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Boxwood Blight: Threat to Ornamentals

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Abstract

Boxwood blight, caused by *Calonectria pseudonaviculata* and *Calonectria henricotiae*, has had devastating effects in gardens since its first appearance in the United Kingdom in 1994. The disease affects two other plants in the Buxaceae: sweet box (*Sarcococca* spp.) and pachysandra (*Pachysandra* spp.). *C. pseudonaviculata* was likely introduced to Europe by nursery trade from East Asia on an ornamental species and then to western Asia and North America. Thus far, *C. henricotiae* has been seen only in Europe. Boxwood, valued at $126 million wholesale per year in the United States alone, is now besieged by an aggressive foliar blight active over a broad temperature range when there are long periods of leaf wetness. Research on inoculum, means of dissemination, cultivar susceptibility, environmental influences, fungicides, sanitizers, and detection methods has vastly improved knowledge of this new invasive disease in a short time. Boxwood with genetic resistance to the disease is critically needed.

BOXWOOD BLIGHT: THREAT TO ORNAMENTALS

Boxwood blight appeared suddenly on the ornamentals scene and was a complete surprise. The disease had never been reported anywhere on the globe until the mid-1990s, when it was first discovered in the United Kingdom. Despite the horticultural havoc it wrought in parts of Europe, it took the United States by surprise in 2011. The Atlantic Ocean is a sizeable barrier, but ornamentals traffic is global, so movement via trade was virtually inevitable. For some countries, boxwood blight has caused immense financial loss from the effects of the disease on horticultural businesses. These are the same countries in which stately historic landscapes of great aesthetic value have been threatened and sometimes destroyed. In some countries in Europe and western Asia, the disease is also a threat to populations of native *Buxus* spp. (61), a threat already compounded in Europe by the recent appearance of the boxwood tree moth, *Cydalima perspectalis*, an aggressive defoliator (48). Boxwood blight's 25-year history in Europe and its 8-year history in North America are short, but it is clear that this disease will change gardening permanently. Management of boxwood blight will require concerted efforts and continued collaboration (79) on the part of plant breeders and plant pathologists: The disease presents an urgent scientific challenge to those who would like to help boxwood retain its exalted position among ornamental garden plants.

BOXWOOD (*BUXUS* SPP.)

Boxwood are in the genus *Buxus*, a name that reflects the long social history of the plant as a source of wood for ornamental boxes (*buxus* meaning box in Latin) (3). Mankind has utilized *Buxus* for many practical, aesthetic, and religious purposes over the centuries (3, 75); its hard, dense wood has been used for highly durable printing blocks, high-quality musical instruments, buttons, boxes, tool handles, rulers, combs, and religious art, including bas-relief carvings and sculptures. *Buxus* is the largest genus in the small family Buxaceae, which also contains *Pachysandra*, *Sarcococca*, *Styloceras*, and *Notobuxus* (95, 96), although it has been suggested that *Notobuxus* should be combined with *Buxus* (96). There are roughly 100 species of *Buxus*, all shrubs or small trees, found on every continent except Australia and Antarctica. Many of the species are tropical. A minority are native in temperate climates; those in the northern part of temperate Europe have fragmented populations (18). There are no native *Buxus* species in temperate North America, but there is a single native *Pachysandra* species, *Pachysandra procumbens*.

The most familiar boxwood to horticulturists is the European native *Buxus sempervirens*, along with its prized dwarf cultivar, *B. sempervirens* 'Suffruticosa,' known in North America as English boxwood. There are approximately 400 named cultivars of *B. sempervirens* (78). Most of the hybrid boxwoods in cultivation are hybrids between *B. sempervirens* and *Buxus microphylla*, a species with obscure Asian origin. *Buxus microphylla* var. *japonica*, the Japanese boxwood, has a number of desirable cultivars. *Buxus sinica* var. *insularis* (syn. *B. microphylla* var. *koreana*) is the cold-tolerant Korean boxwood. There are also some relatively recent *B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis* hybrids.

Boxwood species, cultivars, and hybrids have high value and popularity in the nursery industry (3). As the number one woody plant sold in the United States, boxwood's annual wholesale value was greater than $126 million in the USDA National Agricultural Statistics Service report in 2014 (higher than arborvitae, azalea, holly, or hydrangea) (7). Boxwood are used for hedges and parterres as well as for grouped or individual specimen plantings, including topiaries (3). Nurseries sell boxwood plants as balled-and-burlapped (field-grown) or container-grown material, either directly to landscaping firms or to garden centers. Large specimens are especially prized: size connotes age and value. The plants have a high tolerance for shearing, which makes them ideal for formal gardens. Clippings from boxwood are also used for winter holiday decorations.

A wealth of genetic resources are available for *Buxus*, including a collection of more than 700 accessions at the US National Arboretum in Washington, DC. Botanical collections such as these are places where it is imperative that boxwood blight is managed by impeccable exclusion policies. Van Laere et al. (95) used amplified fragment length polymorphism (AFLP) markers to characterize European and Asian *Buxus* species. Thammina et al. (93) developed and characterized 23 genic simple sequence repeat (genic-SSR) markers to allow fingerprinting of boxwood in germplasm collections. Such genetic clarification of *Buxus* cultivar relationships has already exposed some long-accepted misidentifications in the horticultural trade (92). These and other genetic studies on boxwood are a valuable resource for those working to identify blight-tolerant cultivars in *Buxus*.

RECOGNITION OF A NEW DISEASE

At a nursery in Hampshire, UK, in late 1994, unusual symptoms were noticed on diseased boxwood (40). At first, the pathogen was thought to be the broad host range pathogen *Cylindrocladium scoparium*. Further samples sent to the Royal Horticultural Society in 1998 were examined closely: The fungus had some morphological similarity to *Cylindrocladium mexicana*, but the internal transcribed spacer (ITS) DNA sequence indicated that a species new to science was responsible for blighting boxwood. Leaf and twig blight on boxwood similar to that seen in the United Kingdom was reported in Auckland, New Zealand in 1998 (82). There the pathogen was at first thought to be *Cylindrocladium spathulatum* or *Cylindrocladium ilicicola* (40).

