Management of bacterial leaf spot of tomato and pepper

What is bacterial leaf spot?

Bacterial leaf spot (BLS) is caused by four species of *Xanthomonas: X. vesicatoria, X. euvesicatoria, X. perforans* and *X. gardneri. X. euvesicatoria* infects mainly capsicum and chilli, while the other species primarily infect tomato. Some accounts of species on multiple hosts is recorded. Yield loss is generally a result of decreased photosynthetic area, though in severe infections direct fruit damage is also seen.

Previously it was assumed *X. campestris* pv. *vesicatoria* (now known as *X. vesicatoria*), was causing BLS in all solanceous crops in Australia. The results from the Hort Innovation project (VG14010), clearly indicate this is not the case, instead there are four different *Xanthomonas* species associated with the disease. Furthermore, the species are largely host specific, where *X. euvesicatoria* essentially infects capsicum, chili and tomato, and *X. vesicatoria* and *X. perforans* typically only tomato. The fourth species, *X. arboricola*, was found in association with BLS symptoms and able to weakly infect tomato only. Given this weak pathogenicity, its ability to cause BLS disease is questionable and as such the bacterium is unlikely to need control. Furthermore, race-typing of *X. euvesicatoria* isolates identified races 1 and 7 in Australia. There are currently several commercial capsicum lines in Australia with resistance to race 1. There are very few lines, readily available with resistance to race 7. Importantly, the diversity of *X. euvesicatoria* races and other Xanthomonads detected in Australia is very low, however, as there is no regulation for these bacterial pathogens it is likely new races and species could be introduced with seed.

Cultural controls

In developing a management strategy to control BLS, it is important to consider sources for introduction of the bacteria into a field, district or country, how long the bacteria is able to survive without its preferred host plant and where it resides in the environment between cropping periods. Survival of xanthomonads causing BLS include:

- within seedlings but causing no signs of disease
- on seed for at least 10 months and in dried leaf material for at least 14 months
- in the rhizosphere of a number of different plant species including the non-hosts sorghum, cucumber, bean, pea and wheat. This has implications for potential crop-rotation strategies.
- in soil, however, this can vary from only 16 days to 18 months, and the transfer of the bacteria from the infested soil to host plants is questionable, thus infested soil may not be an important source for new disease outbreaks

Xanthomonads are reported to be seed-borne in a number of hosts, including capsicum and a linkage between infested seed and field disease was established many years ago. To mitigate this risk, seed can be heat treated to eliminate the bacteria; however, germination rates may be compromised. Preliminary studies have looked at treating seed with essential oil to combat BLS pathogens.

Ongoing high-volume international trade in seed, together with the high seed-transmissibility of xanthomonads, increases the risk of co-importation of new races into Australia. This represents the highest risk entry pathway for exotic BLS species and/or races to enter the country. No formal mitigation for this risk exists. Seed disinfestation strategies would help mitigate local disease outbreaks each season and more importantly prevent introduction of new disease agents.

Hort Innovation Strategic levy investment | VEGETABLE FUND

This project has been funded by Hort Innovation using the vegetable research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Queensland Government

Crop rotation and/or fallow periods may contribute to better disease management by reducing the numbers of bacteria surviving between cropping seasons. There is limited information, however, on the length of rotation and the useful alternative crops for the rotations.

Genetic host resistance

BLS of tomato and pepper has been reported in all major tomato growing regions of the world and as early as 1912. Studies from around the world detail race shifts and changes in the distribution of BLS species, e.g. pepper race 6 was found in Ohio in 1994, marking the first time in that region that Xcv overcame the established resistance genes. An outbreak of this race was later recorded in Florida.

Although the diversity of the xanthomonads causing BLS is quite high and in many regions largely unknown, studies have identified a number of host resistance genes for incorporation into commercial lines. To this end, breeding activities by commercial seed companies have incorporated many resistance genes for BLS in capsicum. The results of the first race-typing of *X. euvesicatoria* done with Australian isolates (Hort Innovation VG14010) will assist greatly in variety selection for capsicum growers to combat BLS outbreaks. The results showed that there are at least two races present in Australia, races 1 and 7, and that no new race-types were detected from the *X. euvesicatoria* isolates. Currently, the varieties of capsicum in Australia have a range of different BLS resistance genes. These are described by the seed companies as resistance to Xcv and include genes Xcv-0, 1, 2, 3, 5, 7, 8 and 9. They are available in varying combinations, typically, 0-3, 1-3, 0-5, 1-5 and 7-9. The resistance gene number refers to the race of the bacterial pathogen which it is effective against, for example, Xcv 1 combats race 1. There are very few chilli and tomato lines listed with BLS or Xcv resistance and often this has no race designation.

