

European collaboration on canker stain of plane trees

A study workshop weekend in Padua, Italy, on canker stain of plane: a new collaboration venture between Treework Environmental Practice and De Rebus Plantarum.

Geoff Monck and Neville Fay

When Lucio Montecchio and Neville Fay met at the Society of Italian Arboriculture (SIA) in Milan last year, they embarked upon a collaboration to advance ecological approaches to the study of tree health and disease.

This would build on Treework Environmental Practice's *Innovations in Arboriculture* seminar series and De Rebus Plantarum, a spin-off of the University of Padua and a centre of excellence in tree disease study. Treework would recruit committed delegates and help organise the programme, and Lucio would provide the study environment to provide a radical, memorable, hot-house learning experience in a pleasurable and culturally enriching environment.

Reconvening in London in autumn they planned the launch of the first study workshop – on *Ceratocystis platani*, a disease with a foothold on mainland Europe responsible for killing thousands of plane trees, known as canker stain of plane (CSP) and also called plane wilt. As the disease has not yet arrived in the UK, the best place to teach UK arboriculturists about the symptoms and pathogenic behaviour is where CSP is currently causing devastating tree loss – in Italy. Thus the first of their partnership study workshops was born and eight months later, a small, lucky band of delegates from the UK flew to Padua.

Over one weekend the delegates experienced the rare privilege of receiving sufficient knowledge and training to return competent to identify the disease macro- and microscopically. The knowledge experience was collaterally enriched with fine Italian wining and dining, and

the pleasure of memorable conversation and debate; that the weekend coincided also with the referendum result made the intense European experience utterly unforgettable.

Canker stain of plane: the nature of the disease

C. platani is a highly infectious fungal wound pathogen of plane trees (*Platanus* sp.). Particularly susceptible are oriental plane (*Platanus orientalis*) and London plane (*Platanus acerifolia*). When infected, 100% mortality can be expected. Taxonomically *C. platani* belongs to the Division: Ascomyceta, Class: Sordariomycetes and Family: Ceratocystidae.

While currently not known to occur in the UK, it has spread from Italy through Europe since World War II. It was first reported in Italy in 1972, in France in 1974 and in Spain in 1977. The disease, were it to become established in the UK, would not only be devastating for our plane tree populations, it would also have serious economic consequences from the high costs of managing it. The impact upon London with its large plane tree population would be particularly severe.

C. platani is a notifiable plant disease in the UK and The Plant Health (Forestry) (England and Scotland) Order 2005, issued to implement EU legislation, gives the UK 'protected zone' status for canker stain of plane disease. This legislation allows the UK to ban the movement of plane plants to the UK unless they are certified in a plant passport to have been produced in an area free from the disease.

The Padua experience

On 24 June a small group of assorted tree people assembled at Stansted airport, curious about weekend they were about to embark on. While it promised a professional and educational experience, it would be far more than that, different from any normal training experience. It would be not only profoundly informative and a socially enriching experience, the environment of knowledge transfer would take place in a beautiful Italian location.

In addition to consultants from Treework Environmental Practice and Tim Moya Associates, delegates also represented the Forestry Commission, the London Tree Officers Association, the City of London, Transport for London, local authorities (Southwark, Islington and Guildford), and Barcham Nurseries. The one thing everyone had in common was a deep concern for the health of our plane trees and the potential arrival of *C. platani* in the UK.

Prior to this event, there was scant knowledge of *C. platani* in the UK. As many internet sources and the academic literature provide misinformation and conflicting information, all delegates were determined to learn from Lucio Montecchio, a recognised international authority, who, having studied and published extensively on *C. platani* since the 1980s, has been involved in the fight against CSP in Europe, developing a wealth of knowledge about the disease and its control. Being widely involved in tree pathology, together with colleagues he is also involved in finding practical therapeutic treatments.

As one of Lucio's many talents also includes being superb host, the first collaborative training event was to prove both truly informative and memorable.

