Photographs: Béatrice Henricot

Box blight rampages onwards

A new fungal disease causing severe dieback of box was first recorded 12 years ago in the UK. As it continues to spread, BÉATRICE HENRICOT reviews the current situation and outlines future research

IN LATE 1994, a new disease on *Buxus* was discovered in a nursery in Hampshire. The fungus was initially identified as *Cylindrocladium scoparium*, a pathogen that causes disease on a wide range of hosts distributed worldwide. This species is also the most misidentified species in the genus, as its morphological characteristics resemble those of other species closely.

In 1998 symptoms of leaf and twig blight caused by a species of *Cylindrocladium* were reported in New Zealand. On this occasion the fungus was identified as *C. spathulatum* or possibly *C. ilicicola*. The same year the RHS received the first samples of *Buxus* showing similar symptoms and it was clear that the species causing the disease was undescribed (Henricot *et al* 2002). The new species status was confirmed using traditional cultural techniques and DNA sequencing and it was named *Cylindrocladium buxicola* after its host (Henricot and Culham 2002).

Box blight causes leaf loss and severe dieback on box plants. In formal plantings the disease has a devastating effect (above)

Symptoms

Infection by the fungus produces leaf spots on the leaves that can be either dark brown or lighter brown surrounded by a dark border. The fungus can also infect the stems ➤

producing characteristic black streaks. Eventually severe defoliation and dieback occur. The fungus has never been recovered from the root system. Death of plants, especially young seedlings, can occur.

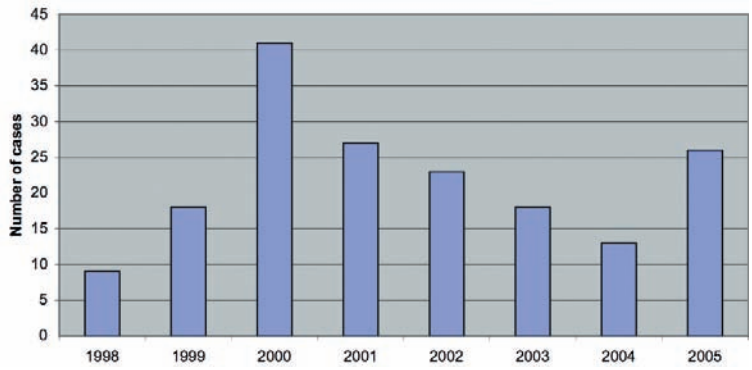
Geographical distribution

The RHS records reveal that the disease is now widespread in the UK. The number of cases recorded has been on the increase since 1998 with a peak of records in 2000. Since then the number of cases has decreased but rose again in 2005. In 2000, the disease was also recorded on Box Hill in Surrey, the only extensive locality in the UK where *Buxus sempervirens* is native. Outside the UK the disease has been recorded in New Zealand in 1998, Belgium in 1998, Ireland in 2001 (RHS records), Germany in 2005 (Brand 2005) and Holland in 2005 (Marcel van Raak, pers. comm.). There have also been unconfirmed reports of the disease in Italy and France.

Species identification

The novel species status of *C. buxicola* was confirmed using a series of morphological characters and DNA sequence data. Species of the genus *Cylindrocladium* are identified mainly on the basis of the shape of their vesicles (inflated cells arising from a spore-bearing structure), and the size and (septation) partitioning of the conidia (asexual spores). However, these characters are not always well differentiated among species and can be influenced by cultural conditions. Therefore DNA sequence data are often used to support species identification.

Morphologically, *C. buxicola* is characterized as having one-septate conidia and ellipsoidal vesicles with pointed or papillate apices. It is also a low temperature fungus as it can grow below 10°C but its growth is inhibited at 30°C and it is killed at 33°C. These characters alone would differentiate *C. buxicola* from morphologically similar species such as *C. ilicicola*, *C. mexicanum*, *C. pauciramosum*, *C. scoparium*, and *C. spathulatum*. In addition, though, the DNA sequences of three different genomic regions, including the ITS, ß-tubulin and *mat*2 genes, were unique compared to other *Cylindrocladium* species.