Copper for BLS control

Are Australian isolates of BLS copper tolerant?

Bacterial isolates of *X. vesicatoria, X. euvesicatoria* and *X. perforans* were tested in laboratory assays for tolerance to copper (VG14010). All of the 58 isolates tested showed tolerance to the lowest copper concentration tested (0.1 mM). Of the 27 isolated from tomato, 13 were rated as highly tolerant to copper with growth up to 1.5 mM, 11 as moderately tolerant (growth between >0.5-1.0 mM) and 3 as tolerant (growth at 0.1-0.5 mM). All most all of the 31 isolates from capsicum and chilli were rated as moderately tolerant to copper, one isolate was rated as tolerant.

The industry standard application of copper is at a concentration of approximately 15 mM, ten times greater than the highest minimum inhibitory concentration (MIC) observed in laboratory testing. The discrepancy between the concentrations of copper required to control bacterial growth *in vitro* compared to in-field is related to the low availability of the bactericidal form of copper (i.e Cu^{2+}) in cropping environments.

How does copper protect plants from bacterial infections?

The evolution of copper tolerance in bacterial populations means they can tolerate higher concentrations of copper than sensitive populations. It doesn't mean the bacteria are resistant and that copper has no effect.

The availability of free cupric ions (Cu^{2+}) is the important component of products to protect against bacterial infections. The concentration of Cu^{2+} on plant surfaces depends on the equilibrium established with the complexed and soluble forms of copper and the chemical reactions releasing the free Cu^{2+} from the soluble forms. There is no strong correlation between the total amount of copper applied and the concentration of Cu^{2+} on leaf or fruit surfaces.

Rainfall or presence of other free water (e.g dew), wind and leaf abrasion, compounds released by the plant and the pH of the leaf surface will affect the amount of soluble copper present. Free water interacts directly with the copper deposits, and indirectly by releasing exudates from the leaf itself, which also interact with the copper. Wind and leaf abrasion can physically remove the copper deposits and/or also release leaf exudates. The spread of the copper on the leaf surface also affects these interactions and this is influenced significantly by the particle size of the product.

As a general rule the half-life of total copper on the leaf surface is about one-month. This is well in excess of the typical application rates of 5 to 10 days. This means more frequent applications will not improve Cu^{2+} availability. Application every 7-10 days is recommended with additional applications if heavy rainfall has occurred or for fast growing plants where additional applications are needed to protect new foliage.

Increasing the total amount of copper applied to the leaves will only give a very minor improvement to the amount of soluble copper present, if any at all. Generally, the amount of total copper is often in excess of soluble copper, thus adding more won't provide additional disease control. Total copper is not a good indicator for the amount of soluble copper present. It is the interaction of the copper product with the plant surfaces that drives solubility of the copper. Furthermore, the amount of free Cu^{2+} present on plant surfaces is only a small fraction of the soluble copper present. In studies on bean, free Cu^{2+} was estimated to be as low as 1% of the soluble copper present. Free Cu^{2+} typically increases with increased amounts of soluble copper.

Application techniques and product type significantly affect the efficacy of copper to protect plants from bacterial infection.

What copper products are commonly used in tomato and capsicum crops?

Products registered for use against bacterial diseases include Bordeaux mixture, cupric and cuprous hydroxide, cuprous oxide, copper oxychloride, copper salts of fatty acids, copper ammonia acetate complexes, tribasic copper sulphate and mixtures of cupric hydroxide and ethylene bis-dithiocarbamates (EBDC, e.g mancozeb).

The product information usually lists the active ingredient in percent metallic copper which is a measure of the insoluble copper salts and not a measure of free Cu^{2+}.

Tank mixes including fungicides such as EBDC (e.g mancozeb) or heavy metals including zinc or iron were shown to improve disease control by increasing the amount of Cu^{2+} in solution. On the other hand, mixing copper products with organic compounds is highly likely to have the reverse affect and reduce availability of Cu^{2+}.

The amount of available Cu^{2+} in a product is a good indicator for efficacy against bacterial pathogens. Commercial products range significantly, from 0.04 to 22.0 µg/ml Cu^{2+}. The concentration of Cu^{2+}, however, is typically not listed on product labels and requires specific measurement. The metallic copper amount listed on the label is not a good predictor of Cu^{2+} concentration. Products with a Cu^{2+} concentration of 1.5 µg/ml or more were most effective against some bacterial species. In comparing products ask for information on the predicted availability of Cu^{2+}.