Day 1: Introductions to the locality and the disease problem

The workshop took place in the medieval Venetian town of Castelfranco del Veneto, in the impressive historic Villa Revedin Bolasco. This inspiring environment has finely landscaped mature gardens, rich in botanical interest. Delegates were warmly welcomed by Dr Mariagrazia Lizza, Culture Councillor for Castelfranco del Veneto, before attending the introductory lecture from Lucio on the biology, symptomology and epidemiology of *C. platani* and relevant Italian quarantine legislation.

C. platani is native to US forests where it is a pathogen of *P. occidentalis*, the less



The spectacular main hall in Villa Revedin Bolasco.

susceptible of the hybrid *P. orientalis* x *P. occidentalis* cross which gives rise to the London plane (*P. acerifolia*). *P. acerifolia* is highly susceptible to *C. platani*, in part attributable to its *P. orientalis* heritage and also to the low genetic variation within the 'species', it being derived from a restricted range of clones.

C. platani is thought to have been introduced into Naples by US troops in 1944 in infected wood packaging material, from where it spread through Italy and into France, Albania, Armenia, Greece and Switzerland. In Italy, it is estimated that in some areas over 60% of plane trees were lost between 1978 and 2004. However, more recently the spread of the disease is slowing down, probably as plane populations decline.

C. platani is primarily a wound pathogen. Unlike some vascular wilt diseases, such as Dutch elm disease, there is no insect vector and the transfer of spores from

an infected tree to a wound on a healthy tree by insects is thought to be rare. In outbreaks in Europe, in predominantly managed, planted populations of trees, *the pathogen is spread primarily by contaminated chainsaws used by tree contractors*. As the spores remain viable on infected sawdust for a long period of time, contaminated contractor equipment is the probable principal pathway of CSP introduction into the UK from Europe. Infection is also readily initiated via root grafts from nearby infected trees as well as from wind-borne sawdust contaminating open wounds.

Open wounds remain susceptible to CSP until occluded. Decay fungi may in a sense be considered 'a type of natural defence' as, when exposed sapwood has been colonised by decay fungi, *C. platani* is unable to compete and cannot initiate an infection. After successful colonisation, the fungus primarily migrates down the phloem, and then moves internally along

the starch-rich medullary rays. This results in a characteristic diagonal 'canker' at the junction between healthy and necrotic tissues advancing around the stem until it becomes completely girdled, ultimately resulting in tree death. There is rarely any sign of callus growth at the canker margin. The colonisation pattern and the resulting tissue necrosis produce a characteristic pattern of crown mortality.

As illustrated in Box 1 (overleaf), the first symptom is usually the death of a whole branch or the side of a crown, with the dead leaves remaining attached. Crown death progressively traverses the crown horizontally until the whole crown is dead. In a mature tree this may take three to five years and in a young tree about 18 months.

Symptoms become obvious within six months of infection. When infection occurs through root grafts, the whole crown usually dies simultaneously as the stem-base becomes girdled. If the canker in an infected tree is less than 3m from the ground, the trunk of any nearby plane tree within 15m will likely be infected and it should therefore also be felled.

There are *Platanus* clones available on the Continent branded as 'resistant' or 'tolerant'. While such trees may be variably CSP tolerant, with the only apparent symptom being a slower growth rate than uninfected trees, a demonstration section of the stem from one such tree showed a dark central column of tissue that had developed since infection (Photo B). CSP-colonised tissue remains infectious for a long time. Thus while CSP-tolerant species may be appropriate in already infected zones, such cultivars in uninfected zones represent a potential biosecurity risk.

