



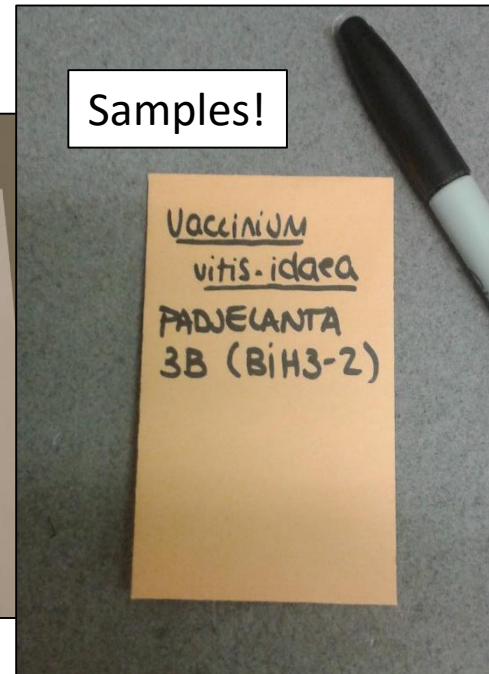
Assessment of invertebrate leaf damage

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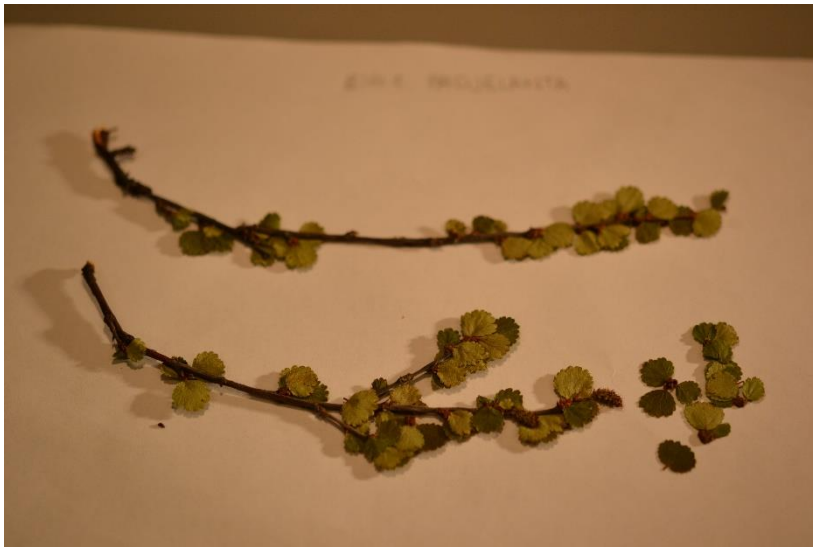
Materials needed

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



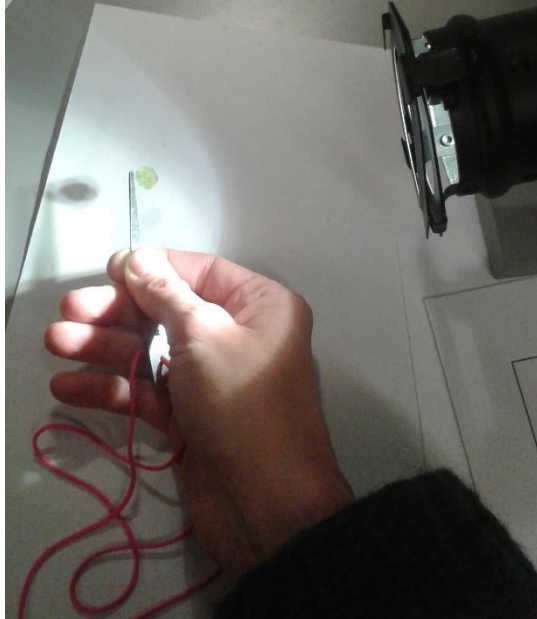
1. PREPARATION OF SAMPLES

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.



Light source
against the leaf

*(look at both sides of
the leaf)*



Light source
through the leaf

(look at both sides of the leaf)