After it appeared on *Buxus* in New Zealand, the pathogen was formally described as *Cylindrocladium pseudonaviculatum* by Crous et al. (13) but also formally described as *Cylindrocladium buxicola* a few months later by Henricot & Culham (40). The analysis in the United Kingdom included isolates obtained from *B. sempervirens* with blight in New Zealand and established they were identical with those isolated from the United Kingdom by examining the ITS, ribosomal 5.8S RNA, β-tubulin, and MAT2 mating-type gene sequences. For several years, publications on the boxwood blight pathogen often referred to the pathogen as *Cylindrocladium buxicola*. However, nomenclatorial rules caused the name to be currently accepted as *Calonectria pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous, after an unsuccessful attempt was made to conserve the name *Cylindrocladium buxicola* (41). The multiple names for the pathogen and the use of box (in the United Kingdom) versus boxwood (in the United States) as the host name may cause some confusion when researchers are looking for information on this disease.

The initial population studies of the pathogen in the United Kingdom, using AFLP and sequencing three DNA markers, were made on 18 isolates: 17 from the United Kingdom and one from New Zealand (40). These isolates were genetically homogeneous, and it was thought that they likely represented a single introduction of the fungus from its (still unknown) point of origin. Over time, *Calonectria* on *Buxus* was reported from a broader geographic area: The disease was detected in many European countries as well as in western Asia and North America by 2011 (19, 31, 47). However, a new layer of complexity lay ahead.

In 2005, a second genetic lineage (G2) became apparent in the previously homogeneous pathogen. This lineage was less common than the one originally observed, termed G1 (30). The G2 lineage made up only 12% of the isolates and was detected in only five European countries. Data suggested that G2 might have been brought into Germany in the mid-2000s in contrast to G1, which appears to have been introduced to the United Kingdom in the mid-1990s (31). In both cases, nursery trade has led to spread between countries. Detection of the disease in the United Kingdom is thought to have been fairly soon after the pathogen's initial introduction, probably as the now widely distributed G1MG1 genotype (30). The genetic variation seen in countries bordering on the Mediterranean Sea indicates more than one independent introduction of the pathogen via international trade (30).

Gehesquière et al. (31) considered 234 samples of *Calonectria* from 15 countries on four continents and produced evidence that the two genetic clades (G1 and G2) were different species. These were separated using multilocus phylogenetic analysis: Four independent nuclear loci were compared using genealogical concordance phylogenetic species recognition criteria. It was also noted that the G1 and G2 types were not able to mate. Thus, the researchers concluded that these were two separate organisms and advised that the original G1 continue as *C. pseudonaviculata* sensu stricto, whereas the G2 clade was proposed to be a new species, *C. henricotiae*, in honor of Beatrice Henricot, who was the first to document and describe boxwood blight. The G2 clade, now known as *Calonectria henricotiae* Gehesquière, Heungens, and J.A. Crouch, has been detected in only five countries in Europe to date (**Table 1**).

No perfect stage (*Calonectria* perithecia) has been detected in nature or induced in the laboratory despite pairings among all combinations of geographically diverse isolates of

Table 1 First records of boxwood blight around the world

Country	Pathogen species detected (year)	Reference
Abkhazia	*Calonectria pseudonaviculata* (2011)	29
Austria	*C. pseudonaviculata* (2010)	25
Belgium	*C. pseudonaviculata* (1998)	11
	Calonectria henricotiae (2010)	31
Canada	*C. pseudonaviculata* (2011)	23
Croatia	*C. pseudonaviculata* (2009)	8
Czech Republic	*C. pseudonaviculata* (2010)	86
Denmark	*C. pseudonaviculata* (2010)	25
France	*C. pseudonaviculata* (2006)	88
Georgia	*C. pseudonaviculata* (2010)	33
Germany	*C. pseudonaviculata* (2005)	4
	C. henricotiae (2005)	31
Iran	*C. pseudonaviculata* (2012)	74
Ireland	*C. pseudonaviculata* (2001)	39
Italy	*C. pseudonaviculata* (2008)	87
Netherlands	*C. pseudonaviculata* (2005)	39
	C. henricotiae (2005)	31
New Zealand	*C. pseudonaviculata* (1998)	82
Norway	*C. pseudonaviculata* (2010)	25
Russia	*C. pseudonaviculata* (2012)	25
Slovenia	*C. pseudonaviculata* (2008)	31
	C. henricotiae (2011)	31
Spain	*C. pseudonaviculata* (2008)	80
Sweden	*C. pseudonaviculata* (2010)	25
Switzerland	*C. pseudonaviculata* (2007)	25
Turkey	*C. pseudonaviculata* (2011)	1
United Kingdom	*C. pseudonaviculata* (1994)	40
	C. henricotiae (2011)	31
United States	*C. pseudonaviculata* (2011)	47

C. pseudonaviculata (31, 40) or *C. henricotiae* (31). Early suspicions that *C. pseudonaviculata* might be heterothallic (40) were confirmed by studies of the mating-type locus (67). Only barren perithecia have been observed in the two species, and these have appeared even when isolates have been paired with themselves (61).

Although *C. pseudonaviculata* was introduced to North America, probably from Europe, sometime during 2011 (or, more likely, earlier), *C. henricotiae* has not yet been found in North America. *C. pseudonaviculata*, however, has appeared in 28 states on boxwood (45, 59, 66, 97, 100; M. Daughtrey, unpublished results) and occasionally on *Sarcococca* (51, 68) and *Pachysandra* (21, 52; M. Daughtrey, unpublished results). The two species may be distinguished by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and real-time PCR–based analyses (31), but there is no obvious difference in the pathogenicity of the two species. *C. henricotiae* is more heat tolerant and is less sensitive in vitro to kresoxim-methyl and tetraconazole fungicides than is *C. pseudonaviculata* (31). Because of these differences that could affect geographic range and management with fungicides, monitoring for the arrival of *C. henricotiae* in North America should be a priority. The US nursery industry was not monitoring for boxwood blight prior to 2011, and this is one reason why *C. pseudonaviculata* was distributed in the US via horticultural trade with great rapidity. Initial identifications were made in October 2011 from North Carolina, Connecticut, and Virginia, followed within three months by detection in Rhode Island, Maryland, Massachusetts, Oregon, New York, Pennsylvania, and Ohio (20).

