The ‘box blight’ pathogen:
Cylindrocladium pseudonaviculatum = Cylindrocladium buxicola (Teleo. *Calonectria pseudonaviculata*)

Scientific Name
Cylindrocladium pseudonaviculatum Crous, J.Z. Groenew. & C.F. Hill 2002

Synonym*:
Cylindrocladium buxicola Henricot 2002
Teleomorph: *Calonectria pseudonaviculata* Lombard, M. J. Wingf. & Crous 2010

*Although most literature refers to this species as *Cylindrocladium buxicola*, the name given to this fungus in the UK where the disease was first observed in the mid 1990s, this pathogen was not formally reported in the literature until 2002, when Crous et al. (2002) published its identity as *Cylindrocladium pseudonaviculatum* after it also became established in New Zealand. Molecular evidence places this species in the teleomorph genus *Calonectria* (Lombard et al., 2010), however, *Calonectria* has never been observed in the field causing disease.

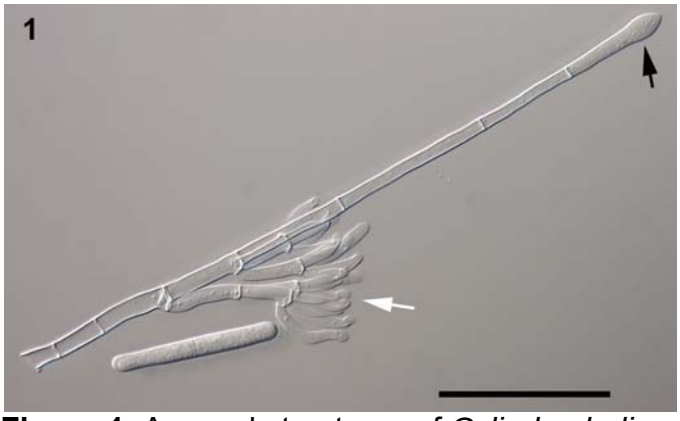

Figure 1. Asexual structures of *Cylindrocladium pseudonaviculatum*. Black arrow at apex of sterile vesicle on the stipe. White arrow at conidiogenous cells. Bar = 50µm (Joseph Bischoff, USDA-NIS).

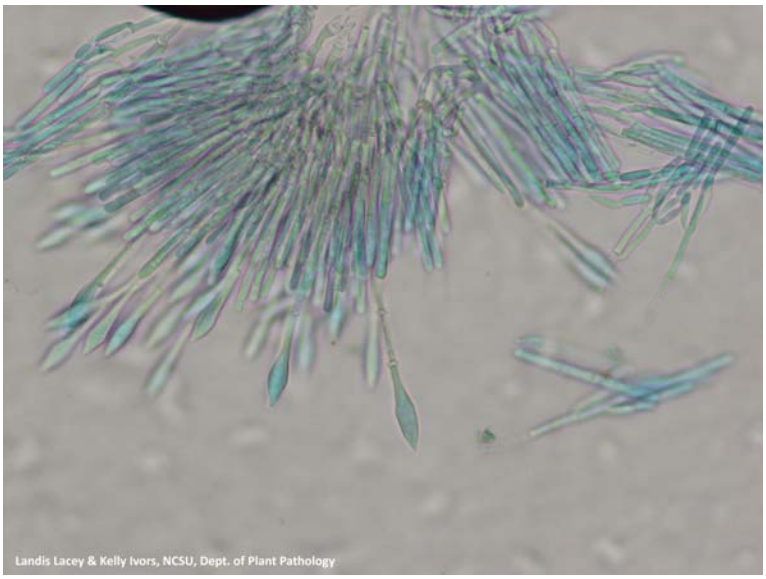

Figure 2. Vesicles of *Cy. pseudonaviculatum* (Landis Lacey and Kelly Ivors, NCSU Department of Plant Pathology)

Common Name(s)
Box blight, Cylindrocladium boxwood blight, blight disease of boxwood, Boxwood leaf drop

