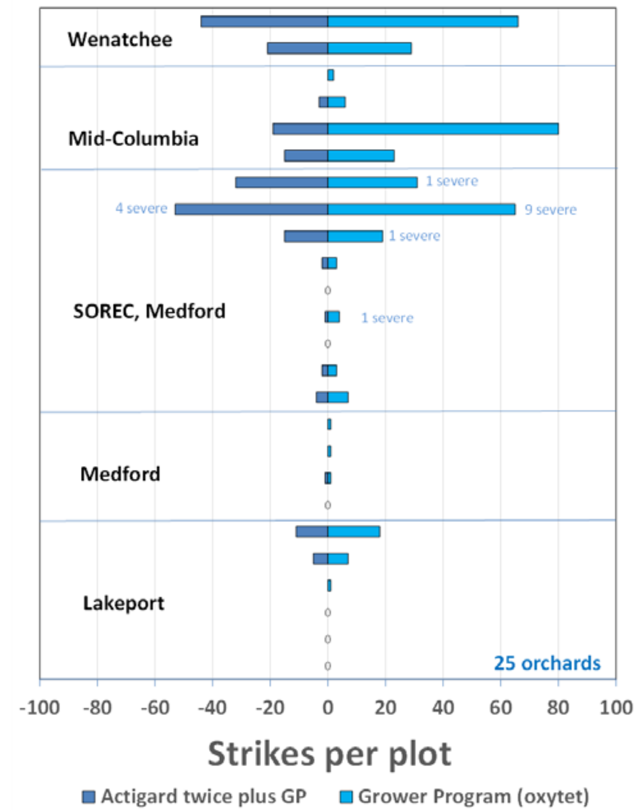






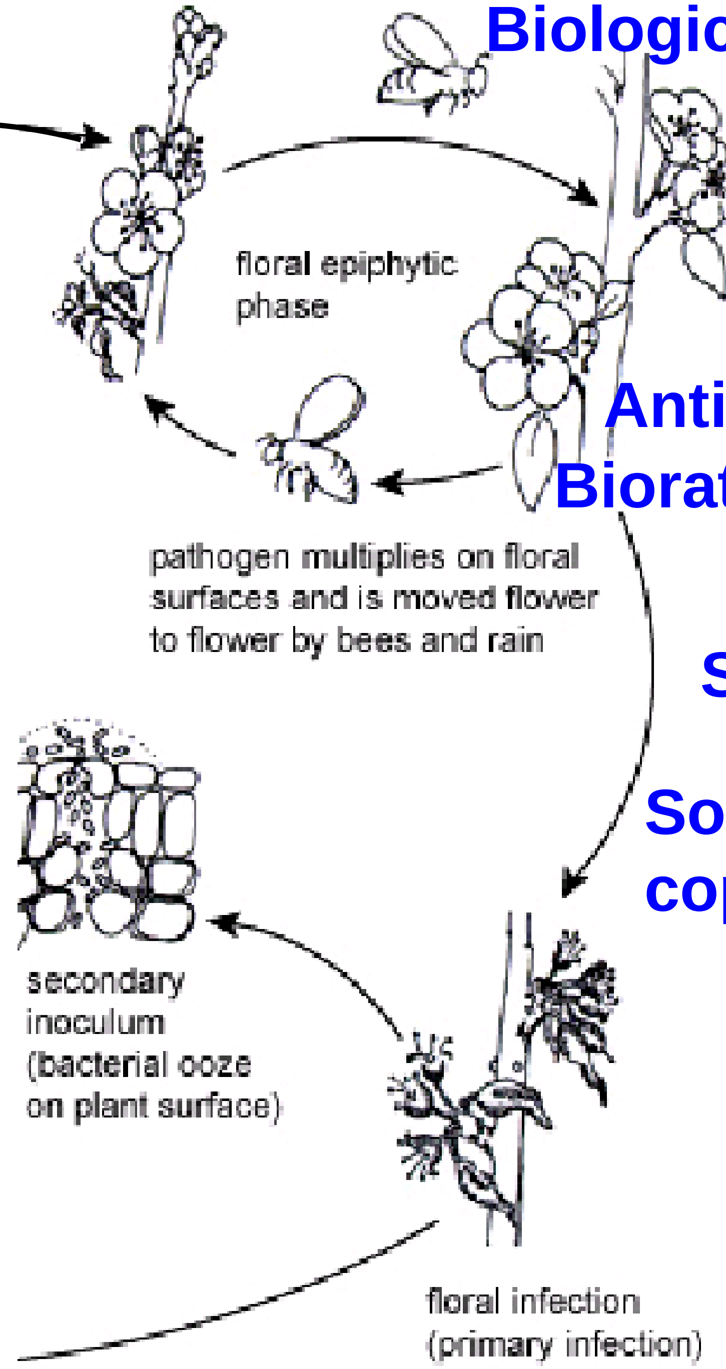
<u>2013 EUP results:</u>

Actigard (twice) plus Grower Program resulted in 37% fewer strikes in pears than Grower Program alone



Concerns about the materials

Crop safety	Fruit russeting: Fixed and soluble coppers Yeast in Blossom Protect Phytotoxicity: (Kasumin)
Efficacy	Bacterial biologicals, Biorationals, Actigard (oxytet, Blossom Protect)
Pathogen resistance	streptomycin (Kasumin)
Inter-compatibility of materials	Lime sulfur with Biologicals Buffer in Blossom Protect with fixed copper Bacterial biologicals with oxytet/Kasumin
Cost of material	Kasumin, Actigard, Blossom Protect



Fixed copper

Biologicals

Antibiotics

Biorationals

SAR

Soluble coppers

Questions ?

Control options for bacterial diseases of cherry and other stone fruit

Control options for bacterial diseases of cherry and other stone fruit

George W. Sundin

MICHIGAN STATE UNIVERSITY

IR-4 Grower Needs and Challenges meeting; September 11, 2014

Economically-significant bacterial diseases of stone fruit in the U.S.

- **Bacterial canker – sweet cherry, apricot, tart cherry**
  - ***Pseudomonas syringae*** **pv. syringae and pv. morsprunorum**
- **Bacterial spot – peach, plum, almond**
  - ***Xanthomonas arboricola*** **pv. pruni**
- **Walnut blight**
  - ***Xanthomonas arboricola*** **pv. juglandis**

Bacterial canker flower infection:

Induced following a freeze

Observed following prolonged periods of cool, wet weather

Certain cultivars with fruit prized by consumers put growers at risk


Michigan, 2012

‘Napoleon’

‘Somerset’

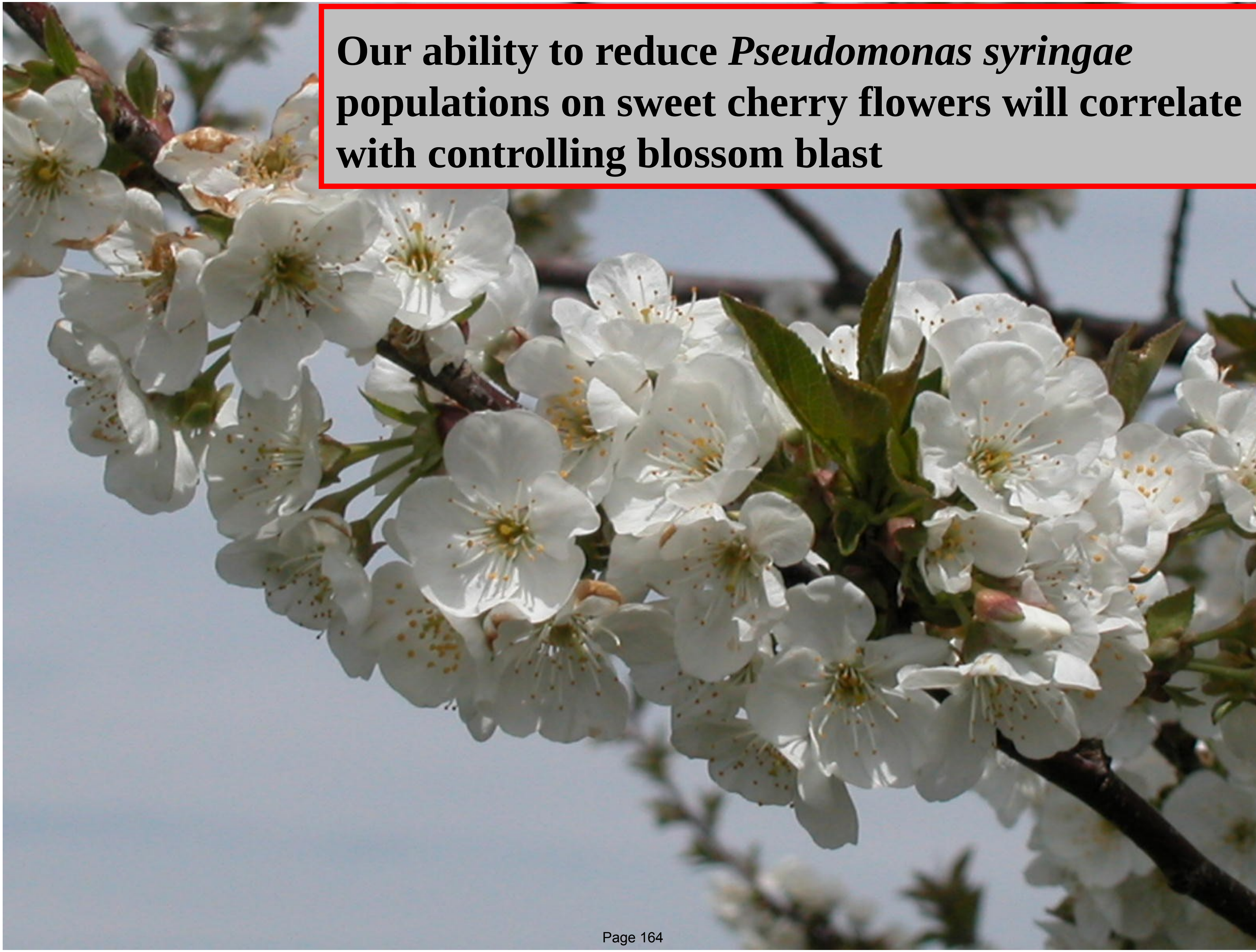
Bacterial canker pathogen
Pseudomonas syringae pv. syringae

- **Excellent colonizer of sweet cherry flowers**
- **Essentially every flower cluster is colonized in MI orchards**

- **Bacterial cells are ice nucleation active**

- **Even low temps close to freezing (~ 31° F) can lead to frost injury induced infection**

Our ability to reduce *Pseudomonas syringae* populations on sweet cherry flowers will correlate with controlling blossom blast



Bacterial canker control:
Coppers – wrong timing
Biologicals? Phosphites?

Bacterial canker summary

- **Bacterial canker epidemics in Michigan since 2002 were induced by freeze events**
- **Prolonged cool, wet weather also leads to bacterial canker infection on smaller scale**
- ***P. syringae* pv. syringae populations on sweet cherry blossoms are high and uniformly distributed throughout Michigan**
- **Copper resistance is present in MI; thus, the role of copper in disease control is debatable**
- **Other products tested – efficacy still in question**

Bacterial canker summary

- ***There is a need for compounds with the capability of reducing P. syringae bloom populations in advance of freeze events or weather favoring infection***

Bacterial Spot

- **Causal agent – *Xanthomonas arboricola* pv. pruni**
- **Symptoms -- spots on leaves, fruit; cankers form on new twigs**

Bacterial Spot

- **Problems for disease management**
  - **Large populations of the pathogen**
  - **Efficacy of chemical controls is not optimal**

Management of Bacterial Spot: Host Resistance

- **Successful management starts with growing varieties with resistance/tolerance**
  - **(however, resistance can be overcome under high disease pressure)**

- **Two main chemical controls**
  - **Copper -- can be limited by phytotoxicity problems**
  - **Oxytetracycline -- can be effective, however:**
  - **successful use requires accurate timing relative to infection events, full coverage**
  - **OxyTc lacks long residual activity**
  - **New concerns about resistance**

Copper Use for Bacterial Spot Management

- **Bottom line from Dave Ritchie, NC State:**
- **"When peach foliage is present, there is no rate of copper that has adequate activity against bacterial spot which will not cause some leaf injury."**

Current control of bacterial diseases of stone fruit

- **Relies on copper**
  - **Trees are highly susceptible to copper**
  - **Can't spray with full rates of copper when it is most needed**
  - **Copper resistance (50+ years of copper use …..)**

- **Potential for biologicals?**
  - **Low because of high pathogen population size, extended periods when control needed, and epiphytic growth of pathogen**

Current control of bacterial diseases of stone fruit

- **Judicious, targeted use of antibiotics:**
  - **Use during bloom when most needed**
  - **Potential for significant reduction of flower populations of *P. syringae***
    - **Control could then potentially carry through season**

  - **More effective alternative for bacterial spot?**


Kasumin for fire blight
control in Michigan

Experience with Kasumin in Michigan

- **Section 18 granted by EPA yearly since 2010**
- **Use timing – bloom through petal fall; 3 applications maximum**
- **Conditions must be predictive of potential fire blight epidemic prior to use**

- **Other conditions:**
  - **First spray of season must be other material**
  - **No animal manure in orchards**

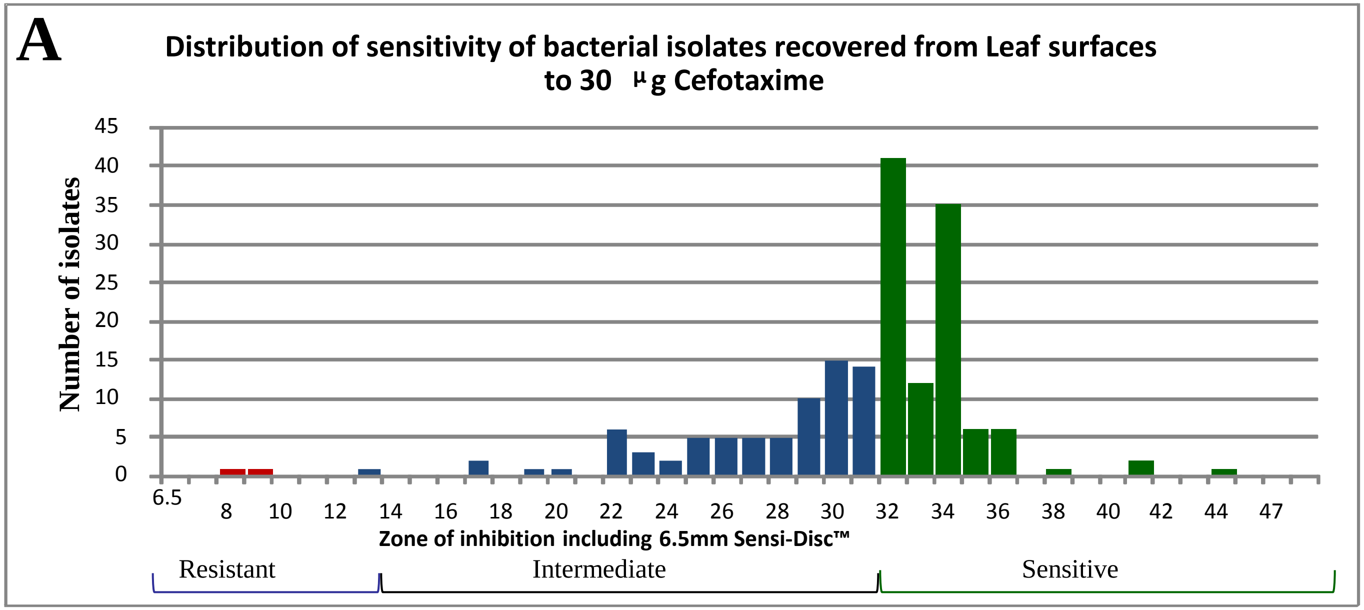
Experience with Kasumin in Michigan

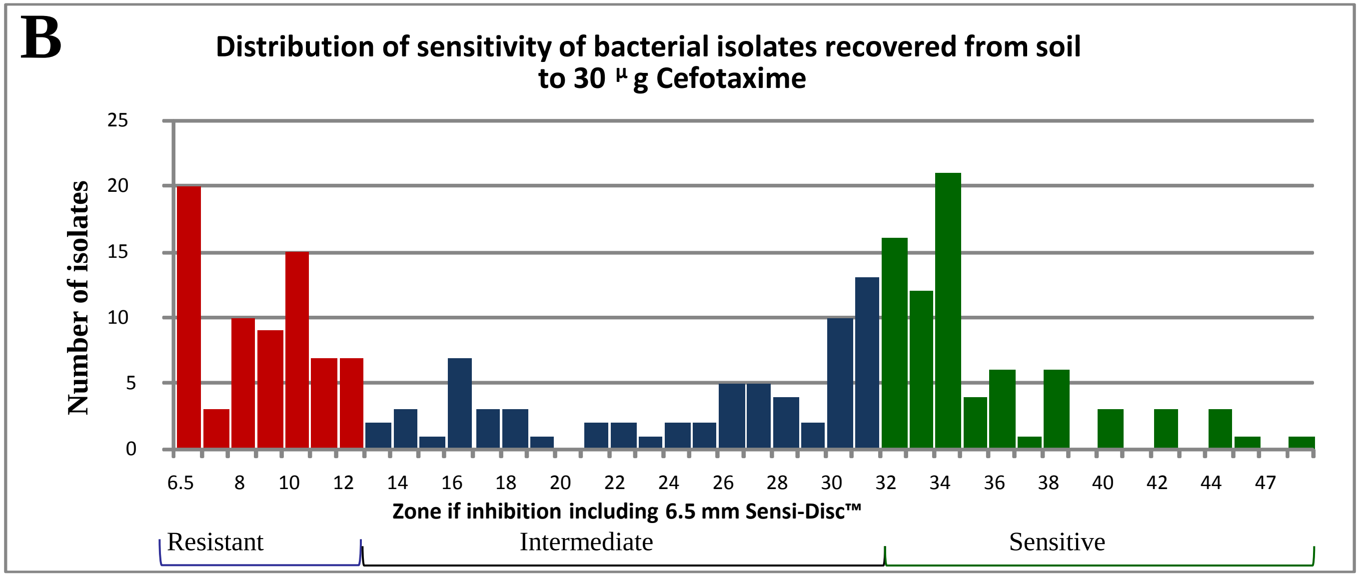
- **Grower adoption increases every year**
- **Control in commercial blocks has been excellent**

Experience with Kasumin in Michigan

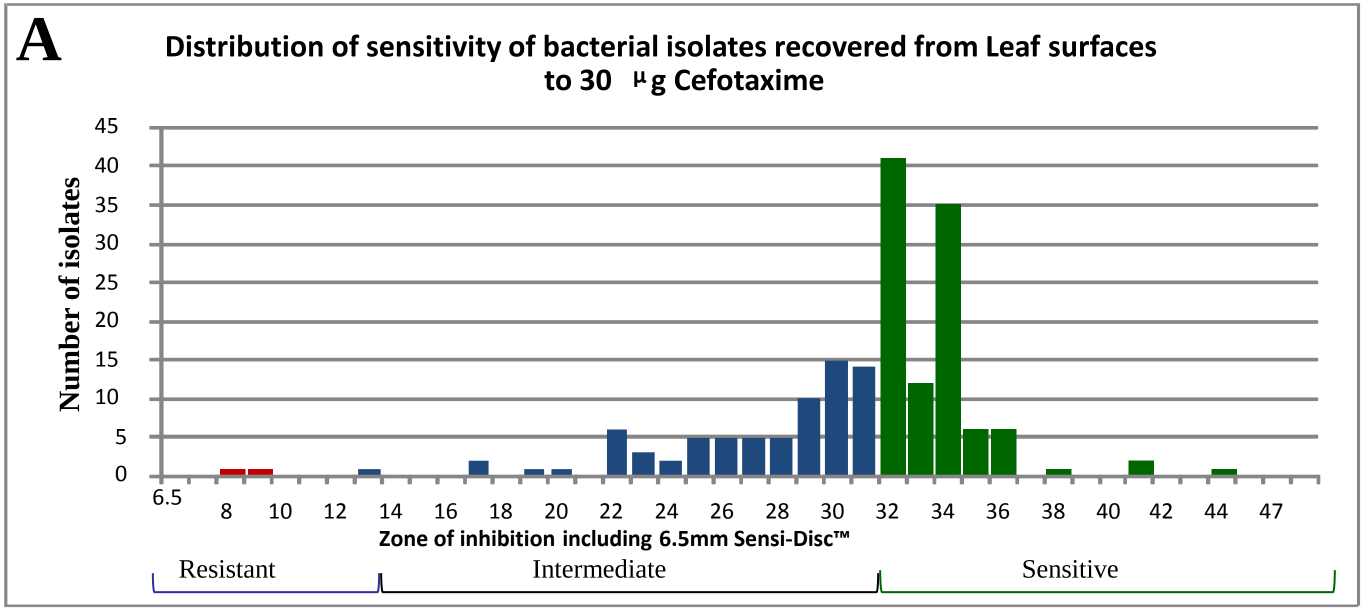
- EPA-mandated resistance monitoring conducted by Sundin lab since 2010
- "Does Kasumin use select for resistance to kasugamycin linked to resistance to other antibiotics?"
  - Sample 10-12 orchards throughout MI, 2 unsprayed controls
  - Quantify culturable bacterial populations
  - Isolate Gram-negative bacteria and assess sensitivity to ampicillin, cefotaxime, gentamicin, streptomycin, tetracycline

