 Protocol for phenological and vegetation sampling on alpine grasslands

PhenoAlp team

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Introduction

PhenoAlp (2009-2011) is a new EU co-funded Interreg Project, under the operational program for cross-border cooperation Italy–France (Alps-ALCOTRA) 2007 - 2013, aiming to get a better understanding of phenological changes in the Alps. The main goals of the project are:

1- The implementation of an observation network in the involved territories (i.e. the Aosta Valley and the Savoies in the Western Alps);
2- The definition of a common observation strategy and common protocols;
3- The involvement of local community members (e.g. through schools) in the observation activities as a way to increase the awareness on the issue of the effects of climate change.

Project leader is the Environmental Protection Agency of Aosta Valley (ARPA Valle d’Aosta – IT) and the partners are the Alpine Ecosystems Research Center (CREA – FR), “Mont Avic” Regional Park (IT), “Massif des Bauges” Natural Regional Park (FR) and the Protected Area Service of the Autonomous Region of Aosta Valley (IT).

The PhenoAlp project is composed by 8 workpackages:
1. PhenoPlantes
2. PhenoDetect (remote sensing)
3. PhenoFlux
4. PhenoZoo
5. Inter_Phrone
6. Meteo_Reseau (Network of automatic stations of temperature)
7. PhenoForm (Training activities)
8. Pheno_Comm/Coord (Communication and coordination)

PhenoPlantes:

The aim of this activity is to develop climate change indicators from plants phenology. Regarding forest species (Picea abies, Larix decidua, Betula pubescens, Betula pendula, Fraxinus excelsior, Sorbus aucuparia, Syringa vulgaris, Corylus avellana, Primula veris, Tussilago farfara) the knowledge and the experience arising from the Phénoclim Project (www.crea.hautesavoie.net/eng/phenoclim/) will be transferred to the Italian partners territories (Aosta Valley). Observation protocols lead to the estimation of both the beginning and the end of the growth season by the monitoring of leaf development, flowering, leaf decolouration and leaf fall events. Observations will be done either by specialist and volunteers.

A new protocol for alpine grasslands is developed in the framework of the project. To be not dependent of the presence/absence of single species in each observation site, the protocol considers seven growth life forms: cyperaceous, poaceae (palatable and non palatable), evergreen and deciduous shrubs, forbs and leguminous. For each group quantitative variables (e.g. leaves length,
plant length, phenophase, ..) will be monitored along the growth season. Observation sites are located along an elevation gradient from 1560 masl (PNRB) to 2580 masl (GP); observation will be carried out on marked individuals located in permanent plots.

Phenological data will be processed to analyse the relationships between vegetation, climate and topography. This data set will allow us the application and evaluation of phenological models aiming to estimate the onset and the duration of the growth season.

The Inter_Phenop activities will allow us to correlate the phenology of the vegetation, animals and climate. Mismatches are expected results!

ALPINE GRASSLAND PHENOLOGY PROTOCOL

One of the aims of the project is to study some alpine grasslands located at different altitudes above timberline in order to determine the impact of climate change on vegetation: analyzing the vegetative and reproductive phenology of several functional growth-life types, and studying productivity thanks to phytomass and cover measurements.

Each zone consists of 2 sites along the elevation gradient. Each one is equipped by temperature stations (4 heights: -5 -soil temperature-, 0, 30 and 200cm) or IBUTTON (Dallas).

**Study zones:**

<table>
<thead>
<tr>
<th>Zone</th>
<th>sites</th>
<th>T° stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR - Chamonix Valley: Vallorcine-Loriaz</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>FR - Regional Natural Park of Massif des Bauges</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IT - Mont Avic Natural Park</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IT - ARPA zone (Torgnon Aoste Valley)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IT - Mont Emilius ZPS (SAP – Aosta Valley)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IT - Gran Paradiso National Park</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Observation Frequency: every week between April and July.

**Common environmental conditions:**

- above the top limit of forest
- southern slope
- absence of pasture or late pasture
- similar slope between the two plots; convex and concave areas are to be avoided (microtopography + snow accumulation in concave zones or early snow melting in convex ones)

**Number and size of plots:**

One study zone is composed by 2 or 1 site. 3 plots of 600m² (20x30m or 24x25m or 40x15m) are delimited for each site (at the same elevation). Three shapes are available to setup the study plot, allowing us to reduce the micro topography effects (rocks, concave and convex micro zones…). Each plot will be subdivided into grids (or subplots) of 4x5m (30 experimental subplots/plot) (Figures 1 and 2). The shape of each plot will depend on the topography and representativeness of a larger area (in adequacy with remote sensing analyses).
Figure 1: Plots and sites distribution by study zone

Figure 2: Shape and size plot
7 Growth life forms are monitored:

List of growth life forms studied in one of the study zones (Vallorcine-Loriaz):