The likely source of *C. pseudonaviculata* is Asia. Imports of boxwood to Europe have recently increased. In 2010, for example, more than a million boxwood plant units were imported by the Netherlands, Denmark, and Italy from China, Taiwan, Indonesia, the United States, Ethiopia, and Turkey (24). As the quantity of imports and the number of sources increase, the chance of importation of new diseases also grows.

Although the pathogens might have been introduced to Europe on imported *Buxus* cuttings from Asia, the absence of any record of this disease in Asia makes this only a supposition. It might just as easily have been introduced on another plant in the Buxaceae, perhaps one of the less susceptible *Sarcococca* species. The movement to *Buxus* as a host might have taken place in a UK nursery where both genera were being grown.

HOST RANGE OF *CALONECTRIA PSEUDONAVICULATA* AND *CALONECTRIA HENRICOTIAE*

Boxwood blight was first found on *B. sempervirens*, and *B. sempervirens* 'Suffruticosa' was noted as very highly susceptible (42). Early investigations showed that multiple species of *Buxus* were susceptible to *C. pseudonaviculata* (42) and additional species and cultivars have been tested (28, 59, 91).

Inoculated *Sarcococca* sp. also developed symptoms in early tests in the United Kingdom, indicating the possibility of other hosts in the Buxaceae (42). In the United States, *Sarcococca hookeriana* naturally infected by *C. pseudonaviculata* was found in landscapes in Maryland (68) and Virginia (51) that also contained infected boxwood.

Susceptibility of Japanese spurge (*Pachysandra terminalis*) to *C. pseudonaviculata* was reported by LaMondia et al. (57) after successfully inoculating plants with an isolate from boxwood. LaMondia & Li (56) also showed the ability of *C. pseudonaviculata* to infect *Pachysandra procumbens*. *Pachysandra procumbens* is the only member of the Buxaceae native to North America and is sometimes grown in nurseries. Detection of naturally infected *Pachysandra terminalis* in landscapes where infected boxwood was growing (21, 52) followed these inoculation studies.

Tests of various cultivars of *Buxus* have not shown any host range differences between *C. pseudonaviculata* and *C. henricotiae*. Gehesquière et al. (31) inoculated 37 cultivars of boxwood with four *C. pseudonaviculata* and one *C. henricotiae* isolates at 16.2 ± 3.5°C and did not see any difference in the interaction between the two fungal species and the tested cultivars. LaMondia & Shishkoff (59) reported on the response of nine *Buxus* cultivars to inoculation with both pathogens in trials at Fort Detrick, MD. Results using 20°C for inoculation and 22°C incubation temperature were parallel for the two fungi. Because the two species are known to have different temperature tolerances, with growth curves different at 20°C and higher (31), trials run at warmer temperatures might have provided different results (59).

Although their known host range is currently limited to the Buxaceae, the *Calonectria* species that cause boxwood blight may be more versatile than we have supposed. Other *Calonectria* species have large numbers of hosts, particularly the familiar *Calonectria cylindrospora* (syn. *Cylindrocladium scoparium*).

SYMPTOMS AND SIGNS

The symptoms of boxwood blight are fairly distinctive but overlap somewhat with those of other boxwood diseases; thus, an experienced eye is needed for sampling, and laboratory testing is important for new outbreaks. The most characteristic features are extremely rapid leaf blighting and defoliation under optimal environmental conditions, which are catastrophic in the eyes of gardeners (**Figure 1*a***). Because of the extensive defoliation, plants often appear moribund after several cycles of infection at conducive temperatures in wet, rainy weather, even though buds, larger stems, and roots remain alive. Boxwood, especially smaller plants, are sometimes killed (39) but may also sometimes be rehabilitated with prompt and continued fungicide treatment if environmental conditions are not continuously optimal for disease development (79; M. Daughtrey, personal observation).

Under conditions highly conducive to boxwood blight, entire leaves are browned or blackened (43). When there are shorter periods of leaf wetness, leaves exhibit more discrete leaf spots. These have a rounded outline and may be light to dark brown at the center, with blackening at the outer edge, often in a zonate pattern (19, 28) (**Figure 1*b***). Lesions often begin at the leaf tip. Another key symptom is seen on the current season's shoots: black elongate streaks (19, 28, 43) (**Figure 1*c***), sometimes referred to as cankers. These run along the axis of the shoot and often do not girdle the stem. The rapid defoliation, leaf spotting, and black stem streaks, taken together, add up to fairly reliable field identification of boxwood blight.

A white bloom of conidia develops on the abaxial surface of the leaf or on the black streaks on shoots (**Figure 2*a***) under humid conditions; conidia are two-celled and covered with colorless slime (40). They are borne in penicillate fashion in cylindrical clusters that appear as white clumps beneath stipe extensions; the stipe extensions end in broadly ellipsoidal vesicles, which are either pointed or papillate at their tips (**Figure 2*b***). These sterile structures extend above the conidia. The white appearance helps to distinguish the boxwood blight pathogen from *Pseudonectria foliicola* and *Pseudonectria buxi* (syn. *Volutella buxi*), which are frequently seen on dead leaves and twigs of boxwood (83). *Pseudonectria* spp. initially have white sporulation, but sporodochia turn pink with maturity.

Microsclerotia develop in infected areas of leaf tissue. These melanized resting structures may be observed with epidermal peels and tape pulls (56) or by clearing and staining (98).

In trials starting with healthy plants of *B. sempervirens* 'Suffruticosa,' a progression of symptoms was noted in the United Kingdom (44). Symptoms began with leaf spotting and progressed to leaf drop, which was followed by black stem streaks and dieback. Dieback was apparent four weeks after inoculation and symptom severity peaked two months after inoculation. Timing of symptom progression might be expected to vary with environmental conditions and also according to the susceptibility of the *Buxus* cultivar.

Figure 1

Damage to boxwood caused by *Calonectria pseudonaviculata*. (*a*) Defoliation in a private garden in Athens, Georgia. (*b*) Leaf spots. (*c*) Black streaks on shoots. Panel *a* courtesy of Jean Williams-Woodward.

Leaves and young twigs are primarily affected by boxwood blight, unlike familiar ornamental diseases caused by *Calonectria cylindrospora* (syn. *Cylindrocladium scoparium*), which commonly involve root rot on many hosts, including azalea and rose. Root infections by *C. pseudonaviculata* and *C. henricotiae* have been observed under laboratory conditions (15, 26). No cases of root rot of boxwood by *C. pseudonaviculata* or *C. henricotiae* under natural conditions have been reported, but the potential exists.

