Pseudomonas syringae pv. aesculi

Scientific Name
Pseudomonas syringae pv. *aesculi* (ex Durgapal & Singh 1980) Young et al. 1991

Synonyms:
None known

Common Name(s)
Bleeding canker of horse chestnut, bleeding canker disease

Type of Pest
Bacterial pathogen

Taxonomic Position
Class: Gammaproteobacteria, **Order:** Pseudomonadales, **Family:** Pseudomonadaceae

Reason for Inclusion in Manual
CAPS Target: Additional Pests of Concern - 2011

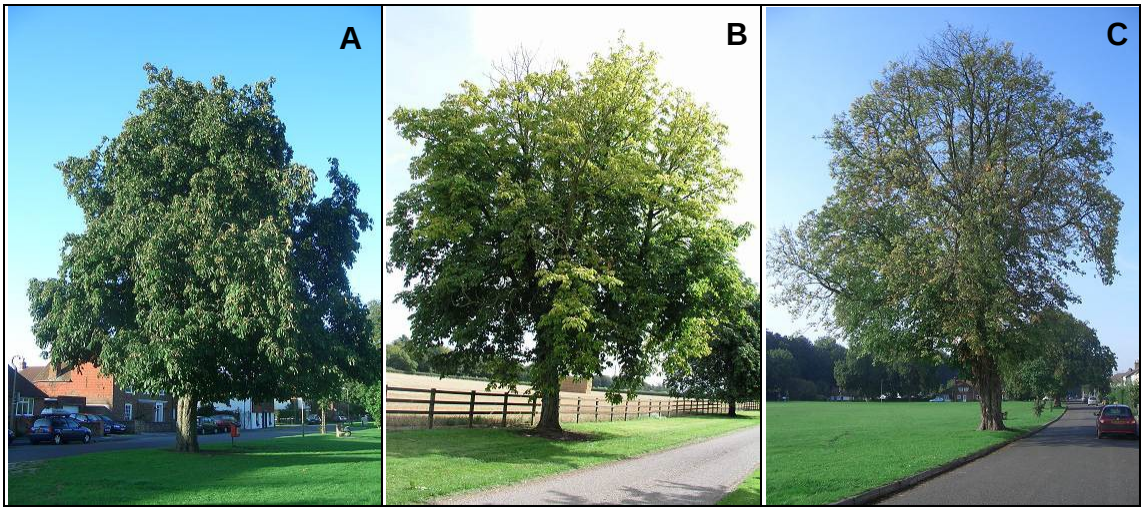

Figure 1: A. Healthy horse chestnut tree. B. Crown dieback and yellowing associated with *P. syringae* pv. *aesculi*. C. Crown dieback and premature leaf fall seen with bleeding canker disease. Photos courtesy of the Forestry Commission.
http://www.forestry.gov.uk/website/forestresearch.nsf/ByUnique/INFD-6L4GBT

Background
Stem bleeding on horse chestnut trees caused by *Phytophthora citricola* and *P. cactorum* was first observed in the early 1970s in the United Kingdom and other European countries (Brasier and Strouts, 1976; Green et al., 2009). Since 2002, the

number of horse chestnuts with bleeding canker, however, has dramatically increased and the disease has become severe in some parts of Europe. Initial assumptions that a *Phytophthora* species was the primary causal agent were refuted when the bacterium *Pseudomonas syringae* pv. *aesculi* was proven to be associated with the recent spread of the disease in the United Kingdom (Webber et al., 2007).

Pest Description

Pseudomonas syringae is a rod-shaped gram negative bacterium with polar flagellae (Mabbett, 2007). *P. syringae* exists as over 50 pathovars (strains) infecting a wide range of plants that fall within nine genomospecies (Gardan, 1999). Pathovars are genetically distinct but morphologically the same and primarily differentiated by host range. Many pathovars were originally considered as separate species within the *Pseudomonas* genus, but molecular biology techniques have shown all to be part of a single *Pseudomonas syringae* species. *Pseudomonas syringae* is named after the lilac shrub (*Syringa vulgaris*) from which it was first isolated (Mabbett, 2007). *Pseudomonas syringae* strains belong to the fluorescent pseudomonad group and most produce siderophores (called pyoverdins) that fluoresce under UV light (Bultreys et al., 2001). Siderophores are iron chelating compounds used by microbes to sequester iron in iron-deficient environments.