- Grazed poacea: *Poa alpina*
- Non grazed poacea: *Nardus stricta*
- Cyperaceae (*Carex* / sedge): *Carex sempervirens*
- Evergreen shrub species: *Rhododendron ferruginum*
- Deciduous shrub species: *Vaccinium myrtillus* or *uliginosum* (present in marshes or in bogs)
- Forbs: (*Asteraceae*) *Arnica montana.*
- Leguminosae: *Trifolium alpinum* (alpine clover)

Sampling and marking efforts:

For each plot, 5 individuals of each growth life are monitored; this leads to: 35 (5 individuals x 7 growth-life form) plants per plot; 105 (35 x 3 plots) plants per site and 210 (105x2 sites) plants per zone. Plants are located in subplots (4x5m) randomly chosen up to reach the considered number of samples. Consequently a minimum of 5 subplots are considered among the 30 subplots available in each plot of 600m² (Figure 3).

**Important:** if a growth life form is not found in one subplot it should be found in the next etc… If a subplot is totally covered with rocks or stones, go to the next one.

The corners of each plot will be indicated by the use of a wooden stick. Location of the subplots and the plants will be done only once at the beginning of the season using a decameter and a plastic band to mark the grid. Each plant is marked thanks a small wooden stick put in the ground adjacent to the plant. Moreover, a cotton or woolen band is attached to the stem or branch.
Phenological measurements

Vegetative phenophases

1) Cyperaceae (Sedge) (Growth life form code: S): The longest five leaves of each plant (indicated with a red band) will be measured in each date. Ten leaves of the previous year will be measured at the beginning of the monitoring. Possible problem: at the beginning of the season there were frequently less than five leaves so the number of measurements taken is often lower than five.

For every visit, the plant length is measured (from base to the apex of stem or flowers). Before the flowering period plant_length = leaf_length mean. Be careful plant_length is different from plant height (some stems are curved)

⇒ Measurement: length of the five longest leaves [mm] + plant length [mm]

2/3) Grazed and non grazed poaceae (Growth life form code: GG and NGG): ratio between the leaf length and the maximum length measured during the season. Length of the five (Nardus stricta) or of less than five leaves (Poa alpina) was taken during the season. Total length of the plants was also measured in Poa alpina. If the phenological reproductive phase is NF, total length of plant is the mean of the five longest leaves.

⇒ Measurement: length of the five longest leaves [mm] and total length of the poaceae [mm].

4/5) Shrub species: Only one branch of the shrub will be marked with a band and observed.

4) Evergreen shrubs (Growth life form code: SE): For one marked branch, we consider only the vegetative phenophase of the marked ramet: without bud (NB), foliar bud (LB), light green leaves (L). Please do not mark one ramet with a floral bud or flower of the previous year.

For one marked ramet, we measure the length of the longest five green leaves (ongoing year “t”) and its phenophase. For the marked branch, we consider the total length of a marked twig, from the base of the part produced during the year to the apex of the leaf. Plant_lengthSE = new ramet length (or new green part). The longest 5 leaves are measured, from the petiole (included) to the apex.

⇒ Measurement: vegetative phenophase of the ramet (NB, LB or L) + length of the five longest leaves of the marked ramet (only new green leaves) [mm] + new ramet length [mm]
5) Deciduous shrubs (Growth life form code: SD): For one marked branch the most advanced vegetative phenophase is indicated: without buds (NB), closed or partially closed foliar bud (LB), completely burst leaf green-light leaf (L), developed dark green-dark leaf (DL), dry or yellow leaf (YL). The phenological phase is indicated as a mean for the whole branch. The ramet length is measured (its growth has been measured from the base of the plant to the apex of the leaf). None leaf length is measured (in the data sheet, we note NA).

⇒ Measurement: most advanced vegetative phenophase + length of the marked branch (from base to the apex of the leaf, including the previous and current produced parts (whole plant).

6) Leguminous (Growth life form code: L) (e.g. Trifolium sp.): Length of the central leaflet of the present leaves (up to the 5 longest leaves). During the season some leaves die and new leaves form. These leaves are counted but not measured. Total plant length from base to flowers is measured at each visit.

⇒ Measurement: length of the central leaflet (maximum 5 central leaflets of the biggest leaves) [mm] + plant length [mm]

7) Forbs (Growth life form code: F) (Astéraceae: e.g. Arnica montana): Length of leaves from the petiole to the apex. We measure five longest leaves. Plant length is measured when the floral stem appears. If the phenological reproductive phase is NF (without flowers), plant length is 0.