The common groundcover Japanese spurge, *Pachysandra terminalis*, was found to be a host through inoculation (57). Plants showed circular lesions 1–4 mm in diameter within 10 days.

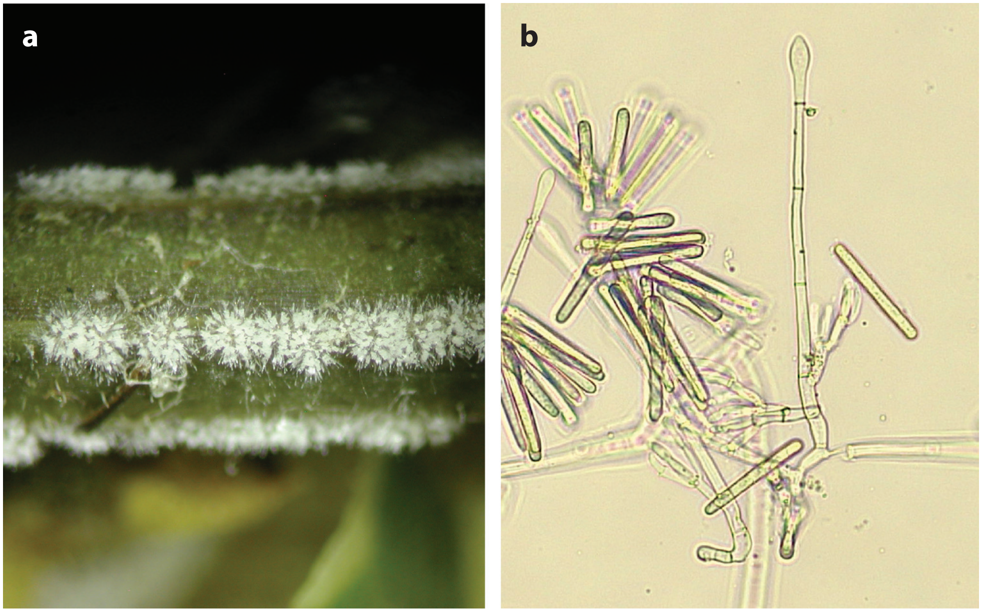

Figure 2

Sporulation of *Calonectria pseudonaviculata*. (*a*) Sporulation on boxwood stem. (*b*) Cylindrical conidia and conidiophore with stipe extensions terminating in a pointed vesicle.

Many of the leaves with lesions turned yellow and abscised three weeks after inoculation and fell to the soil, where conidia and microsclerotia developed on the fallen leaves. Kong et al. (52) observed symptoms in naturally infected Japanese spurge: Leaf lesions were watersoaked, brown to black, and round to irregular spots, 3–10 mm in diameter. Sporodochia developed on these after a 24-h incubation at high humidity. Brown, diffusely haloed spots, 1–10 mm in diameter, were also observed in Connecticut (21) (**Figure 3*a***).

Allegheny spurge (*P. procumbens*) has not shown the disease in the wild, but inoculations by LaMondia & Li (56) have shown it to be susceptible. Within seven days of inoculation, plants showed elongated necrotic stem lesions and 1–4-mm circular leaf lesions with dark margins and

Figure 3

Other Buxaceae with symptoms caused by *Calonectria pseudonaviculata*. (*a*) Japanese pachysandra (*Pachysandra terminalis*) showing necrotic leaf lesions. (*b*) Necrotic lesions on sweet box (*Sarcococca hookeriana*). Panel *a* courtesy of Yonghao Li. Panel *b* courtesy of Chuan Hong.

chlorotic halos. Girdling stem lesions and consequent shoot death were seen in *P. procumbens* in one trial in which *P. terminalis* inoculated under the same conditions did not develop girdling lesions. Microsclerotia were seen in epidermal peels from infected leaves.

S. hookeriana with a natural infection of *C. pseudonaviculata* acquired from boxwood growing nearby in a Virginia landscape showed lesions on leaves, petioles, and stems as well as defoliation (51) (**Figure 3*b***). Spotting ranged from 1-mm diameter speckling to >3-mm diameter, irregular, brown, watersoaked spots, which coalesced. Sporodochia developed on the abaxial side of leaves. In a Maryland landscape where boxwood blight was present, *S. hookeriana* showed round, dark brown lesions with lighter coloring at the center on leaves, dark spots on stems, and twig dieback (68). Ryan et al. (85) reported watersoaked leaf spots on inoculated *Sarcococca* of various species.

IMPACT

This pair of new diseases caused by *C. pseudonaviculata* and *C. henricotiae* are unprecedented in their impact on boxwood, more destructive than any previous disease (44). Although Volutella blight caused by *P. foliicola* and *P. buxi* is very common, stress or wounding is a prerequisite for disease development (83), whereas *Calonectria pseudonaviculata* infects healthy plants under conducive conditions. Boxwood blight has led to garden closures, removal of entire boxwood plantings, and huge dollar losses for nurseries, garden centers, and landscape gardening businesses. It has spurred the investment of the US government and US nursery industry in boxwood blight research and led to international conferences and several intensive plant breeding efforts in Europe and the United States. The NewGen^{TM} boxwood that are being previewed in 2019 are the first fruit of plant breeding efforts in the United States directed at solving the problem of boxwood blight (71).

Buxus has had centuries of use at different scales, from rimming tiny herb gardens or lining brick walkways to creating elegant parterres and mazes for castles and mansions. One of the impacts of the new blight has been the immeasurable loss caused by injury to historic gardens in Europe and North America (34, 76). Additionally, boxwood blight has been found in Williamsburg, Virginia, just a few miles from the boxwood gardens maintained by the Colonial Williamsburg Foundation (C.X. Hong, personal communication). Landscape aesthetics in public and private gardens are destroyed when boxwood blight is unchecked (**Figure 1*a***) (17, 42, 47). Boxwood already established in the landscape today are primarily *B. sempervirens* and *B. sempervirens* 'Suffruticosa,' which are among the most susceptible hosts known for boxwood blight. The replacement cost for boxwood in large blight-stricken gardens can be staggering. Château de Villandry in France, for example, has 7 km of small *B. sempervirens* 'Suffruticosa' edging the immense kitchen garden and has been gradually replacing these with *B. microphylla*. In 2019, they will also introduce new blight-resistant hybrids (H. Carvallo, personal communication).