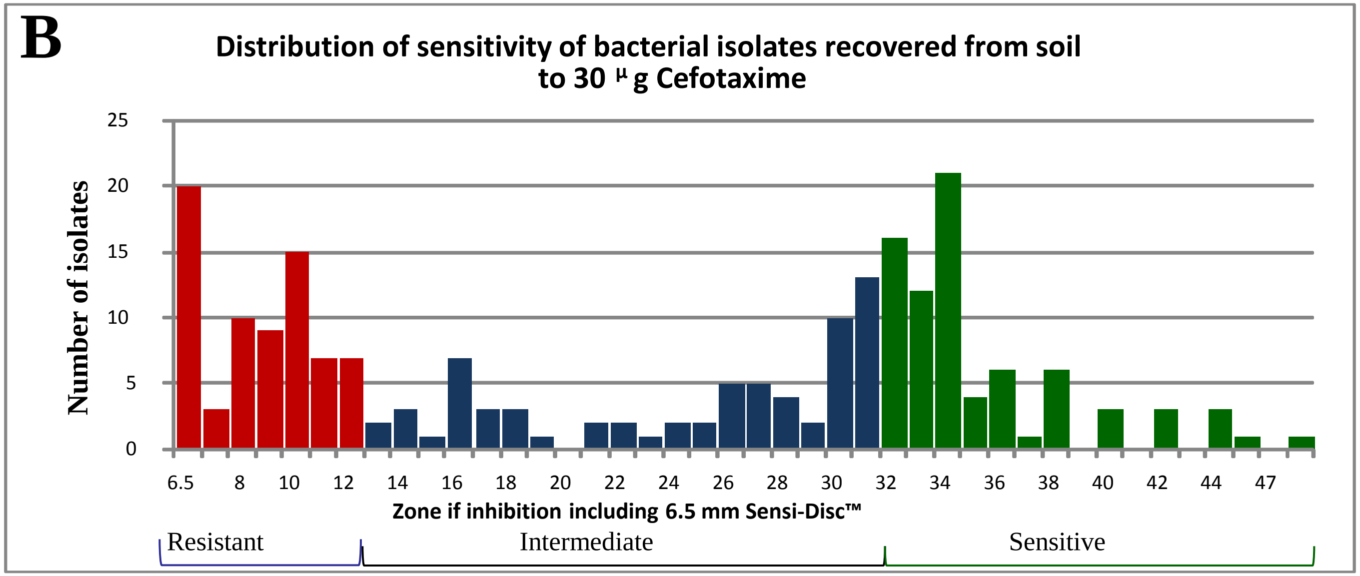
Antibiotic resistance monitoring





Antibiotic resistance monitoring





No differences between Kasumin-sprayed and nonsprayed orchards

Experience with Kasumin in Michigan

- **Excellent antibiotic for fire blight management**
- **Stewardship is critical**
  - **Limited # of applications**
  - **Limited timing to bloom**
  - **Fire blight predictive model must indicate potential epidemic conditions prior to use**

- **No evidence of any effects of Kasumin use on resistance to other antibiotics**

Creating Transgenics for Resistance to Huanglongbing

Creating Transgenics for Resistance to Huanglongbing

Ed Stover
Greg McCollum
Kim Bowman
Randy Niedz

Ute Albrecht
Lesley Benyon
Malu Oliveira
Ric Stange

Principle Collaborators:
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David Hall
Bob Shatters
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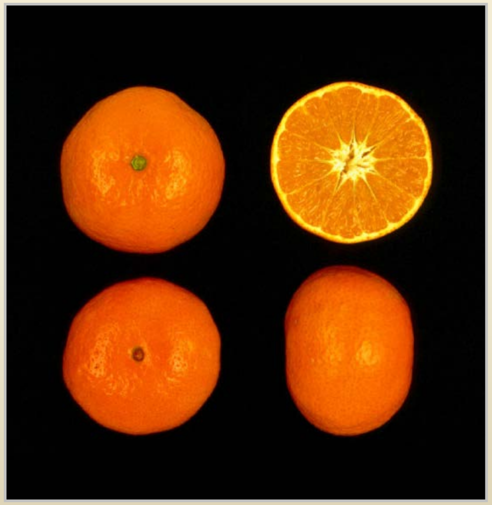
Jesse Jaynes
Gloria Moore
James Thomson



Huanglongbing (HLB, AKA Greening) World Experience (Bové, 2006)

- "Probably the most serious disease of Citrus"
- First unambiguous report in China 1940s
- "Practically all commercial citrus species and cultivars are sensitive, regardless of rootstocks"
- Has caused elimination or contraction of citrus production in several citrus growing regions
- Has latent period with few symptoms for several years
- May completely debilitate trees within two years of first symptoms
- Research supports HLB-management by aggressive ACP spraying, regular scouting and roguing of infected trees, and use of disease-free replants

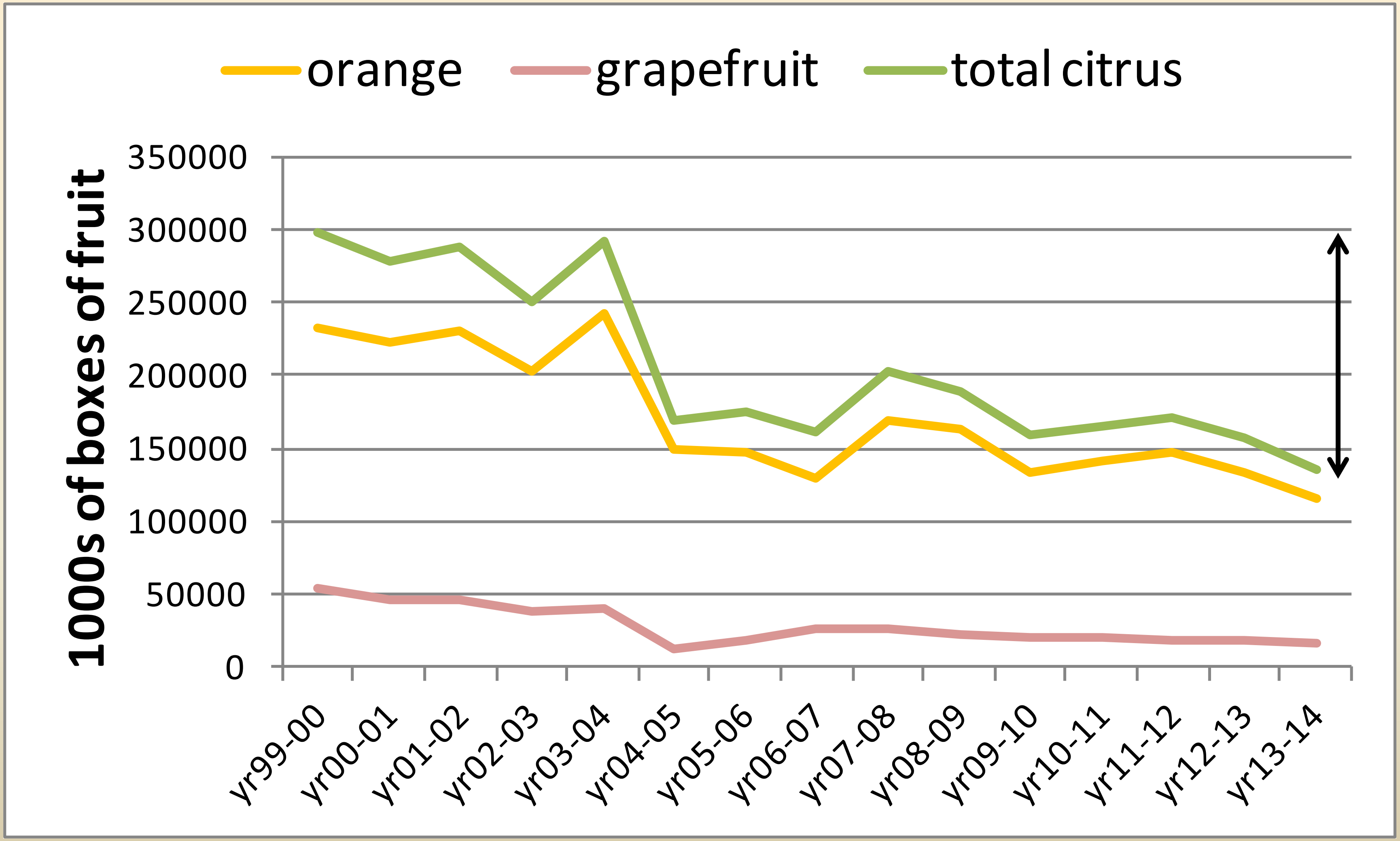
Huanglongbing, AKA Citrus Greening

- First identified in Florida in August 2005
- Estimated that ~40-70% of FL citrus trees are infected, but some groves no longer productive
- Associated with a phloem limited bacterium, Liberibacter asiaticus (Las), vectored by the Asian Citrus Psyllid (widespread FL &TX, in CA, finds in AZ),
- Within a few years of infection, many citrus trees become weak, have poor quality fruit, with lots of fruit drop, and trees may die or become useless
- SERIOUSLY BIG PROBLEM!!!!

Industry $$ and now federal funds directed at finding solutions
>$200 million so far

Photos Bove, 2006

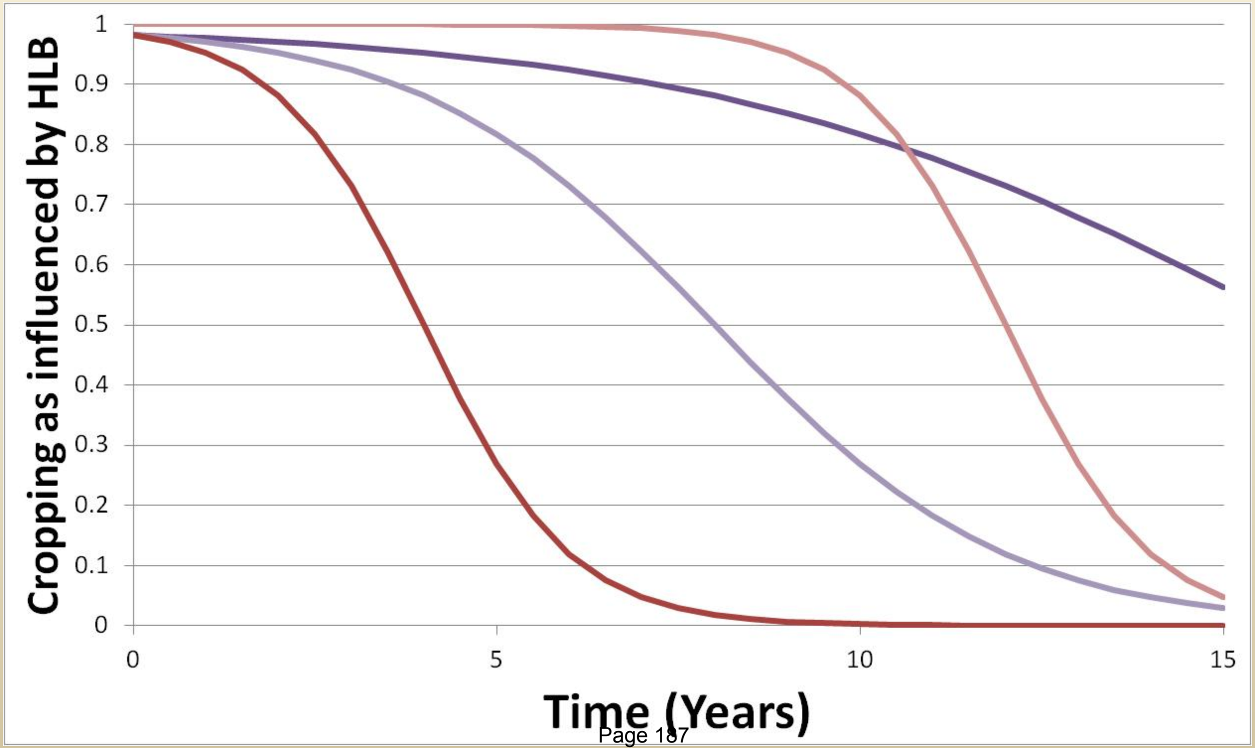
Trend in Florida Citrus Production



We may be living with HLB in FL forever:

- There are numerous areas of research that offer great promise for dealing with HLB
- However, nothing seems likely to “make it go away” any time soon
- Host resistance or tolerance to HLB offers the promise of a sustainable long-term solution to maintain economic citrus production
- Tolerant conventional cultivars may provide acceptable solution until immune citrus or pathogen / psyllid elimination are prime time

- In time truly immune trees will be found… in the meantime
- If resistance or tolerance is confirmed, how may this benefit citrus industries?
- How much of a delay in symptom development / compromise of cropping is needed to be useful?



Transgenics for HLB- Resistant Citrus

- Tolerance and resistance in "conventional citrus is great….. IF you have decided to live with HLB
- Transgenics appear to be the most promising solution for strong HLB resistance and perhaps <u>immunity</u>
- Another major advantage is ability to improve an existing cultivar with essentially no other changes: HLB-resistant Washington Navel,Tango etc.

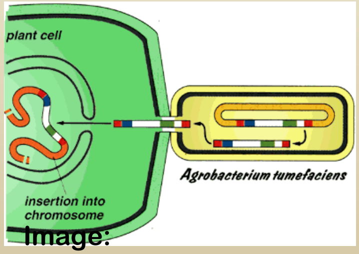
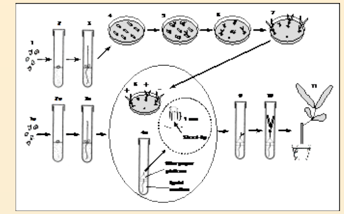


Image: www.plantsci.cam.ac.uk/.../GFP/plantrans.html

Future of transgenic Citrus

- Genetically engineered (GE) cultivars deregulated for commercial use in ~25 different agricultural crops
- GE crops are grown on ~12% of global arable land, mainly four field crops: soybean, maize, cotton and canola
- Several GE horticultural crops are being produced commercially since they provide solutions to otherwise intractable threats, much as HLB seems for citrus
- Commercial GE citrus is likely inevitable and GE crop concerns will likely decline with time
- NO released transgenic in any crop for bacterial resistance

Transgenic Project: Parallel Tracks



- Fastest track- possible “home run” using best available technology on rootstocks, sweet orange and grapefruit- high throughput.
  - Goal is earliest possible resistant variety in field
  - Emphasizing components which are deregulated in crop plants
- Experiments to overcome transformation bottlenecks
- Identifying new targets for transgenes
- Exploring other promoters, constructs, etc. first with easily transformed rootstock types

Categories: Transgenic Strategies

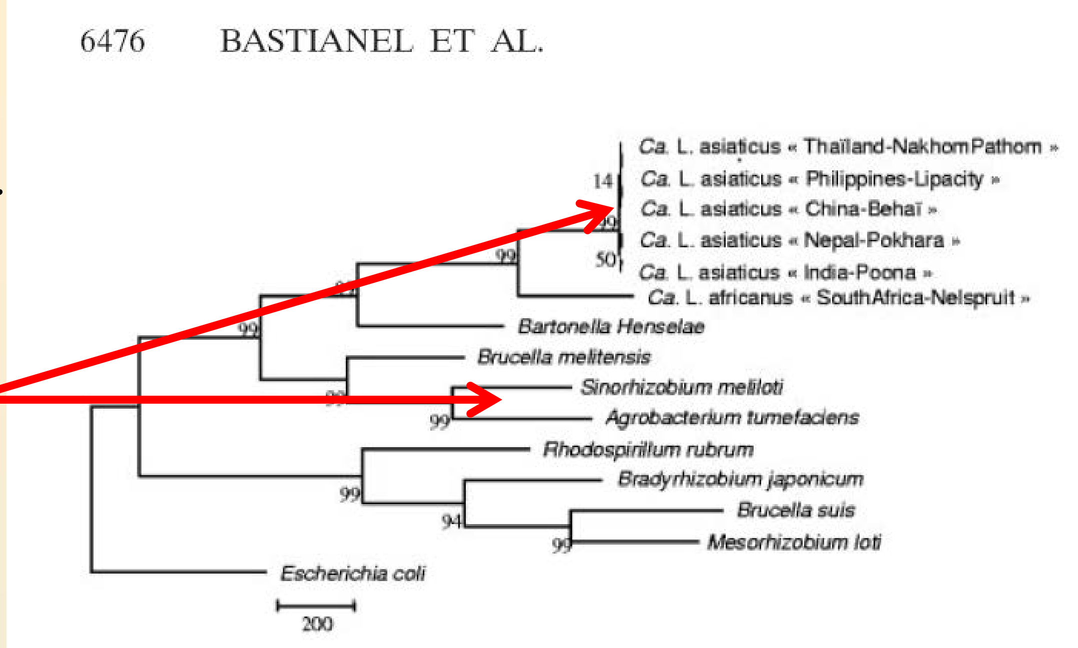
- Direct attack on the pathogen
  >Antimicrobials
  >ScFV for external pathogen epitopes
- Host pathogen interactions
  >Basal defense genes
  >CLas gene products that target host (nuclear localization protein, flagellin etc.)
- Citrus physiology
  >Possibly overactive defense response
  >Deciduousness
- Psyllid targets

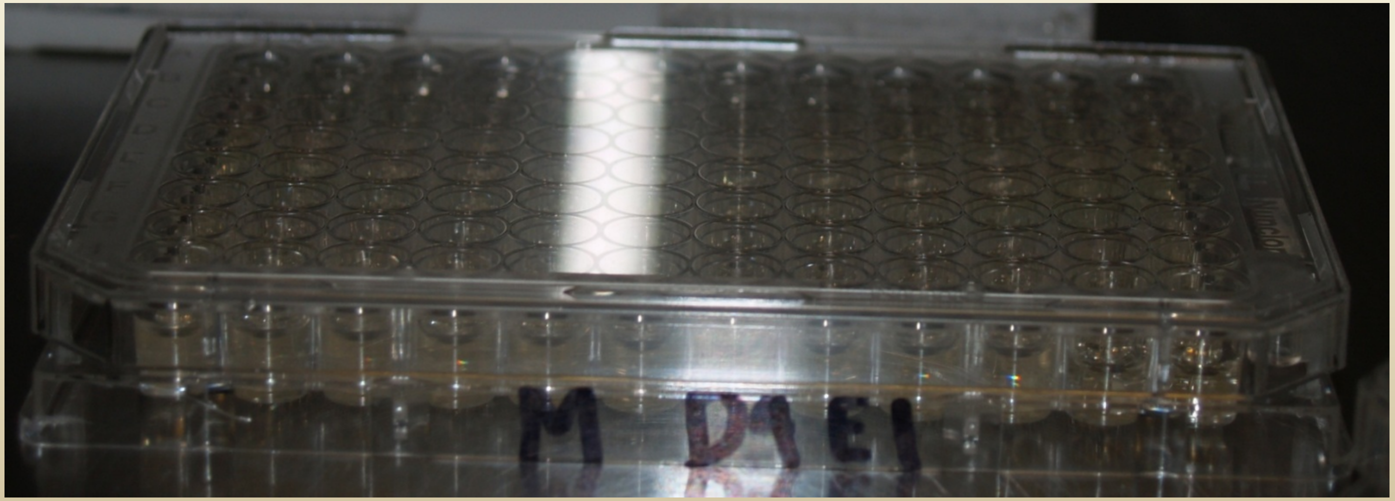
Antimicrobial Peptides

- First line of active defense to combat infection in multicellular organisms
- Broadly active against groups of micro-organisms- may suppress HLB, canker, etc.
- Mode of action primarily intercalation into membranes and depolarization
- Most are very small molecules, MAY move systemically- possible GM rootstock solution?
- Results in microbial death or prevents growth