Pseudomonas syringae pv. *aesculi* is fluorescent on King's B medium, produces **L**evan on sucrose nutrient agar, is negative for **O**xidase, is non-**P**ectolytic (does not cause rot in potato), is negative for **A**rginine dihydrolase, and induces a hypersensitive response on **T**obacco (LOPAT Group I, +---+) (Webber, 2007; Young, 2010). Fatty acid analysis and partial sequencing of the gyrase B (gyr B) gene indicated the pathogen from horse chestnut was identical to *P. syringae* pv. *aesculi* previously isolated from foliage (leaf spots) of *Aesculus indica* from India (Durgapal, 1971; Durgapal and Singh, 1980). *Pseudomonas syringae* pv. *aesculi* produces coronatine, a chlorosis inducing phytotoxin produced by several other pathovars of *P. syringae* (Bereswill et al., 1994; Bultreys et al., 2008). *Pseudomonas syringae* pv. *aesculi* also produces indole-3-acetic acid (IAA, auxin) (Glickmann et al., 1998).

Biology and Ecology

So far, the epidemiology of *P. syringae* pv. *aesculi* remains largely unknown. *P. syringae* can be isolated from the surfaces of horse chestnut leaves and branches, as well as flowers and various parts of the fruits (EPPO, 2010). *Pseudomonas syringae* pv. *aesculi* is able to infect aerial woody parts of its host directly (Steele et al., 2010). Lesions develop during the host dormant session and are centered mainly on lenticels, leaf scares, and nodes (Steele et al., 2010). Recent comparative genomic studies have shown that *P. syringae* pv. *aesculi* fell within a statistically well-supported clade of *Pseudomonas syringae* adapted to woody hosts. This clade was comprised of pathovar *aesculi* and pathovars *morsprunorum*, *myricae*, *savastanoi*, and *mori*, which infect horse chestnut, apricot, bayberry, olive, and mulberry, respectively (Green et al., 2010). The pathogen is not known to be seed transmitted but is known to be moved around in infected nursery stock (plants for planting).

Bacteria were also detected in rainwater in the vicinity of diseased trees (EPPO, 2010). More studies are needed, however, to determine the possible role of water, insects, or even human activities (*e.g.,* pruning) in disease transmission (EPPO, 2010). Pathovars of *P. syringae* are generally known to be spread via water splash and airborne water droplets. Most enter trees via wounds, but some are known to infect plants through intact and unbroken tissues (Mabbett, 2007).

Trees of all ages have been affected by the recent disease upsurge. Young trees with a stem diameter of only 10 cm (4 inches) have been found with advanced symptoms (Forestry Research, 2011). The impact, however, on the environment can be profound when large, maturing trees are infected and disfigured by the disease. If the disease is severe and the areas of bark that are killed are extensive, large trees can undoubtedly be killed. Younger trees (10-30 years old) are at a greater risk and can succumb to the disease in just a few years (3-5) as the smaller diameter of their trunks means that they can be girdled more quickly (Forestry Research, 2011).

Figure 2. Active 'bleeding lesions' on horse chestnut infected with *P. syringae* pv. *aesculi*. Note: Bark cracking in photo on the right. Photos courtesy of the Forestry Commission. http://www.forestry.gov.uk/website/forestresearch.nsf/ByUnique/INFD-6L4GBT.