⇒ Measurement: length of 5 leaves [mm] + plant length [mm]

Reproductive phenophases

To monitor the reproductive phenology, 5 phases are used for all the growth life forms. Depending on the species and difficulties to distinguish the phenological phases, some intermediate phases can be considered. Possible problem: some of the individuals chosen at the beginning of the season could not reach the reproductive phases (Arnica montana, Trifolium alpinum, Carex sempervirens)

Only the most advanced and observed phenophase is considered

<table>
<thead>
<tr>
<th>Reproductive phenophases</th>
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<tbody>
<tr>
<td>Without flowers: No flowers</td>
<td>NF</td>
</tr>
<tr>
<td>Flowers in the sheaths, closed spikelets, closed flowers</td>
<td>C</td>
</tr>
<tr>
<td>Open flowers, Stigmatas developed, open spikelets, stigmatas receptive</td>
<td>O</td>
</tr>
<tr>
<td>Dry flowers or fruits, beginning fruit development, developed seeds, fruits</td>
<td>F</td>
</tr>
<tr>
<td>Loss of fruits or seeds</td>
<td>LF</td>
</tr>
</tbody>
</table>

For shrubs, we note the most advanced reproductive phenophase observed for the marked branch.

More details like, receptive and withered stigmatas, dry flowers, beginning of fructification, and ripe fruits can be noted. For each growth life form, except shrub species, the length of the floral stem is measured: Sedge, Poaceae grazed and non grazed, Asteraceae and Leguminosae. This measurement combines Vegetative and Reproductive approaches.
Images of the reproductive phases for some example species of each growth form

*Trifolium* sp.

Vaccinium myrtillus

Carex sempervirens

Nardus stricta

Arnica montana
Update: 14 – Jan - 2010

**Rhododendron ferrugineum**

Phenophases correspondence:
- **NF** without floral buds
- **C** Stages 1 – 3
- **O** Stages 4 – 5
- **F** Stages 6 - 7
- **LF** Loss of seeds

Different phases can be adopted for *Rhododendron ferrugineum*:

- **Stage 1**: gemma floral shules, caryle strettamente erboticola

- **Stage 2**: gemma floral stems, caryle diagoatole, cocule in distensione

- **Stage 3**: cocule diagoato, fuso clusima

- **Stage 4**: cocule po fuso apoita

- **Stage 5**: cocule affoita

- **Stage 6**...

- **Stage 7**...
**Senescence**

Senescence observations will be done only in two zones (Vallorcine and Torgnon). The protocol is still under development. Variables are not well defined for this first year. It seems possible to quantify the percentage of senescent tissues, for example number of partially or totally yellow and dry leaves on each plant. Measurements will be done after the second half of August.

**Cover and phytomass estimations** (once a year at the peak of productivity)

Vegetation cover will be calculated thanks the point contact method for each plot. Two measurements are done for each subplot (maximum 5 subplots) where phenological monitoring is done. Each contact and specie is registered to supply a diversity index. The point contact square is randomly located in the subplot (Figure 4). Using, 2 squares per subplot, we obtain 2 squares x 5 subplots x 4 spines = 40 points.

Alternatively, for each plot a linear transect has been positioned: 50 points for 25 m (one point every 50 cm). Each contact and species is registered to supply a diversity index. This method avoids to consider the spatial heterogeneity of the plot (600m²).

In order to estimate the plot phytomass value, we use relationships established previously for each growth life form between the vegetation cover and the phytomass. We correlate phytomass, collecting the phytomass of one species between the four iron sticks of the Point contact quadrat, with its cover estimation. Monospecific patches are used. A minimum of 30 measurements for each species are required and distributed in the range of variation.

![Figure 4: Design of the Point Contacts measurement](image)

**Greening measurements** (each week)

Greening measurements are done only in two zones (Vallorcine and Torgnon). Digital pictures are taken weekly on the four corner of each plot (12 images every week). At the same time a visual estimation of the green and dry vegetation cover is carried out (10, 20, 30, 40%...). The images are taken from fixed points. Automatic mode (exposures and time), high resolution,
fixed height (1-1.6 m) and diffuse light (to avoid as much as possible shading effects) are the criteria followed to take digital pictures. The images will be elaborated building a time series of greenness indexes (built from the RGB channels). The indexes will be calculated considering the pixels located inside the area delimited by a PVC tube frame of 50x50 cm (Figure 5). The grey color of the PVC frame or a plastic test pattern is used for the color calibration. From the time series of the greenness indexes we will derive the phenological phases of the grassland. Be careful with the shade of the camera.

Figure 5: Site Loriaz-Vallorcine; Plot X; Corner n° 1

**Biomass and LAI measurements** (each 15 days)

Torgnon zone: we evaluated the biomass in 12 squares (the four corner of each plot) of 30 x 30 cm considering both dry and green biomass (dry matter after twenty four hours in the oven at 60 °C). on the same samples LAI measurements are done (scanner)

Please find enclosed the field data sheet. This format allows us to use the same sheet to digit data at the office and to process it with R.

GIS projection: WGS84 UTM zone 32

<table>
<thead>
<tr>
<th>1 ZONE</th>
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</thead>
<tbody>
<tr>
<td>2 SITES</td>
</tr>
<tr>
<td>3 PLOTS (600m²)</td>
</tr>
<tr>
<td>30 SUBPLOTS (20m²)</td>
</tr>
</tbody>
</table>