Type of Pest
Pathogen (fungus)

Taxonomic Position
Kingdom: Fungi, **Phylum:** Ascomycota, **Class:** Sordariomycetes, **Order:** Hypocreales, **Family:** Nectriaceae

Pest Description

Anamorph stage—the only stage found infecting host tissue. Description taken from Henricot and Culham (2002):

Perithecia of *Cylindrocladium* have not been found. Macroconidiophores are comprised of a penicillate arrangement of fertile branches, the conidiogenous phialide, a stipe, a sterile elongation, and a vesicle (Fig. 1). The stipe is septate ranging in length from 95 to155 µm long, hyaline, terminating in a lanceolate or broadly ellipsoidal vesicle with a pointed or papillate apex. The vesicle is typically 6.5–11 µm in diameter (Fig. 2). In the vast majority (97%, 168/174) of vesicles measured, the widest part was above the midsection. Primary philalide branches are uni-septate or aseptate, (5–) 15–41 (–66) x 3–5 µm, with secondary phialide branches aseptate (11–) 13–25 (–35) x 3–5 µm, tertiary phialide branches are rare, each terminal branch produces 2–5 reniform, hyaline, non-septate phialides; phialides. Conidia are cylindrical, rounded at both ends, straight, hyaline, 42–68 x 4–6 µm, uni-septate held in cylindrical clusters by a clear slime-like matrix (Fig 3). Microconidiophores have not been observed.

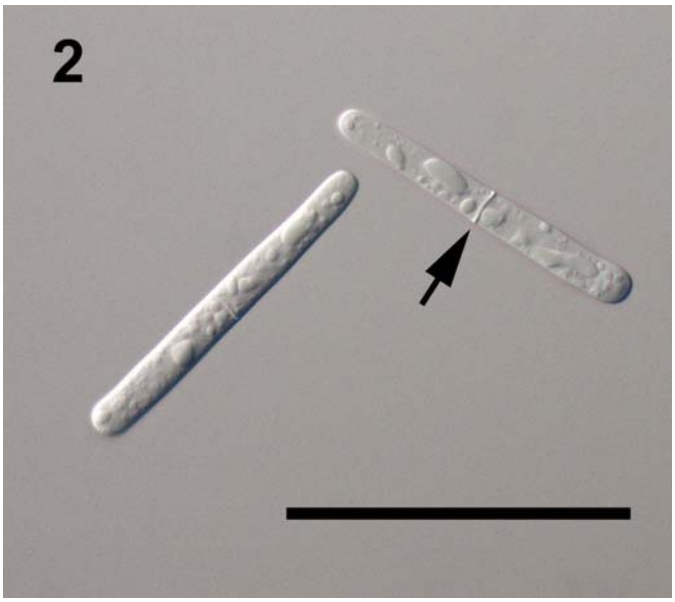

Figure 3. Hyaline conidia of *Cy. pseudonaviculatum*. Conidia are rounded at both ends with one septum. Black arrow at septum. Bar = 50µm (Joseph Bischoff, USDA-NIS).

Figure 4. Sporodochia of *Cy. pseudonaviculatum* on plant tissue as well as stem streaks (Landis Lacey and Kelly Ivors, NCSU Department of Plant Pathology)

When present, chlamydospores are dark, brown, thickened, and formed in moderate numbers on carnation leaves, not on agar, aggregated to form microsclerotia common to the genus (Henricot and Culham, 2002). Although observed on carnation leaves in culture, neither chlamydospores nor microsclerotia have ever been observed in host tissue; the

occurrence and role of these structures in the disease cycle is unknown at present (B. Henricot, personal communication, October 28, 2011).

Cultural characteristics: Colony color (reverse) on Malt Extract Agar at 25°C (77°F) after 7 days is fuscous black at the middle which fades to sienna with a halo of pale luteous (Rayner, 1970). The growing mycelium at the margin is white. Minimum temperature of observed growth is above 5°C (41°F); with a temperature maximum below 30°C (86°F). The optimum temperature for growth is 25°C (77°F). This appears to be a low temperature species, with *Cy. pseudonaviculatum=Cy. buxicola* killed after 7 d at 33°C [91.4°F] (Henricot and Culham, 2002).