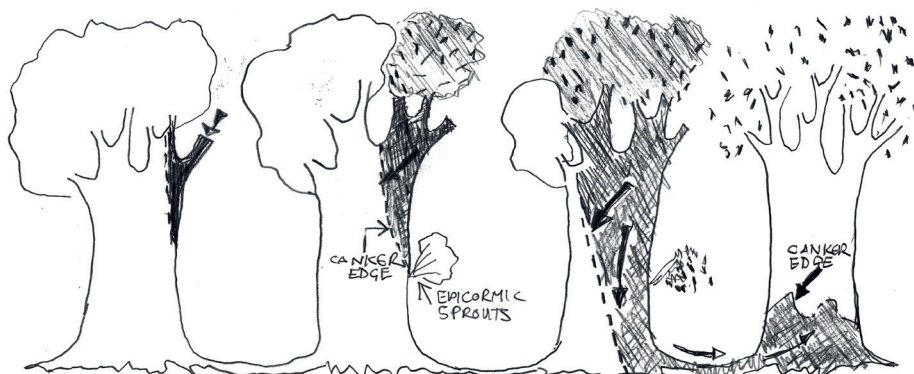
There is no known chemical or biological control for *C. platani*. While *Bacillus subtilis* and *Trichoderma harzianum* have proved effective *in vitro*, this proved not to be the case in the field. Additionally 30 years of research into fungicidal injections have so far proven fruitless.



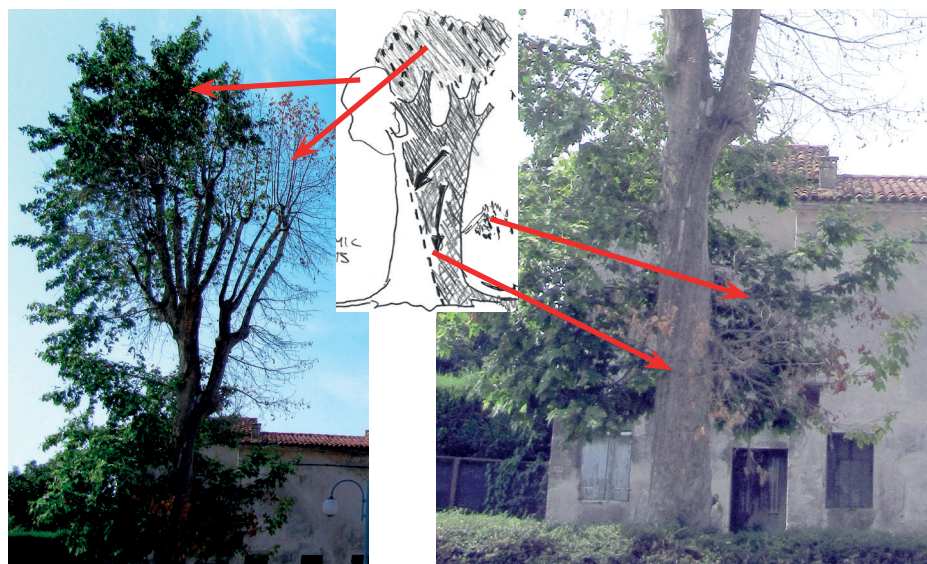
Photo B: CSP-tolerant clone: B1 (left) longitudinal section, B2 (right) cross-section of same stem of infected tree.



Box 1: Crown symptoms. (Illustrations copied from Lucio Montecchio; photos: Neville Fay)



From an infected wound the canopy desiccates branch-to-branch.
From a root graft the canopy desiccates suddenly.



Photos C (above) and D: External bark stem canker indicators (white ellipses) and the junction between internal necrotic and healthy tissues exposed with a timber scribe. (Neville Fay)



Day 2: Learning about false and real disease indicators, and dealing with infected trees

Delegates attended various sites with decline symptoms, some directly due to *C. platani* infection and others due to non-CSP-related decline symptoms (from biological and chemical causes). Such observations are invaluable in learning familiarity with symptoms caused by common non-CSP agents inducing decline or low vitality in plane, for comparison with those caused by *C. platani*.

C. platani infection symptoms progress through the crown and present on the stem with clues in the outer bark associated with the junction between internal necrotic and healthy tissues (Photos C and D, white ellipses).

Using a timber scribe to scrape away the outer bark to expose the phloem reveals the characteristic, clearly defined margin

between dark brown (infected) and white to pink (healthy) tissue. (Note the stain is *not* blue as commonly reported in internet sources, see Photo D). This distinct margin beneath the bark helps to give confidence in the identification of *C. platani*. Final confirmation can then be made back at the lab by culturing core samples taken with an auger from the stem and inspecting them under a microscope.