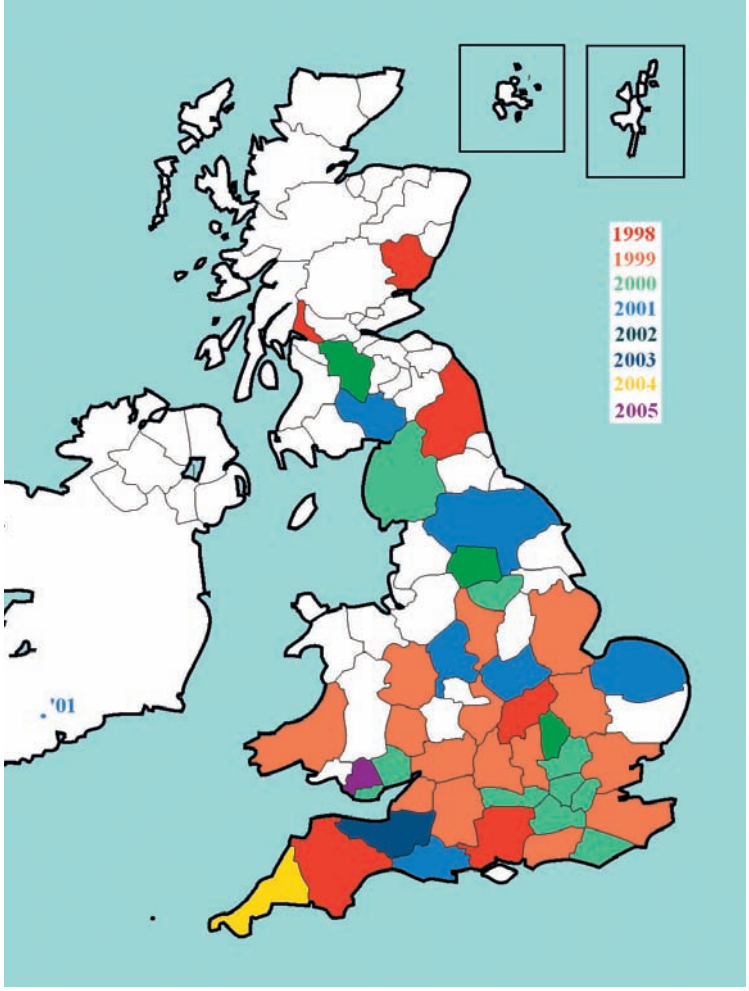


Cases of box blight reported to the RHS declined after 2000 but rose last year. This may reflect publicity

Origin of the pathogen

The geographic origin of *C. buxicola* is unknown. Its aggressiveness on *Buxus* and its relatively limited geographic range in relation to the distribution of its hosts suggests it is an exotic species introduced recently. Indeed, the genus *Buxus* includes 91 species distributed over 4 continents with 10 species occurring in Africa, 45 in America,

Box blight infection produces leaf spots that can be pale brown with a darker magin (left) or dark brown (right). Also, black streaks may appear on the stems



County records of box blight in the UK compiled by the RHS show a steady expansion of range since 1998

34 in Asia and 2 in Europe (Batford 2004). So far the disease is present only in Europe and in Australasia, which is the only continent where *Buxus* is not native. In addition, DNA evidence from amplified fragment length polymorphisms (AFLPs) indicates that *C. buxicola* has a clonal population structure. All the isolates seem to belong to one mating type as suggested by the mating experiments which failed to produce any fertile perithecia (sexual fruiting structures).

These data support the hypothesis of an exotic origin. Another hypothesis is that this new species has arisen through interspecific hybridisation. Cases of new pathogens emerging through hybridization are rare, but might have been overlooked. It can occur when closely related but previously geographically isolated taxa come into contact (Brasier *et al* 1999), for instance, in nurseries or gardens. The threat has largely increased as a result of present trade practices, which have led to more physical contact between exotic species (Brasier 2005). However, the phylogenetic analysis combining the sequences of the ITS and ß-tubulin showed that *C. buxicola* forms a distinct clade (a group consisting of a common ancestor and all its descendants) in the genus. This seems to rule out the possibility of a hybrid, as a more intermediate grouping would have been expected if it was a hybrid (Henricot & Culham 2002). Therefore the most likely explanation is that *C. buxicola* has been introduced to Europe from a geographically isolated region on apparently innocuous plant 'carriers' – a situation referred to as the Typhoid Mary syndrome (Brasier 2005).

Life cycle of the fungus

The infection by the fungus is rapid in warm (18–25°C) and humid conditions. The spores of the fungus are sticky so primary infection is unlikely to occur from wind-borne spores. Water-splashed spores or spores carried by a vector such as insects, birds or plants are the most likely source of primary inoculum.