In –Vitro AMP Screening: but Liberibacter is unculturable

- *Agrobacterium* and *Sinorhizobium* are related to *Liberibacter*
- Also used *Xanthomonas citri*, the pathogen that causes citrus canker





AMP	Source	MIC (μM) At	Sm	Xcc	Hemolytic Activity (%)
Tachyplesin I	crustac	0.3	0.3	0.3	3.0
SMAP-29	sheep	1	0.3	1	3.2
D4E1	synth	1	0.3	1	3.6
D2A21	synth	1	0.3	1	8.4
LL-37	human	1	1	1	5.1
Melittin	bee	1	1	1	100.8
Cecropin A	insect	3	3	10	1.1
Cecropin B	insect	10	3	10	1.2
Indolicidin	cow	10	3	3	2.0
Apidaecin IA	insect	>30	1	>30	1.6
Drosocin	insect	>30	3	>30	1.6
α-Purothionin	plant	30	10	1	22.5
Pyrrhocoricin	insect	>30	10	>30	1.9
Magainin I	frog	>30	>30	>30	1.3
Magainin II	frog	>30	>30	>30	1.5
Histatin-5	human	>30	>30	>30	1.8
Ib-4	plant	>100	100	>100	
Cn-1	plant	>100	>100	>100	
P4c	plant	>100	>100	>100	

In-vitro assays: broad group

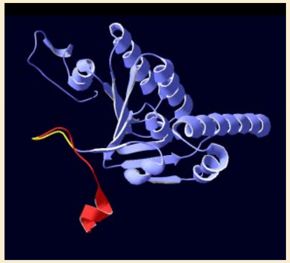
- **Quite repeatable across multiple runs**
- **"Best" AMPs in trial were animal or synthetic**
- **Tachyplesin from Horseshoe crab was "best", but several similar**

AMP	MIC (µM)			Hemolytic Activity (%)
	At	Sm	Xcc	Mean
AGM 155	1	0.1	1	1.6
D4E1	1	0.3	1	3.6
AGM 156	1	0.3	1	5.7
D2A21	1	0.3	1	8.4
AGM 152	1	0.3	1	9.3
AGM 153	1	0.3	1	10.5
AGM 154	1	0.3	1	18.2
AGM 151	3	0.3	3	21.4
AGM 178	1	1	1	1.2
AGM 176	1	1	1	1.3
AGM 175	1	1	1	16.3
AGM 174	1	3	1	17.6
AGM 179	3	3	3	2.1
AGM 157	10	1	3	19.4
AGM 158	10	0.3	10	29.0
AGM 159	10	0.3	10	32.3
AGM 170	>10	1	10	39.4
AGM 180	30	30	30	0.6
AGM 172	>10	>10	>10	43.1
AGM 173	>10	>10	>10	50.9
AGM 177	>30	>30	30	0.4
AGM 171	>30	>30	>30	36.8

Focus on Agromed synthetic AMPs to try to enhance activity- Jesse Jaynes designed

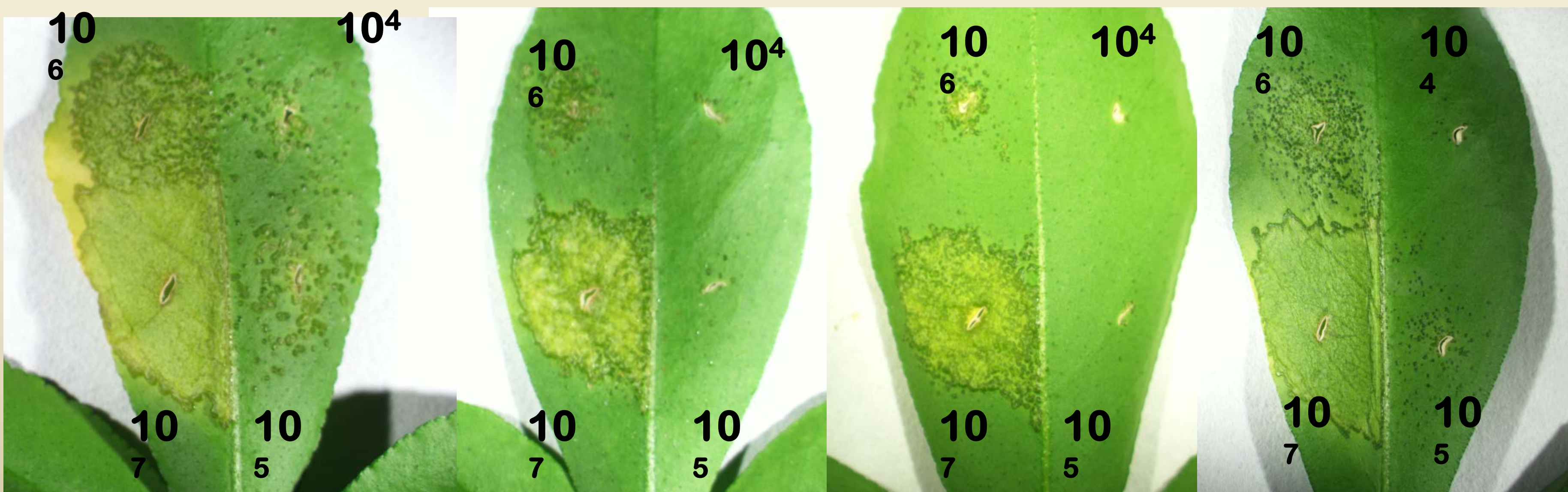
>hit a ceiling in MIC????

Chimeral antimicrobial peptides, designed by Goutam Gupta (Los Alamos National Laboratory)

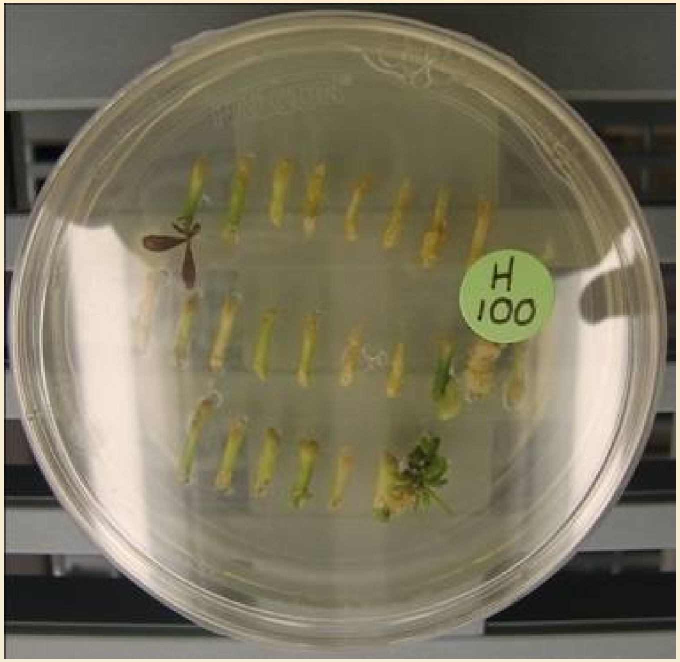


Chimera of a thionin (cyan) joined to the lytic D4E1 peptide (red) by a GSTA linker (yellow)

Xcc Infiltration results with transgenic plants containing thionin, D4E1 and chimera



Non transformed control **Thionin-C12** **Chimera-C9** **D4E1-C20**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100

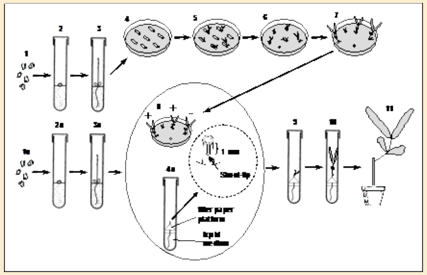
Aggressive challenge begins with no-choice exposure to CLas infected psyllids- led by D. Hall

Trees in greenhouse with free-flying CLas infected psyllids plus source plants-led by D. Hall

Numerous Transgenics Being Tested Under Field permits- USDA Farm Site with HLB pressure Open to all Researchers: UF, UC, TA&M, and USDA
- So far only modest effectiveness against HLB

N

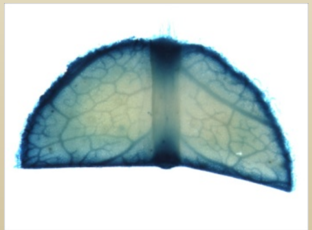
Transgenic Project: Challenging with HLB etc.



- Lots of plants of transformed with D35S/D4E1, AtSS/D4E1 etc. have been replicated challenged in field

- No major differences, **so far**, in HLB symptoms or Liberibacter levels. Some D4E1-GE plants have better growth.

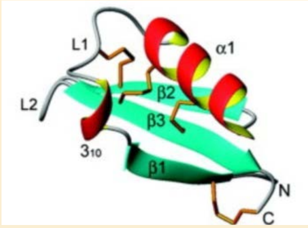
- Many more plants in pipeline with different promoters as well as different transgenes



d35S:GUS Activity in most tissue

SS:GUS activity <u>limited to phloem!</u>

Spinach Defensins for HLB resistance: Erik Mirkov Texas A&M University and Southern Gardens Citrus

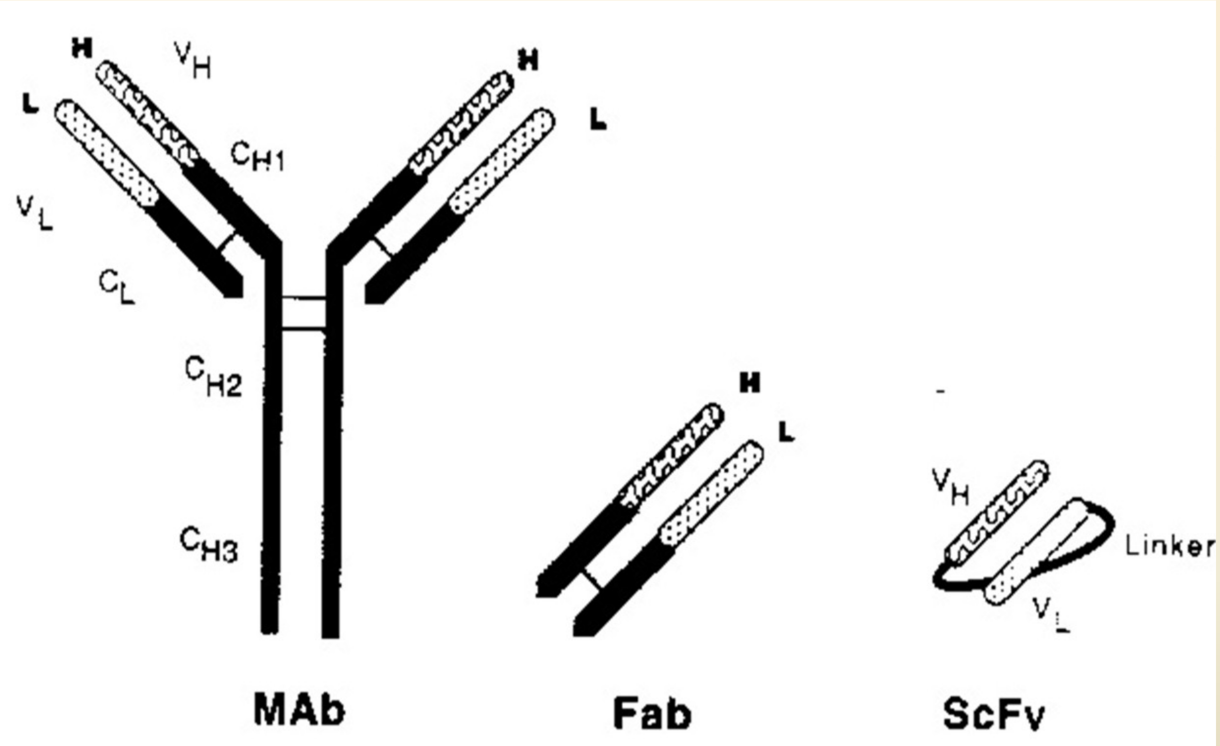


- <u>Furthest along in deregulation- data package well along</u>
- Trees being tested in field -Red Grapefruits and Sweet Orange
- Indicate have stronger HLB-resistance with <u>*two different*</u> spinach defensins expressed in same trees, in greenhouse

Transgenic production of ScFv against 'Ca. Liberibacter asiaticus'

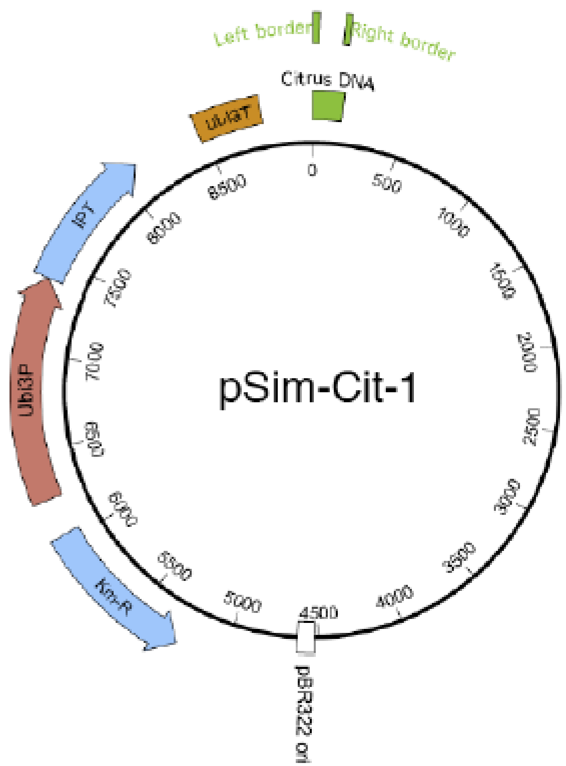
Hartung, Yuan, Stover

Hartung and Yuan also have scFv against psyllid targets



Transgenics using only DNA from Citrus Genepool- Belknap lead

Citrus Intragenic Binary Vector



Right Border

CACTACAACTCAATATATCCTGTACGAGATATCACAG
AAAGACANCAANATATATCCTGTCA (Consensus)

Left Border

GGGGCAGGATATATCCAAATGGAAAGACTAATCTGTCAGAAGGAAAAAGAAGAAGGACCTGTGTCAG
NNGGCAGGATATATNNNNNTGTAAA (Consensus)

Simplot
Caius Rommens and Kathy Swords

Whole Genome Assembly and Annotation of Citrus Sinensis (JGI) | Citrus Genome Database - Windows Internet Explorer

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Favorites | tides.info Ocean and river ti... | Agricultural Marketing Reso... | Mandarin - Tangerine Varieties | Free Hotmail | Web Slice Gallery

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Whole Genome Assembly and Annotation of Citrus Sinensis (JGI)

Downloads

Please note: if you download and use the JGI whole genome assembly and annotation please abide by the requirements for this data as specified on phytozome.org's Citrus sinensis download page.

Citrus sinensis Genome Assembly (JGI v1.0)

Scaffolds (FASTA file, 83Mb compressed)	Csinensis_v1.0_scaffolds.fa.gz
Scaffolds w/ masked repeats (FASTA file, 83Mb compresseD)	Csinensis_v1.0_scaffolds_RM.fa.gz
Scaffolds (GFF3 file, 78 Mb compressed)	Csinensis_v1.0_scaffolds.gff3.gz

Citrus sinensis Genome Annotation (JGI v1.0)

Coding sequences--CDS (FASTA file, 11Mb compressed)	Csinensis_v1.0_cds.fa.gz
Transcript sequences--mRNA (FASTA file, 15Mb compressed)	Csinensis_v1.0_transcript.fa.gz
Protein sequences (FASTA file, 7Mb compressed)	Csinensis_v1.0_peptide.fa.gz
Gene models (GFF3 file, 4Mb compressed)	Csinensis_v1.0_gene.gff3.gz
Alternate genes (GFF3 file, 3.5 Mb compressed)	Csinensis_v1.0_alt_gene.gff3.gz

Resources

- Genome Browser
- Assembly Details
- Downloads

A Race to Save the Orange by Altering Its DNA

Trees that are infected by disease are cut down and burned in Clewiston, Fla., at groves owned by Southern Gardens Citrus. Richard Perry/The New York Times

By AMY HARMON JULY 27, 2013 778 COMMENTS

CLEWISTON, Fla. — The call Ricke Kress and every other citrus grower in Florida dreaded came while he was driving.

"It's here" was all his grove manager needed to say to force him over to the side of the

Mother Jones

In Which I Actually Endorse One Use of GMOs

—By ***Tom Philpott*** | Wed Aug. 7, 2013 3:00 AM PDT

Carl Kiilsgaard/ZUMA

In a July 27 feature article that set the interwebs aflame, *New York Times* reporter Amy Harmon told the tale of a bacterial pathogen that's stalking the globe's citrus trees, and a Florida orange juice company's effort to find a solution to the problem through genetic engineering.

An invasive insect called the Asian citrus psyllids carries the bacteria, known as Candidatus Liberibacter, from tree to tree, and it causes oranges and other citrus fruits to turn green and rot. "Citrus greening," as the condition has become known, has emerged as a pest nearly wherever citrus is grown globally. Harmon reported that an "emerging scientific consensus" holds that only genetic engineering can defeat it.

Meanwhile, Michael Pollan, a prominent food industry and agribusiness critic, tweeted this:

Thanks!

- <u>Florida Citrus Research & Development Foundation</u>
- New Varieties Development and Management Corp
- Florida Citrus Research Foundation (Whitmore)
- California Citrus Research Board
- DPI Budwood Office (especially Peggy Sieburth)
- USDA/ARS Funding and USDA/APHIS

Jodi Avila	Robyn Baber	Abby Bartlett
Greg Brock	Wayne Brown	Fede Caro
Scott Ciliento	Ellen Cochrane	Jacqueline Depaz
Belkis Diego	Lynn Faulkner	Amber Holland
Diane Helseth	Scott Hyndman	Chris Lasser
Angel Ledger	David Lindsey	Philip Matonti
Steve Mayo	Kathy Moulton	Jerry Mozoruk
Luc Overholt	Sean Reif	Mike Rutherford
James Salvatore	Matthew Sewell	Jeff Smith
Regina Tracy	Ashley Witkowski	Jon Worton

Genetic Improvement of Citrus for the development of HLB (greening disease) bacterial resistance

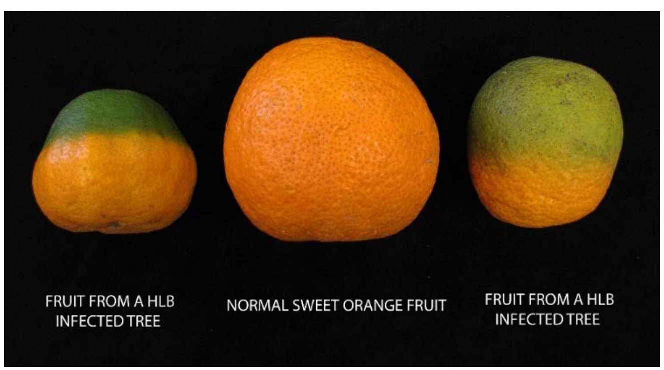
Genetic Improvement of Citrus for the development of HLB (greening disease) bacterial resistance

Manjul Dutt and Jude Grosser
Citrus Research and Education Center,
University of Florida,
Lake Alfred, FL, 33850

The problem!