Much of the dollar loss caused by boxwood blight falls on the nursery industry. Affected plants are unsellable and must be destroyed: One North Carolina grower burned 15,000 infected boxwood prior to going out of business (47, 72). In Connecticut alone, the nursery industry saw a $5.5 million loss to boxwood blight in the first five years after the new problem was identified (59). Disease losses of *Pachysandra* and *Sarcococca* are also meaningful, as both plants serve important horticultural roles, but the value of the crops is much smaller than that of boxwood.

This review focuses on the impact of a new disease on cultivated boxwood and other ornamental hosts, but it is important to note that boxwood blight has also had a devastating impact on native *Buxus* (*B. sempervirens* and *B. colchica*) forests in Europe and Asia, disrupting natural ecosystems (1, 33, 43, 61, 62, 75).

PATHOGEN BIOLOGY

After inoculation of boxwood with *C. pseudonaviculata*, germination began in 3 h on either upper or lower leaf surfaces (39, 42). Direct penetration through the cuticle on the upper surface of the leaf was seen at 5 h. Penetration through stomata on the undersurface of the leaf, without appressorium formation, was noted 1 day after inoculation. After intercellular growth of hyphae, hyphae began to emerge through the stomata on the undersurface of the leaf 2–3 days after infection; conidia were formed by 7 days after inoculation. LaMondia & Shishkoff (59) described infection of the abaxial leaf surface of *Buxus* predominantly via stomatal penetration, but with some instances of appressoria and direct penetration. They also noted infection of the adaxial leaf surface, although this was less common. On 'Green Velvet,' direct penetration of one cell on the (apparently stomate-free) adaxial surface was observed, followed by a conidiophore and sparse conidia; no symptoms developed. Nine of eleven of the least susceptible accessions in the greenhouse trial showed no infection through the adaxial leaf surface in the detached leaf assay (59), suggesting that absence of stomata on the adaxial surface may be associated with resistance.

Less is known about the interaction of *C. pseudonaviculata* with hosts from genera other than *Buxus*. Kong & Hong (49), however, compared conidial germination on leaves of *Buxus*, *Pachysandra*, and *Sarcococca* and found it occurred within 6 h of inoculation. After germination, differences became apparent: Hyphal growth was greatest and penetration was seen after only 12 h on boxwood, whereas it required 72 h for *Pachysandra* and *Sarcococca*. Lesion size was greater for boxwood than for *Pachysandra* and *Sarcococca* at six days post-inoculation. Conidium production was also higher on boxwood: 129,000 conidia/cm^2, almost seven times that on the other hosts.

Henricot et al. (42) noted that fewer conidia were produced on boxwood cultivars that were less susceptible than *B. sempervirens* 'Suffruticosa.' Avenot et al. (2) found that >5,000 conidia/mL were needed for infection of some of the less susceptible boxwood such as *B. sinica* var. *insularis* 'Nana,' whereas 1,250 conidia/mL could cause some infection on the highly susceptible *B. sempervirens* 'Justin Brouwers.' Changing which boxwood cultivars are widely grown in the future will have an effect on inoculum production as well as on the potential impact of that inoculum.

Leaf age has an effect on boxwood blight susceptibility, but the effect varies with cultivar. The assumption that younger leaves are more susceptible is not always true. Young leaves were more susceptible than mature leaves for *B. sempervirens* 'Justin Brouwers,' but young leaves were less susceptible than old leaves for 'John Baldwin,' and there was no difference in susceptibility between old and young leaves for 'Green Mound' (2).

Weeda & Dart (98) found clear histological evidence for the aggregation of chlamydospores into microsclerotia by observing cleared and stained leaf and stem tissue infected with *C. pseudonaviculata*. A single leaf may contain as many as 3,600 microsclerotia (15). Boxwood tissue infected with *P. buxi* (syn. *V. buxi*) or *Dothiorella candollei* (syn. *Macrophoma candollei*) did not show microsclerotia, only darkly colored hyphae (98). The microsclerotia of *C. pseudonaviculata* and *C. henricotiae*, apparently important for sporulation and survival, tend to develop in substomatal cavities. They are sometimes uniform in size and in other cases fuse with adjacent microsclerotia to form larger individuals; they may also erupt through the epidermis to form a larger mass (89). Microsclerotial size ranges for three isolates of *C. henricotiae* and two isolates of *C. pseudonaviculata* inoculated onto *B. sempervirens* 'Suffruticosa' overlapped and were not distinctive for the two species (89). Henricot et al. (42) documented survival of *C. pseudonaviculata* in buried and surface leaves for at least five years, and this was presumably through the survival of microsclerotia even though the structures were not noted at the time. Ganci (27) saw overwintering survival of *C. pseudonaviculata* on diseased leaves either at the surface of the growing medium or buried 5 cm below the surface and concluded that the fungus could overwinter via microsclerotia under western North Carolina climate conditions. Microsclerotial numbers and sporulation were both higher in subsurface treatments. Shishkoff & Camp (90) showed survival of *C. pseudonaviculata* in infected boxwood twigs and leaves at constant 0, 10, and 20°C for more than two years, whereas five months at 30°C and seven months at −10°C caused mortality. Although temperature extremes can kill the pathogen, these data suggest that survival from one season to the next in plant debris is likely in temperate climates.

Conidia do not possess the survival capacity of microsclerotia: They have been observed to maintain infectivity for approximately 2–3 days in water at 20°C, 4–6 days at 12°C, and 1 week at 4°C (30). Only nongerminated or recently germinated conidia are infectious; infectivity is reduced one day after germination begins (30). Almost all conidia germinated at 20°C within 24 h, whereas lower proportions of conidia germinated more slowly at lower temperatures (30).

ENVIRONMENTAL INFLUENCES

C. pseudonaviculata was seen to grow at less than 10°C but was inhibited at 30°C and killed at 33°C (39) and was thus described as a low-temperature fungus. Avenot et al. (2) undertook a study to expand the knowledge of the behavior of boxwood blight at higher temperatures than the 22.4°C studied in Belgium. Boxwood blight infection incidence on two-year-old inoculated *B. sempervirens* ‘Suffruticosa’ increased with increasing temperature between 18°C and 25°C, and then decreased to zero at 29°C. Modeling of the disease resulted in the estimate of a 23.7°C temperature optimum. Similarly, the estimated threshold temperature, beyond which no infections could occur, was 28°C.