Copper plus manganese-zinc ethylene bisdithiocarbamate (mancozeb) is consistently better than other copper only products in field studies of a range of bacterial diseases, including bacterial spot and speck of tomato. This is attributed to the ability of the bisdithiocarbamate anion to chelate copper and transport the Cu^{2+} into the bacteria. As stated above it also improves the concentration of Cu^{2+} in solution.

Ferric chloride combined with cupric hydroxide improved bacterial disease control in walnut. The ferric chloride increased the sensitivity of the bacterium to the copper. It also increased availability of Cu^{2+} on leaf surfaces by lowering the pH and cation exchange between Cu^{2+} and Fe^{3+}. However, lowering the pH with hydrochloric acid or adding a range of other metal ions ($MnSO_4$, $MgCl_2$, $MgSO_4$, $CaCl_2$, NaCl and KCl) did not increase availability of Cu^{2+}.

Zinc used instead of or in combination with copper is effective in disease control in walnut. However, further work is needed as again different combinations of product give very different results. Research into alternative chemicals for disease control in capsicum and tomato is underway; however, no products are yet available.

Suggested spray program

What can be mixed with what in the spray tank?

A tank mix of copper plus an EBDC (ethylene bis-dithiocarbamates, e.g mancozeb) will give the best disease control compared to copper alone. Additionally, avoid mixing the copper with other products that will complex the copper reducing its solubility and ultimately the availability of Cu^{2+}.

What products are likely to perform best and how often/when to use?

There are various different forms of copper registered for use. These include Bordeaux mixture, cupric and cuprous hydroxide, copper salts of fatty acids, copper ammonia acetate complexes, tribasic copper sulphate and mixtures of cupric hydroxide and ethylene bis-dithiocarbamates (EBDC). There are no strict rules as to which form of copper works best.

Several studies have reported the combination of copper and EBDC (ethylene bis-dithiocarbamates, e.g mancozeb) work best to control bacterial speck of tomato and bacterial spot of capsicum. It is recommended to use this combination early, before harvest as mancozeb has withholding periods which will affect harvest times. During harvest, other copper products or alternative control methods should be used.

Products come as wettable powders, wettable granules, liquid flowable suspensions or aqueous liquids. The particular formulation will affect coverage of the product which is an important factor to consider. The formulation may affect solubility of the copper and availability of Cu^{2+}. Additivities to products could also potentially affect solubility and/or Cu^{2+} availability. Consult your local supplier for more information about the solubility and Cu^{2+} availability of specific products

A typical application rate of 7 to 10 days should be adequate as the average half-life of total copper on leaf surfaces is one-month. Applications more frequently are unlikely to improve Cu^{2+} availability and thus disease control. However, in fast growing crops, additional applications might be required to ensure newly developed foliage is protected.

Alternative chemical control

In 2008, a study published findings from evaluations of a range of potential control products for BLS on tomato. These included chemicals such as famoxadone plus cymoxanil (E.I. du Pont de Nemours and Company), the defence response activator acibenzolar-*S*-methyl (Syngenta Crop Protection) and the biocontrol agent *Bacillus subtilis* QST713 (AgraQuest Inc.) with and without copper. Although these products looked promising, there were no definitive recommendations from the study. The products have undergone further research and in some instances commercial trials, in particular products using *Bacillus amyloliquefaciens* QST713 (previously known as *B. subtilis* QST713). A product containing QST713 was released for use to supress BLS in Australia in 2018.

A subsequent study in 2012 further investigated the efficacy of famoxadone plus cymoxanil in the management of two *Xanthomonas* spp., which included *X. perforans*. Neither of these components alone had *in vitro* or *in vivo* activity against the bacterial species, however, there was a synergistic effect when the product was used with copper hydroxide to control a copper-sensitive species of *X. perforans*. The authors suggest this product could be used in replacement of mancozeb to enhance control of bacterial pathogens with copper during the production cycle where mancozeb is excluded due to withholding periods. Further work is required to confirm these results before this recommendation is adopted.

