

To

Date

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4 pages de Mario Lanthier



The “11th International Workshop on Fire Blight” was held in Portland (Oregon) August 12 to 17.

The meeting was attended by about 100 persons, mostly scientific researchers from USA and Europe.

From British Columbia:

Dr. Sholberg, Dr. Hampson, J. Boulé (Agriculture Canada, Summerland), also G. Jespersen (BC Ministry Agriculture, Kelowna). From Alberta: no one.

This meeting is held every 3 years. The 2004 meeting was in Italy. The 2010 meeting will be in Warsaw, Poland.

Current status of fire blight

The disease is present in most of North America and some parts of Europe.

- In Europe, the 2007 epidemic in Switzerland has cost so far 1.8 million Euros.
- The disease is present in New Zealand, but not in Australia, China and Japan.
- There are concerns for disease spread with commercial movement of nursery stock.

There is extensive research happening at the molecular level.

- Researchers are looking for genes that give resistance to some apple rootstock (B9).
- Other researchers are looking for genes that give virulence to the fire blight bacteria.
- The results could be used in breeding programs to generate more resistant *Malus*.

Nature is more complex than first thought!

- Fire blight was thought to be caused by the bacteria *Erwinia amylovora*.
- Researchers are finding different strains of *E. amylovora*, or other *Erwinia* species.
- The species and strains behave slightly differently and may require different controls.

MANAGEMENT WITH SPRAYING

“Best” spray program

One spray of biofungicide, then spray of streptomycin, then spray of biofungicide.

- First application: first bloom (most of the flowers are poised to open) + surfactant.
- Streptomycin: early to full bloom (to prevent bacteria build-up), effective 48 hours.
- 3rd spray: 50 to 70% bloom (to recover antagonist bacteria), to manage resistance.
- Field studies indicate “better results” when the spray program includes a biofungicide.

Other products can be used in rotation with streptomycin.

- Research in California: Captan (6.7 mg / L) and Maneb (8.3 mg / L) are effective.
- Copper is harsh on native competitive bacteria / induces a “dormant” state in *Erwinia*.

About streptomycin

Streptomycin is the best material for blossom blight management.

- Field studies show consistent 80 to 90% reduction in flower infection.
- Under very high pathogen pressure, disease reduction is still 75% versus untreated.
- Excellent when applied 1 day before infection, ineffective when applied 4 days before.

Resistance is widespread in BC, Washington, California, Utah and Michigan.

- Resistant bacteria will alter the target site, or secrete enzymes to inactivate product.
- Target site alteration, common in Washington State, is not overcome by a higher dose.
- Resistant isolates in New York in 2002 were sourced to nursery stock from Michigan.

Registration of streptomycin is uncertain because it is an antibiotic.

- In Canada, registration is currently year-to-year while waiting for other products.
- In Europe, product is banned for concerns with resistance in public health products.

New pesticides

New biofungicides are based on specific strains of competitive bacteria.

- *Pantoea agglomerans* is a bacteria found on apple flowers everywhere in the world.
- It is an excellent colonizer of flowers, outcompeting *Erwinia* for space and nutrients.
- Products “Blightban” and “Bloomtime” became available in Canada this year.
- Field studies show 35 to 50% disease control, but highly variable from trial to trial.
- Coming and similar effectiveness: “Serenade” (*Bacillus subtilis*), “Actigard” (harpin).

Kasumin 2L is a new antibiotic with great potential.

- The active ingredient (kasugamycin) is a fermentation by-product of *Streptomyces k.*
- Not used in medicine and mode of action is different than public health antibiotics.
- Field tests in Oregon, California, Utah: excellent results when applied during bloom.
- Already registered in Europe and Asia. Tested in Canada in 2007 for registration.

UPDATED INFORMATION

Epidemiology (spread of disease)

The following are accepted definitions regarding fire blight.

- The bacteria is a highly virulent, necrogenic, vascular pathogen of *Rosaceous* plants.
- Disease is severe since 1) destructive character of bacteria, 2) lack of effective control.
- The bacteria gains entry via undamaged flowers, or damaged mature xylem vessels.
- Symptoms appear after multiplication in the intercellular space of cortical parenchyma.

