Figure 1. Yellow boxwood leaves infected with Volutella blight

Figure 2. Black streaks on petioles and stems caused by Volutella blight

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Volutella blight of boxwood

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In 2008, an outbreak of boxwood blight was seen in southern Ontario. We received funding from Landscape Ontario to research this. Diseased plants had symptoms ranging from yellow leaves on green stems (Figure 1) to entirely dead shoots. Pink spots were found on abaxial (lower) surfaces of leaves (Figure 3) and some on stems. Infected leaves and stems became yellow and dried out, but usually other parts of the infected plants remained green. Black streaks were also sometimes observed on petioles and stems (Figure 2).

Five boxwood cultivars commonly grown in Ontario nurseries, 'Green Mound', 'Green Gem', 'Green Mountain' and 'Green Velvet', were all found to be susceptible to this disease. In 2009 and 2010, the same disease was also found in some nurseries in British Columbia. In addition to 'Green Velvet', the following cultivars commonly grown in B.C. were also found to be susceptible: 'Chicagoland Green', and 'Green Beauty'.

To determine the cause of the blight, symptomatic boxwood plants were obtained from nurseries in southern Ontario. From over 80 samples collected in 2008 and 2009, more than 300 fungal strains were isolated, and among these, eight major fungal cultural types were observed. These cultural types differed in growth characteristics and colour in Petri plates, and were separated into groupings called morphotypes. Representatives of the eight morphotypes were subjected to DNA sequencing of a conserved genetic region (ITS of ribosomal DNA) to confirm identity. The morphotypes, their frequency of isolation, and tentative DNA identification were as follows: pink-orange (46 per cent, *Volutella buxi* also known as *Pseudonectria buxi*), red (seven per cent, *Fusarium tricinctum*), black with black dots (three per cent, *Phoma herbarum*), pure white (11 per cent, *Acremonium* sp.), red-grey (eight per cent, *Colletotrichum gloeosporioides*), white-yellow (seven per cent, *Bionectria ochroleuca*), light purple (10 per cent, *Fusarium oxysporum*) and yellow-orange (eight per cent, *Epicoccum nigrum*). Representatives of each group were then tested on detached boxwood leaves for their ability to cause disease. Only *V. buxi* was found to be pathogenic, confirming its role as the causal agent of Volutella leaf and stem blight, hereon referred to as Volutella blight.

The optimal temperate for growth of nine isolates of *V. buxi* was tested in the lab; they were found to grow fastest between 20 to 25° C. On detached wet leaves, spore germination tubes were seen 18 hours after inoculation on the leaf surface. After three days, verticillate branching structures of the fungus were seen on leaf surfaces. The practical implication is that at the optimal temperature for growth,

V. buxi needs at least 18 hours under wet conditions to germinate on leaf surfaces, and infection can occur over the next two days while the leaf surface remains wet.

To better understand the interactions of boxwood with Volutella blight, inoculation tests were conducted. We found that: (1) one-month-old boxwood leaves were more susceptible to infection than one-year-old leaves; (2) 'Green Gem' was the most susceptible cultivar, 'Green Velvet', 'Green Mound' and 'Green Mountain' were moderately susceptible, and 'Pincushion' was the least susceptible among these tested; (3) wounding was required for infection; and (4) adaxial leaf surfaces were much less susceptible to infection than abaxial (upper) surfaces.

The life cycle of *Volutella buxi* and the disease cycle of Volutella blight have not been thoroughly investigated. Based on published details of the disease cycle of *V. pachysandrae* (Safrankova 2007, *Plant Protection Science* 43:10), and observations of Volutella blight and *V. buxi* from the current work, the putative life cycle of *V. buxi* in an outdoor environment is described as follows. As temperatures warm up in the spring, spores of *V. buxi* are produced by overwintered mycelium in dead tissues such as fallen leaves and attached dead tips and shoots. These spores are produced throughout the spring and dispersed by air or splashing water (rain or irrigation). They can infect boxwood tissues, especially younger, new foliage, which is more susceptible than older, overwintered foliage. The fungus will grow through foliar tissues and into woody tissue. In summer with drier, warmer conditions, spore production is reduced, but with wet weather of several days' duration, the fungus can grow out from infected tissues, produce spores, and cause more infections when plant surface wetness periods exceed 18 hours. In the fall, with wetter, cooler conditions, the fungus is again active and will produce spores to cause more infections. In winter, the fungus becomes dormant in fallen leaves and in stems of dead tissues until the following spring.