Biology and Ecology

This pathogen has a rapid disease cycle that can be completed in one week (Henricot et al., 2008). Infection can occur very quickly in warm (18-25°C, 64.4-77°F), humid conditions (Henricot, 2006). Fungus growth can occur at low temperatures (<10°C, 50°F) with pathogen death occurring at 33°C or above (91.4°F) (Henricot, 2006).

High humidity levels or free water are needed in order for infection to be successful (Henricot et al., 2000). Germination of spores occurs 3 hours after inoculation, with penetration occurring in as little as 5 hours (Henricot, 2006). Hyphae can penetrate plant cuticles without appressorium formation (Henricot et al., 2008) and may enter through leaf stomata (Henricot, 2006). Fungal growth can occur intercellularly in the plant mesophyll (Henricot, 2006). Conidia borne on conidiophores cover the underside of the leaf after 7 days (Henricot, 2006; Saracchi et al., 2008).

Cy. pseudonaviculatum survives as mycelium on fallen leaves and will produce spores when environmental conditions become suitable (RHS, 2011). *Cy. pseudonaviculatum* can survive at least 5 years on decomposing fallen leaves of *Buxus sempervirens* 'Suffruticosa' (Henricot et al., 2008).

The entire foliage typically becomes blighted, causing the leaves to turn 'straw' to light brown in color. These leaves will defoliate, making the plant unsalable. Often the stems of blighted boxwoods will remain green under the outer bark until a secondary invader or opportunistic pathogen attacks this tissue, eventually killing the entire plant. That's why *Cy. pseudonaviculatum* is often associated with the

Figure 5. Leaf spots caused by Cylindrocladium blight of box (RHS)

secondary pathogen *Volutella buxi* (teleomorph *Pseudonectria rousseliana*), known to cause Volutella blight (Henricot et al., 2000). *V. buxi* is associated with plant wounds, whereas *Cy. pseudonaviculatum* does not need a wound to infect, but can penetrate directly through the cuticle (Henricot et al., 2000; Henricot et al., 2008) *Cy. pseudonaviculatum* is considered the more damaging of the two as it also infects young stems, causing dieback. Both can also occur independently (Henricot et al., 2000).

Figure 6. Infected prop bed (Landis Lacey and Kelly Ivors, NCSU Department of Plant Pathology)

Isolates can be grown on either potato-carrot agar (PCA) or carnation leaf agar (Henricot et al., 2008). On carnation leaf agar, sporulation will occur around 7-10 days after incubation at 25°C (77°F) under near-ultraviolet light (Henricot et al., 2008). The minimum temperature for growth is approximately 5°C (41°F) and maximum is 30°C (86°F); the optimal temperature for growth is 25°C (77°F) (Henricot and Culham, 2002).

Symptoms/Signs

Symptoms caused by this species include dark or light brown spots on leaves with dark borders (often in a circular pattern) (Fig. 5), black streaks on stems (Fig. 4), straw to bronze colored blighted foliage, and defoliation (Henricot and Culham, 2002, Henricot, 2006). In some cases, blighting and defoliation can occur suddenly (Saracchi et al., 2008) with complete leaf loss in severe cases (Henricot et al., 2000).

Figure 7. *Buxus* spp. infected with *Cy. pseudonaviculatum* with healthy root system (Landis Lacey and Kelly Ivors, NCSU Department of Plant Pathology)

Disease spreads rapidly throughout infected plants when conditions are warm and humid, and in shady areas (Henricot et al., 2008). Unfortunately, most boxwood cuttings are initially rooted and propagated in humidity chambers or tents, and young boxwood liners are purposely grown under shady conditions (Fig. 6). These production conditions are also conducive to the pathogen; therefore young boxwoods are more at risk to infection.