The auger used to obtain core samples for culturing in the lab needs to be aimed tangentially, i.e. across the sapwood, to increase the likelihood of passing through more than one necrotic zone. The outer (external) section of the core sample is discarded before culturing, as it will contain other non-target fungi and bacteria. As on average only about 25% of core samples cultured will produce the diagnostic fruiting bodies called 'perithecia' (see Box 3), a minimum of four samples are taken.

In the UK suspect trees should be reported to the Forestry Commission (FC) via Tree Alert (www.forestry.gov.uk/treelalert) and, as the movement of infected tissue is a serious biosecurity risk, the FC will be responsible for the extraction of cores.

The final site visit of the second day was to observe the dismantling of an infected tree and the demonstration of practical sanitation precautions required by law in Italy. Tarpaulins and plastic sheeting were spread over an area of about 15m × 20m beneath the tree to catch the bulk of the arisings and sawdust. Dismantling was primarily by hand-saw to minimise the amount of infected wind-dispersed sawdust, particularly when climbing. Naturally, major aerial sections were chainsaw-dismantled, and the trunk was felled near ground level in the largest possible remaining section, all intended to minimise the production and spread of infected sawdust. Inevitably chainsaw use dispersed some infected sawdust in



Box 2: Misleading symptoms. (Photos: Lucio Montecchio)

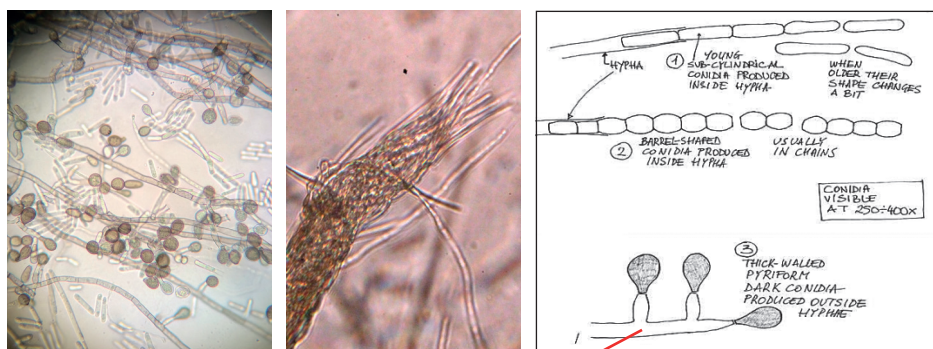


Above: twigs and leaves affected by *Apiognomia veneta*.

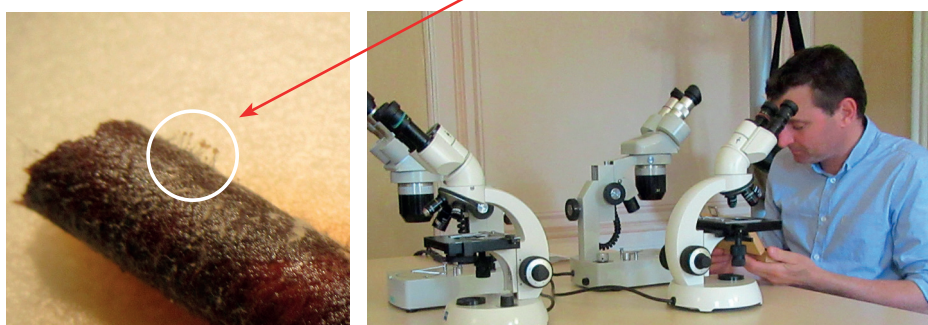


Left and centre: physiological cracks. Right: an abnormal bulge.

Box 3: Illustration of perithecium and conidia, and photos easily obtained by delegates using smartphones.



Conidia are released from hyphae as: sub-cylindrical, truncated; barrel-shaped; thick-walled, pyriform.



the breeze. Delegates wore biohazard suits (to minimise the risk of transporting infected particles to a non-infected zone). After use the suits were sealed in a plastic bag and sent with the arisings for incineration.

