Technical review TR-PE 002

Protected Edibles

Tomato bacterial wilt and canker (*Clavibacter michiganensis* subsp. *michiganensis*)

A summary of recent studies on the causal bacterium, symptom development and disease spread.

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New insights on spread of tomato bacterial canker

Bacterial canker is the most serious bacterial disease of tomatoes worldwide. In recent years there have been several outbreaks of this sporadic, seed-borne disease in Europe, with outbreaks in England in 2008, 2009, 2010 and again this year. Once established in a crop it can be extremely contagious and difficult to eradicate. Tim O'Neill and Sarah Mayne of ADAS summarise the latest research findings from around the world and discuss how new insights on disease epidemiology influence the risk of secondary spread in the light of current crop management practices.

Causal agent

Bacterial wilt and canker is caused by *Clavibacter michiganesis* subspecies *michiganensis* (Cmm), a unicellular, rod-shaped bacterium. Non-pathogenic strains of *C. michiganensis* also occur on tomato and recent molecular studies suggest that some of these should be considered a separate subspecies. The Cmm genome was sequenced in 2008. Cmm has a single chromosome and two small circular plasmids of DNA. A number of pathogenicity factors have been identified which are essential for the bacterium to cause disease with at least two on the chromosome and one on each of the plasmids. Isolates which lose one of the plasmids can colonise tomato but do not cause severe disease.

Host range and symptoms

Bacterial wilt and canker is primarily a disease of tomato although natural infection also occurs in pepper, aubergine and some other *Solanum* species (e.g. black nightshade). Isolates from pepper showed greater pathogenicity to pepper than tomato, and in both hosts these isolates only caused cankers, not wilting.

All the recent outbreaks in England have caused losses through wilting and plant collapse (Fig. 1). This is the result of systemic infection which starts as transient unilateral wilting of occasional leaves, progressing to interveinal leaf windows (Fig. 2), permanent wilting of most leaves and plant collapse. Yellow to brown vascular staining develops in the stem, especially at the nodes. The crop affected in April this year was a grafted crop grown on rockwool slabs with run to waste irrigation; there was no recent history of the disease on the nursery. Disease spread was very rapid, progressing from a few plants affected in two rows to many plants in many rows in just three weeks.

1. Severe wilting and plant death resulting from systemic infection.

2. Leaf symptoms showing windows of interveinal necrotic tissue; can be confused with leaf scorch.

Due to the potential for rapid spread and the lack of effective plant protection products for use during crop production, it is essential that there is rapid communication between parties when a bacterial canker risk is identified: from propagator to growers, and from Plant Health authorities from the country of origin of the plants to UK PHSI. In the UK it is a statutory requirement for propagators, but not growers, to notify the PHSI when bacterial canker is suspected.

Why sudden outbreaks?

Bacterial canker nearly always causes surprise by its sudden appearance partway through the season and usually on a nursery with no recent history of the disease. This is generally due to a long period of latent, systemic infection in young plants. Infected seed or young plants are the most common source of infection. Cmm enters plants through natural openings and wounds on roots, stems and leaves. The bacterium spreads both upwards and downwards in the xylem (including into the roots), and remains largely restricted to the xylem. Microscopy studies have shown accumulation of the bacterium at stem nodes. Cmm exists initially as a non-pathogenic 'endophyte' within the nutrient-poor xylem of tomato stems, generally for at least two and sometimes many weeks. It needs to establish a population of at least 10^8 cfu/g (100,000,000 cells/gram) plant tissue before disease symptoms develop; even at this high population level, symptoms do not always develop. Factors other than population size recently shown to induce a shift from endophytic to pathogenic status include a warm temperature (much faster at 28°C than 15 or 35°C), a marked fluctuation in day/night temperatures and high humidity. The stress of a high fruit load also seems to trigger onset of the wilting phase.

New insights on disease spread

The use of grafted plants and closed irrigation systems, and the occurrence of leaf guttation, are all areas for increased scrutiny with regard to Cmm outbreaks – in certain situations each could have a large impact on development of bacterial canker. The use of grafted plants, now widespread practice for maintaining plant vigour, inherently doubles the risk as two seed sources are used. Moreover, the grafting process provides wounds for direct pathogen entry; and high humidity maintained to achieve graft union was shown to encourage movement of Cmm within plants. Symptomless grafted seedlings may harbour latent infection or carry Cmm on leaf surfaces, escaping inspections at the propagation stage.