Boxwood blight has been seen to be most severe in sites with high precipitation and high humidity during the summer, such as in the state of Georgia in the United States (J. Williams-Woodward, personal communication). The amount of rainfall was correlated with incidence and severity of disease in a study at a residential property in Richmond, VA: Total rainfall and disease levels during the observation period (April 29 to November 7) were both higher in 2016 (total rainfall: 706.2 mm) than in 2017 (total rainfall: 372.4 mm) (63).

Natural landscape situations include fluctuation in both temperature and moisture, making beneficial models challenging to develop from data collected under controlled conditions. To address this problem, the effects of dry interruption on infection were examined (2). Short dry periods of 0.5 h had no effect. However, a dry period of 3 h or longer significantly reduced the probability of infection. This probably reflected a deleterious effect on germinated spores that had not finished the infection process.

Information developed by Gehesquière (30) and Avenot et al. (2) has been incorporated into a boxwood blight forecasting model created by Leonard Coop of Oregon State University, available as an Android or iOS app and also as a mobile-friendly web version (**https://uspest.org/risk/boxwood_app**) and a full web version (**http://uspest.org/risk/models?mdl=bxwd_s**). A wide-area (continental US and southern Canada) synoptic version of the model is available at **http://uspest.org/risk/boxwood_map**. This version shows the current risk level for hundreds of locations, with links to the full model for each.

A description of the 1.0 version of the model is available (10). The initial model had a threshold dryness period of 8 h to stop the infection process, and this probably provided an overestimate of disease risk; the latest calibration of the model conservatively assumes that five hours of dry conditions will halt the infection process. This 5-h dry period assumption allows for varying canopy conditions. A shorter 3-h dry period was found to underestimate infection in some field situations (L. Coop, personal communication).

Light may also have an effect on disease development. Shaded plots saw significantly more severe disease than full-sun plots in a Virginia study (63). Marine et al. (69) tested the effect of the length of the daily darkness period on disease development but found no effect on boxwood blight severity.

PATHOGEN DISSEMINATION

The two-celled conidia of *C. pseudonaviculata* are large and sticky and thus not well suited for dispersal by air currents (30). Samples collected from simulated nursery run-off water contained a much higher concentration of inoculum than air samples from a Burkhard spore trap (32).

Infected leaves typically fall from the plant (63) and land on the soil surface. The use of leaf-blowers by landscape gardeners has been suggested as one means of distribution of inoculum within a garden (6). There are also many other potential means of human-mediated dispersal (30). As many as 100 conidia/cm^2 of working surface were found when shears, boots, gloves, and jeans were tested. A trial using shears showed 64% of the *C. pseudonaviculata* lesions spread by pruning were found on the first-pruned detector plants, falling to 29% and 7% on subsequently pruned plants (30). Movement by birds, cats, dogs, and deer and other wildlife has been suspected as well. The appearance of boxwood blight in sites where no new boxwood plants have been purchased for decades has given credibility to the assumption of spread via horticultural tradespeople or animals. The possibility of long-distance spread by wind-driven rain, including hurricane-force winds, should also be considered.

One unusual method of dispersal is through the sale of winter holiday greenery using clippings collected from nurseries. Boxwood wreaths being distributed (often interstate) were found to be carrying *C. pseudonaviculata* in North Carolina in 2014 (77); Indiana (84), New York (M. Daughtrey, unpublished results), and South Carolina (37) in 2017; and Michigan in 2018 (70). Disposal of the wreaths after their display period presents the greatest danger, as wreaths may be discarded near landscape boxwood.

MANAGEMENT OF BOXWOOD BLIGHT

Fungicides

Once boxwood blight entered the picture, it was important to develop chemical control information as soon as possible. The nursery industry needed to halt the spread of the pathogens on boxwood being sold to garden centers and landscapers. Fungicide management held the promise of prompt—if not ultimately sustainable—solutions. The effects of fungicides on inhibition of mycelial growth and/or germination of *C. pseudonaviculata* observed by Henricot et al. (42), Brand (5), and LaMondia (53) are thoroughly covered in the review of Palmer & Shishkoff (79), along with field trial results. Benefits have been seen from both protectant (e.g., prochloraz, fludioxonil, mancozeb, and chlorothalonil) and systemic (e.g., strobilurin, benzimidazole, and demethylation inhibitor) materials, with different fungicides working against different parts of the life cycle of *C. pseudonaviculata*. Researchers pointed out advantages of tank mixing or rotating materials with complementary effects (79). From early in the North American experience with chemical control of *C. pseudonaviculata*, the pathogen was shown to be sensitive to a wide range of fungicides (46), but nurseries and landscape care companies find the two-week treatment interval used by researchers and indicated on product labels difficult to impossible to sustain over time. The management dilemma is delivery of fungicides frequently enough to keep plants protected against infection throughout each season (M. Daughtrey, personal observation). *C. pseudonaviculata* can infect at temperatures 5°C or above, which makes it a winter–spring–summer–fall pathogen in parts of the United States. In especially rainy years, the challenge is increased. Even if possible, treatment every few weeks during the long boxwood blight season in some localities requires what some might consider an unconscionable quantity of pesticide applications and all consider expensive. To manage the disease in an integrated fashion, without sole reliance on fungicides, a number of states have developed Best Management Practices and many nurseries have adopted strict sanitation procedures.

EC_{50} values have been calculated for many fungicides (79), and this allows monitoring of changes in sensitivity over time. Already, fungicide sensitivity in *C. henricotiae* has been shown to be lower for kresoxim-methyl and tetraconazole than it is in *C. pseudonaviculata* (31). Deploying fungicides according to refinements of an environmental risk model (10) holds high promise for minimizing the number of fungicide applications in the future. But innovative management tools with longer periods of effectiveness than fungicides can currently provide are sorely needed.

Biocontrols

Biological control of boxwood blight is in its infancy, but initial explorations with both fungal and bacterial antagonists have been encouraging. The rhizosphere of *Buxus* species/cultivars in two arboreta was mined for microorganisms that might help provide sustainable solutions to boxwood blight (38). The growth of *C. pseudonaviculata* was reduced by as much as 99.4% by *Trichoderma* spp. isolates in dual-culture experiments.