Infected leaves can be aggregated in expanded patches. Infected leaf tissue will eventually expand to the petiole and shoot of the leaf (Saracchi et al., 2008). Leaf spots can coalesce, covering the entire leaf surface. Infected shoots can have multiple dark brown or black lesions, either linear or diamond in shape. The black streaks found on stems progress from the bottom of the plant up (Henricot et al., 2000). New young shoots continue to develop on healthy twigs (Saracchi et al., 2008). The fungus has not been recovered on the root system (Henricot, 2006), and often the root systems remain healthy and intact (Fig 7).
Sporulation of the pathogen can be seen on the underside of the infected leaves (Henricot, 2006; Saracchi et al., 2008). Under high humidity, white fuzzy spore masses (called sporodochia) containing large numbers of conidia are sometimes visible to the naked eye or with a hand-lens on infected stem and leaf tissue (Fig 8).

Figure 8. (Image courtesy of Landis Lacey and Kelly Ivors, NCSU Department of Plant Pathology)

Pest Importance

This species was discovered in the United Kingdom during the mid-1990s causing a blight disease on boxwood (*Buxus* spp.) (Henricot and Culham, 2002). An outbreak of the disease occurred in the United Kingdom in 1998 (Henricot et al., 2000) and is now considered widespread there (EPPO, 2004). The disease can seriously impact the appearance and aesthetics of the host plants (EPPO, 2004) and can also kill plants, especially young seedlings (Henricot, 2006). This fungus could have an impact on nurseries, parks, and gardens as the hosts are used widely as ornamental species (EPPO, 2008).

Buxus spp. are not native to the United States, but were brought in as an ornamental plant (Batdorf, 1995). In general, *Buxus* spp. are used in the United States as ornamentals in landscapes (Bir et al., n.d.; Batdorf, 1995); boxwood tips and branches are also commonly used in Christmas holiday adornments such as wreaths, garlands, and floral arrangements. Infection by the fungus causes unsightly symptoms including

leaf spots, stem streaks, lesions, straw-colored blighted foliage, and defoliation, which can be severe (Henricot et al., 2000; Saracchi et al., 2008).

This disease could lead to negative impacts on the nursery industry as infested plants become unsalable due to symptoms. *Buxus* spp. are produced in two main areas within the United States, the first being the West Coast of Washington, Oregon, and north central California, and the second being the East from Maryland to South Carolina and west to Tennessee (Bir et al., n.d.). This disease could also lead to negative impacts on tourism for attractions that use *Buxus* spp. There are many gardens throughout the United States that have boxwoods displayed prominently, several of which are part of historic sites and buildings (Batdorf, 1995).

Although this species is no longer listed on the EPPO Alert List, some countries have taken harsh actions when this pathogen has been found. Austria immediately destroys all infected *Buxus* spp. every time *Cy. pseudonaviculatum* is detected (EPPO, 2009; EPPO, 2010). In 2010, Austria prevented export of plants from companies that had been linked to an outbreak of the disease (EPPO, 2010). Based on recent export data, an introduction of this pathogen would have some impact on international trade as over 400,000 plants were exported in the past 3 years, most of which were sent to Canada (L. Campbell, personal communication, October 26-27, 2011).

Known Hosts

Cy. pseudonaviculatum has only been observed on leaves and shoots of *Buxus* spp. under natural conditions. The full host range of this species is not currently known (RHS, 2011) but there appears to be no published evidence of resistance to this fungus in any of the commercial boxwood species available. Some varieties of boxwoods are be more susceptible than others, possibly due to physical features of the plant, such as water retaining foliage. *Buxus semperviens* 'Suffruticosa' ('English' boxwood) appears to be especially susceptible to *Cy. pseudonaviculatum* (EPPO, 2004; Henricot et al., 2008), although *Buxus semperviens* ('common' or 'American' boxwood) is also quite susceptible. Pathogenicity assays conducted in the laboratory determined that a *Sarcococca* species, another member in the family Buxaceae, is also susceptible to this fungus (Henricot et al., 2008), however this plant has never been found with infections under natural field conditions. It is unknown whether other members of the family Buxaceae, such as *Pachysandra*, are also susceptible.

