Assessment of invertebrate leaf damage

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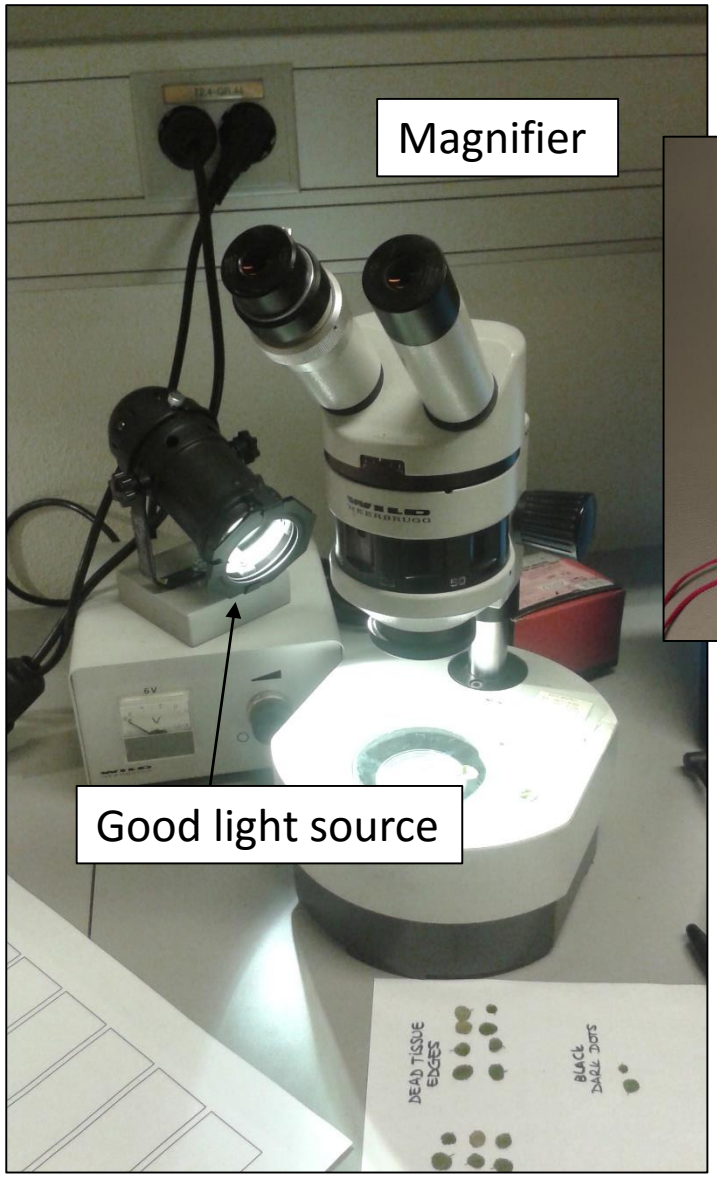
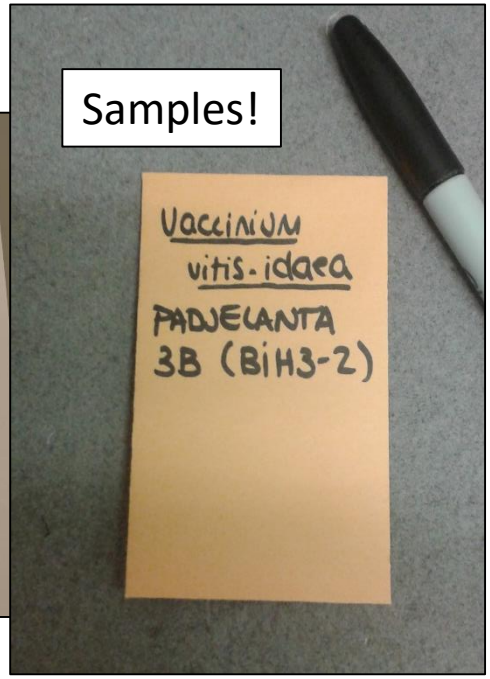
Materials needed

The **magnifier** is needed to check some of the samples; especially in the beginning, most of samples need to be checked. It is useful if it has a back light I was using 6x-50x