Closed hydroponic growing systems increase risk by providing an extra transmission route. Work in Canada showed that infectious Cmm is released from the roots of infected plants into the nutrient solution and provided direct evidence of disease transmission between plants via the hydroponic solution. Disease spread was found to be faster in an NFT crop, where the solution is recycled, than in a run-to-waste rockwool crop. It is believed that Cmm in nutrient solution infects healthy plants at the natural wounds where lateral roots emerge, and possibly also through dead roots associated with the physiological root dieback that occurs at fruit loading stage.

Leaf guttation droplets were shown in Israel to be a potent source of Cmm, resulting in rapid secondary spread along a row when symptomless, systemically infected wet plants were handled. Guttation occurs at times of high humidity when root pressure at night forces water to exude through the hydathodes (natural openings found primarily at the tips of leaves) (Fig. 3). Leaf margin guttation droplets, which are rarely seen in UK substrate-grown glasshouse tomato crops, are most likely to occur in the morning and following poor irrigation management. Guttation fluid is essentially xylem sap and any endophytic bacteria in the xylem will be secreted with the guttation fluid. Large numbers (10^5 cells per droplet) were found in guttation fluid from infected plants in Israel. Touching a row of plants exhibiting guttation drops at dawn, including a single symptomless plant deliberately infected with Cmm by inoculation of the roots, resulted in spread to more than 20 adjacent downstream plants. In comparison, no spread occurred when all plants in a similar row with a single symptomless infected plant were touched at midday when dry. In another treatment, when dry infected plants were de-leafed using scissors without disinfecting them between plants, there was spread to four plants in the row.

3. Tomato plant with guttation droplets, a potential infection source for bacterial canker.

Guttation fluid from infected plants can lead to a high incidence of Cmm on the leaf surfaces of adjacent plants. This 'epiphytic' Cmm can invade leaves through stomata, leaf hairs, hydathodes and pruning wounds. The resultant secondary infection on leaves usually becomes visible as localised yellow or necrotic areas of leaf margins within 3-5 days. If contaminated guttation fluid on a healthy plant is withdrawn back into the leaf, systemic infection may result. Alternatively, systemic infection can arise if hands or tools contaminated with Cmm (from guttation fluid or from epiphytic Cmm on leaves) then touch fresh stem wounds during de-leafing and side-shooting. Systemic symptoms arising from localised infection generally develop between one and 12 weeks later.

Young plants most at risk

In further work in Israel, on tomatoes grown in the soil in polytunnels, it was shown that plant age has a marked effect on host response to infection. The duration of the incubation period to first symptoms increased, and disease severity decreased, with increasing plant age. There was a critical period of vulnerability from planting to around first flowering (16-17 true leaves present) during which time secondary infection generally developed into systemic infection and led to plant wilting and death. When older plants were inoculated at fresh petiole stubs (29-30 leaves present) only 40% of plants developed bacterial canker symptoms and no plants wilted. Such changes in susceptibility with plant age have been reported previously for others diseases, a phenomenon described as 'adult plant resistance'. This increased host resistance may be associated with physiological changes at onset of fruiting. Inoculation of leaves of different age showed no difference in susceptibility. It should be noted that although this work indicates a fall-off in infection as plants age, it is likely to be gradual and incomplete.