2. LEAF INSPECTION

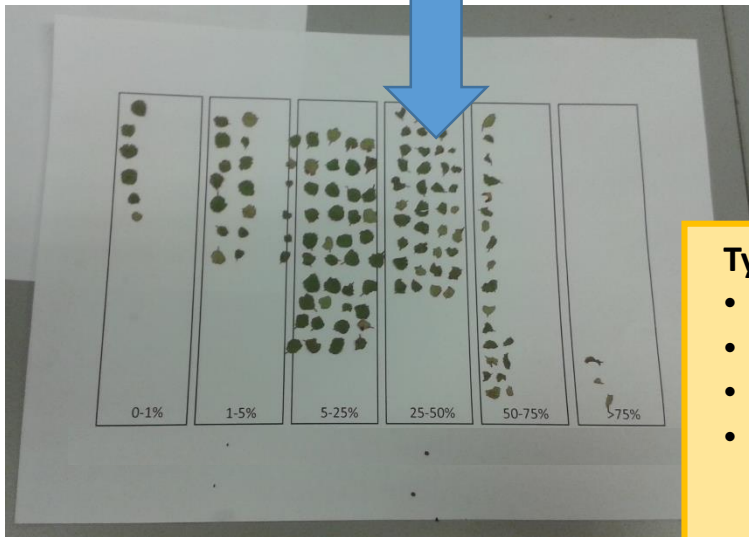
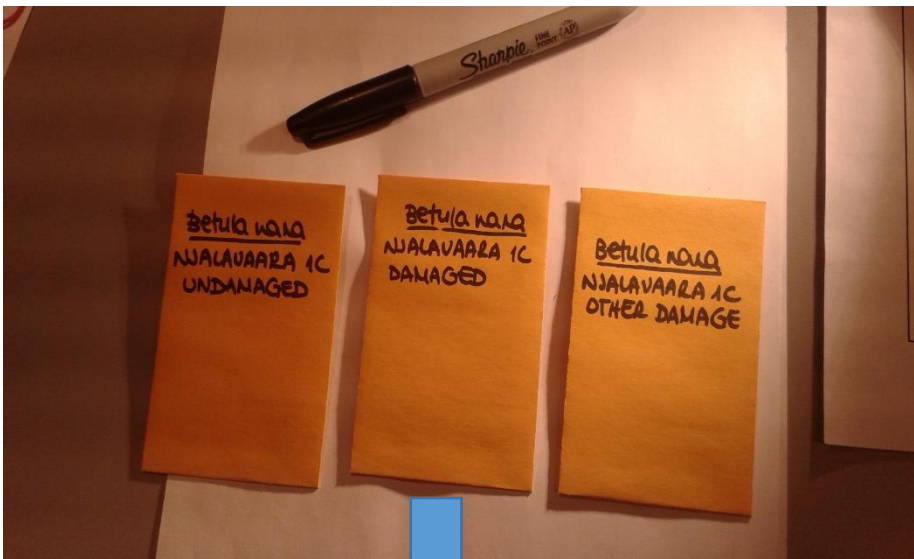
Inspect each leaf on both sides, first with a light source against the leaf to see damage on the surface, and then with the light through the leaf, to detect potential damage inside the leaf. In total, we are looking at each leaf **four times**. For example, some types of leaf damage might only be visible 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

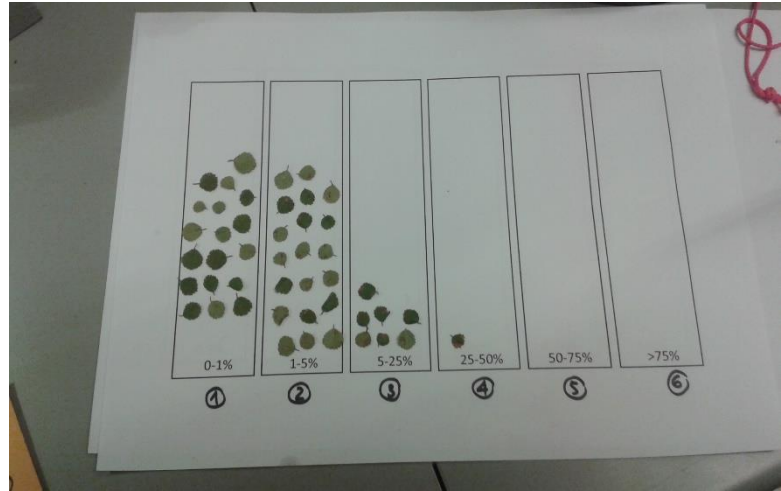
- Leaf chewers
- Skeletonized leaves
- Leaf galls
- Leaf miners
 - Serpentine mines
 - Other mines

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.

UNDAMAGED LEAVES LEAVES WITH INVERTEBRATE DAMAGE



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

[illegible]

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

[illegible]

4. DATA ENTRY

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

leaf_damage_Betula_nana - Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW

Clipboard Font Alignment Number Styles

AD1

Assessment of leaf damage

Number of leaves in each category

DAMAGE BY INVERTS

OTHER DAMAGE

undamaged leaf chewers

leaf mine (serpentine)

leaf mine (window)

skeletonized

galls (leaf)

galls (petiole) -- presence/absence

dark spots (lower part of leaf)

dark spots (upper part of leaf)

black dots (well defined)

tiny black dots

brown fungus

pink fungus (hyphae)

yellow fungus (rust?)

white fungus (surface)

necrosed tissue (mostly upper part)

edge fungus

long piercing

dead tissue along edges

necrosed spot (edge/tip, reddish)

necrosed spot (piercing)

mechanical damage

malformation

scraping

TOTAL NR LEAVES IN SAMPLE

LEAVES DAMAGED BY INVERTS

PROPORTION LEAVES AFFECTED

AREA AFFECTED BY LEAF CHEWERS

AREA AFFECTED BY MINES (SERPENTINE)

AREA AFFECTED BY MINES (WINDOW)

AREA AFFECTED BY SKELETONIZATION

AREA AFFECTED BY GALLS

Sample ID

EXAMPLE_1

25 4 2 1

4 2

42 7 0.2 0.7 0 0 0 0

template data_entry_sheet sample_metadata

Different categories for leaf classification, similar to the ones in the template

Each sample goes in a different row

Number of leaves in each category

Information for each sample (GPS coordinates, dominant habitat type... and any relevant additional information) is recorded in a different sheet

Summary columns