- Huanglongbing (HLB), also known as citrus greening disease, is a bacterial plant disease that is fatal for citrus trees.
- HLB is caused by the fastidious, phloem - limited, gram-negative bacterium *Candidatus* Liberibacter asiaticus (CLas) which is transmitted by the Asian citrus psyllid.
- Diseased trees produce bitter, hard, misshapen fruit and die within a few years of being infected.
- HLB is considered to be one of the most serious plant diseases in the world and currently there is no permanent cure.

Source: http://californiacitrusthreat.org/huanglongbing-citrus-greening.php



http://www.dawn.com/news

Where are we right now?

- We have produced over 1000 independent transgenic events (containing one of 11 different gene constructs) in over 15 different citrus rootstock and scion varieties, both diploid and tetraploid. Many of them are in current field trials.
- Several antimicrobial peptides have shown initial promise but long term studies have not been fruitful for the majority of them.
- We have identified two Systemic Acquired Resistance (SAR) genes that have potential in combating HLB.

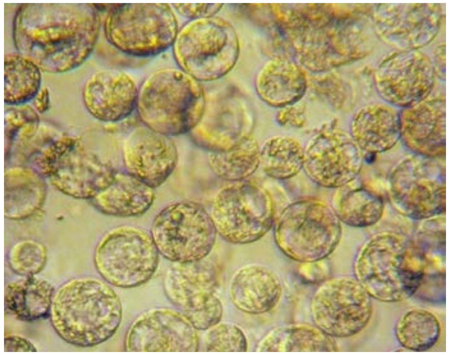
Our research priorities

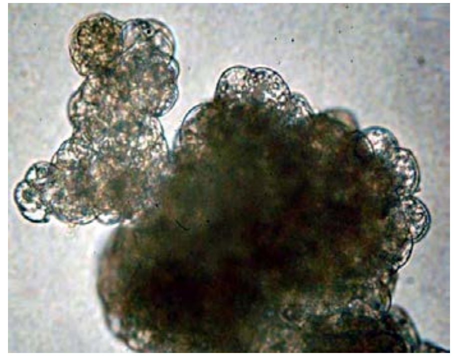
- Produce consumer acceptable transgenic plants (both rootstock and scions) containing only plant sequences and our plant derived gene(s) of interest without virus or bacterial components to target Clas.
- Utilize RNAi (troponin, anti wing development protein or wingless) and insecticidal gene(s) to create trap plants and target the Asian Citrus psyllid vector.

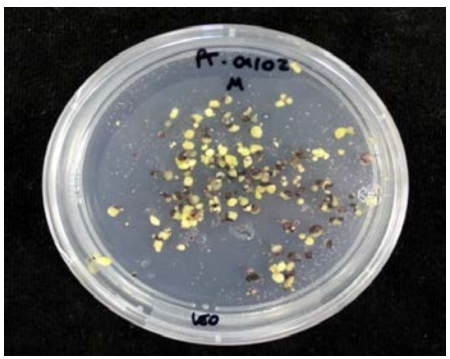
Components of an “all plant“ system

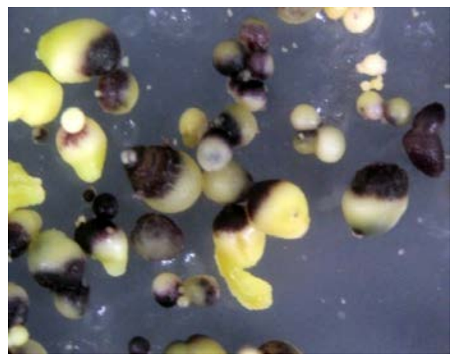
- A plant derived selectable marker.
- A set of plant derived promoters, genes and terminators.
- A robust transformation and regeneration system.

Protoplast mediated Transformation







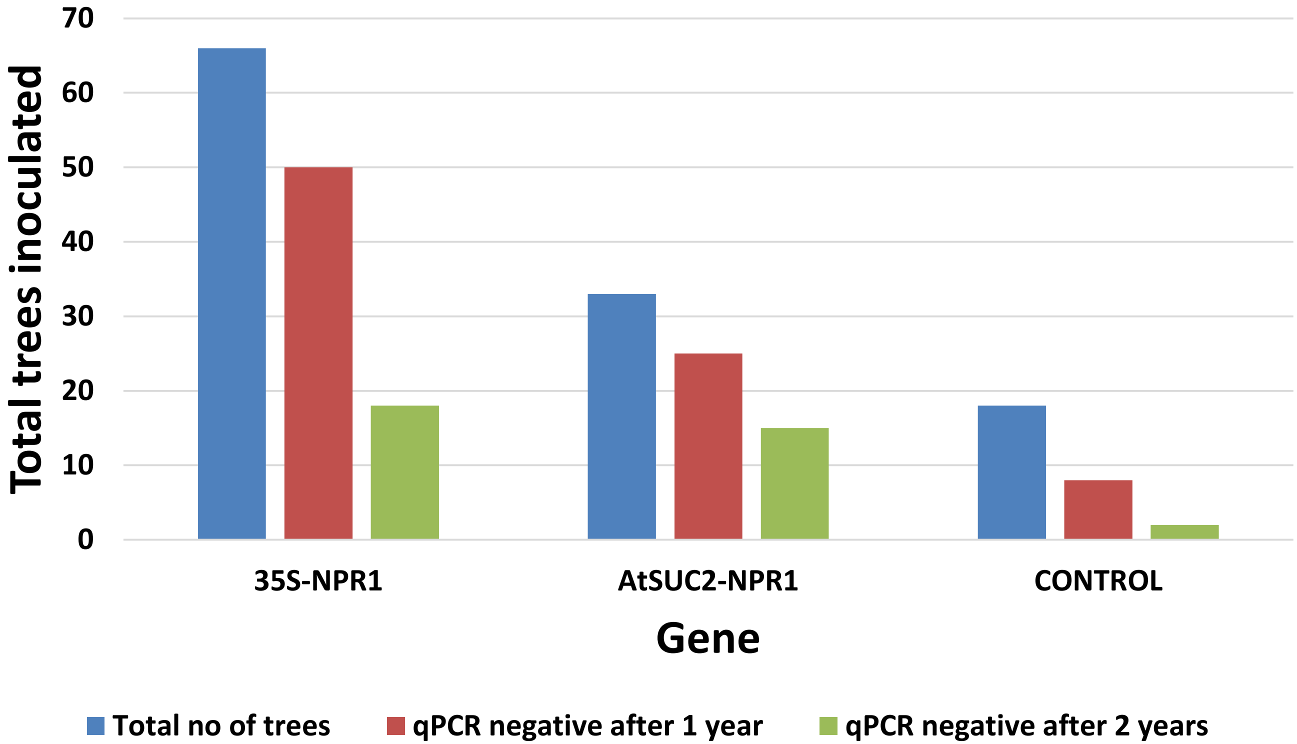


Protoplast transformation offers the ability to bypass the *Agrobacterium* mediated transformation process.

It also enables the use of linear DNA pieces.

The Anthocyanin overexpressing RUBY gene obtained from the ‘Moro’ Blood Orange is expressed only in the somatic embryos using a citrus derived embryo specific promoter

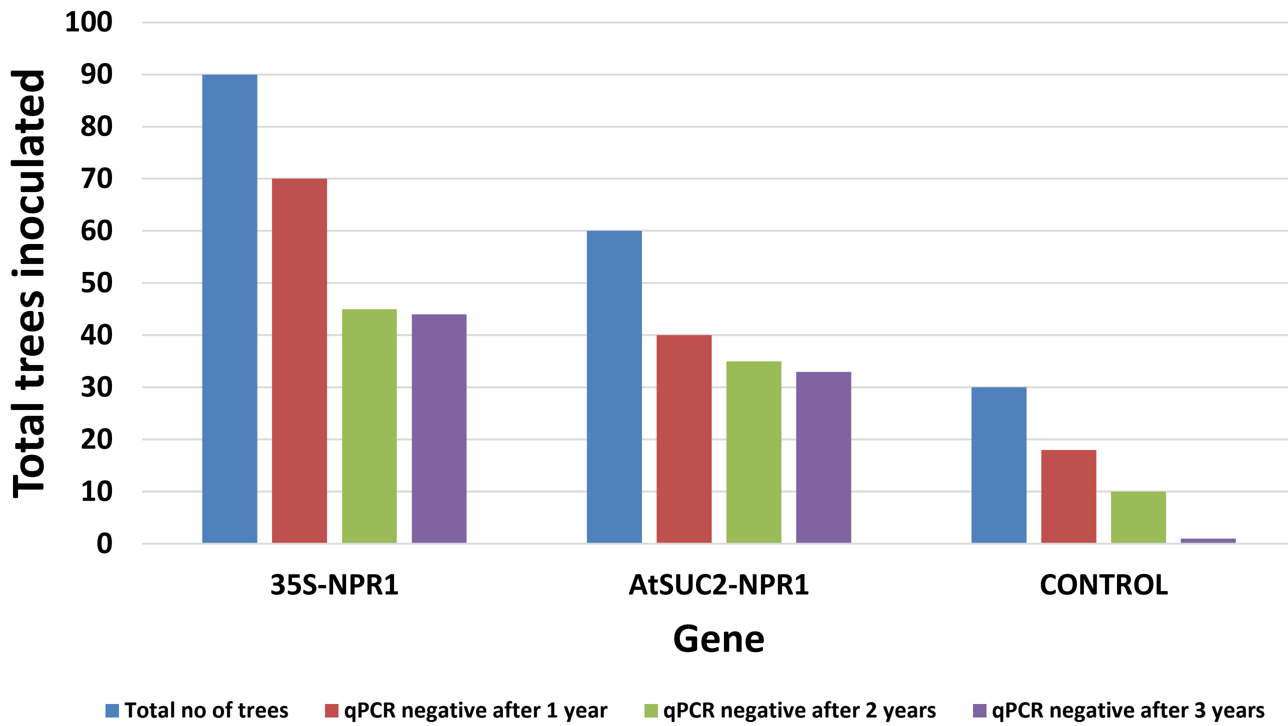
Results from greenhouse experiments



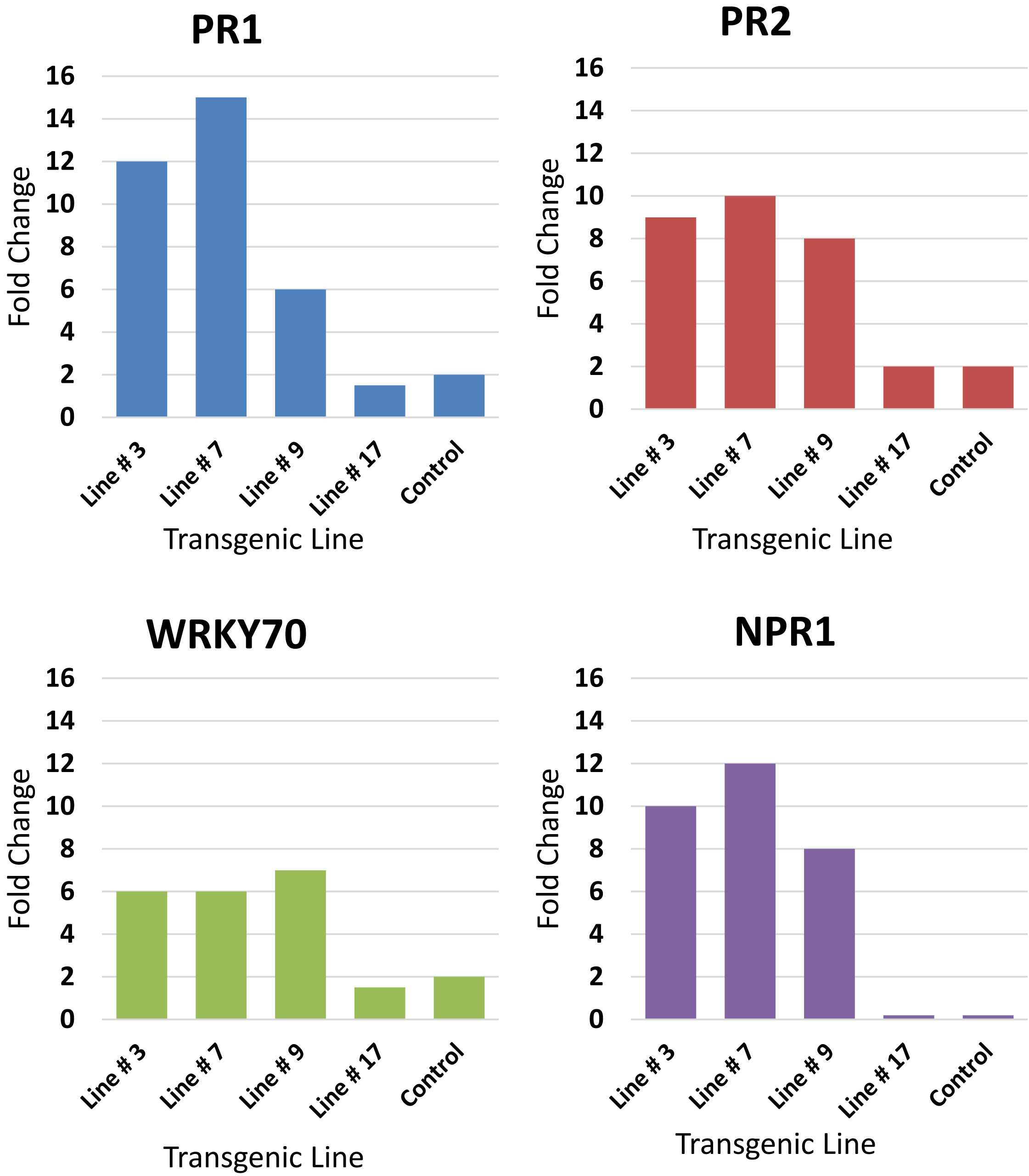
A NPR1 (HLB-) tree planted in the field after 2 years in the greenhouse

- Transgenic trees and controls were exposed to free flying potentially CLas+ psyllids in a greenhouse.
- Psyllids were randomly tested for the presence of CLas.
- Data was collected at yearly intervals.

Results from Field trials



- Transgenic trees and controls were planted in two sites, both in South Florida counties with a 80 - 90+ HLB infection rate.
- Samples were collected at yearly intervals and analyzed using qPCR.

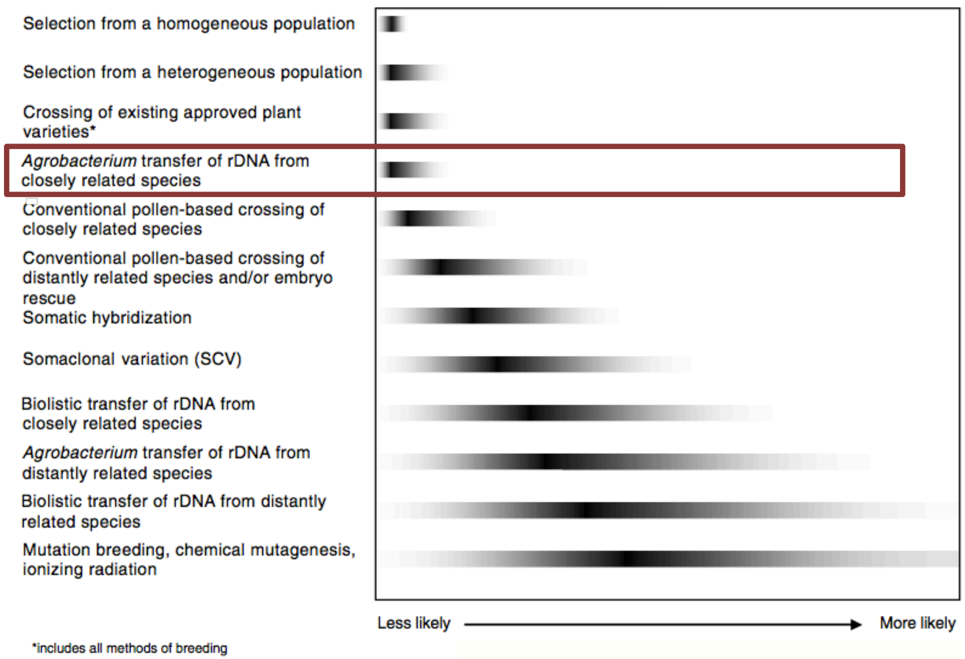


Relative quantification of transgene activity using the $2^{-\Delta\Delta Ct}$ method and RT-qPCR a year after infection

HLB (–) tree expressing the NPR1 SAR inducing gene.

Unanswered Questions?

- Resistance or Avoidance?
- Consumer acceptance?
- Long term transgene stability
- Nutritional assessments



Source: National Research Council. *Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects*. Washington, DC: The National Academies Press, 2004.

Acknowledgements

CRDF Citrus Research and Development Foundation, Inc.

UF | Citrus Research *and* Education Center
Institute *of* Food and Agricultural Sciences

USDA

Registering Antibiotic Pesticides for Use on Crops

Registering Antibiotic Pesticides for Use on Crops

Susan Jennings
Public Health Coordinator
Office of Pesticide Programs
US Environmental Protection Agency
September 11, 2014

Antibiotics on Crops: Today's Topics

- Background and Regulatory Authority
- Registration Process for Antibiotics on Crops
  - Data Needs
  - Data Interpretation
- Risk Assessment
  - Potential for Antimicrobial Resistance
  - Risk Mitigation

EPA's Mission for Pesticides

- Make best possible regulatory decisions to protect public health, non-target species, and the environment
- Be consistent with core principles
  - Sound science
  - Overwhelming transparency
  - The rule of law
- Applies to conventional, biological and antimicrobial pesticides

Antibiotics as Pesticides

- Antibiotics on crops are pesticides under FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act)
  - FIFRA requires EPA to determine that any registered pesticide is not expected to cause unreasonable adverse effects on human health or the environment
- For antibiotic pesticides, antibiotic resistance is part of this determination

Background and Regulatory Authority

- Pesticides can be approved under FIFRA Section 3 or Section 18 [or even 24(c)]
  - Section 3 broadest section
  - Section 18 used for emergency situations
  - Section 24(c) for special local needs

Antimicrobials on Crops: Data Needs

- Registering pesticides, including antibiotics requires, an extensive data set
- Data requirements for active ingredient:
  - Toxicity (short-term and long-term effects for mammalian, avian, and aquatic species)
  - Environmental Fate Data (how long it will last in the environment)
  - Exposure or monitoring data, if available

Antimicrobials on Crops: Data Needs

- Data requirements may be satisfied using different sources:
  - Studies conducted for registration according to EPA published protocols and guidelines
  - Information published in the general literature
  - Surrogate data conducted with other methods
- Studies not performed according to EPA guidelines will need to be approved before submission

Antimicrobials on Crops: Assessing Resistance

- Resistant bacteria is a unique and increasing threat to human health across the globe
- Number of antibiotic pesticides registered for use on crops is fairly limited
- EPA's process first used during reregistration process for streptomycin and oxytetracylin
  - During 2005 decision, consulted with CDC and FDA several times

Antimicrobials on Crops: Assessing Resistance

- Resistance can develop through several routes
  - Residues on foods
  - Workers exposed to antibiotics
  - Exposure to bacteria populations in the environment

Antimicrobials on Crops: Assessing Resistance

- Typically, registrant submits analysis of the potential resistance using FDA's Guidance for Industry #152
  - Guidance created to assess risk for resistance among bacteria of human health concern from treating food-producing animals
- EPA may also assess potential for drug residues in/on food to cause adverse effects on the intestinal microflora of consumers

FDA's Guidance #152

- Designed to evaluate potential for the transmission of bacteria of human health concern from consumption of food products
  - Helps define the degree of concern
  - How much the use of pesticide in food-production would be expected to result in resistant food-borne bacteria that adversely impact human health

FDA's Guidance #152

- Qualitative risk assessment for release, exposure, and consequence of use
  - Risk estimation for the release and exposure (ranked low, medium or high)
  - Consequence finding (ranked important, highly important or critically important)
  - Results in estimate of the overall risk (ranked low, medium or high) for proposed antibiotic use

Assessing the Risk: A Collaboration

- EPA consults with CDC before taking regulatory action on a pesticide used to control a pest of public health importance
  - Initially, used for vector control pesticides
- To further protect public health, EPA consults with interested federal partners on antibiotics
  - For antibiotics, this includes CDC and FDA

Assessing the Risk: A Collaboration

- CDC and FDA are involved throughout the risk assessment and management phases
  - Results of assessment conducted according to Guideline #152
  - Potential for refining the risk assessment through special studies or other means
  - Risk Mitigation/Monitoring options

Mitigating Risks of Concern

- EPA and registrants may identify changes in use patterns that may mitigate any risks of concern
- For antibiotic resistance, these may include:
  - Application method (injection vs. air blast)
  - Application timing (pre-bloom vs. post-harvest)
  - Application rates
  - Application frequency
  - Use a formulation that reduces the risk of resistance (will vary with a.i.)
  - And others…..