Impact on control

Control measures are described in detail in HDC Factsheet 01/10. The research results described above underline the value of various hygiene and cultural techniques to reduce the risk of bacterial canker outbreaks:

Propagation

- Check that seeds of both rootstock and scion have been tested and found free of Cmm
- Maintain strict hygiene/sanitation during grafting
- Note: propagators must notify Plant Health if Cmm is suspected in propagation

From planting to first fruit

- Do not handle plants when they are wet during this period of higher susceptibility
- Consider switching from twisting of heads to the use of clips to train stems
- Manage irrigation/ventilation to minimise occurrence of any guttation
- Do not wet spray if bacterial canker is suspected or confirmed

During crop handling

- Strict hand, glove and knife hygiene until first pick (e.g. change gloves between rows; disinfect knives by heat or hypochlorite, especially if the disease is confirmed)
- Deleaf only when crops are dry

Hydroponic crops

- Ensure any recycled solution is adequately disinfected
- Reduce root zone pH (e.g. to 5.0-5.5) at night where this is possible without detrimental effect on crop nutrition or growth; this reduces Cmm survival and consequent spread via the hydroponic solution

Removal of infected plants during cropping

- Place them in bags *in situ* rather than dragging them out
- Pick up all fallen fruit and debris
- Dispose of them into a covered skip
- Replace drippers, bobbins etc.
- Disinfect crop wires, trolleys etc.
- If bacterial canker is widespread and infected plants are not removed, manage fruit load to delay onset of the more severe wilt symptoms

Re-planting of rows cleared due to a bacterial canker outbreak

- Disinfect or renew drippers, bobbins, crop wires, trolleys etc.
- Use new slabs; if replanting is done on slabs from which systemically infected plants were removed, there is a real risk of infection of the new young plants from old roots remaining in the slabs
- If only small numbers of plants are removed in a row, consider taking extra shoots rather than replanting

Future developments

Genetic resistance to Cmm would be the ideal solution for a disease where no effective crop treatment options are available. Tolerance to Cmm has been identified in wild *Solanum* species but no immunity has been identified. Recent work at Wageningen University in the Netherlands has focussed on the development of new varieties with a high tolerance and no transmission via the seed (Fig 4). If this was achieved, the use of healthy seed could have a large beneficial effect over time.

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4. Cross section of infected fruit showing infected vascular tissue, a possible route for seed infection.

Cmm 'factfile'

Causal bacterium

Bacterial wilt and canker is caused by a rod-shaped plant pathogenic gram positive actinomycete (a group of bacteria that form branching filaments like fungi). This is notable as most plant pathogenic bacteria are gram negative, having a thinner, lipid based membrane rather than the thick slime envelope of Cmm. The genome of the sub-species infecting tomato, Cmm, has been sequenced and is 3.4 Mb in size (published in 2008). Cmm has a single circular chromosome and also has two circular plasmids (pCM1 is 27.5 kb and pCM2 72 Kb). All of the gene functions required for infection, successful colonisation and evasion or suppression of plant defences are carried on the chromosome; the plasmids carry genes essential for pathogenicity (symptom production). In this respect Cmm is similar to root mat disease of tomato and cucumber where the Ri plasmid is essential for *Agrobacterium* to cause root proliferation symptoms. Plasmids can be lost and gained from donor Cmm bacterial strains in the environment, and their loss results in less virulent strains. A prominent feature of Cmm is the presence of numerous genes coding for production of extracellular enzymes, especially proteases and cellulases. In culture the tomato infecting sub-species appears yellow, while the pepper infecting strain appears orange.

It has been speculated that Cmm is a 'recent' pathogen which has evolved from plant associated *Microbacteriaceae* and is still in the process of proper adaptation to its host plant. This is based on the finding that Cmm carries most biosynthetic pathways, indicating a versatility similar to that of soil bacteria. In many other pathogenic bacterial species, genome reduction occurs as an adaptation to a stable environment provided by the host. However, survival of Cmm in soil is limited due to defects in biosynthetic pathways for nitrate and sulphate reduction.