Ambient temperature is the #1 driver of fire blight development.

- Bacteria population must be high to trigger tissue damage leading to visible symptoms.
- High bacteria population is reached after a few consecutive days of hot temperatures.
- High bacteria population can be reached at 10°C, but it takes much longer.

The bacteria can remain alive 2 years in symptomless trees.

- The bacteria was detected in closed flowers of a tree with fire blight the year before.
- The bacteria was detected in tissues of symptomless trees inoculated 2 years earlier.
- In resistant trees, bacteria was detected 1 year after inoculation, but only at that site.
- Thus, the bacteria can likely survive at low levels in the vascular system of trees.

Management practices

Pruning of infected branches should be into 2 or 3-year old wood.

- Disease progression is *fast* in newer wood and *slow* in older wood.
- Prompt pruning may or may not reduce plant death, depending on situation.

Disinfection of pruning tools should be done regularly, but it is not mandatory.

- Prompt removal of strikes is more important than disinfection of tools.
- Disinfection of tools is important when pruning is done by uneducated staff.

Growth regulators that reduce shoot extension help reduce fire blight.

- Recent study, treated trees had 19% of diseased shoots, versus 61% for untreated.
- Apogee must be applied at least 5 days before infection to reduce shoot blight.
- Other practices that stabilize tree growth will also increase resistance to fire blight.

Identification methods

Current methods to confirm fire blight are based on laboratory procedures.

- Methods include plating into a nutrient agar, instrument detection of genetic materials.

Coming: on-site diagnostic with immuno-strips (similar to "pregnancy" test).

- Flowers are suspended in a buffer solution, then placed on antibody-specific paper.
- Commercial product "Agri-Strips" will be available in Europe in 2008.

LABORATORY PROCEDURES

History

Diagnostic methods have become reliant on sophisticated equipment.

- 1970s: development of ELISA method (based on antibody, takes 2 days).
- 1980s: development of PCR (polymerase chain reaction, targets DNA-RNA).
- 1990s: development of real-time PCR (allows quantification, same day results).
- Currently: attempts to develop on-site methods (presence / absence in 10 minutes).

Field sample protocol is available from a European document (EPPO 2004).

- Example: ten flowers placed in a plastic bag with distilled water, kneaded gently.
- Suspension is spread on plates, incubated for a week, colonies examined.

Upcoming methods

Immunochromatographic lateral flow strips, available in Europe in 2008.

- Based on migration of gold-antibody complex down to a specific line.
- Sensitive for moderate to high population, not effective for small populations.

Direct real-time PCR is being developed by Agriculture Canada in Ontario.

- Based on development of specific primers and TaqMan probes for their targets.
- Promoted as cost effective and rapid, allowing 400 to 500 samples per day.
- A "secret" direct pathogen extract buffer will be available commercially from AgDia Inc.

Impact for our company

The simplest approach would be to do plating of suspected samples.

- Plating is still used commonly as step #1 to grow and isolate fire blight bacteria.
- Cost of plating is about \$1.50 per sample. Cost of PCR is \$3 to \$5 per sample.
- To confirm id: serological technique ELISA (Enzyme-Linked Immunoabsorbent Assay).

Media recipe appears standardized among laboratories.

- For *Erwinia*: SNA (sucrose-enriched nutrient agar) or NAS (nutrient sucrose agar).
- SNA: nutrient agar 2.3% concentration + sucrose 5% (equal glucose and fructose).
- For *Pseudomonas* (a different bacteria): King's B agar.
- Protocol: sample extraction / plating / incubation 48 hours at 25°C / visual or ELISA.
- "Typical" colonies are creamy white, raised, non-fluorescent.

Pathogenicity is also used for pathogen confirmation.

- Isolated disease is inoculated into host plants and should develop typical symptoms.
- Example: using sliced immature pear fruit, spots inoculated with 20 ul of suspension (10^6 cfu/ml), then incubated at 25°C for one week.