Follow-up Post Registration

- EPA sometimes requires monitoring data when an antibiotic is registered to:
  - Evaluate whether the use is likely to convey resistance to bacteria in the environment or workers;
  - Detect any cross-resistance to other compounds that may be problematic for public health
  - If resistance does become a problem, detect it before it becomes too expansive.

EPA Decisions

- Although EPA has sole regulatory authority when making these decisions, FDA and CDC have extensive expertise in this area
  - EPA first started looking at resistance to these compounds as a public health element in 2005
- Since then, we've looked at several new applications, both under Section 18 and 3, and our process has been fairly robust

EPA Decisions

- EPA must adhere to its regulatory mandate by making a:
  - Determination under FIFRA (expected to pose no unreasonable adverse…)
  - Determination under FFDCA to establish tolerances (reasonable certainty no harm from aggregate exposures ...)
- For antibiotics on crops, the potential for resistance to human pathogens is a concern

In Closing ……

- Antibiotics are pesticides (when used on crops) and EPA regulates them as such
- During risk assessment, EPA consults with CDC and FDA on potential for use to cause resistance of public health concern
- Decision processes are transparent and scientifically sound

Questions??

Susan Jennings

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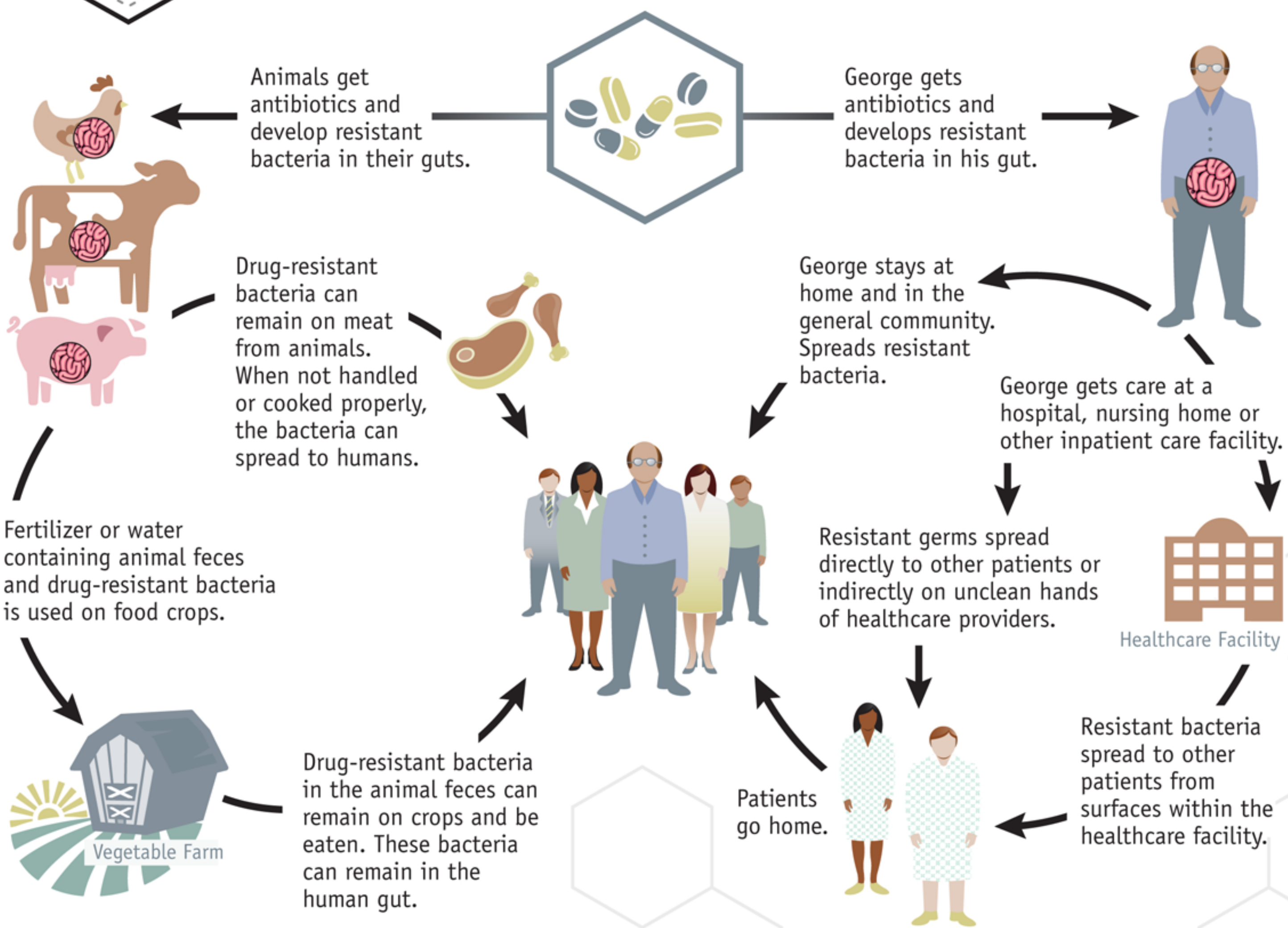
The Threat of Antibiotic Use in the Environment on Human Health

The Threat of Antibiotic Use in the Environment on Human Health

September 11, 2014

Jean B. Patel, PhD, D(ABMM)
Deputy Director
Office of Antimicrobial Resistance
Division of Healthcare Quality Promotion

Examples of How Antibiotic Resistance Spreads



Simply using antibiotics creates resistance. These drugs should only be used to treat infections.

The Risk of Pesticide Antibiotic Use for Increasing AMR Infections in Humans

Potential pathways:

1. Pesticide antibiotic use selects for resistance to a human antibiotic.

   A. The pesticide antibiotic could select for new resistance and this also confers resistance to human antibiotics

   B. The pesticide antibiotic selects for amplification of a pre-existing resistance and a resistant human pathogen is amplified

2. Pesticide antibiotic use disrupts a animal or human microbiome and creates a niche for amplification of a human AMR pathogen

Important Scientific Questions

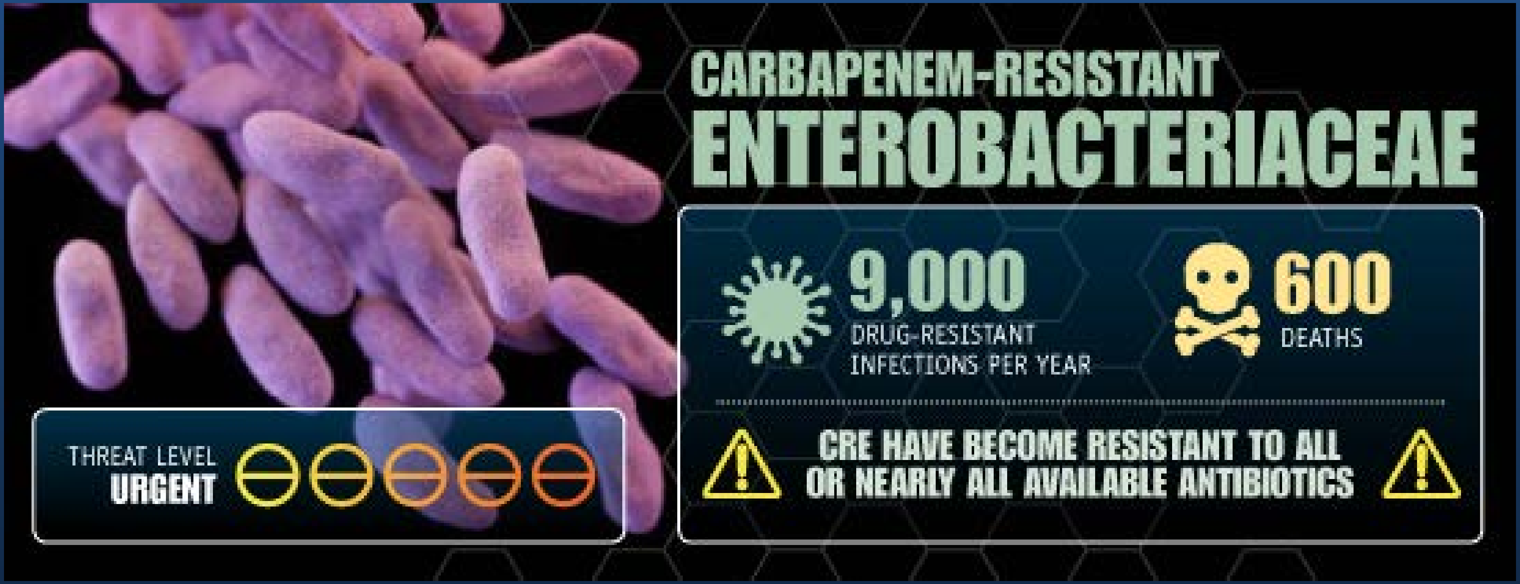
- Does use of the pesticide antibiotic select for resistance determinants that confer cross-resistance to human antibiotics?

- Do resistance mechanisms common in human pathogens confer resistance to the pesticide antibiotic?

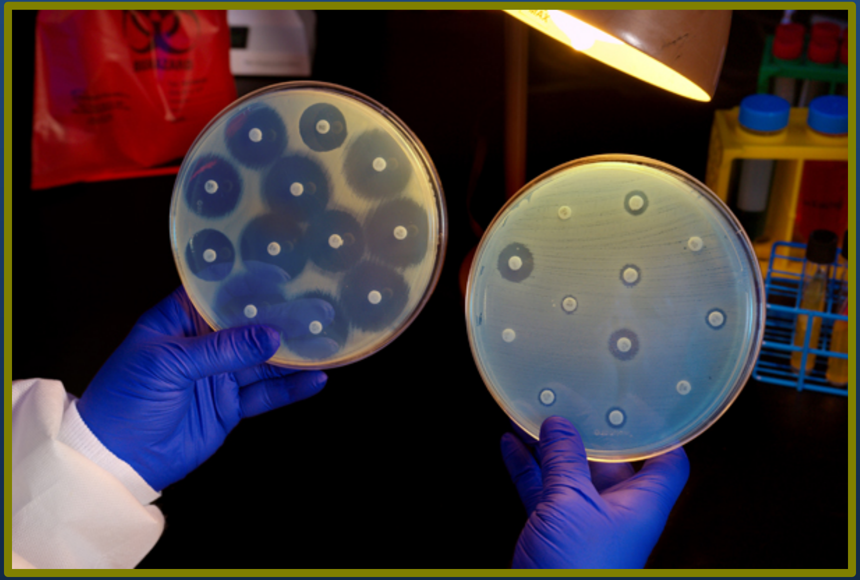
- Does the pesticide antibiotic pathogen disrupt animal or human microbiomes.

A Hypothetical Scenario:
CRE Amplification from Pesticide Use

The pesticide antibiotic selects for amplification of a pre-existing resistance and a resistant human pathogen is amplified


CARBAPENEM-RESISTANT
ENTEROBACTERIACEAE
9,000
DRUG-RESISTANT
INFECTIONS PER YEAR
600
DEATHS
THREAT LEVEL
URGENT
CRE HAVE BECOME RESISTANT TO ALL
OR NEARLY ALL AVAILABLE ANTIBIOTICS

Carbapenem-Resistant Enterobacteriaceae (CRE)

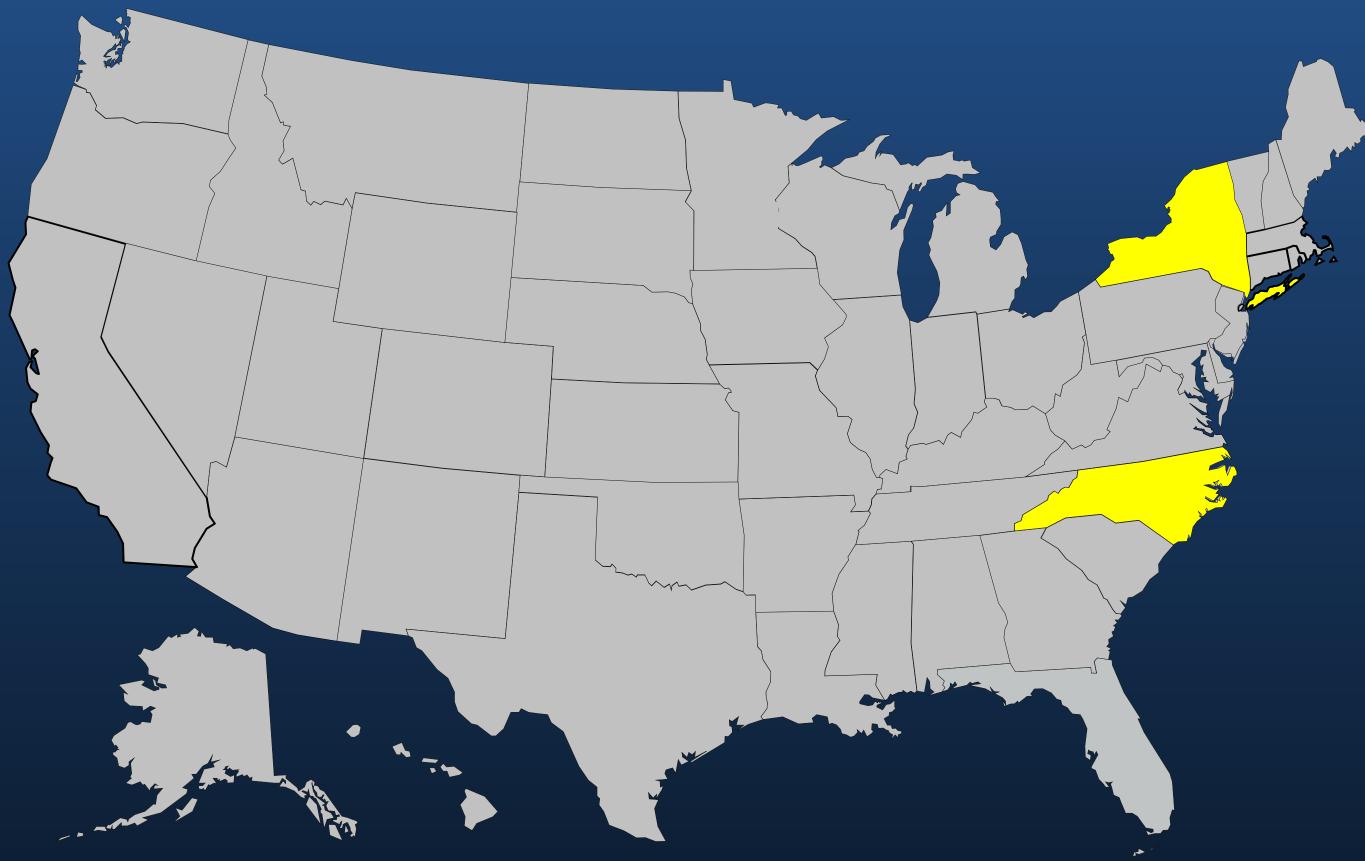


Carbapenemase

KPC
NMD
VIM
IMP
OXA-48

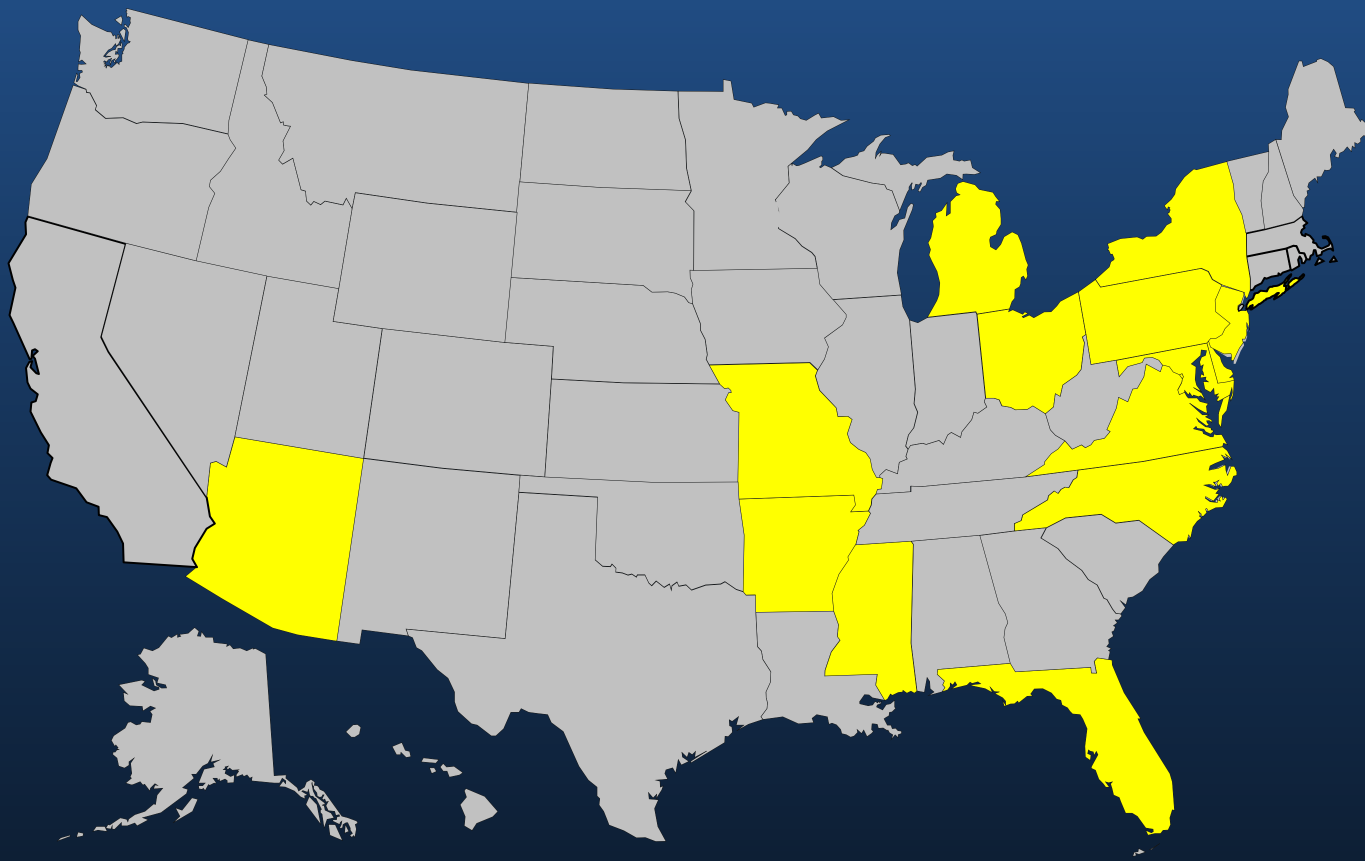
- Nearly pan-resistant pathogens
- Multiple carbapenemases
- Carbapenemases are carried on multiple drug-resistant plasmids

