4

Pathogen sampling and sample preservation for future analysis

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General recommendations

- 1) A diseased plant may be affected by more than one pathogen and display various symptoms. Thus:
 - check the whole plant and do not consider only a specific organ (e.g., stem);
 - if a plant shows a general crown decline without specific above-ground symptoms, check the roots;
 - take samples from all symptomatic organs;
 - the collected samples should represent all the symptoms observed.
- 2) Do not collect dead plants or plant organs, as these may have been colonized by saprotrophic organisms, they are not useful for the diagnosis.
- 3) Do not collect samples in rainy weather; wet or humid samples may become colonized by other organisms, making the correct diagnosis more difficult.
- 4) Collect the whole plant whenever possible (e.g., seedlings, saplings and bonsai).
- 5) Place the samples in paper or plastic bags of appropriate size: if you use plastic bags, wrap the sample first in a paper towel, especially when samples are humid or wet. If you suspect a quarantine organism, double wrap the samples.
- 6) Label the samples properly (simple and understandable) right after collection. Use preferably a pencil, as it withstands water. Provide as much information as possible (e.g., name of the collector, date of sampling, coordinates, elevation, exposition, plant species, origin of the plants; outdoor or potted plants, plant age, how spatially distributed the symptomatic plants are, e.g., regular, random or aggregated pattern, incidence and severity of the symptoms, environmental conditions; management history in case of forest/plantation/arboretum).
- 7) Send the samples immediately after collection to the diagnostic laboratory; storing the samples will reduce the chances of isolating the original pathogen and promote the development of saprotrophic organisms. In case immediate shipping is not possible, store the samples at a cool temperature (at 3-5 °C) and do not allow them to dry out.

- 8) If you use a tool for sampling (e.g., secateurs, pruning saw), clean and disinfect it after each sample, e.g., with 70% ethanol or a biocide. This simple measure will considerably reduce the risk of cross contaminations.
- 9) Take good quality pictures of the whole plant (general view) and close up of each symptomatic part of the tree.
- 10) **Remember:** sampling can be time consuming, but a good sample considerably increases chances of a correct diagnosis!

Sampling of bark

Symptoms visible on the bark include outer depression, discoloration, and cracks (cankers), commonly indicating the presence of a necrotic lesion. For collecting samples of bark lesions on stems or branches, the following procedure should be adopted:

- 1) Identify the margins of the lesion (e.g., change of colour of the bark, end of cracks).
- 2) Using a knife or a chisel, carefully remove the outer bark to definitively reveal the front of the lesion in the inner bark (if the lesion is clearly identifiable, this step may not be necessary). In this region, the pathogen causing the lesion is most likely active, increasing the chances for its successful isolation. By contrast, in the middle of the lesion the pathogen may not be active anymore and the dead bark already colonized by saprotrophs.
- 3) Cut and remove a piece of bark of a few cm² including both healthy and symptomatic tissue (see Fig. 4.1.a.). The incision should reach the cambium and, in case the wood underneath is also discoloured, a layer of wood should also be removed. To increase the chances of isolating the pathogen, you can collect a few bark samples per lesion. When the lesion is located on a small branch or sprout (see Fig. 4.1.b.), you can remove the whole section of the branch/sprout containing the lesion by sawing 3-5 cm in the healthy tissue beyond the lesion with a pruning saw or cutting with secateurs. In the case of seedlings or saplings, you can cut and collect the whole plant. If stromata or fruiting bodies are present on the surface of the lesion, a bark sample with them should also be taken.
- 4) Label, pack and send the sample as described above.

Sampling of wood

Symptoms of a wood infection by a pathogen may include branch wilting to different extents (from localised to general on the crown), leaf size reduction, and discoloration.

For collecting samples of infected wood, the following procedure should be adopted:

- 1) Choose a declining branch not yet completely wilted. In case of small branches (up to 3-5 cm diameter), cut the whole branch; in case of large branches, collect a cubic sample with a chisel being sure to include a relevant part of the wood. When sampling bark, try to include both symptomatic (discoloured) and healthy wood tissue.

- 2) In case no discoloration is evident in the wood, proceed as for sampling of roots.
- 3) Label and pack the sample, and send it to the diagnostic laboratory.