A *Trichoderma koningiopsis* isolate recovered from a decaying wild mushroom inhibited the mycelium of *C. pseudonaviculata* (50). This isolate reduced infection on cuttings of *B. sempervirens* 'Suffruticosa' by 85% and on container-grown *B. sempervirens* 'Justin Brouwers' by 54%–63%.

Some biocontrol products available in the United States were tested for effectiveness against boxwood blight (99). Root Shield Plus+ used at 0.5 lb/100 gal both two weeks and six hours before inoculation gave a 44.4% reduction in disease severity. However, a chlorothalonil treatment in this same trial (Daconil DF at 1.4 pt/100 gal) completely controlled the disease, which is an indication of the current gap between fungicide and biocontrol effectiveness.

In another study, 1,547 strains of bacteria recovered from recycling irrigation systems in nurseries were tested for inhibition of *C. pseudonaviculata* in vitro and in planta (100). Indications of biocontrol potential were seen in 153 strains. The three isolates with the most potential for biocontrol were all *Pseudomonas protegens*; these gave 93%–100% inhibition of mycelial growth in a multiwell (48-well) plate assay, presumably from antibiosis.

Cultural Controls

Educational material has been developed by governmental agencies, botanical gardens, and horticultural businesses to assist the public and professional gardeners in dealing with boxwood blight. Some excellent examples may be found online, including pages of the Royal Horticulture Society (81), the Connecticut Agricultural Experiment Station (9), and Virginia's Boxwood Blight Task Force (94).

Management of boxwood blight in gardens starts with pruning back areas of dieback to healthy tissue, then removing fallen leaves and prunings as well as the surface of the soil (42) before beginning a spray program. Mulching at the base of plants reduces reinfection from dropped leaves (63, 64). A joint North Carolina–Virginia study of the effect of mulching showed more boxwood blight overall on nonmulched than mulched plants during two growing seasons with various environmental conditions (63). Gardening experts recommend that boxwood be shaped with a convex rather than a flat top to aid the canopy in shedding water; overhead irrigation of boxwood is also to be avoided (64, 81). Modifying the plant canopy to improve air circulation is also suggested by LeBlanc et al. (61) and others, but this recommendation is in conflict with centuries of horticultural tradition in which boxwoods have been sheared tightly and shaped artistically, with no attention to disease-promoting side effects.

To avoid introducing boxwood blight to healthy gardens, it is suggested to keep new *Buxus* in quarantine for at least six weeks and up to six months if there is a valuable collection at stake (64). Ideally, new plants should be propagated on-site from clean plants already on the premises (81). The ability of disinfestants to eliminate inoculum has been studied (22, 89). In some cases, different kinds of inoculum were vulnerable to different sanitizers. Although hydrogen dioxide, for example, is effective against conidia, microsclerotia are more sensitive to ethanol at 70%. Gardening tools and clothing should be disinfested after work in contaminated boxwood garden areas, which is a major inconvenience for commercial landscape gardeners.

The organic debris beneath infected boxwood is loaded with inoculum because of the many microsclerotia in the fallen leaves. Dart et al. (16) utilized a propane push flamer moving at about 1 m/45 sec to treat a field following removal of infected plants. Nontreated soil averaged 25.2 cfu/10 g soil in contrast to 4 cfu/10 g soil in the flamed areas. Although leaf flaming was not 100% effective, it had a significant effect and is helpful toward eliminating inoculum from a contaminated nursery. This technique, or any other intended to physically remove infected leaves, should be done as soon as possible after disease is detected, before wind, water, or animals redistribute the leaf debris (16).

Hot water treatment has been tested as a means of sanitizing cuttings. Conidia and microsclerotia are both sensitive to hot water but cultivar, isolate, and exposure time all affected the results (73). *C. pseudonaviculata* germination was decreased significantly more than that of *C. henricotiae* at 47.5°C and 50°C, but the microsclerotia of both species were killed quickly at 55°C (73).

Cultivar Resistance

Comparing disease susceptibility of *Buxus* cultivars already in the nursery trade is important to assist the green industry with choosing which ones to install in the intervening years before plant breeders develop highly resistant boxwood. Henricot et al. (42) used cuttings dipped in inoculum to compare the susceptibility of *Buxus* cultivars and observed high susceptibility of *B. sempervirens* and *B. sempervirens* 'Suffruticosa.' Shishkoff et al. (91) also used cuttings dipped in inoculum to assess the susceptibility of 42 accessions from the US National Arboretum, and found 32 that were less susceptible than English boxwood. Spotting developed on 6.3%–36.2% of the leaves of these more resistant plants, in contrast to 74.2% of infected leaves on English boxwood and 71.5% of infected leaves on *B. sempervirens*. A later greenhouse trial on whole plants grown from a duplicate set of cuttings gave largely similar results (59). Of the cultivars currently in the nursery trade, fewer lesions per plant (normalized for plant size) were seen on *Buxus* × 'Green Mound,' *B. microphylla* 'John Baldwin,' *B. sempervirens* 'Decussata,' *B. sempervirens* 'Dee Runk,' *B. sempervirens* 'Edgar Anderson,' *B. sempervirens* 'Handsworthiensis,' *B. microphylla* var. *japonica* 'Jim Stauffer,' *B. microphylla* var. *japonica* 'National,' *B. microphylla* var. *japonica* 'Winter Gem,' *B. sempervirens* 'Scupi,' and *B. sinica* var. *insularis* 'Pincushion.'

Cultivars were evaluated in two trials in Mills River, North Carolina, in 2013 (28). Plants seen to have low leaf spot susceptibility included *B. microphylla* var. *japonica* 'Green Beauty,' *B. microphylla* 'Wedding Ring,' *B. sinica* var. *insularis* 'Nana' and 'Franklin's Gem,' and *B. sempervirens* 'North Star.' In additional NC trials, no complete resistance was found but, in general, the Asiatic cultivars showed a higher degree of resistance (27).

In Connecticut trials, 'Korean' and 'Winter Gem' were the least susceptible, 'Common' and 'True Dwarf' were highly susceptible, and 'Green Mountain' and 'Green Velvet' were intermediate (54). Nontreated 'Green Velvet' showed more disease than 'Tide Hill' (58).