Geographical Distribution of KPC-Producers



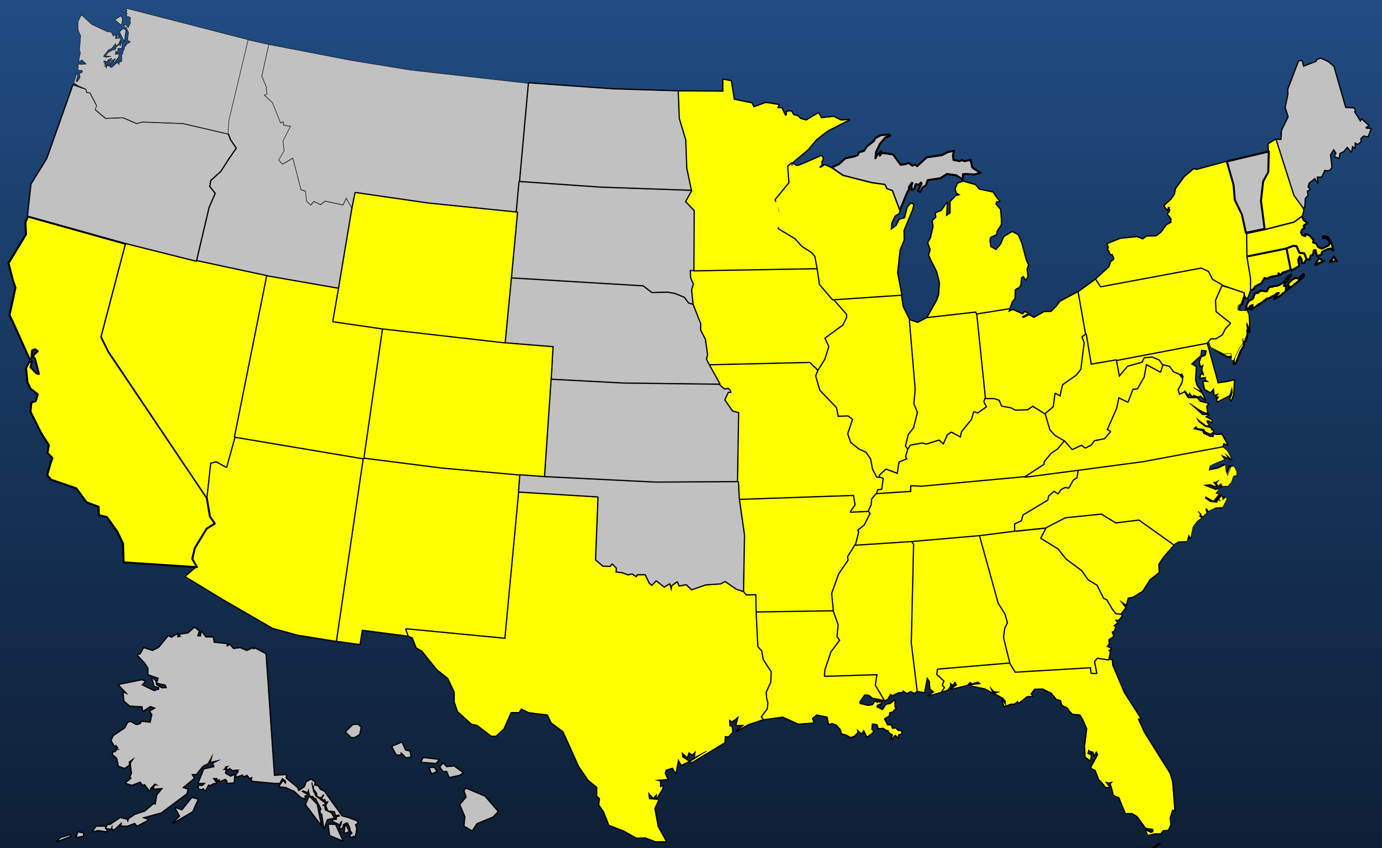
2001

Geographical Distribution of KPC-Producers



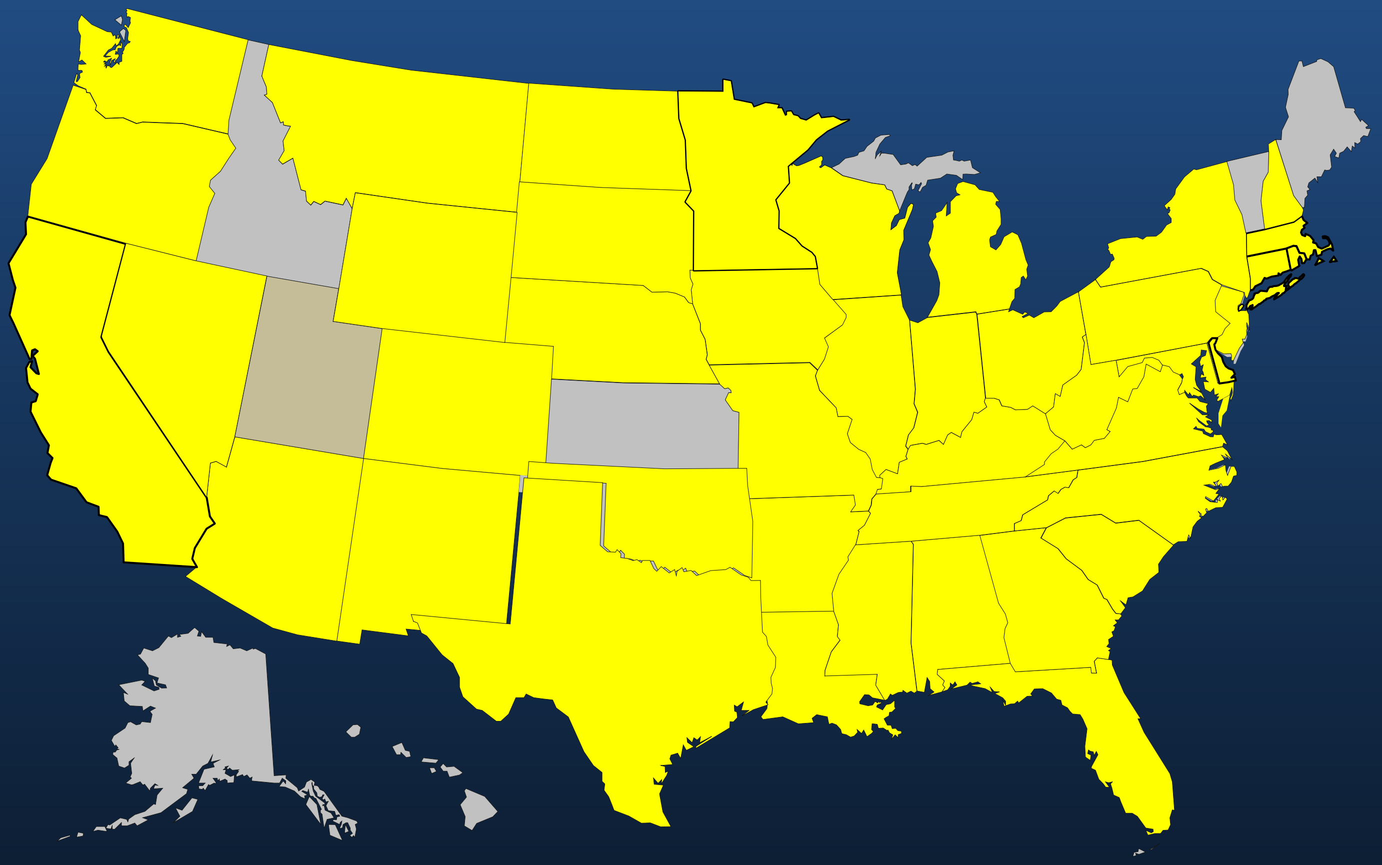
2006

Geographical Distribution of KPC-Producers



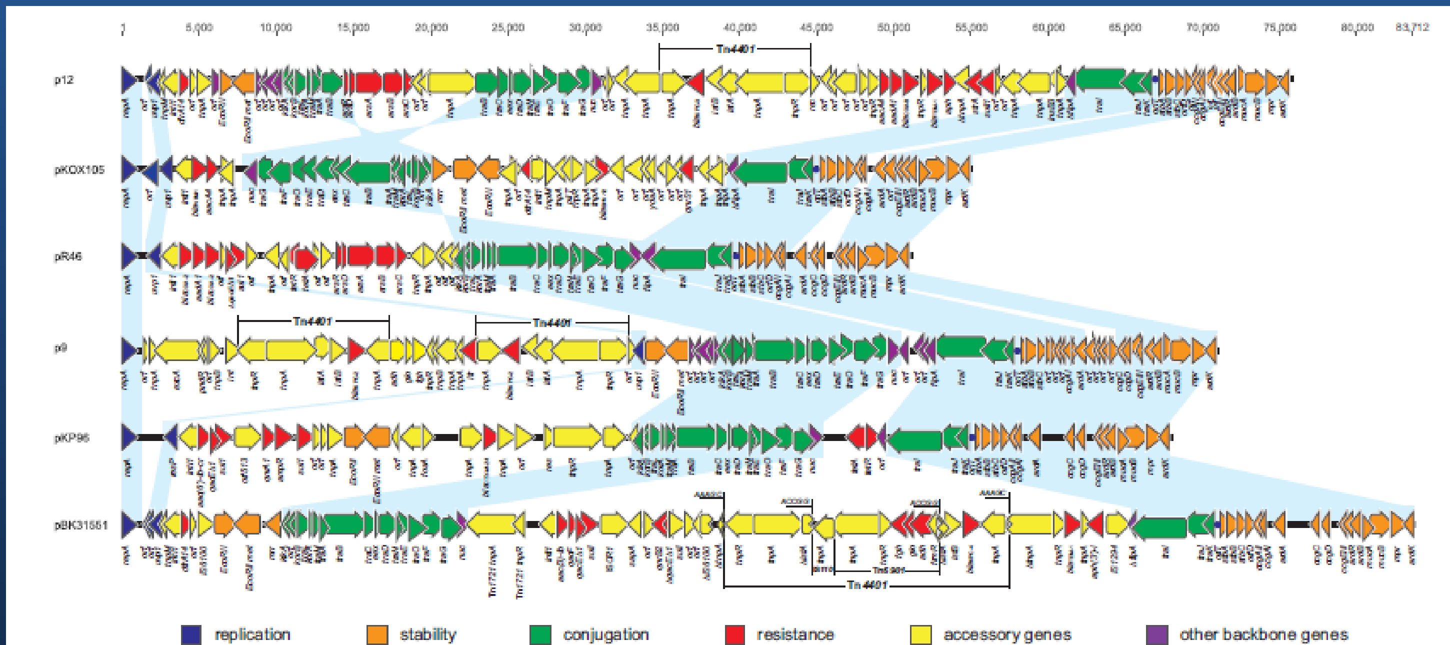
2010

Geographical Distribution of KPC-Producers



2014

CRE Plasmids



Resistance genes are indicated in red arrows and include:

- aminoglycoside resistance genes aph (3=)-I, aac(3)-Ib, aac(6=)-Ib-cr, aph, aadA1, and aacA4,
- beta-lactamase genes blaKPC-2, blaKPC-3, blaKPC-4, blaKPC-5, blaVIM-1, blaCTX-M-24, blaOXA-2, blaOXA-9, blaTEM-1, blaSHV-12, and ampR
- quinolone resistance genes qnrA1, qnrB2, and qnrS1
- arsenic resistance genes arsA, arsB, arsC, arsD, and arsR
- tetracycline resistance genes tetA and tetR
- trimethoprim, and sulfonamide resistance genes sul1, qacF, qacE1, and dfrA14

L. Chen, et al. ***Antimicrob. Agents Chemother.* 2013, 57(1):269**

A Question of Exposure

- If CRE is a cause of healthcare-associated infections would a pesticide antibiotic ever come in contact with the resistant pathogen?

MAJOR ARTICLE

Wastewater Treatment Plants Release Large Amounts of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* Into the Environment

Caroline Bréchet,[1] Julie Plantin,[1] Marlène Sauget,[1] Michelle Thouverez,[1] Daniel Talon,[1] Pascal Cholley,[1] Christophe Guyeux,[2] Didier Hocquet,[1] and Xavier Bertrand[1]

[1]Service d'Hygiène Hospitalière, UMR 6249 Chrono-environnement, Centre Hospitalier Régional Universitaire, Université de Franche-Comté, Besançon; and [2]Département DISC, Institut FEMTO-ST, UMR 6174 CNRS, Université de Franche-Comté, Belfort, France

(See the Editorial Commentary by Griffiths and Barza on pages 1666–7.)

CRE Colonization

ORIGINAL ARTICLE EPIDEMIOLOGY

Gastrointestinal colonization by KPC-producing *Klebsiella pneumoniae* following hospital discharge: duration of carriage and risk factors for persistent carriage

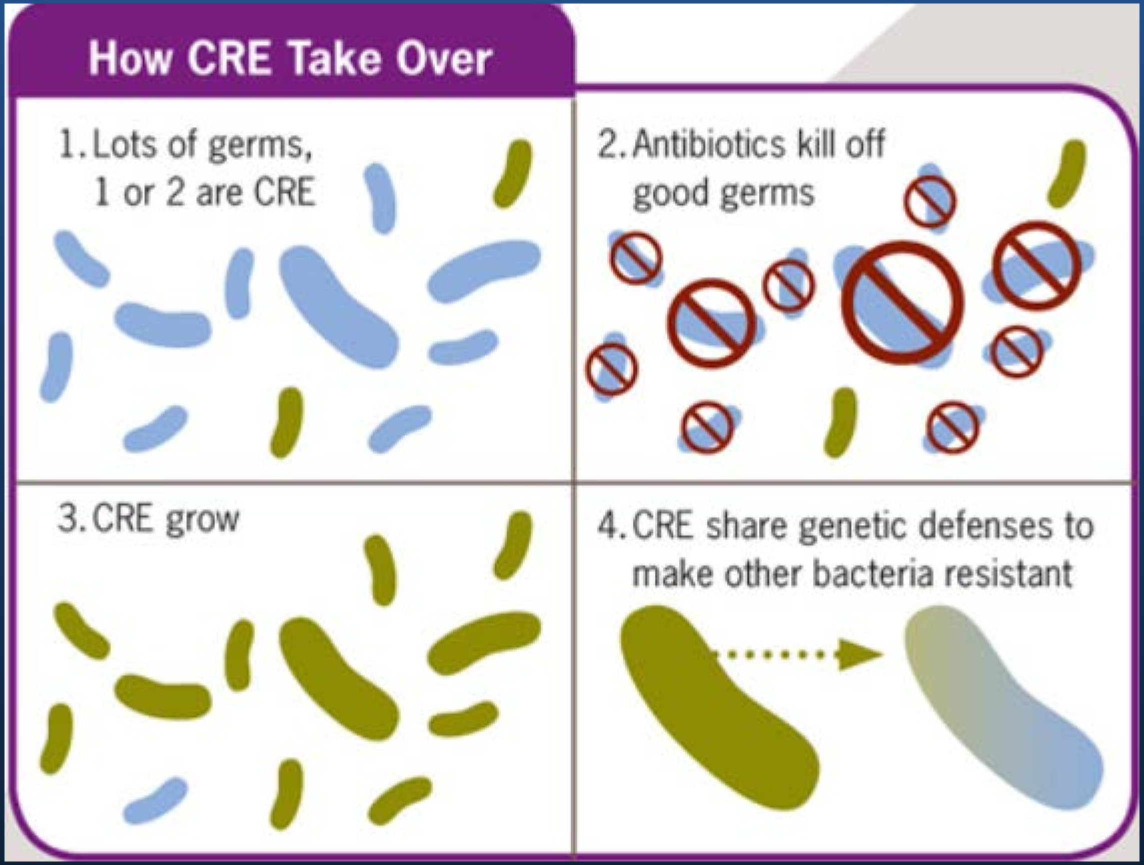
N. Feldman[1†], A. Adler[2†], N. Molshatzki[1], S. Navon-Venezia[2], E. Khabra[2], D. Cohen[1] and Y. Carmeli[2]

1) *Sackler Faculty of Medicine, School of Public Health, Tel-Aviv University* and 2) *Division of Epidemiology, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel*

A few things to know about colonization:

- Colonization can persist for > 6 months
- There is no treatment to eradicate GI colonization
- Colonization is a source of person-to-person transmission
- Colonization increases the likelihood of infection for an individual

How CRE Colonization Leads to Infection



Hypothetical Event

- Human resistant pathogen like CRE is present in the environment.
- The pesticide antibiotic selects for and amplifies the pathogen because the pathogen carries a determinant that confers resistance to the pesticide antibiotic.
- The amplified human pathogen colonizes humans or animals that come into contact with it.
- The increased colonization results in AMR infection and/or transmission.

The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria

"Antibiotic resistance develops through complex interactions, with resistance arising by de-novo mutation under clinical antibiotic selection or frequently by acquisition of mobile genes that have evolved over time in bacteria in the environment."

Wellington, EMH et al., Lancet ID., February 2013, Pages 155–165

From the same article…

"The reservoir of resistance genes in the environment is due to a mix of naturally occurring resistance and those present in animal and human waste and the selective effects of pollutants, which can co-select for mobile genetic elements carrying multiple resistant genes."

Other Concerns

- Antibiotic residue in food
  - Selection of resistance
  - Allergic reaction

Thank You

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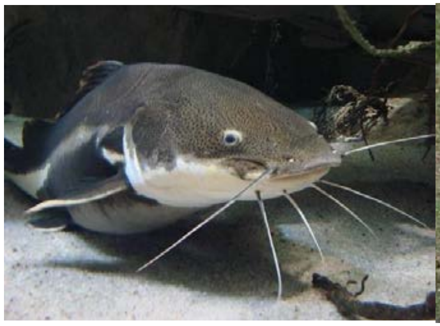
Centers for Disease Control and Prevention
1600 Clifton Road NE, Atlanta, GA 30333
Phone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov   Web: www.cdc.gov



Microbial Food Safety Risk Assessment and Regulatory Decision-Making




Comments by
Heather Harbottle, Ph.D.
Microbial Food Safety Team (HFV-157)
Office of New Animal Drug Evaluation
Center for Veterinary Medicine

Uses of Antimicrobials in Food-Producing Animals

- Therapeutic uses – treatment, control, and prevention of animal diseases

- Production uses - to increase feed efficiency (improve feed efficiency, increase rate of weight gain)
  - may be a contributing factor in the rise of antimicrobial resistance due to their typical administration to entire herds or flocks of food animals at low doses and for prolonged durations.
  - FDA thinks that such production uses of medical important antimicrobial are not judicious.

Uses of Antimicrobials in Food-Producing Animals

Production uses

- all production uses of medically important antimicrobials were originally approved prior to the implementation of Guidance for Industry (GFI) #152.