Buxaceae - *Buxus colchica*, *Buxus microphylla* (littleleaf boxwood), *Buxus microphylla* var. *japonica* (Japanese boxwood), *Buxus sempervirens* (common or American boxwood), *Buxus sempervien* 'Suffruticosa' (English boxwood), *Buxus sinica* (Korean boxwood), and *Buxus sinica* var. *insularis* (Korean boxwood) (Henricot and Culham, 2002, Henricot et al., 2008; Gorgiladze et al., 2011; RHS, 2011).

Experimental hosts

Buxaceae - *Buxus balearica*, *Buxus bodinieri*, *Buxus glomerata*, *Buxus harlandii* (Harland's box), *Buxus macowanili* (Cape box), *Buxus riparia*, and *Sarcococca* sp. (Henricot et al., 2008). *Buxus balearica* was the most resistant to infection. This could

be due to the very thick, leathery texture of the leaves of this particular species (Henricot et al., 2008).

Henricot et al. (2008) states that this pathogen can infect several cultivars of the three main boxwood species, *Buxus microphylla* (littleleaf boxwood), *Buxus sempervirens* (common, American and English boxwood), and *Buxus sinica* var. *insularis* (Korean boxwood).

Known Vectors (or associated insects)

Spores of this fungus are contained in a gelatinous matrix and may be moved by insects, birds (Henricot, 2006), or contaminated tools.

Known Distribution

The disease caused by this pathogen was first observed in the United Kingdom in the mid-1990s and was widespread by 1998 (Henricot and Culham, 2002). It was most likely introduced into New Zealand (Henricot and Culham, 2002) where it was first reported in 1998 (Riley, 1998). The origin of this species is unknown (Henricot, 2006; RHS, 2011) but it is believed to have been introduced into the United Kingdom before being introduced into New Zealand (EPPO, 2008). Although some information available online indicates that the pathogen is endemic to Central America, the Caribbean, and Mexico, these claims have not been substantiated by scientific research. The original origin of Cy. pseudonaviculatum is unknown at present.

Europe: Belgium, Croatia, France, Georgia, Germany, Ireland, Italy, the Netherlands, Slovenia, Spain, Switzerland, United Kingdom; **Oceania:** New Zealand (Henricot and Culham, 2002; Crepel and Inghelbrecht, 2003; Brand, 2005; CABI, 2007; Henricot et al., 2008; Saracchi et al., 2008; Vincent, 2008; Benko Beloglavec et al., 2009; Varela et al., 2009; Cech et al., 2010; Gorgiladze et al., 2011).

This species has been detected several times in Austria, but the population of the fungus is considered local and under eradication (EPPO, 2010).

Potential Distribution within the United States

Buxus spp. (boxwoods) are not native to the United States (USDA-NRCS, 2011), but are widely cultivated as ornamentals. In the United States, there are two major production areas for *Buxus* spp., the first being the West Coast of Washington, Oregon, and north central California, and the second being the East from Maryland to South Carolina and west to Tennessee (Bir et al., n.d.). The three main species grown as ornamentals in the United States are *Buxus sempervirens*, *Buxus microphylla*, and *Buxus sinica* var. *insularis* (Bir et al., n.d.), all of which are known to be hosts for *Cy. pseudonaviculatum*. Known hosts of this fungus can be found from USDA Zone 10 (*Buxus balearica*) to USDA Zone 4 (*Buxus sinica* var. *insularis*) (Batdorf, 1995). This fungus can grow successfully at low temperatures (<10°C, 50°F) and will be limited at higher temperatures (Henricot, 2006).

Buxus sempervirens (common or American boxwood) and *Buxus semperviens* 'Suffruticosa' (English boxwood) are the most susceptible and are also the most widely grown in North Carolina and throughout the United States, especially in the Northwest region of North Carolina where this pathogen was recently found (K. Ivors, personal communication, October 26, 2011).