Symptoms/Signs

Trees that have been affected for some years may show crown symptoms (Fig. 1). The early symptoms, however, tend to be limited to 'bleeding' lesions (Fig. 2); scattered drops of rusty-red, yellow-brown, or almost black, gummy liquid ooze from small and large patches of dying bark on the stems or branches of infected horse chestnuts (Bultreys et al., 2008). Bleeding patches may be associated with the base of the tree at the soil surface or may start higher up the trunk at about one meter, and then extend upwards. Early in the year (spring), the exudates from bleeding patches are a dark color but transparent. As the weather becomes warmer, bleeding from infected tissues

becomes more copious and runs some way down the tree. At this time, it is often a conspicuous rusty-color and no longer transparent but cloudy or opaque. Under dry conditions (summer), this exudate dries to leave a dark, brittle crust near the point of exit in the bark (Fig. 3). Renewed bleeding may be seen later in the year, often in autumn. This suggests that the pathogen activity is greatest under moist, mild conditions of spring and autumn. After some months, the center of the bleeding bark patch may become cracked (Forestry Research, 2011). Cracking of bark and disfigurement of trees is common (Fig. 4)

Figure 3. Bleeding spot drying out in the summer and becoming crusted. Photo courtesy of the Forestry Commission.

In time, fruiting bodies of wood rotting fungi often appear on the surface of the dead bark, protruding out of the bark cracks (Fig. 5). Over three-to-four years and particularly if a tree has multiple bleeding cankers, the areas of dead phloem and cambium underneath the bleeding areas may coalesce and extend until they encircle the entire trunk or branch. When this happens, crown symptoms become visible, typically consisting of yellowing of foliage, premature leaf drop, and eventually, crown death (Fig. 1) (Webber et al., 2007). Sometimes, part of the crown will fail to flush, and later in the year the remaining foliage withers and dies. In trees with chronic dieback caused by the disease, the leaves may be smaller, and seem thinner and more flaccid than the foliage of healthy trees (Forestry Research, 2011). The inner bark (phloem) under the bleeding patches is usually necrotic or dead, with an orange-brown color, which is often clearly mottled or zoned (Fig. 6). Underneath, the wood may be stained blue-black (Forestry Research, 2011).

Figure 4. Bark cracking and disfigurement of trees infected with *P. syringae* pv. *aesculi.* Photo courtesy of the Forestry Commission.

Sometimes white fungal mycelium (Fig. 7) can be seen under the dying bark, but this is usually indicative of *Armillaria* or other decay fungi, which invade the moribund tissue, and not the original cause of bark death. In these instances, it is rarely possible to isolate the agent that originally caused the bleeding canker. Sometimes *Armillaria* does attach healthy trees and causes stem bleeding, as it invades via the tree root system. However, in such causes the stem bleeding is likely confined to the root collar and lower stem (Forestry Research, 2011).

Figure 5. Fungal fruiting bodies protruding through bark cracks of an old canker. Photo courtesy of the Forestry Commission.

Pest Importance

Horse chestnut is native to Europe but is grown as an ornamental tree in temperate regions of North America. There are several *Aesculus* species that are native to North America, as well as the introduced, cultivated *A. hippocastanum* that has naturalized in the eastern United States (Eggert, 2010). Horse chestnut is an attractive tree that is frequently planted along roadways and avenues, as well as parks in Europe. They can also be found in woodlands.

Figure 6. Left: The Edge of an extending necrotic lesion in phloem with crusted exudate on bark surface. Right: Mottled appearance of necrotic tissue, caused by bleeding canker. Photo courtesy of the Forestry Commission. http://www.forestry.gov.uk/website/forestresearch.nsf/ByUnique/INFD-6L4GBT.

In the United Kingdom, on the basis of survey carried out in 2007, it was estimated that 35,000 to 50,000 trees were affected and probably a few thousand have already been felled as the result. Over 70% of those trees surveyed in parts of England had bleeding canker symptoms, with 36 and 42% of surveyed trees showing symptoms in Wales and Scotland, respectively (Green et al., 2009). In the Netherlands, recent surveys estimated that 20% of the horse chestnut trees are infected with the pathogen (Eggert, 2010).

Figure 7. *Armillaria* (white mycelium) extending into a canker lesion. Photo courtesy of the Forestry Commission.