- To address concerns FDA published:

  - GFI # 209 *"The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals"*

  - GFI # 213 *"New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for voluntarily Aligning product use conditions with GFI # 209"*

Uses of Antimicrobials in Food-Producing Animals

- GFI # 209 provides:
  - a framework to ensure the judicious use of antimicrobial in food animals
  - Strategies for reducing antimicrobial resistance:
    - ✓ Limiting medically important antimicrobials to uses in food-producing animals that are considered necessary for assuring animal health
    - ✓ Limiting such drugs to uses in food-producing animals that include veterinary oversight or consultation
- GFI # 213 provides:
  - recommendations on how to implement strategies outlined in GFI 209, specifically for those medically important antimicrobials approved for use in water or feed for food-producing animals.

http://www.fda.gov/antimicrobialjudicioususe

Microbial Food Safety Analysis

- Reviews of Microbial Food Safety of New Animal Drugs in the following categories:
  - Guidance for Industry #152 – Analysis of risk of development of antimicrobial resistance among bacteria of human health concern in/on treated food-producing animals
  - Guidance for Industry #159 – Microbiological ADI
- Food Additive Petitions
- GRAS Notifications

Guidance for Industry #152: Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern

- Qualitative risk assessment approach
- Assess antimicrobial drugs intended for food-producing animals regarding the development of resistance
- Address human exposure to antimicrobial resistant microbes through ingestion of animal-derived food

http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052519.pdf

Hazard Characterization

Qualitative Risk Assessment

Step 1. Release Assessment

Step 2. Exposure Assessment

Step 3. Consequence Assessment

↓

Risk Estimation

Hazard Identification

The **hazard** has been defined as human illness

- caused by an antimicrobial-resistant bacterium
- attributable to an animal-derived food commodity
- treated with a human antimicrobial drug of concern.
- In some instances, a hazard characterization is sufficient for a particular antimicrobial drug

Qualitative Risk Assessment

Step 1: Release Assessment

Describes factors related to an antimicrobial drug and its use in animals that contribute to the emergence of resistant bacteria or resistant determinants in the animal

Release Assessment

Release parameters (examples)	Release assessment
Mechanism of activity	High, medium, low
Spectrum of activity	
Pharmacokinetics	
Pharmacodynamics	
Resistance mechanisms	
Resistance transfer	
Selection pressure	

Qualitative Risk Assessment

Step 2. Exposure Assessment

Describes likelihood of human exposure to food-borne bacteria of human health concern through animal-derived food products

Exposure Assessment

- Probability that humans consuming animal derived foods will be exposed to resistant bacteria of public health concern
- Evaluation based on relative consumption and contamination of those commodities
- Variety of data sources – all welcome to better address the concern
  - NARMS, CIPARS, DANMAP, AFSSA FARM Report, etc

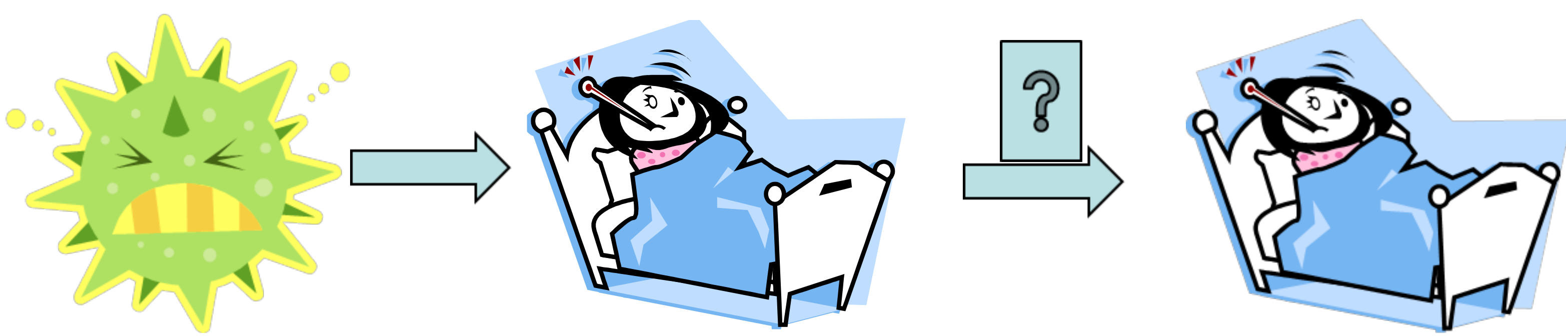
Exposure Assessment

	Per capita consumption of the food commodity		
Probability of food commodity contamination	High	Medium	Low
High			
Medium			
Low	Medium		

Qualitative Risk Assessment

Consequence Assessment

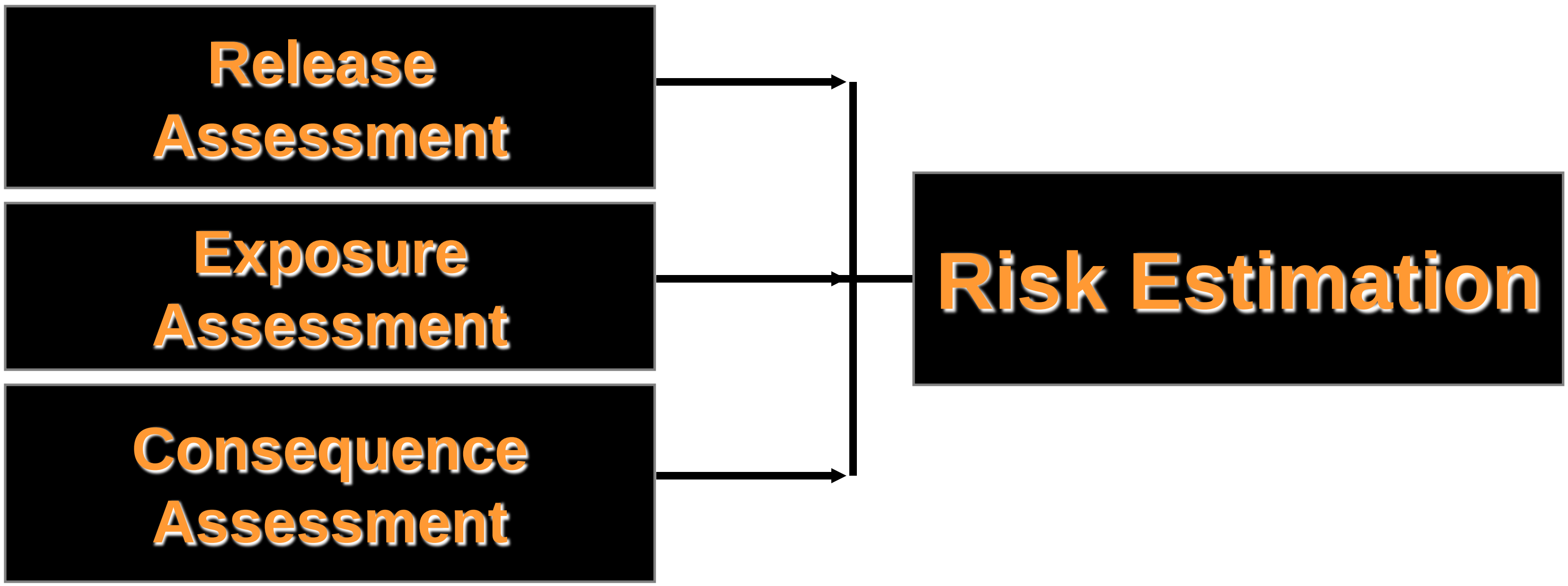
Describes human health consequence of exposure to resistant bacteria based on importance of drug (or related drugs) to humans (ranking of antimicrobials)



Drug Rankings and Examples

- Critically Important
  3rd Generation cephalosporins, macrolides, fluoroquinolones
- Highly Important
  aminoglycosides, clindamycin
- Important
  monobactams, quinolones

Qualitative Risk Integration



Risk estimation integrates results from release, exposure and consequence assessments to produce overall measure of risk associated with hazards.

Examples of Possible Risk Management Strategies Based on the Level of Risk (H, M, or L).

Approval conditions	Risk Category		
	Category 1 (H)	Category 2 (M)	Category 3 (L)
Marketing status	Rx	Rx/VFD	Rx/VFD/OTC
Extra-label use	ELU restriction	Restricted in some cases	ELU permitted
Extent of use	Low	Low, medium	Low, medium, high
Post-approval monitoring	NARMS	NARMS	NARMS
Advisory committee review	YES	In certain cases	NO

GFI #152, Table 8, pp. 25

Foodborne Pathogens Commonly Addressed in GFI 152 Risk Assessments

- Top pathogens transmitted by food (MMWR): *Salmonella enterica* serotypes and *Campylobacter* spp.
  - Ground beef, Pork chops, Chicken breast, Ground turkey,
- *Enterococcus* spp. (Gram+ resistance marker)
- Generic *E. coli* (Gram- resistance marker)
- Other non-foodborne bacterial species if human therapy may be compromised by veterinary use of a particular drug

Example: β-lactam -3rd Generation cephalosporin

- Release Assessment: HIGH
  - Many resistance genes detected (*bla* family) and associated with mobile elements (plasmids, integrons, transposons)
- Exposure Assessment: Medium
  - <5% prevalence *S. enterica* in meat, 10-20% resistance prevalence
  - 70-80% prevalence in *E. coli*, 5% resistance prevalence
- Consequence Assessment: HIGH
  - Critically Important drug
- Risk Estimation: HIGH

NARMS 2010 Executive Report
http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM312407.pdf

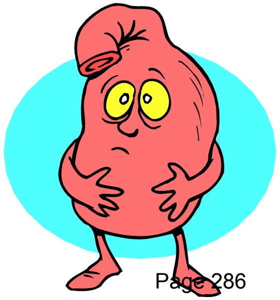
GFI #159/VICH GL36: "Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI

- Aimed to assess the acceptable daily intake (ADI) per day of drug that can be consumed by humans in animal-derived food product
- Assess the risk of disruption of the colonization of the human gut
- Assess the risk of the development of human gut microbe antimicrobial resistance

http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM124674.pdf

Objectives of the Guidance

- To outline steps in determining the need for establishing a microbiological ADI
- To recommend *in vivo* or *in vitro* test systems and methods for determining no-observed adverse effect concentrations/levels (NOAEC/Ls) for endpoints of human health concern
- To recommend a procedure to determine a microbiological ADI from the NOAECS/Ls



Assessing the need to calculate a mADI

Step 1: Are residues of a drug (and/or its metabolites) microbiologically active against representative human intestinal flora?

Recommended data to answer the question:

Examples of selected intestinal flora including:

E. coli, and species of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*, *Peptostreptococcus*/*Peptococcus*.

Example MIC data from 10 strains of 10 genera from healthy human donors

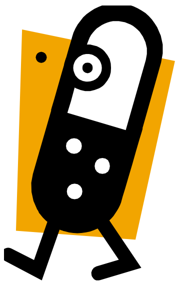
Bacterial group	Summary of MIC parameters (μg/ml)		
	MIC_{50}	MIC_{90}	MIC range
Bacteroides fragilis group	2	2	All 2
Bacteroides, other species	2	2	1-2
Bifidobacterium spp.	2	2	1-4
Clostridium spp.	2	4	1-4
Enterococcus spp.	2	2	All 2
Escherichia coli	8	8	4-16
Eubacterium spp.	1	2	1-2
Fusobacterium spp.	0.5	2	0.25-4
Lactobacillus spp.	4	8	4-8
Peptostreptococcus spp.	1	2	0.5-4
All isolates (total # of 100)	2	8	0.25-16

Step 2: Do residues enter the human colon?

Recommended data to answer the question:

- Drug's absorption, distribution, metabolism, excretion (ADME)
- Bioavailability
- or similar data may provide information on the percentage of the ingested residue that enters the colon.

Step 3: Do the residues entering the human colon remain microbiologically active?



Recommended data to answer the question:

Data demonstrating loss of microbiological activity from *in vitro* inactivation studies of the drug incubated with feces, or data from *in vivo* studies evaluating the drug's microbiological activity in feces or colon content of animals.

Scenario - 1

If the answer to any of questions in steps 1, 2, or 3 is "**NO**"-

- the ADI will **not** be based on microbiological endpoints and remaining steps need **not** be addressed

The ADI will be determined using a NOEL derived from traditional toxicology studies.

Scenario - 2

However, if the answer to questions in steps 1, 2, and 3 are "**YES**" -

- then proceed to Steps 4 and/or 5.
- Address one or both microbiological endpoints of concern.

Endpoints of Human Health Concern

1. Disruption of the colonization barrier, and
2. Increase in the population of resistant bacteria in the human colon.

Addressing the endpoints of concern

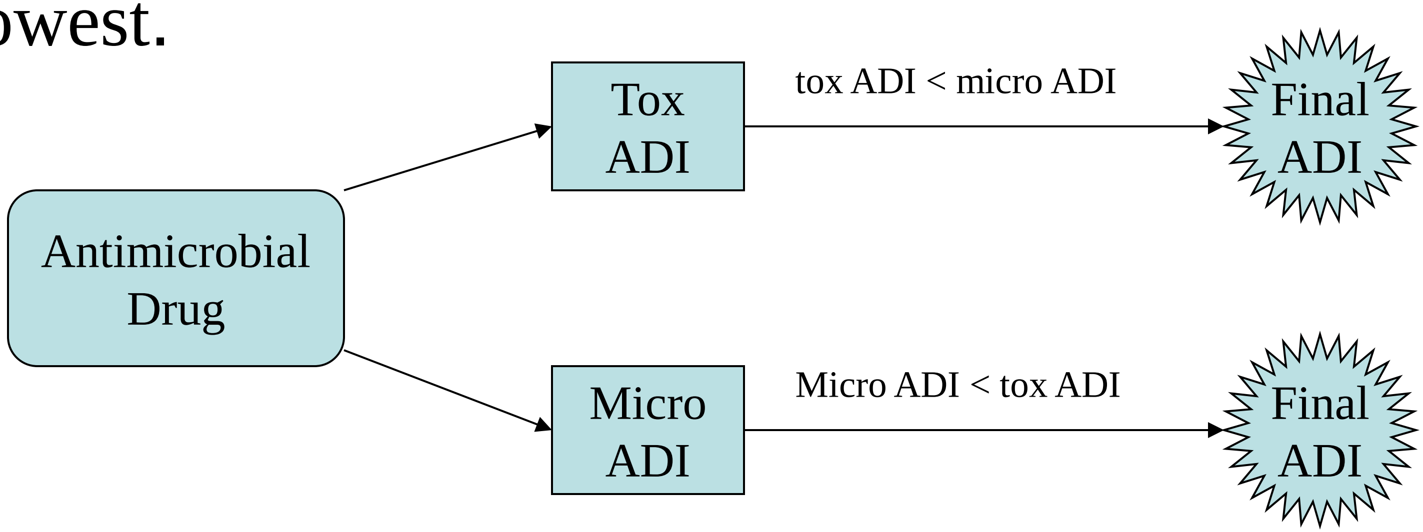
Step 4: To assess any scientific justification to eliminate the need for testing either one or both endpoints of concern.

Step 5: Determine the NOAEC/NOAEL for the endpoint(s) of concern.

The most appropriate NOAEC/NOAEL is used to determine the microbiological ADI.

Final ADI for an Antimicrobial Drug

- The final ADI for total residues of an antimicrobial drug in edible animal tissues will be the toxicological ADI or the microbiological ADI, whichever is the lowest.



Conclusions

- Qualitative Risk Assessments aid in science-based decision-making for new animal drug approvals to preserve and protect human health
  - Using existing surveillance system and research data
  - Using literature reviews of previous studies
  - Sponsors voluntarily conducting studies to address concerns
- Mitigation for risk can be achieved by
  - Limiting extra-label use, requiring oversight by a Veterinarian, modifying delivery method, and/or extending withdrawal periods
- Microbiological ADIs mitigate the risk of antimicrobial residues effecting the human intestinal flora

Acknowledgements

ONADE

- Dr. Jeff Gilbert
- Dr. Karen Ekelman
- Division of Human Food Safety
- Microbial Food Safety Team

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Recent Registration Example: Kasugamycin

Recent Registration Example: Kasugamycin

Shaunta Hill, Ph.D.
Registration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency

September 11, 2014

Pesticide Registration

- Office of Pesticide Programs has designed the registration process to support Agency objectives while meeting statutory requirements to act on registration applications. These include:
  - Protecting human health
  - Protecting the environment
  - Providing benefits for society

Registration Process

Front End Processing:

Mail Room Sort, PRN 86-5 Screen, Product Manager Screen, Science Screen

Science Reviews

Health Effects Evaluation, Eco. & Enviro. Effects Evaluation, Lab Validations

Peer Review and Risk Assessment

Establish Endpoints of Concern, Establish RfD (ADI) and Safety Factors, Assessment of Hazards and Risk

Risk Management and Regulatory Decision

Public Process

Risk Assessors Review of Risk Mitigation Measures, Risk Management Coordination with Registrants and Risk Assessors, Management Decision

Federal Register Publication

Risk Manager Develops Document, Program Wide Concurrence, Management Signature, Public Dissemination

Public Participation Process

- Allows for public comment on draft risk assessments, impact assessments, labels, and proposed registration decisions.
- Public participation process applies to registration actions involving:
  - New pesticide active ingredients
  - For currently registered active ingredients
    - First food use
    - First outdoor use
    - First residential use
  - Any registration action determined to be of significant interest to the public

Kasugamycin

- Kasugamycin belongs to the aminoglycoside class of compounds.
- Kasugamycin has a different mode of action from both streptomycin and oxytetracycline, both of which are used for agricultural purposes.
- Kasugamycin has never been approved as a human drug.
- Kasugamycin represents the first antibiotic registered (Section 3) in +35 years.

U.S. Regulatory History

- Import tolerances
  - Tomatoes and Pepper, 2005
- Section 18 use
  - Apples, 2008
- Section 3 application, 2010
  - Pome Fruit
  - Walnuts
  - Fruiting Vegetables

Antibiotic Considerations

Human Health:

- The use of antibiotics on crops, although minor relative to total antibiotic use, can result in situations that impact the buildup of resistant bacteria.

- Antibiotics used as agricultural pesticides, can be applied over large areas of land to densely vegetated fields and orchards. This can lead to the proliferation and rapid spread of resistant genes in the bacterial population.

- Although the probability is low, there is a potential for bacterial resistance to cross between plant bacteria and human bacteria.

Antibiotic Considerations

Environmental:

- Bacteria serve an essential role in cycling nutrients and energy in the environment (*e.g.*, through decomposition of organic materials and Nitrogen-fixation).
  - The effects of the potential reduction or alteration of the microorganism community from antibiotic uses are unknown.

Evaluating Potential for Resistance

EPA:

- Reviewed a qualitative antibiotic resistance risk assessment based on FDA's "Guidance for Industry #152

- Collaborated and communicated with other government agencies:
  - Center for Disease Control
  - U.S. Food and Drug Administration
    - Center for Veterinary Medicine
    - Center for Drug Evaluation and Research

- Consulted OPP's Science Policy Council

FDA's Guidance for Industry #152

Outlines a risk assessment approach for evaluating the microbial food safety of antimicrobial animal drugs by considering:

Applicable to EPA's evaluation of agricultural antibiotics

- Release Assessment - emergence or selection or resistant bacterial.
- Exposure Assessment - likelihood of human exposure to food-borne bacteria of human concern.
- Consequence Assessment - likelihood that human exposure to resistant bacteria results in an adverse health effect.
  - Includes a ranking (important-highly important-critically important) for antimicrobials according to their importance in human medicine.
- Risk Estimation – integrates assessments into an overall estimation of risk (low-medium-high) associated with the proposed conditions of use of the drug.

Human Health Summary

- Complete toxicology database for the assessment.
- There were no toxic effects associated with a single dose.
- There was no systemic toxicity associated with dermal exposure.
- Chronic dietary risk estimates are below EPA's level of concern (LOC) for all population subgroups.

Human Health Summary

- Based on the hazard and exposure data, the FQPA safety factor (10X) was reduced to 1X.
- Kasugamycin exhibits low acute toxicity. There was no evidence of neurotoxicity, carcinogenicity and mutagenicity.
- Based on the overall weight of the evidence, kasugamycin was classified as "not likely to be carcinogenic to humans".

Ecological Summary

- Kasugamycin is practically nontoxic to freshwater organisms, birds, mammals, and terrestrial invertebrates on an acute exposure basis.
- Chronic exposure resulted in no significant effects at the highest concentration tested.
- Direct effects to birds, reptiles, terrestrial-phase amphibians, fish and aquatic plants are not expected.