Hosts

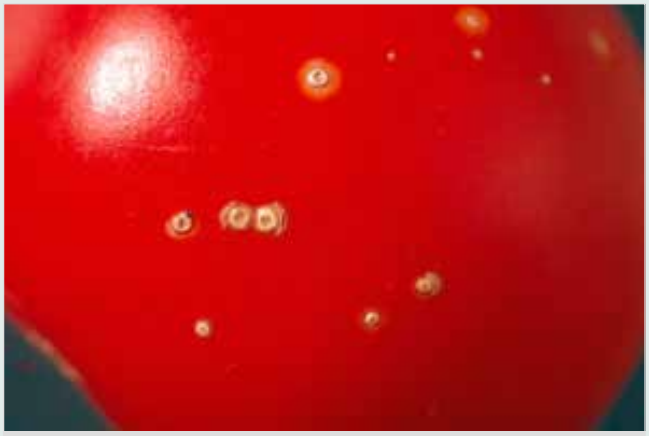
The Genus Clavibacter contains only one species (Cm) containing 5 sub-species of plant pathogenic bacteria affecting tomato, potato, maize, wheat and lucerne. Cmm is known to infect tomatoes, and other members of the Solanaceae including peppers, aubergine and tobacco as well as solanaceous weeds such as black nightshade. It can infect seeds and also has an epiphytic lifestyle. Cmm can maintain large epiphytic populations on leaves of tomato and smaller populations on various other solanaceous and non-solanaceous species. Natural infection of aubergine and pepper is rare, and isolates that infect pepper are less pathogenic to tomato than tomato isolates, but more pathogenic to peppers. They have no plasmids and it appears that they have evolved into a separate population from tomato-infecting Cmm strains. Bacterial canker on pepper has been reported in Italy, Israel, Korea and the USA. Plants rarely die and damage is limited, and it is possibly a new sub-species as it is genetically dissimilar to tomato isolates.

Symptoms

The complex of bacterial canker symptoms is highly variable, dictated by factors such as plant age, infection site, inoculum concentration, varietal susceptibility and environmental conditions. Symptoms in recent outbreaks have mostly been those of systemic infection. The first symptoms usually seen are unilateral wilting of leaves. Wilting is initially reversible, and leaves recover in the cooler part of the day. However, as the infection progresses the wilt will persist. Wilting is not due to blockage of xylem vessels by high bacterial populations or by the extracellular polysaccharides it produces, but by plasmid encoded pathogenicity factors. Leaves of mature plants may become stressed rather than wilt, and may develop windows of white and then brown interveinal necrotic tissue. This may resemble leaf scorch caused by bright sunny weather after a dull period. In both cases dead leaves remain attached to the stem. Young petioles may show curved or distorted growth. Vascular tissue of infected stems is discoloured yellow, later turning brown, which is especially noticeable at the nodes if the stems are split vertically. Stems may split (Fig. 5). Severe vascular infections result in the epidermis and outer cortical tissue coming away from the inner stem if stems are squeezed between the thumb and forefinger. A yellow bacterial ooze may occasionally accompany necrotic and discoloured areas of vascular bundles, even within fruit. Fruit may develop abnormally, fall prematurely or ripen unevenly. New, vigorous shoots may be produced from the base of affected plants. Cankers on the stem and petioles are actually relatively rare.

5. Stem splitting caused by bacterial canker.

Systemic infection may spread up, down or through side shoots via the vascular system, and Cmm is known to extensively colonise to the apical tip in 15 days. The mechanism by which it moves down the stem against the flow of water is unknown, as the bacteria are unflagellated; it has been suggested that the bacterium attaches to the xylem wall and moves by a twitching motion. Cmm accumulates at nodes where there is more vascular tissue. Microscope analysis using fluorescent-tagged Cmm has shown that many vessels are not infected, a strategy to keep the plant alive to maintain nutrition. Bacteria enter via natural openings and wounds on the roots or stem. Bacterial enzymes destroy the xylem wall, providing nutrients from the macerated tissues and allowing localised spread of the bacterium.



6. 'Bird's eye spots' on red fruit, an uncommon symptom of epiphytic infection.

7. Stem symptoms showing pale, bubbling, 'mealy' stem.

Localised (non-systemic) infection causes leaf margin yellowing and necrosis due to infection of water-excreting glands (hydathodes), and white blister spots (bird's eye spots) may develop on fruit following infection via natural openings on young fruit, usually the base of broken hairs (Fig. 6). These fruit spots appear as raised, pale green or whitish pustules which develop a light brown centre and a chlorotic halo as the young fruit expands. Stems, leaves and calyces may also develop a mealy appearance, exhibiting raised, creamy white spots, often on the side of the plant that has been exposed to a spray (Fig. 7). On the upper surfaces of leaves, roughly circular and slightly raised white spots about 1 mm in diameter may develop, which may be more numerous near the midrib and main veins of leaflets. Spots may expand and merge, and portions of the lamina may disintegrate to give the leaves a tattered appearance. This may be more noticeable after a spray, as affected pieces of leaf may be blasted away. These localised symptoms were the main symptoms of bacterial canker in the UK in the 1980s, when hosing down a house, spraying plants with water to move flowers to aid pollination, and application of high volume pesticide sprays, were all common practice. The lack of localised symptoms in outbreaks occurring in the last decade probably reflects reduced water splash with the new cropping practices.