Due to the severity of the disease, the Animal and Plant Health Inspection Service (APHIS) issued a federal order prohibiting the importation from all countries, except Canada, of *Aesculus* spp. plants for planting (excluding seed) (Eggert, 2010).

Known Hosts

Major Hosts:
Aesculus spp. (horse chestnuts; buckeyes), *Aesculus hippocastanum* (horse chestnut (white flowered)), *A. indica* (Indian horse chestnut), *Aesculus* x *carnea* (horse chestnut (red flowered)), and *A. flava* (sweet or yellow buckeye).

Aesculus hippocastanum cultivar Baumanii appears to be extremely susceptible (Forestry Research, 2011).

Known Vectors (or associated insects)
Pseudomonas syringae pv. *aesculi* is not known to be a vector, is not known to be vectored by another organism, and does not have any associated organisms.

Known Distribution
Asia: India. **Europe:** Belgium, France, Germany, Italy, Netherlands, and the United Kingdom (Schmidt et al., 2008; Green et al., 2009).

Potential Distribution within the United States
Pseudomonas syringae pv. *aesculi* is not known to occur in the United States but presents a significant risk to horse chestnuts in the United States. The organism can be easily moved in plants for planting. Particular attention should be given to horse chestnuts naturally occurring in your areas (Table 1) or from ornamental plantings. A recent risk analysis by USDA-APHIS-PPQ-CPHST indicates that portions of Alabama, Arkansas, California, Georgia, Illinois, Indiana, Kansas, Kentucky, Michigan, Mississippi, Missouri, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, and West Virginia have the greatest risk for *P. syringae* pv.

aesculi establishment based on host availability and climate within the continental United States. The remaining states have low levels of risk for establishment of *P. syringae* pv. *aesculi.*

Table 1: The native range of *Aesculus* spp.

Species of *Aesculus*	Common Name	Native Range
A. hippocastanum	Horse chestnut	Bulgaria, N. Greece, S. Albania
A. x carnea	Red horse chestnut	Hybrids between *A. hippocastanum* and *A. parva*
A. turbinata	Japanese horse chestnut	Japan
A. californica	California buckeye/horse	California, United States

	chestnut	
A. parryi	Parry buckeye, lower California buckeye	California, Mexico
A. flava	Sweet or yellow buckeye	Southeast United States
A. glabra	Ohio buckeye	Southeast United States
A. parvia	Red buckeye	Southeast United States
A. parviflora	Bottlebrush buckeye	Southeast United States
A. sylvatica	Painted buckeye	Southeast United States
A. assamica	Assam horse chestnut	N. Siam, NW Indo-Chino, S. China, NE Pakistan, Bhutan
A. indica	Indian horse chestnut	NW Himalayas
A. chinensis	Chinese horse chestnut	China
A. wilsonii	Wilson's horse chestnut	Central China

Survey

CAPS-Approved Method: Visual survey is the approved survey method for *P. syringae* pv. *aesculi* . For visual survey, collect symptomatic plant material.

Literature-Based Methods: In Germany, branch and stem sections were sampled from *Aesculus hippocastanum* with typical symptoms of horse chestnut bleeding canker (Schmidt et al., 2008). After sampling, part of the material was frozen at -18°C (O°F). Discs of approximately 5 cm thickness showing necrosis of the bark were cut from the frozen samples and used for bacterial isolations.