Spore germination starts three hours post-inoculation. Penetration can be observed as quickly as five hours after inoculation. It occurs through the stomata on the lower surface of the leaves or directly through the cuticle on the upper surface. No specific penetration or feeding structures are formed. The fungus can grow intercellularly in the mesophyll layers. Re-emergence of the fungus through the stomata occurs two or three days after infection. After seven days the lower leaf surface is covered with a layer of conidiophores producing conidia (asexual spores). The leaves are eventually killed, probably by general stress resulting from colonisation of the intercellular spaces and deregulation of the stomata.

In comparison with other species of *Cylindrocladium*, the fungus can form resting structures called microsclerotia, which can survive in the soil in the absence of a susceptible host. Our experiments so far have shown the fungus can survive on decomposing leaf material for at least five years but no microsclerotia development has been observed.

Host range

To date the disease has been detected on three different *Buxus* ➤

species and many of their cultivars. These are *B. sempervirens*, *B. microphylla* and *B. sinica* var. *insularis*. These three species are predominantly grown in the UK and other European countries. The full list of species and cultivars infected naturally since 1998 is:

B. sempervirens
B. sempervirens 'Angustifolia'
B. sempervirens 'Blauer Heinz'
B. sempervirens 'Compacta'
B. sempervirens 'Elegantissima'
B. sempervirens 'Latifolia Maculata'
B. sempervirens 'Memorial'
B. sempervirens 'Suffruticosa'
B. sempervirens 'Suffruticosa Variegata'
B. sempervirens 'Variegata'
B. microphylla 'Faulkner'
B. microphylla var. *japonica* 'Morris Midget'
B. microphylla var. *japonica* 'National'
B. sinica var. *insularis* 'Justin Brouwers'

It is not yet clear whether these species and cultivars are particularly susceptible to the fungus or whether they are more likely to be affected because of their widespread use as ornamentals.

Pathogenicity assays carried out at RHS Garden Wisley on detached stems showed that the host range in the genus was in fact quite extensive, including *B. balearica*, *B. bodinieri*, *B. glomerata*, *B. harlandii*, *B. macowanii* and *B. riparia*. All these species occur naturally over four continents. In addition, *Sarcococca* (in the same family, *Buxaceae*) was found to be a host for the disease but it has never been found naturally infected. This means that potentially other members of the *Buxaceae* such as *Pachysandra* could be host for the disease, although this was not tested.

Although no *Buxus* species was immune to the disease, there was some variation in the susceptibility to the pathogen. Based on leaf symptoms and the number of conidia produced by the fungus once it re-emerged from the stomata, it was found that *B. balearica* was the most resistant and *B. sempervirens* 'Suffruticosa' was the most susceptible. The apparent resistance of *B. balearica* could be due to the texture of its leaves which are more leathery than some of the other species; this could disadvantage a fungus that penetrates directly through the cuticle.

Control measures

Control measures include cutting back infected twigs to healthy tissue and removing fallen material and topsoil. Badly affected plants should be disposed of. Making sure the foliage is kept dry to create a microclimate unfavourable to the growth of the fungus limits the spread of the disease.

There are no fungicides specifically labelled to control this disease available to amateur gardeners. However, products labelled to control any disease on ornamentals may be used to control box blight at the owner's risk. These products include myclobutanil and penconazole. Similarly, there are approved commercial products that can be used on ornamental crops in nurseries but no data is available on their efficacy, or the dosage and rate to control the disease on box plants. Preliminary *in vitro* experiments carried out at the RHS showed that penconazole was a more effective product than myclobutanil in inhibiting mycelium growth and spore germination. They were, however, much less effective than some of the commercial products available. Of 10 fungicides tested, carbendazim, prochloraz and kresoxim-methyl completely inhibited the mycelium growth while kresoxim-methyl was the most effective at inhibiting spore germination. However none of the fungicides killed the fungus. This finding has important consequences in understanding how the disease has and continues to spread in gardens.

