

Appendix 5:

A) Symptoms of Box Blight Infection on Boxwood and Pachysandra Plant Material

C. buxicola Symptoms Guide

Use the following websites to view pictures of plant symptoms and spore characteristics.

ANLA – Box Blight

<http://www.anla.org/knowledgecenter/premium/index.cfm?view=player&colid=112&cid=324&mfid=5311&StartNum=1>

Royal Horticultural Society, UK

<http://apps.rhs.org.uk/advicesearch/Profile.aspx?pid=96>.

Disease Symptom Description

(Excerpt from ‘*Box Blight – Best Management Practices*’ CNLA publication, August 2012)

- On Boxwood plants, infected leaves will have circular spots that are purplish-brown in colour and have dark borders, the plants may also exhibit complete defoliation, and/or the stems may have black cankers or lesions. Photographs are available on the internet (see www.boxwoodblight.org).
- Pachysandra symptoms include small necrotic regions (1-10mm in diameter) with diffuse yellow haloes.
- If the disease is suspected then contact your local diagnostic laboratory to submit samples for confirmation.

B) Sampling and Testing Protocols

This section provides guidelines on sampling of incoming plant shipments and on-site sampling of plants with disease symptoms (leaves with circular leaf spots that are purplish-brown in color and have dark borders, leaves that are totally blighted, as well as stems with black cankers/lesions). This sampling may be carried out by qualified nursery employees or contractors. See Appendix 4 for photographs of *C. buxicola* symptoms and spores.

General Sanitation Procedures

- Use a hand-sanitizer between sample collections, and before leaving an isolation block.
- Disinfectant all tools using a spray bottle filled with an appropriate disinfectant.
- Clean all soil or growing media from boots or shoes and then spray boots with disinfectant before leaving a restricted area or an isolation area.
- Follow decontamination procedures before and after taking each sample.

I. Preparing for Sampling Equipment needs:

- Ziploc bags with paper towel
- Permanent markers and labels
- Disinfectants (e.g. Zep Pine Disinfectant, Lysol, Virkon, Chemprocide or a 10% bleach solution),
- Hand sanitizer (e.g. OneStep Extra Strength 70% alcohol hand gel sanitizer by Belvedere)
- Hand-held pruning shears (optional)
- Cooler

See *C. buxicola* symptoms guide at the end of Appendix 5

II. Sample Collection and Packaging Sample Selection:

- Take one sample of plant materials showing disease symptoms, taking 1-2 twigs with leaves for each sample from the same block of plants.
- If possible, take twigs showing symptoms on several leaves from each plant sampled.
- Symptomatic leaves *recently* fallen in the immediate vicinity or on the potting soil can also be collected in a sample. Collected fallen leaves must still be in good condition.
- When possible, samples should contain leaves with a range of symptoms (green, lesions, brown).
- **Use flagging tape to identify the block/plant** where the plant sample was taken and write the sample # on the flagging tape corresponding to the number assigned on the sample bag.

III. DO'S AND DON'TS OF SAMPLE COLLECTING

Do:

- Sanitize sampling tools after each sample collection.
- Spray boots with bleach or other disinfectant between host blocks or fields.
- Attach labels on the outside of bags since labels inside the bag may deteriorate and become illegible.

Do not:

- Do not add extra moisture to the sample to keep it fresh. The extra moisture will actually speed deterioration of the sample.
- Do not leave samples in sunlight, allow them to dry out, or allow them to get hot.
- Do not shake or disturb the sample or source plant while you are in the field – use caution to avoid spreading any spores

IV. MOISTURE CHAMBER METHOD

For sampling of boxwood plants during initial diagnosis of symptomatic plants:

1. While following sanitation protocols, collect whole plants in their pots (where feasible). If the plants are too large or field planted and cannot be managed in a bag, take cuttings (at least 2-3, 6" branches if possible without affecting the saleability) from the large plants and place them upright in a disinfected stainless steel bucket to avoid water-soaking.
2. Bag the plants or bucket of branches on-site in strong, clear plastic bags (e.g. contractor garbage bag size). Clear bags make it easier to see in the bag without opening it! Label the

outside of the bag with the crop identification number and date. Small plants (#1-3 pot size) can be bagged together, larger ones separately to avoid getting holes in the plastic. Tie the top tightly.

3. Bring the bagged boxwood material into a stable, warm environment, preferably 19-23°C out of the direct sun. Place the bagged material into a tray (in case you get a hole in the bag and it leaks).
4. Before and after opening each bag, sanitize your hands.
5. Open the bags one at a time and “water” the plants, providing enough water to moisten the leaves and wet the growing media or have an inch or so of water in the bucket. Be careful to avoid touching the inside of the bag or the plants with your hands or the watering can. Seal the bag back up and repeat with any other bags.
6. Check the bags every 2 days to see if you need to add more water. The plants should start to flush, and will use up some of the moisture in the bag.
7. After 5 days, start looking daily for disease symptoms (e.g. lesions, spots, fungal growth). Where there is evidence of fungal growth, the nursery may use a microscope (magnification of 200x or 400x) to diagnose *C. buxicola* based on the resource materials or may send the sample to a lab.
8. Pending the results, the forced plants should be put back in the production block or destroyed as outlined in the nursery’s Biosecurity policy.

V. Readyng Samples for Further Laboratory Testing

1. When collecting tissue samples, put the sample and a dry paper towel in a Ziploc bag.
2. Label each bag clearly on the outside of the bag with a waterproof marker to include the following information:
 - a. Sampler’s name
 - b. file #
 - c. full host name (genus, species and variety)
 - d. block #
 - e. date collected
 - f. sample #
3. Flatten the bag (removing air) and close the zip.
4. Place all sample bags into a second larger bag and then into a cooler.
5. Label the package “*C. buxicola* SAMPLES” and include your nursery name and contact information.
6. Deliver the samples to the diagnostic service provider.
7. Ensure that transportation has been arranged such that samples will be delivered to the laboratory as close to 24-48 hours after collection as possible. (Send courier samples before Wednesday noon.)