A national delimiting type survey was conducted in Great Britain for bleeding canker. The protocol is available at http://www.forestresearch.gov.uk/pdf/bleedcankersurveyrep020408.pdf/$FILE/bleedcankersurveyrep020408.pdf.
A summary of the survey is provided: Great Britain was divided up in to 11 regions reflecting a broad geographical spread. The purpose of the survey was not to estimate bleeding canker infection rates for the whole of Great Britain but to provide a snapshot of the extent of the disease using a subset of horse chestnut sites obtained from the initial data gathering exercise. From these subsets, counties, which had sites where 10 trees or more were reported as present, were identified. Each region, except Scotland and Wales, was made up of a number of counties. Within each region a minimum of 10 rural and 10 urban sites were selected for inspection. The sites to be visited were independently selected prior to the start of the survey to prevent bias. Surveyors operating from each field station could inspect more than the minimum number of locations providing the time taken to inspect the additional sites could be accommodated within the time scale agreed. A minimum of 10 trees were inspected for signs of the disease at each location. If there did not appear to be any symptoms of the disease on the horse chestnut, the site was recorded as being free from the disease. A standard *pro forma* was completed for all sites visited. This was used to record basic environmental variables (grid reference; height above sea level; etc.) and will be used

for possible future analysis. The diameter of all horse chestnut trees inspected was measured at breast height to the nearest 0.1cm. Both the stem and the branches were inspected for signs of the disease. Four categories of symptoms characteristic of bleeding canker were assessed. These were bleeding on the main stem; bleeding on the branches; cracks in the bark on the branches; dieback in branches. No samples were collected although photographs were taken of symptomatic trees to enable subsequent investigation.

Key Diagnostics/Identification

CAPS-Approved Method:

Molecular: Green et al. (2009) developed a real-time PCR for detection of *Pseudomonas syringae* pv. *aesculi* in plant tissues. This PCR assay can be used as a preliminary screen for *Pseudomonas syringae* occurring on horse chestnut.

Literature-Based Methods:

Strain Isolation: *Pseudomonas syringae* pv. *aesculi* is fluorescent on King's B medium. Strain isolations were performed onto King's B medium (King et al., 1954) from the margins of fresh or dry lesions found on horse chestnut trees (Bultreys et al., 2008).

Biochemical: *Pseudomonas syringae* pv. *aesculi* produces **L**evan on sucrose nutrient agar, is negative for **O**xidase, is non-**P**ectolytic (does not cause rot in potato), is negative for **A**rginine dihydrolase, and induces a hypersensitive response on **T**obacco (LOPAT Group I, +---+) (Webber, 2007; Young, 2010. *Pseudomonas syringae* pv. *aesculi* produces coronatine, a chlorosis inducing phytotoxin produced by several other pathovars of *P. syringae* (Bereswill et al., 1994; Bultreys et al., 2008). *Pseudomonas syringae* pv. *aesculi* also produces indole-3-acetic acid (IAA, auxin) (Glickmann et al., 1998).

Identification: Bultreys et al. (2008) identified *Pseudomonas syringae* pv. *aesculi* through pyoverdin-based tests, including visual, spectrophotometric and high performance liquid chromatography, the induction of potato rot, and extragenic palindromic (REP)-PCR. The modified PCR test detecting the *cfl* gene involved in coronatine synthesis was also used in identification (Bultreys et al., 2008).

Schmidt et al. (2009) developed a 'fast' molecular detection method, which included DNA extraction, polymerase chain reaction (PCR), and electrophoresis until gel documentation. The PCR primers were derived from the gyrase B gene. This primer, however, also amplified pathovar *aptata* (foliar blight of sugarbeet, bellpepper, cucumber, sunflower, lettuce, bean, eggplant, faba bean, and cowpea) and pathovar *morsprunorum* (bacterial canker of stone fruit) due to great similarity in the gyrase B gene sequence of closely related pathovars. The authors thought that the differing host ranges would aid in the identification of these pests from pathovar *aesculi*, because the other pathovars should not occur near a lesion of a horse chestnut tree. The test identified the pathovar from a tree sample within 6 hours of starting laboratory work.

Easily Confused Pests

Bleeding on the trunk and branches, necrotic phloem, foliar discoloration, and dieback of horse chestnut has also been seen with *Phytophthora* spp. (*cactorum* and *citricola*) in Europe. In the United States, the same pathogens have also been found to cause bleeding cankers on maple, birch, sweet gum, oak, and willow. *Phytophthora ramorum* also has been found to cause bleeding cankers on certain broadleaf species in England, including a single specimen of horse chestnut.

References

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