Factors influencing symptoms

For Cmm to cause wilt symptoms a population of more than 10^8 cfu/g plant tissue must be established in the stem. Population size is the primary influencing factor, however factors other than population size induce the shift from an endophytic to pathogenic lifestyle. This shift is also affected by temperature, being rapid at 28°C, but occurring 2 weeks after if temperatures are closer to 15 or 35°C. Temperature continues to affect disease development throughout the whole season. Bird's eye spot (BES) on fruit is an inconsistent symptom that may occur if a flower or small, green, young fruit is infected. High relative humidity (above 83%) contributes to the occurrence of BES and other secondary symptoms caused by water splash. Varietal susceptibility, plant age, crop culture practices, crop nutrition and nutrient solution pH may all also influence how the disease develops and spreads.

Severity and presence or absence of symptoms is also determined by which plasmid(s) the specific strain has. The *CelA* gene on pCM1 encodes a 746 amino acid glucanase enzyme that degrades cellulose and is involved in plant cell wall degradation. The *Pat-1* gene on plasmid pCM2 encodes a 290 amino acid protein, a secreted protease that has been shown to be necessary for production of wilt symptoms. Isolates of Cmm which have lost one or both plasmids may still be able to infect and colonise the xylem of tomato plants, but no bacterial wilt symptoms develop. Other isolates which show poor stem colonisation may cause cankers and no wilting. Plasmid-free strains can be generated by increasing the temperature from 26 to 32°C, and plasmids may be lost under stress conditions. Some field strains are known to be pCM1+ but pCM2- (e.g. 20% of strains in a survey of crops in Israel). The fluctuation of plasmids in the natural population modulates virulence, which may allow an equilibrium to develop enabling maintenance of host viability. Non-virulent strains may also be a result of loss of part of their chromosome, meaning that the bacteria are poor colonisers and population size never reaches high enough levels to cause disease.

Sources and survival

- Contaminated seed – a major source of outbreaks and a major cause of long distance spread
- Latent (symptomless) infection in young plants; usually arising from contaminated seed
- Soil – survival of unprotected Cmm cells in soil is poor
- Debris - Cmm can survive in plant residues in soil for at least 2 years
- Nutrient solution – Cmm survived at least 24h in nutrient solution at pH 5.5-6.5 but for less than 6h when solution pH was <5.0
- Symptomless *Solanum* spp. including weeds
- On tools, wires, irrigation pegs, bobbins, equipment – left over from previous outbreaks in a dried bacterial ooze

The bacteria may survive months on tools etc. due to its ability to tolerate desiccation and cold temperatures. Extracellular polysaccharides produced by the bacteria function to promote survival. Their roles are considered to include the generation of water-soaked tissues to prevent dehydration of the bacteria, protecting the bacteria against toxic compounds, preventing recognition by the plant, promoting adhesion to surfaces, and exploiting plant mineral and carbon sources.

Spread

- Seed and young plants – both grafted and non-grafted
- Water splash of Cmm – from epiphytic asymptomatic populations, from localised mealy spot infections, and in guttation droplets on plants
- Air – in small particles of debris and water droplets
- Hands/tools – contact spread
- Hydroponic solution – spread between plants via the roots
- Insects and birds – there is no evidence, but nor have they been excluded as a possible vectors
- Where infection arises from the soil, only a small proportion of infected plants may show symptoms, leading to unnoticed spread during crop handling

Genetic studies show there is persistence of specific isolates on a nursery. Symptomless seedlings may harbour latent infection or carry Cmm epiphytically. These asymptomatic seedlings escape inspections at the propagator stage. Mechanical wounds at grafting may promote dissemination of Cmm directly into vascular tissue.