Once felled, the basal stump-section showed clear demarcation and canker stain pattern across the stem (Photo E). Also removed were two asymptomatic plane trees on either side of the subject tree. All trees, stumps and arisings were transported to an approved incineration site.

As the tree removal operations had taken place at 30°C, delegates needed to rehydrate and freshen up before attending the afternoon lab session. The methods involved in culturing the samples previously collected were demonstrated in the lab, along with how to look for and recognise *C. platani* fruit bodies and spores.

C. platani has both vegetative and sexual reproductive strategies. When two compatible mycelia join sexually, a fruit-body (perithecium) is formed, just visible to the naked eye (ca. 0.8–1mm long). Under a light microscope at 200× magnification, the long, thin and hollow 'flask'-like structure is clearly visible, containing a liquid infused with spores created from sexual recombination (ascospores) that emanate from the tip (Box 3). Under magnification the diagnostic fringe-like perithecium tip (in Latin *fimbriatum*) is the key microscopic diagnostic feature of *C. platani*, being the only example of the *C. fimbriata* species-complex to occur on plane. While perithecia will develop in around 25% of *C. platani* samples cultured, mycelium and vegetative conidial spores are observable in all successful cultures. There are three types of vegetative conidiophores produced by *C. platani*. The smallest two types are produced within the hypha and are either cylindrical or barrel shaped, occurring in chains (Box 3); these



Photo E: Stained cross-section of CSP-infected tree, showing circumferential progression of infection and radial incursion along carbohydrate-rich rays. (Neville Fay)



appear transparent under a light microscope due to their thin cell walls. The third type of conidia is more persistent than the other two, and being produced outside of the hypha is therefore larger, appearing pear-shaped (pyriform) and dark brown due to thicker cell walls (Box 3).

This truly enlightening laboratory session was followed by relocating to the spectacular main hall of the villa, where all were served a delicious array of seafood, while being serenaded from the minstrels' gallery by a Gondola singer performing to great appreciation and applause.

Day 3: Disease controls, legislation and practical prevention

Day 3 started with a lecture from a leading Italian arboricultural consultant, Dr Paolo Pietrobon, who explained the bureaucratic context and background to decision- and policy-making in Italy, and how these have shaped (relatively inefficient) legislative processes in the wake of *C. platani* outbreaks. In Italy, an effective nationwide biosecurity system was eventually adopted by ministerial decree in 1987, though this proved too late to secure disease eradication, let alone containment. The system, summarised in Box 4, divides infected areas into different zones with different control measures based on the risk of spread of *C. platani*.

In the event of a *C. platani* outbreak in the UK, Paolo suggested that our cooler climate may slow the spread of the disease – a position supported in some of the literature, which suggests that the risk of infection is reduced at colder temperatures. Optimum mycelium growth occurs at 25°C, with a functional range between 10°C and 35°C. According to available literature, temperatures of 40°C are lethal. This picture is confused in that, while *C. platani* has yet to spread into the coastal regions of south-eastern Italy that are buffeted by the cold Bora trade winds blowing in off the Adriatic, the European Food Safety Authority (EFSA) considers there to be no eco-climatic factors limiting the potential establishment or spread of *C. platani* to regions wherever the *Platanus* host is present. Furthermore, in the UK, increased levels of humidity associated with climate change may favour *C. platani*.

A lunchtime group discussion in the garden provided the opportunity to gain deeper insights on *C. platani* in Italy. An interesting discussion focused on potential therapeutic treatments for *C. platani*. Thirty years of experimentation with fungicidal injection have proved stubbornly

Box 4: Italy divides infected areas into different zones with different control measures based on the risk of spread of *C. platani*.