Fig. 4.1. Collecting samples from bark lesions. (a) Lesion on the main stem: pieces of bark including both healthy and symptomatic tissue of a few cm² should be removed (yellow rectangles); (b) Lesion on a young stem (or on thin branches): the entire section of the stem/branch containing the lesion can be removed. WSL.

Sampling of shoots

Visible symptoms may include wilted or necrotic shoots, flagging, discoloration and ‘shepherd crook’ curved shoot tip. To collect samples from symptomatic shoots, the following procedure should be adopted:

- 1) Collect the whole shoot by cutting through the healthy tissue about 3 to 5 cm below the symptomatic part
- 2) As shoots are fragile, it is imperative (more important than for bark samples) to collect them dry and send them as fast as possible to the diagnostic laboratory.

Sampling of leaves and needles

Symptoms visible on the leaves (and needles) consist mainly of necrotic spots of different shapes and colour. For collecting samples from symptomatic leaves (and needles), the following procedure should be adopted:

- 1) Collect the whole leaf and not only parts of it: when sampling simple leaves, include the petiole and when sampling compound leaves include the leaflets, rachis and petiole.

- 2) If different types of necrotic spots are present on the leaves of a single plant (e.g., different colour), collect leaves with all representative symptom types.
- 3) If all leaves on a small branch or twig are wilting, include a piece of the adjacent branch/twig in the sample. Check for a lesion on the branch/twig and if present, cut the branch/twig 3-5 cm in the healthy tissue beyond the lesion (see Sampling of bark) and collect it. In this case, the branch/twig, and not the leaves, may be infected.
- 4) If symptoms are visible on different foliar stages (e.g., young vs. old leaves), collect symptomatic leaves from all stages.
- 5) If stomata or fruiting bodies are present on individual leaves, these particular leaves should also be collected.
- 6) In the case of seedlings or saplings, you can cut and collect the whole plant.
- 7) As leaves are fragile, it is imperative (more so than for bark samples) to collect them dry and send them as fast as possible to the diagnostic laboratory.

Sampling of roots

Symptoms on the roots include bark lesions, resin flow, or decay. In contrast to symptoms on stem, branches, twigs and leaves, which are easy to detect, symptoms on the roots, except main roots, superficial roots or shallow roots that are partially visible, and root collar, are frequently not visible. Therefore, root sampling can be more laborious.

To collect samples from symptomatic roots, the following procedure should be adopted:

- 1) If no roots are visible, remove the soil to expose at least the main superficial or shallow roots.
- 2) Remove the soil from the root surface using a brush.
- 3) Inspect the individual roots for the presence of lesions, decay or exudates. If something is visible, proceed as for bark sampling. As for branches, in the case of small roots you can remove the whole section containing the lesion by sawing the healthy tissue 3-5 cm beyond the lesion with a pruning saw or by cutting with secateurs. In the case of seedlings or sapling, you can collect the whole root ball.
- 4) As roots are mostly infected by soil-borne organisms, it is recommended to collect also soil samples from the rhizosphere of trees with symptomatic roots. For this, select four points at a distance of 50-100 cm from the tree (each point representing the corner of a square, with the trunk in the centre). At each point remove the litter from the soil surface and with a spade collect soil until a depth of 20-25 cm (total of 200-300 g of soil for each tree). Pool the soil of the four samples in a plastic bag or container. Remove stones or litter from the sample, fine roots or small roots of the sampled tree can be included.

Further reading

- Nelson, S.C. and Bushe, B.C. (2006) Collecting plant disease and insect pest samples for problem diagnosis. *Soil and Crop Management* 14. College of Tropical Agriculture and Human Resources, Hawai'i, US.
- Sancisi-Frey, S. (2015) *Observatree Volunteer Sampling Guide*.
<http://www.observatree.org.uk/wp-content/uploads/2015/10/Observatree-sampling-guide.pdf>
- Schubert, T.S., Bremanm, L.L. and Walker, S.E. (1988) Basic concepts of plant disease and how to collect a sample for disease diagnosis. *Plant Pathology Circular* No. 307. Florida Department of Agricultural & Consumer Services, Tallahassee, FL, US.