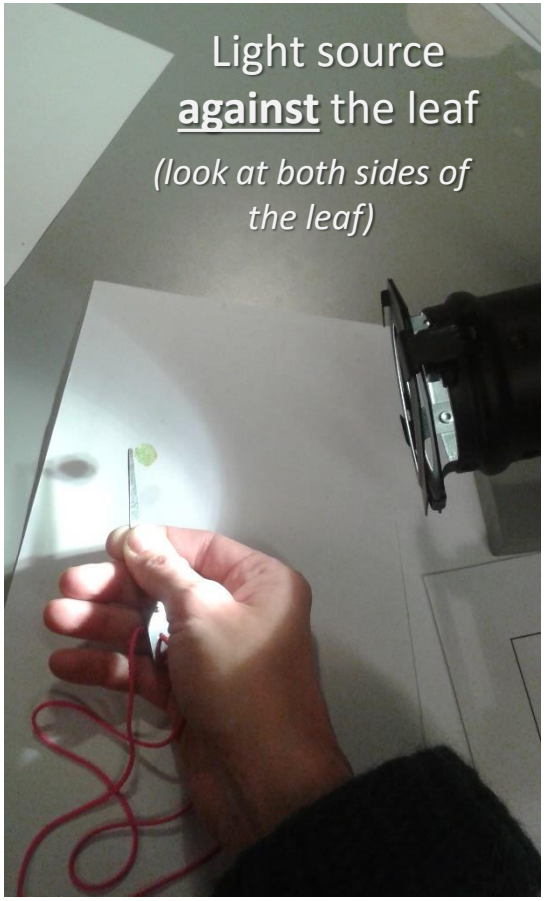


Steps of the protocol

1. PREPARATION OF SAMPLES
2. LEAF INSPECTION
3. LEAF CLASSIFICATION
4. DATA ENTRY

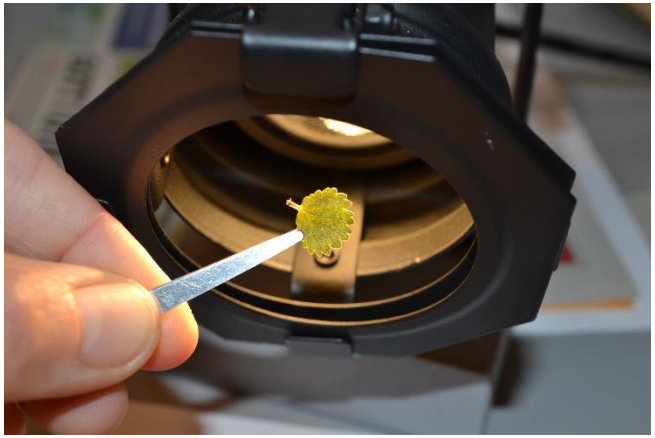
1. PREPARATION OF SAMPLES

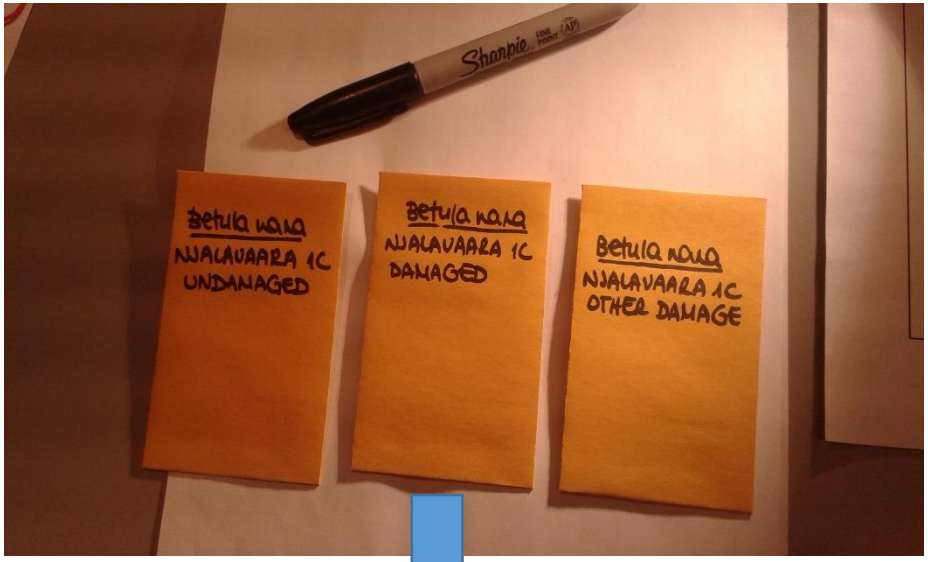
If leaves are still attached to stems, we need to **detach** them, as we will need to inspect each leaf carefully on both sides. Usually this can be safely done by hand, but soft tweezers can be handy. Be careful not to miss petioles of leaves that have been nearly ‘completely’ eaten.



2. LEAF INSPECTION

Inspect each leaf on both sides, first with a light source against the leaf to see damage on the surface, and then with the light through the leaf, to detect potential damage inside the leaf. In total, we are looking at each leaf **four times**. For example, some types of leaf damage might only be visible on one side of the leaf with the light source against it, such as some leaf mines, while others may only be evident when looking at the leaf through the light.