If this pest becomes established in the United States, it could be further dispersed through natural spread (rain splash, movement of spores in flood/irrigation water) (RHS, 2011), as well as animal vectors, contaminated tools and equipment, or through movement of contaminated asymptomatic nursery stock (Henricot et al., 2008; Saracchi et al., 2008).

Pathway

Cy. pseudonaviculatum has not been intercepted on imported commodities by U. S. agriculture inspectors; however the genera *Calonectria* and *Cylindrocladium* have each been intercepted 7 times. All instances were on plant materials (AQAS, 2011; queried October 24, 2011). Buxaceae is not a regulated family for nursery stock, but one species, *Buxus vahlii*, is a regulated propagative material (USDA, 2011). The United States does import a small amount of *Buxus* spp. from the Netherlands, which is known to have the disease (C. Katsur, personal communication, October 27, 2011).

This disease has likely spread throughout Europe via the movement of infected nursery stock. In Italy, infected plants were originally imported from Belgium as symptomless cuttings (Saracchi et al., 2008). Short distance dispersal can occur through water (splash dispersal), contaminated tools, and possibly birds and other animals as spores are sticky (Henricot, 2006; RHS, 2011). Spores are unlikely to travel long distances by wind (RHS, 2011). Human activities, such as pruning, may also spread the fungus.

Control

Methods for control have not been studied in detail for this specific fungus. Pruning of infected twigs and/or removal and destruction of fallen leaves and topsoil may help reduce inoculum of *Cy. pseudonaviculatum* (EPPO, 2004; Henricot et al., 2008). Sanitation of pruning equipment may be helpful in preventing spread of the disease. Control of water and humidity levels may also be useful in controlling this fungus. Badly infected plants should be removed and destroyed. RHS (2011) states that alternative ornamentals outside of the *Buxus* family may be used in place of susceptible hosts to prevent infection by the disease.

In general, Cylindrocladium diseases are very difficult to control with fungicides. Henricot et al. (2008) looked at the effect of 13 ornamental fungicides on *Cy. pseudonaviculatum*. None killed the fungus, although some did inhibit spore germination and mycelium growth. However, the active ingredient fludioxonil was not tested because of its inavailability to the ornamental industry in the UK. Studies conducted on other ornamental hosts in the U.S. have shown that fludioxonil is one of the most effective chemicals against other *Cylindrocladium* species (Haralson et al., 2007). Fungicides should be applied on both sides of the leaves to prevent both germination

and penetration of the fungus (Henricot et al., 2008). Due to the tight nature of boxwood foliage, it may be difficult to get good coverage within the plant canopy. RHS (2011) suggest holding commercially sourced plants in quarantine for at least three weeks before planting since it may take up to three weeks for the fungicide application to wear off, allowing better detection of the pathogen.

Volutella buxi is currently present in the United States (Batdorf, 1995) and methods to help control this pathogen may be helpful in controlling *Cy. pseudonaviculatum*.

Survey

Visual survey:

A visual survey should be completed to look for symptomatic plant tissue.

Survey Site Selection

It may be useful to visually survey host species that are known to be particularly susceptible to this fungus, like *Buxus semperviens* 'Suffruticosa', which is hardy to USDA Zone 5 (Batdorf, 1995). Surveys should occur wherever host plants are abundant. As the hosts are ornamentals, surveys could occur in large scale container and field nurseries, gardens, parks, or residential areas. As this fungus may move through international trade (through ornamental plants for planting), nurseries are a logical survey site.

It is unknown whether other members of the family Buxaceae, such as *Pachysandra*, are also susceptible so any suspicious samples from these plants should be collected as well.

Sampling

Submit adequate amounts of suspect leaf and stem material when possible. This helps ensure that there is sufficient material if downstream diagnostic techniques are required. Twelve or more leaves per sample are desired.

Storing

Refrigerate samples while awaiting shipment to the diagnostic laboratory. Place leaves without paper towel in a sealed and labeled ziplock bag.