1. **'Free Zone'** – an area where CSP has not been found or where it has been officially declared eradicated.
 - In this zone it is still necessary to give notice to the Regional Plant Protection Service (RPPS) to do any work on plane and follow any specific orders relating to the work.
2. **'Outbreak Zone'** – a circle of radius no less than 300m around any infected tree.
 - Within this zone all work on plane trees is banned until the infected trees have been eliminated.
 - This sanitation felling must be notified to the RPPS, specifying the sanitation methodology, and site and means of disposal of all arisings. The work can be carried out 30 days after notice has been given; the authority may specify additional specific measures that must be complied with.
 - Where infected trees are identified on private land, the owner is served notice that they must fell the tree (at their own cost), with the contractors working to the above protocol. The penalty for non-compliance is a short prison sentence!
 - All symptomatic trees and immediately adjacent trees must be felled.
 - Replacement planting with plane trees is prohibited.
 - An 'Outbreak Zone' can become either a 'Free Zone' again where the outbreak has been confirmed as eradicated, or a 'Containment Zone' once the criteria are met (see below).
3. **'Containment Zone'** – an area where canker stain has persisted in time and its spread is such that eradication is not possible in the short term.
 - All works on plane trees are subject to a 30-day notice period to the RPPS, again detailing the site and means of disposal of arisings and having to comply with any additional measures specified.
 - Arisings must be disposed of within the same area unless notified otherwise by the RPPS.
4. **'Buffer Zone'** – an area of at least 1km distance separating a 'Free Zone' from an 'Outbreak Zone', or a 'Free Zone' from a 'Containment Zone'.
 - These areas are subject to systematic monitoring and to the notification process and restrictions of adjacent Outbreak Zones.

unsuccessful due to the fungus colonising not only the vascular system but also the starch-rich, non-vascularized rays. Lucio has, however, encountered promising *in vitro* results from stem injections using plant extracts.

This summary session enabled delegates to put questions to the whole group, and to reflect on what had been gained from the extraordinary study weekend. There was unanimous agreement that all had gained a deep and thorough understanding of the biology, symptomology and epidemiology of *C. platani*, and particularly of control and sanitation requirements.

Reflections on the study experience

The study experience with fellow professionals was one of convivial learning, and the warm and generous hospitality of our hosts gave an extra dimension to our sense of enjoyment and privilege. Sunday afternoon before the

return flight involved exploring the beautiful medieval town, accompanied by the mayor, with someone on hand as a guide to explain the significance and history of the sites. All too soon we departed to the airport for the return journey and to whatever was unravelling in our new post-Brexit home.

We arrived home motivated to initiate work on updating and expanding current CSP contingency plans and to continue the vital job of raising awareness of *C. platani*. Despite the prospect of the disease arriving in the UK, we feel empowered with the knowledge required for containment and, critically, aware of the need to raise awareness of *C. platani* to ensure that the risk of its transportation is minimised. Given that humans (and arguably particularly arborists) are primary vectors of the disease, with effective planning and dissemination of information on *C. platani* within our sector and beyond, we stand a good chance of preventing its spread to the UK.

UK context, CSP control and new published guidance

Since 2014 the London Tree Officers Association (LTOA) has been in the front line leading on this work for the Forestry Commission.

EU Directive 2000/29/EC, Annex II/ A2 prohibits the movement of trees for planting and non-squared wood from countries where the disease occurs. Since October 2014 the UK has held EU Protected Zone Status (PZS) for *C. platani*, requiring robust controls relating to importations of plane trees and ensuring that planes can only be imported from other areas which have

been designated free of the disease. In order to maintain PZS it is necessary for the UK to demonstrate that the pathogen is being actively looked for and has not been confirmed as being present.

In November the LTOA, in association with Treework Environmental Practice, jointly published a second edition of Lucio Montecchio's booklet *Detecting and Identifying Canker Stain of Plane*, which has been revised and updated by John Parker and Neville Fay. It is available from the LTOA (cost £5, www.ltoa.org.uk).

Treework Environmental Practice and De Rebus Plantarum thank local organisations in Italy who generously contributed to our



not-for-profit hot-house study event – they are Comune di Castelfranco Veneto TV, Comune di Bressanvido VI, Demethra Biotech, BM Medical, Silvatica and Nautilus Fish and Wine.