Seed infection

This can occur both externally (on the seed) and internally (within the seed or endosperm). With plants systemically infected, seeds in developing fruit may become infected via the vascular tissue. Cmm can also develop and grow to the seed from a bird's eye spot, spreading into the fruit as they ripen. Cmm enters fruit through trichome bases on green fruit. In experimental studies in the USA, the highest infection of fruit (78%) occurred following inoculation of dead flowers/emerging fruit. It was also found that Cmm can access the xylem and seed on plants with no external fruit or plant symptoms, making it difficult to identify diseased plants with potentially contaminated seed.

Seed testing

Although tomato seed is routinely tested for Cmm, epidemics of bacterial canker continue to occur in various countries. The importance of seed transmission has been highlighted by genetic studies showing the same strain occurring in highly diverse geographic locations, and by the stability of strains in a single location over long periods of time (>30 years) once introduced into an area. Studies of pathways to seed infection show that infection can occur both via the xylem and through the base of leaf hairs on green fruit; these infections may occur with no external fruit or plant symptoms of bacterial canker, consequently making it difficult to identify (and thereby avoid) diseased plants with potentially contaminated seed in seed crops. This helps to explain why outbreaks from contaminated seed continue to occur.

Verifying that a seed lot is pathogen-free is complex, and depends on sample size, level of inoculum and the efficiency of the seed health test. Diagnostic tools need to be specific and extremely sensitive. Identification of Cmm can be done via various methods, including antibody detection and PCR tests. Definitive diagnosis can be difficult due to false negative and positive results, especially as non-pathogenic Clavibacter-like strains occur on tomato, which may cross-react with Cmm identification tools, despite being distinct. New developments in molecular methods which permit more rapid, extremely sensitive and very specific plant and seed testing for Cmm should reduce the risk of future outbreaks arising from infected seed.

The recommended seed health assays are based on plating seed extracts onto selective media, selecting strains based on colony morphology, and using confirmatory tests such as pathogenicity, immuno-fluorescence (IF) or PCR with specific primers. Use of tomato plants in pathogenicity tests has the disadvantage of a potentially long interval (3 days to several weeks) between inoculation and symptom expression. The '4 o'clock plant', *Mirabilis jalapa*, can also be used to test for pathogenic Cmm, as it elicits a distinct hypersensitive reaction within 48 hours of inoculation. Commonly, 10,000 seeds are tested. The inoculum density per seed is also important, and not yet assessed, as higher inoculum per seed is more likely to result in disease.

Seed treatment

The source of Cmm in UK glasshouse crops grown out of the soil is most commonly untreated or ineffectively treated seed; for soil-grown crops survival in tomato debris in the soil is also a high risk source. Seed-borne infection occurs despite protocols to minimise risk of Cmm including good seed production practices, hydrochloric acid seed treatment and rigorous seed health tests. Hypochlorite seed treatment reduces Cmm populations on the external coat of treated seed but is not a treatment that guarantees elimination.

Control measures between crops

Infected crop should be disposed of to landfill. For plants without symptoms but which may possibly be infected or contaminated with Cmm experimental work has shown that Cmm can be eradicated from crop debris by aerobic composting with turning. Temperatures should reach 55°C throughout the stack to ensure eradication, as high temperatures are needed to kill the pathogen. It would be sensible to dispose of such compost onto land not intended for tomato or other solanaceous crops. There is direct evidence that overwintered debris on and in soil can cause infection in a subsequent soil-grown crop. Decline of bacterial populations was faster in debris incorporated into the soil than when it was left on the surface.

Glasshouses and any other areas that may have come into contact with the bacterium should be thoroughly sanitised before a new tomato crop is planted. This includes trolleys, irrigation equipment, support wires, bobbins, flooring, concrete pathways and the glasshouse structure. Recent experience in the UK where infection has re-emerged in the same location in one glasshouse for several successive years strongly indicates that Cmm can persist from one season to the next on bobbins and drippers, so these should be thoroughly disinfected or renewed after an outbreak.

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