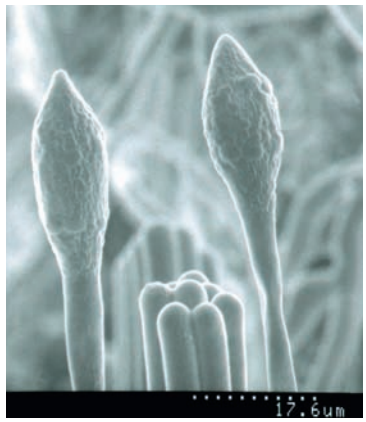
BOX BLIGHT ON BOX HILL

In 2001, *Cylindrocladium buxicola* was confirmed on native *Buxus sempervirens* growing wild (right) on Box Hill, a site of special scientific interest in Surrey. At the time the disease was mainly confined to the north side of the hill, affecting young seedlings and mature plants. Since then the disease has been left unmanaged and no scientific survey on its impact has been carried out.

During a visit in 2006, it did not appear that the fungus had spread further. The damage seen in 2001 was still obvious as dead branches on the lower parts of the older trees. However, regeneration seemed to have been reduced as not many seedlings were seen on the affected site and a high proportion of those present had fresh lesions on the stems. If these infections spread throughout the plants then the seedlings will die, posing a threat to this unique population and habitat.

Future work

After a primary screen of fungicides *in vitro*, those that showed significant levels of inhibition of spore germination and mycelium growth need to be tested on whole plants. The RHS will carry out a two-year trial evaluating fungicides for the control of *Cylindrocladium* blight at the ADAS Arthur Rickwood site (Cambridge) that is partially funded by English Heritage. The work will assess the protective and eradicative action of seven different fungicides on *Buxus sempervirens* 'Suffruticosa'. These include the three professional fungicides which were the most effective *in vitro*: carbendazim, prochloraz and kresoxim-methyl. Also included is penconazole which was the most effective fungicide available to amateur gardeners. The three remaining fungicides that will be tested were reported by box growers as being effective at controlling this disease (Moran 2005, Karel Goossens, pers. comm.).



The vesicles of box blight fungus are ellipsoidal with a mucilaginous surface and acute apices

These include azoxystrobin (Amistar), chlorothalonil (Repulse) and epoxiconazole, kresoxim-methyl, pyraclostrobin (Opponent). The fungistatic action (ability to inhibit growth and reproduction without killing the fungus) of the fungicides will also be assessed.

Conclusions

The use of fungistatic chemicals in nurseries has allowed the introduction of the disease through apparently healthy material to many gardens in the country. This way of transmitting disease is well documented by Brasier (2005), where he calls it Trojan horse syndrome, and is not limited to the spread of *Cylindrocladium*. Still, the nursery trade (suffering from what I would refer to as ostrich syndrome) has made the economic choice of spraying to suppress symptoms. The failure to eradicate the disease has perpetuated the problem for future generations of box lovers. The disease is now too extensively distributed to be eliminated by cultural measures alone and will continue to wreak devastation to new and established box plantings wherever it is introduced. ■

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ALTERNATIVES TO BOX

List 1 includes a traditional selection of plants suitable for hedge planting and trimming. **List 2** includes less commonly used plants for hedges, but planted in the right conditions they should be suitable for that purpose.

Plants indicated as * can withstand temperatures down to -5°C but may still be damaged by less severe frost, while plants indicated as † require an acid soil.

List 1

Berberis buxifolia 'Pygmaea'
B. x *stenophylla* 'Corallina Compacta'
B. x *stenophylla* 'Irwinii'
B. x *stenophylla* 'Nana'
B. thunbergii 'Atropurpurea Nana' (deciduous)
Euonymus fortunei cultivars
E. japonicus 'Microphyllus' *
Hebe cupressoides 'Boughton Dome'
Ilex crenata
Lavandula angustifolia cultivars
Lonicera nitida
Osmanthus delavayi
Rosmarinus officinalis
Santolina chamaecyparissus *
Taxus baccata 'Semperaurea'

List 2

Azara microphylla *
Camellia x *vernalis* 'Yuletide' †
Corokia x *virgata* *
Erica erigena 'W.T. Rackliff'
Escallonia 'Compacta Coccinea' *
Hebe rakaiensis, *H. topiaria*, *H.* 'Emerald Gem'
Hedera helix 'Glymii'
H. helix 'Walthamensis'
Ligustrum delavayanum *
Olearia x *haastii*
Pieris japonica 'Little Heath Green' †
Pittosporum tenuifolium *
Rhododendron dwarf cultivars †
Teucrium x *lucidrys*
Vaccinium delavayi * †

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