Key Diagnostics/Identification

Cylindrocladium spp. are morphologically differentiated mainly by the shape of the vesicle and characteristics of the conidia (Crous and Wingfield, 1994), but misidentifications can occur, mainly due to cultural conditions that may influence these characters (Crous et al., 1992; Henricot and Culham, 2002).

Unfortunately, molecular diagnostic techniques have not yet been developed for rapid detection of this pathogen. In the UK, the disease is mainly confirmed through morphological examination of the fungal spores and isolation of the pathogen *in vitro*. In order to correctly identify *Cy. pseudonaviculatum* as a new species, Henricot and Culham (2002) used a variety of methods including morphological characteristics,

sequencing the ribosomal 5.8S RNA gene and the flanking internal transcribed spacers (ITS), the β-tubulin gene, and the high mobility group (HGM) of the *MAT*2 mating type gene.

Isolation: Henricot and Culham (2002) incubated diseased leaf and stem pieces of *Buxus* spp. in damp chambers at 20°C (68°F) to induce sporulation. "Isolates were single spored and subcultured weekly on potato carrot agar (PCA) (carrot 20g/L, potato 20g/L, agar-agar 20g/L, ampicillin 30 mg/L, streptomycin 133 mg/L)" (Henricot and Culham (2002).

To determine morphology "Single spores of 14 isolates were plated onto carnation-leaf agar (CLA) (Fisher et al 1982, Crous et al 1992) and incubated at 25°C [77°F] under near-ultraviolet light. The plates were examined after 7 d or until sporulation occurred (no later than 9 d) and only conidiophores on the carnation leaves were examined. For each isolate, mounts were prepared in lactic acid with aniline blue (0.2 g/100 mL), and measurements of at least 30 conidia, vesicles, stipes, branches and phialides were made at 1000x magnification with an optical microscope (Zeiss)" (Henricot and Culham, 2002).

Easily Confused Pests

This species was initially misidentified as *Cylindrocladium scoparium* in the United Kingdom (Henricot and Culham, 2002). *Cy. scoparium* is the most misidentified species in the genus most likely due to variability in morphological characteristics used in identification (Polizzi and Crous 1999, Schoch et al 1999). *Cy. pseudonaviculatum* can be differentiated from *Cy. scoparium* as it has "one-septate conidia and ellipsoidal vesicles with pointed or papillate apices"; *Cy. scoparium* tend toward globiose to ob-pyroid vecicles and lacks the pointed, lanceolate vesicles (Henricot and Culham, 2002).

In New Zealand, this species was initially misidentified as *Cylindrocladium spathulatum* (Ridley, 1998).

Cy. pseudonaviculatum can occur with another species *Volutella buxi* (teleomorph *Pseudonectria rousseliana*) known to cause Volutella blight (Henricot et al., 2000). *V. buxi* produces pink to orange spore masses on infected tissues (Fig. 9); under the microscope it is fairly easy to distinguish these fungi apart. Care must be taken to rule out the presence of *Cy. pseudonaviculatum* when *Volutella* is observed, as both

Figure 9. Spores of *Volutella buxi* (Landis Lacey and Kelly Ivors, NCSU Department of Plant Pathology)

pathogens may be present in the tissue.

Other pathogens that affect *Buxus* spp. in the United States are *Phytophthora parasitica*, *Macrophoma candolleri* (Macrophoma leaf spot), and nematodes. *Phytophthora parasitica* can be found on all cultivars of *B. sempervirens* (American boxwood) and causes wilting and discoloring of the foliage. *Macrophoma candolleri* is considered a secondary invader and causes raised tiny black spots on the underside of leaves. Nematodes cause wilting, stunting, and yellowing of the foliage (Batdorf, 1995).

All of these pathogens, as well as *Paecilomyces buxi* (=*Verticillium buxi*), may cause Boxwood decline. This complex is poorly understood, but can lead to poor plant growth, small leaves, and defoliation or dieback (Batdorf, 1995).

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