The disease cycle under controlled environment conditions may differ, because enclosed environments offer protection from desiccation and provide more humid or wet and warmer conditions that are favorable for plant growth, but also for fungal growth. During this propagation cycle, there are various stages where Volutella blight might enter the system and even become enhanced by cultural operations:

(1) For rooted cuttings, mother plants that have non-symptomatic infections may carry hyphae of *V. buxi* inside plant tissues, even if care is made to remove visibly diseased tissue.

(2) When cuttings are dipped in fungicides before propagation, the foliar portions are not protected. However, *V. buxi* is almost completely restricted to entry through wounds, so if wounding of above ground parts is minimized during these controlled environment operations, infection levels might be decreased.

(3) With latent or non-symptomatic infections, *V. buxi* still can grow out from infected live tissues, produce spores, cause infections, and be transported by air and splashing water. The infection process is faster in controlled environments between 20 and 25° C and under high humidity in propagation rooms, than it is in the external environment. In lab studies with detached leaves, the entire disease cycle from infection to spore production can occur in three days. Furthermore, we found that from dead dried leaves, the fungus is still viable after six months under dry storage conditions at room temperature, and hence debris can be a source of infection, in addition to infected non-symptomatic live tissues.

Figure 3. Pink dots found on lower surfaces of leaves of infected boxwood associated with Volutella blight

(4) In cases where there is a high risk of disease, repeated applications of fungicides are likely needed, because spores will be constantly produced and released under the moist, warm growth conditions in controlled environments.

No fungicides are registered for control of Volutella blight on boxwood. The following six fungicides, which are commonly used against woody ornamental diseases in Ontario, were tested on whole boxwood plants: Phyton-27 + Nova 40W (0.3 g/L water + 0.3 g/L water); Dithane DG 75WP (3 g/L water); Daconil 2787F (2.4 mL/L water); Rovral Green 240 g/L (24 g/L water); Senator 70WP (0.14 g/L water); and Banner MAXX 14.3 per cent (0.35 mL/L water). Whole plants were treated with the solutions until runoff, and 1.5 ml was applied per leaf for detached leaves. For the pre-inoculation treatments (i.e., fungicide applied before the fungus), the fungicides all reduced disease by 80 to 95 per cent, while fungicide treatments at seven or 14 days after inoculation gave disease reduction between 57 and 88 per cent. All six fungicides tested here have the potential to be used in an integrated control program to reduce infection by *V. buxi* and losses due to Volutella blight. However, more field and growth room fungicide testing is required under commercial operating conditions. **LT**

Box blight *(Cylindrocladium buxicola)*

Another disease called box blight, caused by the fungus *Cylindrocladium buxicola*, has recently been found in British Columbia and Ontario, after first causing issues in the American eastern states and Oregon. This box blight disease differs from Volutella blight in being much more aggressive, attacking, infecting and killing plants that are not wounded (unlike Volutella blight, which requires wounds for infection, and has slower disease progression). However, both diseases can be found together on the same plant tissues, and even the fungal growth and spores of both can be mixed together. There are similarities between the two in symptoms in terms of black streaking, and white fungal growth on leaves, but Volutella blight has pink-orange fungal growth often in clots, rather than white, spikey growth with box blight. Also, the black streaking with Volutella blight is much less frequent and found in much longer lines along flat or rounded portions of the stems, whereas box blight causes more abundant, shorter, black streaks, particularly along the square edges of stems.

Note: Landscape Ontario is funding research at the University of Guelph on this disease .