Top 10 points to take home on CSP

1. CSP is a highly infectious fungal wound pathogen with 100% mortality rate for existing clones/cultivars of London plane (*Platanus acerifolia*) and oriental plane (*Platanus orientalis*).
2. Infection has been shown to be initiated by as few as 20 spores introduced to a wound.
3. With no natural insect vector, it is spread almost exclusively on the infected chainsaws and tools of arboricultural contractors who have been working on infected trees. Therefore contractors who have been working on plane trees on the Continent represent a critical biosecurity risk if they do not thoroughly clean and disinfect their chainsaws and other equipment or clothing that could have come into contact with infected sawdust BEFORE returning to the UK. All local authorities with contracts that include management of plane trees should require contractors to notify them of any employees who have been working outside the UK and to ensure that equipment including PPE is non-contaminated.
4. CSP can survive and continue to be infectious on contaminated sawdust for a long and indeterminate period of time.
5. CSP causes a recognisable, wound-mediated pattern of crown death, starting in one side of the crown then moving across the crown horizontally, with the dead leaves being retained on the tree.
6. CSP can also spread from an infected tree to an uninfected, adjacent tree via root grafts. This mode of infection results in the whole crown dying at once, with the leaves retained.
7. Anyone observing these symptoms in a plane in the UK should report it immediately to the Forestry Commission via Tree Alert (www.forestry.gov.uk/treealert)

- and under no circumstances (apart from absolutely essential safety works) should arboricultural works be carried out without first notifying them.
8. The cost of felling infected trees works out to be around three times more expensive than normal felling costs, due to strictly necessary sanitation procedures including the containment of all infected arisings and their removal for incineration. In addition any non-symptomatic adjacent trees within 15m also have to be felled, using the same sanitation measures, due to the risk of root graft transmission. Therefore the potential costs of responding to an outbreak in the UK could be extremely high, particularly in London.
9. London plane clones may be available on the Continent now or in the future, marketed as 'resistant' while yet presenting asymptotically with CSP infection. UK purchasers therefore need to be aware that in such cases, disease-affected wood within the tree retains the capacity for infection. Any such plane clones branded as *C. platani* 'resistant' or 'tolerant' represent a biosecurity hazard to the UK.
10. It is not known whether the UK climate will help or hinder the establishment and spread of the pathogen should it arrive here. Colder temperatures may limit its spread while humid conditions may favour its growth. The potential for *C. platani* to establish and spread throughout the UK will increase with the effects of global warming.

6 key necessary actions

1. Legally enforceable clearly defined contingency protocols to be followed in the event of an outbreak: these are being formulated and must be communicated throughout the industry.
2. Development of biosecurity protocols: key to reducing the risk of CSP introduction and spread in the UK, protocols must

- become part of common working practice, and disseminated across the entire arboricultural sector, including tree nurseries.
3. Raising awareness of the danger of clonal imports: the nursery trade must be made aware of the danger of clonal imports branded as *C. platani* 'resistant' or 'tolerant'. As such trees pose an unacceptable biosecurity risk, importation banning is recommended.
4. Quarantine of imported nursery stock: imported plane trees should be grown on by the importing nursery ideally for 12 months before being sold on. Any stock which becomes symptomatic should be reported immediately to the FC. Ideally this would be made a statutory requirement.
5. Maintaining and extending current monitoring programmes: monitoring programmes already underway for *C. platani* in key areas of London and the south-east to be maintained, and further rolled out nationally, accompanied by CSP disease awareness initiatives alerting to key symptoms. This should be a core sector objective to ensure that any outbreaks that do occur are immediately identified and eliminated.
6. Sector training and certification: while the FC 'Keep It Clean' campaign is a good starting point, there is need for a drive within the tree sector to commit to the adoption of these and other biosecurity protocols for controlling pathogens as standard practice for all arboricultural contractors and ensuring that these are not just subject to voluntary uptake. Certification to show 'competence in biosecurity', to cover all significant threats to the UK's trees, as a priority, is recommended. Holding and regular updating such certification should be a pre-requisite to obtain work within the industry and ideally should be made a condition of insurance.