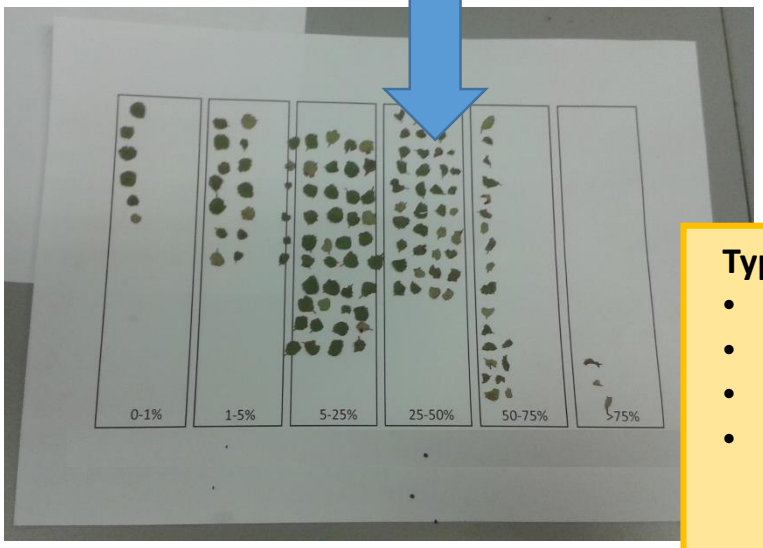




3. LEAF CLASSIFICATION

Basically we are classifying leaves into **3 broad categories,** as:

- 'undamaged',
- 'damaged by invertebrate herbivores'
- with 'other types of damage'



For leaves 'damaged by invertebrate herbivores' we will assess the **type** and **extent** of damage, using a scale of percentage leaf area affected.

Types of leaf damage by invertebrates

- Leaf chewers
- Skeletonized leaves
- Leaf gallers
- Leaf miners
  - Serpentine mines
  - Other mines

All these damage types are further classified according to the **extent of damage** (percentage leaf area affected) using the scale:

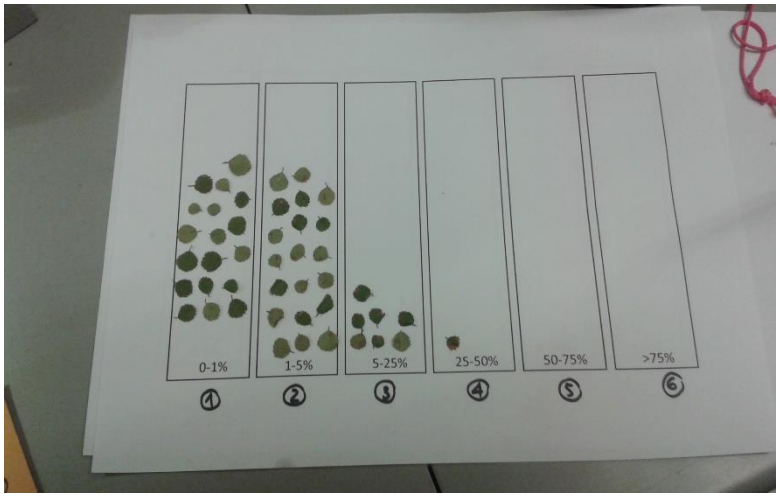
undamaged	damaged	
0 0%	**1** <1%	**50** 25-50%
	5 1-5%	**75** 50-75%
	25 5-25%	**100** >75%

Classifying leaves needs a bit of familiarization with the leaves of the focal plant species, their natural variability, and the types of damage that frequently affect them. For example, changes in coloration (e.g. browning) might not be good indicators, as they might be more related to issues during processing of samples or leaf senescence.

I found particularly useful to arrange leaves on sheets of paper separating them by categories, so that they are easier to count at the end.

UNDAMAGED LEAVES

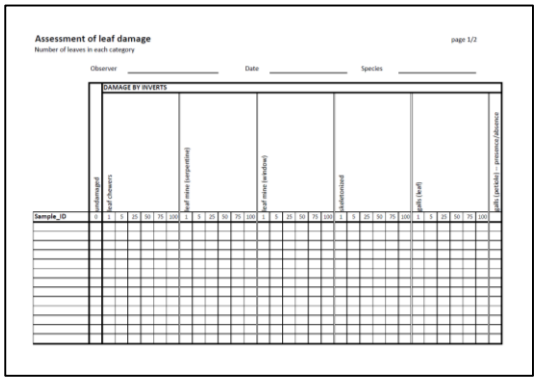
LEAVES WITH INVERTEBRATE DAMAGE



The data we are collecting from each sample are the **numbers of leaves** in each category

All this information is to be collected in the **datasheets** (template provided), and then, entered into the computer.

Having paper copies ensures you have a hard copy! and avoids some errors while typing in…



LEAVES WITH OTHER TYPES OF DAMAGE

I also distinguished different types of damage within the "other types of damage" class. This step is not critical, as we are mostly interested in damage by invertebrates, but can provide additional information (see the document on leaf damage on *Betula nana* for more details)

4. DATA ENTRY

From the datasheets, the last step is to enter the data into an Excel file