Resistance Risk Summary

Release	Exposure	Consequence	Overall
Low	Medium	Important	Low*
*Initial registration period			

Based on:

- Registrant's 152 document
- Available monitoring/resistance screening data
- Incidence of food borne illness associated with consumption of the crops
- Proposed crops (crop cycle, acres treated, residues, PHI, and RTI)

Proposed Regulatory Decision

- Based on Eco, Human Health and Resistance Risk assessments
- Reviewed by several levels of management
- Published March 18, 2014
  - Unconditional term registration (4 years)
  - Pome Fruit
  - Yearly submission of qualitative summary (#152)
  - 30 day comment period

Risk Reduction Measures

- Modification of agricultural practices
  - Restriction of animal grazing
  - Restriction of animal manure use
  - Restriction of alternate tree-row applications
- Specification of application rates/timing
  - Full strength applications
  - Applications prior to petal fall
  - 4 applications per year
  - Maximum of 2 applications consecutively
- Inclusion of resistance management language
  - MOA information
  - Disease forecasting systems
  - Consultation if reduced efficacy is suspected

Tolerances and Registration

- Tolerances published on 8/29/14
  - Pome Fruit Crop group
- Final Regulatory Decision posted 8/29/14
  - Unconditional Registration
  - Term-limited (4 years)
- Registration Notices (Section 3(c)(5)) and labels issued on 9/8/14

Post Registration Considerations

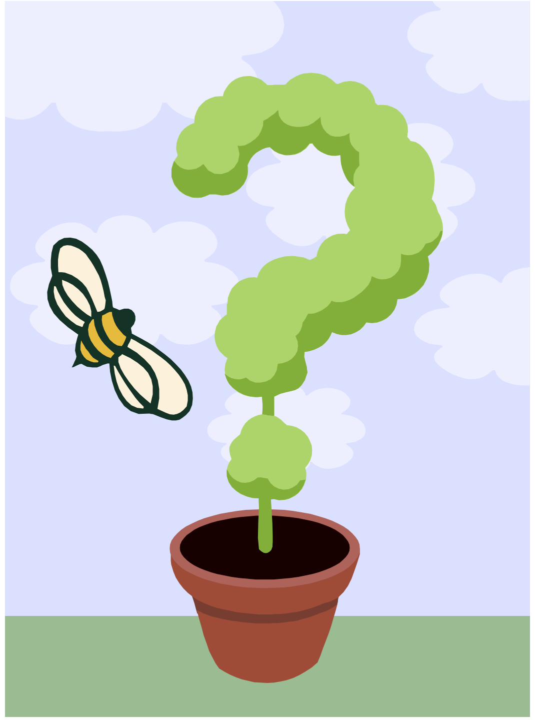
- Compliance
  - Monitoring of safe use
  - Proper disposal
- Clinical use
- Research, training, and extension needs
- Label modification
- Product Stewardship

Regulatory Docket

www.regulations.gov

Docket ID: EPA-HQ-OPP-2010-0297

Questions



Speaker Biographies

Speaker	Topic Being Covered	Biography
Dr. Jim Dukowitz, Commercial Products Manager, Citrus Research and Development Foundation, Inc.	Grower Needs and Challenges / New and Existing Options for Control Experiences of setting up CRDF and research efforts of control options for HLB	Jim Dukowitz is currently serving as a Program Manager in support of the Commercial Product Delivery Committee of the Citrus Research and Development Foundation. Jim brings to this position more than 35 years of high-tech industry experience in the areas of technology commercialization, strategic partnerships and planning, business and organizational development, communications and international marketing. He is a Principal with Technology Innovation Group, and in this capacity has developed and implemented programs with governments, NGOs, universities and companies around the world in the areas of innovation, entrepreneurship, and technology commercialization. He has served as an officer and director of several companies spanning electronics, biomedical technology and education. Prior to joining TIG he worked for over 20 years in executive positions at Texas Instruments, where he served as President of Texas Instruments Asia and China, as well as several corporate staff vice presidencies with responsibilities for strategic planning, business development, communications and international marketing. He also served in the Pentagon as a senior program analyst in the Office of the Secretary of Defense. He holds a BA in mathematics from the University of Iowa and PhD from MIT where he studied science, technology and public policy.
Dr. Jim Graham Soil Microbiologist, University of Florida	Grower Needs and Challenges / New and Existing Options for Control Research efforts of control options for Citrus Canker	Dr. James Graham studies citrus canker epidemiology and control by integrated management, as well as management of health of trees affected by belowground pathogens and pests and the systemic bacterial disease, HLB, or citrus greening. Graham's research focuses on the area of microbiological relationships of citrus and citrus soils, and the advancement of his findings for development of management tactics that sustain tree health. Thus far, Graham's research has led to improved integrated management strategies for above- and below-ground diseases and pests that reduce productivity of citrus. Graham's accomplishments include identifying best seasonal management tactics with conventional and new materials for control of bacterial citrus canker, demonstrating that fruit grown in canker-prone areas are not an avenue for transmission of the bacterium, opening movement of fresh fruit into canker-free markets, as well as utilizing systemic acquired resistance as a tool to control the disease in groves. Graham is also a leader in HLB (citrus greening) research. His work identified the root damage associated with HLB pathogen infection of fibrous roots and the increased susceptibility of roots to Phytophthora infection, and defined control strategies to maintain root health on HLB-affected trees. He also documented and extended research from large-scale trials in Brazil and Florida that demonstrated tree rouging as a viable strategy to control HLB. Belowground, Graham is participating in research leading to new understandings about the role of entomopathogenic nematodes on citrus insect pests such as Diaprepes root weevil. To complement his research leadership strengths, Graham is active in interdisciplinary programs across IFAS units, and among research agencies and industry groups worldwide. He is well known in the citrus industry and works regularly with individual growers and stakeholders.
Dr. Jim Adaskaveg Professor Dept. of Plant Pathology and Microbiology University of California, Riverside	Grower Needs and Challenges / New and Existing Options for Control Research efforts of control options for bacterial diseases of almond, olive, walnut	Jim's research program includes investigations on the biology, ecology, epidemiology, and management of diseases on tree crops grown in California. Specifically, his research focuses on the epidemiology and management of foliar diseases caused by fungal and bacterial tree pathogens. Research on cell biology of host-pathogen interactions, biological and molecular techniques for the detection and identification of fungi and bacteria, and development of innovative management practices for pre- and postharvest disease control of fruit crops. Jim and his collegues have developed exciting new strategies for the identification, detection, and management of pre- and postharvest diseases of tree fruit using reduced risk fungicides, new antibiotics, and disease forecasting programs in California.

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Dr. Ronald French Extension Plant Pathologist and Diagnostician Department of Plant Pathology and Microbiology Texas A&M AgriLife Extension Service Amarillo, TX	Grower Needs and Challenges / New and Existing Options for Control Research efforts of control options for zebra chip of potato	Dr. Ronald D. French, is an Assistant Professor and Extension Specialist at the Department of Plant Pathology and Microbiology at Texas A and M AgriLife Extension Service in Amarillo, TX, the Coordinator of the Texas Plant Diagnostic Clinic/Texas High Plains Plant Diagnostic Laboratory in Amarillo, TX and an Adjunct Professor at Texas Tech University, Lubbock. He received his Bachelor of Science from Cornell University in Plant Science, his Master's degree in Plant Pathology from North Carolina State University and his PhD in Plant Pathology from the University of Florida, Gainesville. He has held professional leadership roles in a number of scientific organizations, conferences and workshops including the APS-Caribbean Division, the Latin American Phytopathological Society, the first Latin American Workshop for the Identification of Oomycetes, the International Oomycete Web Symposium and the International Phytophthora capsici Conference, among others. Ron also serves or has served on several National and international professional organizations including a reviewer for Plant Disease Journal, the Organizing Committee for the Biannual Latin American Phytopathological Society Meeting and a Reviewer for several agricultural and pathology journals. He belongs to the American Phytopathological Society, the Latin American Phytopathological Society and the American Phytopathological Society-Caribbean Division. He currently has 21 Peer-Refereed Publications including The First Report of 'Candidatus Liberibacter solanacearum' on Field Tomatoes in the United States" in Plant Disease. Multiplex real-time PCR for detection, identification and quantification of 'Candidatus Liberibacter solanacearum' in potato plants with zebra chip in the Journal of Microbiological Methods And the First Report of the Detection of 'Candidatus Liberibacter' Species in Zebra Chip Disease-Infected Potato Plants in the United States in Plant Disease (First Disease Report)
Dr. Ken Johnson Professor, Plant Pathology, Oregon State University	Grower Needs and Challenges / New and Existing Options for Control Research efforts of organic, biopesticide and conventional control options for fireblight on pome fruit	Dr. Ken Johnson is a Professor of Plant Pathology at Oregon State University in Corvallis, OR. He has been at OSU since 1988 and teaches courses in introductory plant pathology and plant disease management. His research program is concerned with economically important diseases of horticultural crops with an emphasis on bacterial pathogens including fire blight of pear and apple. With fire blight, Dr. Johnson's recent projects have focused on integrated non-antibiotic control, improved pathogen detection, and induction of acquired resistance in fruit trees to mitigate the damage caused by this disease.
Dr. George Sundin Professor and Extension Specialist, Dept. Plant, Soil, and Microbial Sciences, Michigan State University	Grower Needs and Challenges / New and Existing Options for Control Research efforts of control options for bacterial diseases of stone fruit including research and experiences with Kasumin in Michigan	Dr. George Sundin received his B.S. at Penn State, his M.S. in Plant Pathology at Michigan State and his Ph.D. in Plant Pathology at Oklahoma State. He is a Professor and Extension Specialist for the Department of Plant, Soil, and Microbial Sciences, Michigan State University. He is a tree fruit pathologist with research and extension responsibilities for bacterial and fungal pathogens of pome and stone fruit.
Dr. Ed Stover USDA/ARS, Research Horticulturalist/ Geneticist, U.S. Horticultural Research Laboratory, Fort Pierce, FL	Biotechnology Options for Control Development of resistant citrus to Citrus Greening bacteria through genetic engineering	Dr. Ed Stover is a horticulturist and plant breeder with the USDA Agricultural Research Service focusing on developing improved citrus varieties. This broad-based breeding effort includes more than 30 collaborators in many disciplines. Resistance to the disease HLB has been the major focus of his work for the last six years and he has 25 HLB-related publications, among 200 total publications. He leads an active citrus transgenic program and has conducted extensive screening for HLB resistance in the greenhouse and field.

Speaker	Topic Being Covered	Biography
Dr. Manjul Dutt Citrus Research and Education Center, University of Florida, Lake Alfred, FL	Biotechnology Options for Control Development of HLB (greening disease) bacteria resistance through genetic engineering	Manjul Dutt is a Research Assistant Scientist at the Citrus Research and Education Center in Lake Alfred, FL with research interests in Citrus Genetics, Breeding and Biotechnology for the development of improved citrus varieties. He received his PhD degree in grapevine biotechnology from the University of Florida. The main emphasis of his research is on the development of biotechnological strategies to combat HLB, a deadly phloem vectored disease of citrus. His current emphasis is on the development of resistant scions and rootstocks that can withstand this disease. He also has considerable experience in the development of genetically modified rootstocks that can resist Diaprepes infestation. Collaborating with Dr. Jude Grosser, he has one of the largest transgenic citrus field trials in the U.S. Dr. Dutt emphasizes the development of genetically modified plants using intragenic technologies to produce a consumer acceptable product. He is also an Adjunct Professor of Biology at Polk State College.
Susan Jennings Public Health Coordinator Office of Pesticide Programs, U.S. Environmental Protection Agency	Regulatory Review Processes and Perspectives Registering Antibiotic Pesticides for Use on Crops	Susan Jennings is the Public Health Coordinator for EPA's Office of Pesticide Programs. As the Public Health Coordinator, Susan serves as the Office's primary contact for public health issues involving pesticides. She works with public health interest groups, other federal, state and local government agencies and the regulated industry to encourage consideration of public health issues in EPA's regulatory processes. Susan also represents EPA on the Interagency Taskforce on Antimicrobial Resistance and is very involved in registration issues involving antimicrobial pesticides intended for use in agriculture.
Dr. Jean Patel, D(ABMM) Deputy Director Office of Antimicrobial Resistance Division of Healthcare Quality Promotion, CDC	Regulatory Review Processes and Perspectives The Threat of Antibiotic Use in the Environment on Human Health	Dr. Jean Patel is the Deputy Director of the Office of Antimicrobial Resistance in CDC's National Center for Emerging and Zoonotic Infectious Diseases. In this role, Dr. Patel works on the coordination of antimicrobial resistance activities within CDC, activities with other federal agencies and activities with international partners. Jean leads a W-H-O Collaborating Centre for International Monitoring of Bacterial Resistance to Antimicrobial Agents and is the chair of the Clinical and Laboratory Standards Institute Subcommittee for Antimicrobial Susceptibility Testing.
Dr. Heather C. Harbottle Microbial Food Safety Team (HFV-157) Office of New Animal Drug Evaluation Center for Veterinary Medicine, FDA	Regulatory Review Processes and Perspectives Microbial Food Safety Risk Assessment and Regulatory Decision-Making	Dr. Heather Harbottle received her Ph.D. in Veterinary Medical Sciences from Louisiana State University in 2004. She went on to complete a Post-Doctoral study at FDA's Center for Veterinary Medicine and served as a Principal Investigator at CVM's Office of Research investigating the molecular mechanisms of antimicrobial resistance. In 2011, Dr. Harbottle joined the Microbial Food Safety Team in CVM's Office of New Animal Drug Evaluation, where she currently serves as a regulatory review Microbiologist for Division of Human Food Safety.
Dr. Shaunta Hill Registration Division Office of Pesticide Programs U.S. Environmental Protection Agency	Recent Registration Example-kasugamycin	Dr. Shaunta Hill received her Master of Science and Doctoral degrees in Plant Pathology from Michigan State University. Currently, she serves as a Plant Pathologist with the Environmental Protection Agency – Office of Pesticide Programs, Registration Division. Her regulatory responsibilities include the review of amendment requests for conventional pesticides, management of joint and global reviews for new conventional pesticides and implementation of re-registration standards. With respect to agricultural antibiotics, Shaunta has managed the registration application for Kasugamycin, to which general aspects of the review process will be discussed.

Attendees

Speaker	Affiliation
Adaskaveg, James	University of California
Allen, Bob	IBM Research
Almodovar, Luis	Univerity of Puerto Rico, Mayaguez ALVARADO-HERNANDE MONICA GOWAN
Anglea, Tim	The Coca-Cola Company & CRDF
Archambault, Shirley	AAFC - Pest Management Centre
Armella, Zena	ABC Organics, LLC
Balint, Barry	IBM
Barbier, Marcel	B&W Quality Growers, Inc
Baron, Jerry	IR-4 Headquarters
Bennett, Niki	Ontario Greenhouse Vegetable Growers
Berger, Lori	Ag Business Resources
Black, Larry	Peace River Packing Company
Bledsoe, Michael	Village Farms International
Boatwright, Megan	Golden Pacific Laboratories, LLC
Bolin, Dave	Arysta LifeScience
Boncristiani, Humberto	Forrest Innovations USA Inc.
Botts, Dan	Third Party Registrations, Inc. / Florida Fruit & Vegetable Association
Brannen, Phillip	University of Georgia
Braverman, Michael	IR-4 Headquarters
Bret, Brian	Dow AgroSciences
Bruss, Bob	Technical Services Manager
Calabro, Jill	Nufarm Americas
Carpenter, Debbie	IR-4 Headquarters
Chastagner, Gary	Washington State University Puyallup REC
Coleman, Krista	IR-4 Headquarters
Conti, Lisa	Florida Department of Agriculture and Consumer Services
Cranney, James	California Citrus Quality Council
Czochor, Lesley	DuPont Crop Protection
Daiker, Davis	Florida Department of Agriculture and Consumer Services
Davis, Joe	Citrus Research and Development Foundation, Inc.
Delaney, Nancy	Bayer CropScience Inc.
Desrochers, Anne	Quebec Horticultural Council
DeYoung, Alan	Van Drunen Farms
Dirks Jr., Richard	SePRO Corporation
Dorschner, Keith	IR-4 Headquarters
Drost, Dirk	Syngenta Crop Protection, LLC
Dukowitz, James	Technology Innovation Group
Dutt, Manjul	University of Florida
Egel, Daniel	Purdue University
Fajardo, Julius	US Department of Agriculture
Farrar, Jim	Western IPM Center
Felix, Joel	Oregon State University
Fernandez, Jessica	Bayer CropScience
Ferrazoli, Cheryl	IR-4 Headquarters
Flanagan, Stephen	UC Davis IR4 Program
Graham, James	University of Florida, Citrus Research and Education Center

Speaker	Affiliation
Grose, Julianne	Brigham Young University
Harbottle, Heather	U.S. FDA, Center for Veterinary Medicine
Hattermann, Dennis	Landis International, Inc.
Hedrick, Brooke	ADAMA
Henn, Alan	Mississippi State University Extension Service
Hill, Shaunta	US. EPA
Homa, Kathryn	IR-4 Headquarters
Howard, Beau	J.G. Boswell Company
Howard, David	Graves Brothers Company
Hunter, Craig	OFVGA
Hurley, Mike	CA Fig Advisory Board
Ingersoll, Tammy	Windset Farms
Ivey, Melanie	LSU AgCenter
Jackson, Sidney	US EPA
Jennings, Susan	US EPA
Johnson, Ken	Oregon State University
Johnson, Nikki	Market Access Solutions
Johnson, Timothy	Marrone Bio Innovations
Kanga, Lambert	Florida A&M University
Kawate, Mike	University of Hawaii
Kleppe, Craig	BASF Corporation
Kress, Rick	Citrus Research and Development Foundation
Kunkel, Dan	IR-4 Headquarters
Lajoie, Cindy	Syngenta Canada Inc.
Lalancette, Norman	Rutgers University - Rutgers Agricultural Research & Extention Center
Liburd, Oscar	University of Florida
Lurvey, Edith	NER IR-4, Cornell
Madden, Barbara	U.S. EPA, Office of Pesticide Programs, Registration Division
Malamud-Roam, Karl	IR-4 Headquarters
Marshall, Maurice	University of Florida
McClure, John P	Evans Properties, Inc.
McGrath, Margaret	Cornell University
McWhorter, Judy	Pace 49, Inc
Miyazaki, Satoru	Michigan State University
Monterroso, Victor	Grower
Moore, Robert	Florida Dept of Agriculture & Consumer Services
Norden, Alan	Australian Pesticides & Veterinary Medicines Authority
Odero, D. Calvin	University of Florida
Palm, Mary	USDA Animal and Plant Health Inspection Service
Palmer, Cristi	IR-4 Headquarters
Patel, Jean	CDC
Porterfield, Dunk	Arysta LifeScience
Rackley, Anderson	A FDACS
Raid, Richard	University of Florida
Rainbow, Rohan	Crop Protection Australia
Richardson, Taw .	AgroSource, Inc
Robles, Wilfredo	University of Puerto Rico, Mayaguez
Ross, Marylee	University of Maryland
Rossi, Lois	U.S. EPA
Ruiz, Roger	Servicio Fitosanitario del Estado
Samuel-Foo, Michelle	University of Florida
Sances, Frank	Pacific Ag Research
Sanson, Charlotte	Bayer CropScience
Scholz, Todd	USA Dry Pea & Lentil Council

Speaker	Affiliation
Schrieber, Alan	Washington State Commission on Pesticide Registration
Scorza, Ralph	USDA-ARS Appalachian Fruit Research Station
Seal, Dak	University of Florida
Simmons, Alvin	USDA-ARS
Simmons, Wayne	CRDF Florida LaBelle Fruit Co
Sisco, Rebecca	Western Region IR-4 Center
Soderlund, David	Cornell University
Starner, Van	IR-4 Headquarters
Stopyra, Thomas	The Packers of Indian River, Ltd
Stover, Ed	USDA/ARS
Sumpter, Sheldon	DuPont Crop Protection
Sundin, George	Michigan State University
Tanner, Berry	National Watermelon Association
Tolson, Mika	Western Region IR-4 Program, University of California Davis
Vallad, Gary	University of Florida
VanWoerkom, Anthony	Michigan State University Trevor Nichols Research Center
Wade, Layne	Arysta LifeScience
Wang, Laixin	PepsiCo
Werner, Scott	USDA/APHIS/VVS/ National Wildlife Research Center
Williams, Ronald	The Coca-Cola Company
Wilson, John	Cranberry Institute
Wofford, Tommy	BASF
Wright, Lesley	Engage Agro Corporation
Yagiz, Yavuz	University of Florida