In 2012, the compound 2-aminoiidazole (2AI) was investigated for control of BLS. The compound was shown previously to inhibit and disperse bacterial biofilms. The compound was trialled alone and in combination with a number of other products including copper hydroxide with and without mancozeb, kasugamycin, Regalia™, potassium phosphite and a nonionic surfactant. In laboratory assays 2AI mixed with copper improved control of a copper resistant isolate. The compound also reduced biofilm formation when tested *in vitro*. Results of the field trials were less conclusive, in some trials it performed better than copper hydroxide alone and in others it was similar or less, thus 2AI still requires further evaluation. The first field trial showed significant reduction in disease when 2AI as used in combination with copper hydroxide and potassium phosphite as compared to either of those products alone and to the untreated control. In the second trial, disease reduction of 2AI with copper hydroxide was similar to copper hydroxide mixed with mancozeb and significantly greater than the untreated control and the two components (i.e 2AI and copper hydroxide) used alone. In the same trial, latron a nonionic surfactant was also tested and improved efficacy of 2AI used alone with disease reduction comparable to treatment with a mix of 2AI and copper hydroxide. In the final field trial, the use of 2AI either alone or in combination with copper hydroxide or the biofungicide Regalia™ was significantly greater than the untreated control. The best treatments for the trial were obtained using copper hydroxide (1.4 kg) with 2AI (100 µM), Regalia with 2AI (100 µM) and the industry standard of copper hydroxide (1.4 kg) with mancozeb.

Further work with both 2AI and Regalia would be useful for the Australian industry, particularly to evaluate their effectiveness against a broader range of xanthomonads and other solanaceae crops such as chilli and tomato. Regalia is a 5% extract from the plant species *Reynoutria sachalinensis* sold by Marrone Bio Innovations. The mode of action for Regalia is listed as an induced systemic resistance (i.e a plant defence response activator; http://marronebioinnovations.com/ag-products/brand/regalia/). It has broad spectrum activity for foliar pathogens.

A complication to evaluating disease control products is the unreliability of performance in field trials. This is often due to the strong linkage between bacterial diseases and weather events. If environmental conditions are not favourable for disease development and spread it is often difficult to get robust results from field trials. Pot trials are useful as a preliminary method for evaluating potential products but cannot give good yield impact data.

Essential oils

Essential oils from a range of different plant species were previously shown to have efficacy in control of xanthomonads, including those causing BLS. Sage, clove and BioZell™ (based on thyme oil) performed better at controlling a range of plant pathogenic bacteria than lavender and lemon balm. A 2005 study reported coriander and hyssop oil were efficacious against *X. campestris* pv. *malvacearum* but not cumin, dill, fennel, mint or anise. The chemical compounds carvacrol and thymol found in a range of essential oils was shown to be antibacterial to a range of plant pathogens including *X. vesicatoria*. Other studies over the past decade have also evaluated essential oils against a range of phytopathogenic bacteria.

Testing essential oils for efficacy against the BLS causal agents was completed in Hort Innovation project, VG14010. Clove, coriander, fennel, lavender, oregano and thyme were trialled in laboratory assays for control of *X. vesicatoria* and *X. euvesicatoria*. All the oils showed significant inhibition of bacterial growth for both species as compared to the control when applied as a contact or through exposure to volatile gases from the oils. Fennel was the least effective in the laboratory trials. Further work is needed to investigate the efficacy of these oils as a seed treatment or foliar management option.

What else needs to be studied to improve BLS disease management?

Improvement in management of bacterial spot of tomato and capsicums will be through improved understanding of disease life-cycles, pathogen diversity and development of novel control products. These products could include formulations which directly interfere with bacterial survivability and/or promote defence responses within the crop plants. Additionally, further plant breeding efforts could identify resistance or tolerance within tomato and capsicum germplasm that could be used instead of or in combination with chemical control methods. The improved understanding of pathogen diversity in Australia will assist with plant breeding efforts and in the development of novel control products. Further research on alternative crops for rotation would also be beneficial. Investigation of different seed disinfestation protocols would be highly desirable.

Ongoing international trade in seed, together with the high seed-transmissibility of xanthomonads, increases the risk of co-importation of new races into Australia. This means existing resistance genes managing disease in Australia may become unreliable in the future. Additionally, overuse of a single management strategy such as a resistance gene or a single chemical can provide strong evolutionary pressure on the bacterial pathogen, leading to mutation and the local emergence of resistance-breaking races or chemically tolerant populations. Multiple management strategies will help to reduce evolutionary pressure on the bacterial pathogens to circumvent resistance genes and also provide options to control disease if exotic races are introduced. A multi-strategy approach to disease management is best. Consideration of other crop management practices is also important for control of any pest or disease. Development of a holistic strategy to control foliar diseases which considers fungal and bacterial pathogens in addition to pests and nutrient requirements would be highly beneficial.

For further information please contact:

Rebecca Roach, La Trobe University/ Department of Agriculture and Fisheries, QLD

roach.r@students.latrobe.edu.au, phone: 07 3708 8495

Cherie Gambley, Department of Agriculture and Fisheries, QLD

Cherie.gambley@daf.qld.gov.au, phone: 0423 200 211

Hort Innovation Strategic levy investment | VEGETABLE FUND

This project has been funded by Hort Innovation using the vegetable research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au