Avenot et al. (2) chose to work with four cultivars with different levels of susceptibility to boxwood blight. In their trials, *B. sempervirens* 'Justin Brouwers' was extremely susceptible. This cultivar was for some time thought to be a *B. sinica* var. *insularis* (Korean boxwood) or *B. microphylla* (Japanese boxwood) but is now reclassified as *B. sempervirens* (95). *Buxus* × 'Green Mound' and *B. microphylla* 'John Baldwin' were of intermediate susceptibility, whereas *B. sinica* var. *insularis* 'Nana' was the least susceptible.

It is important to be able to screen seedlings and young plants of *Buxus* for susceptibility to boxwood blight in an efficient and reliable manner. Studies at the US National Arboretum by Guo et al. (35, 36) have carefully compared inoculation techniques with this goal in mind. LaMondia & Shishkoff (59) caution that whole-plant studies should be coupled to lab assays, as some components of resistance such as systemic acquired resistance and plant form are not expressed in detached plant parts. *Pachysandra* trials to compare susceptibility to *C. pseudonaviculata* were made by using whole plants and detached leaves of different species and cultivars (55). *P. procumbens* was generally more susceptible than *Pachysandra axillaris* and *P. terminalis*. *Sarcococca* species and cultivars were tested for susceptibility to *C. pseudonaviculata* at the University of Georgia (85). There was a high percentage of leaf spotting in *Sarcococca confusa* but almost none in *Sarcococca ruscifolia* and *S. ruscifolia* var. *chinensis* 'Dragons Gate.'

DISEASE IDENTIFICATION AND PATHOGEN DETECTION

Typical diagnostic lab procedure for blight on buxaceous hosts is to incubate symptomatic plant material at high humidity for 24 hours to several weeks. Normally sporulation occurs within a few days, but samples from fungicide-treated plants may not sporulate with characteristic rapidity. Spotted leaf tissue is most helpful for obtaining the characteristic sporulation, but some stem streaks will also form conidia. Culturing on agar is usually a more difficult route to diagnosis than incubation because of competing fungi, particularly *Pseudonectria* spp. Molecular diagnostic techniques may be helpful in many situations, such as when only stems without leaves are available for diagnosis (30).

Molecular methods have been developed for use in epidemiological research for detecting latent infections and distinguishing the two species of *Calonectria* that cause boxwood blight on Buxaceae. Different quantitative PCR (qPCR) assays were developed by Gehesquière et al. (32) to aid in detection. A highly sensitive TaqMan ITS assay targeting the multiple-copy ITS locus was developed for detection in water and air samples for epidemiological studies; it could detect 10 fg of genomic DNA. One conidium equivalent was calculated to contain 151 ITS copies, allowing quantification of inoculum. Even 1 conidium/mL in a 10-mL water sample and as few as 10 conidia on a tape piece associated with 12-h air sampling in a Burkard volumetric spore trap could be detected. Although this assay showed high sensitivity, it yielded false positives for a nontarget species of *Calonectria*. For diagnostic applications needing less sensitivity but more specificity, a SYBR Green–based assay targeting the single-copy beta-tubulin 2 (*TUB2*) gene was developed that was four times less sensitive but did not yield false-positive results. Isolates can be identified as *C. pseudonaviculata* or *C. henricotiae* using DNA sequencing of phylogenetic markers, analysis of microsatellites (60) or with a PCR-RFLP profile from the *TUB2* gene (31). Two quantitative real-time PCR assays are also available to distinguish the two species (31, 61).

A comparative genomics approach was used to identify marker regions for loop-mediated isothermal amplification assays for both *C. pseudonaviculata* and *C. henricotiae*. By screening other common saprotrophic and pathogenic fungi in the Nectriaceae, as well as boxwood rhizosphere soil DNA characterized using meta-barcoding, the assay was shown to be specific for the targeted pathogens (65). Portable instrumentation could make this approach practical in the field (61). Whole-genome sequencing was employed by Malapi-Wight et al. (68) to ascertain the identity of a *C. pseudonaviculata* isolate obtained from naturally infected *Sarcococca* in Maryland in 2014. Whole-genome sequence assemblies are being collected at a website developed by the Crouch lab (12) and will aid in identifying genome-wide genetic variants for detection of emerging pathogen genotypes. Alternative protein-based detection techniques are also under development: These have some advantages over DNA-based identification, including reduced cost and potentially easy incorporation into tools for field use (61).

Dart et al. (14) developed a detection protocol for microsclerotial inoculum in soil. The most efficient detection was with a leaf disc bioassay, using flooded soil (1,000% field capacity) in Petri dishes incubated for 96 h. A single microsclerotium/g of soil could be detected. A soil-plating assay was tenfold less sensitive but allowed quantification of soil inoculum at high inoculum levels. Azalea leaf discs allowed a low level of detection but were much less effective than boxwood discs.

FUTURE NEEDS

Real progress in the management of boxwood blight requires plant material that is more resistant than what is currently available in the industry. *Buxus* breeding is underway in Europe and the United States with the goal of improved resistance to *Calonectria* spp., but *Sarcococca* and *Pachysandra* also need to be improved to avoid the menace of diseases caused by *Calonectria* spp. Until this more resistant material is available, gardeners require guidance on which existing cultivars of buxaceous ornamentals perform reasonably well, and they need to fight their desire for monoculture, which exacerbates the problem for boxwood. Changes in cultivar choices, garden design, and pruning, shearing, and mulching practices are all needed, and this requires research-based information. Fungicide application and other practices need to be assessed on the more resistant cultivars, not just on *B. sempervirens* and other extremely susceptible cultivars. Treatment actions that are effective for a month or longer are the goal for best management practices in nurseries and landscapes. Fine-tuning our forecasting expertise based on knowledge of host–pathogen–environment interaction is critical for knowing when it is necessary to deploy fungicides on less-susceptible cultivars. A better understanding of how to inactivate inoculum in a nursery or garden is also important. Although our knowledge of factors influencing this disease is still incomplete, it is important to move forward to incorporate what we do know into integrated pest management programs that are more effective and more sustainable for the nursery and landscape industries as well as for home gardeners. Further understanding of physical and chemical boxwood traits that reduce susceptibility, and the genes associated with these, will ultimately allow us to design better boxwood to meet the challenge of boxwood blight.

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