PLANT RESPONSE VARIABLES

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Introduction

This chapter deals with monitoring of the plants' responses to ITEX temperature enhancement with Open-Top Chambers (OTCs) or ITEX Corners. In this context, controls in non-manipulated situations are essential. Furthermore, permanent tagging, also of control plants during the implementation of ITEX, could prove to be extremely valuable in the future as biological monitoring stations during an anticipated climatic change. Therefore, careful tagging of the selected plants using long-lasting materials is important and worth the effort. Note that all ITEX monitoring should be non-destructive, thereby maintaining the informative value of the selected plant individuals for many years.

After the general recommendations for sampling, tagging, etc., the eight ITEX Group 1A species or species groups are presented (one by one, in alphabetical order), and the selected phenological and quantitative response variables defined. The present selection and definition of response variables was approved during the Fourth ITEX Workshop at Oulu, Finland, in December 1992, and updated during the Fifth ITEX Workshop at St. Petersburg, Russia, in March 1994. For each species there is a protocol (ITEX report form) in the Appendices to the Manual (VII–XVI). Each sheet can accomodate eight different phenological dates (P1–P8; all to be given as day numbers [see Appendix 1]), and eight quantitative measurements (Q1–Q8) for 20 experiment plants (in OTCs or ITEX Corners), and 20 control plants. If more than 20 + 20 plants are monitored, just add an extra protocol page and re-number the plants (left column). The blank protocol (Appendix XVII) can be used for Group 1B species, where any suitable response variable can be defined, and for more detailed studies of 1A species if additional variables are desired.

The selected response variables cover events during most of the vegetation period. This does not imply that sites operating over shorter periods of time are excluded; reports on smaller numbers of response variables are equally valuable for among-year and inter-site comparisons as long as the 20 + 20 minimum sampling design applies. Try to include as many of the ITEX species as possible in your monitoring program; even if you are not able to carry out the warming experiment for all of them, your monitored unmanipulated controls may turn out extremely valuable in a near future if the anticipated warming of the Arctic proceeds according to the IPCC prognosis.

All ITEX sites should communicate their results to the respective "species co-ordinator". The resulting publications will be of the multi-author type, including all active collaborators.

Please communicate experiences of the methods, particularly the bad ones, and suggestions for improvement to the authors or to the ITEX secretariat. Comments and additions to the Manual will appear in the ITEX Update newsletter.

General Recommendations

Sampling

Random sampling of study plants is recommended, but in homogeneous habitats with relatively even distribution of plants, a systematic design (e.g., grid-net) is equally adequate and more easy to overlook and manage. Sampling at regular intervals along random transects is often the best method. In cases of sparse distributions, every possible plant will be involved in the monitoring. Controls should be selected in the same way as the typically 20 experiment plants (genets or ramets depending on species, habitat, and growth form); they should be of the same number and represent a similar distribution of size classes and developmental stages, and they should be situated as close as possible to the OTCs or corners without being influenced by their presence (i.e., at least 1 m apart in the case of OTCs, less for ITEX Corners). In order to avoid pseudoreplication (see Hurlbert, 1984) make sure that each plot, OTC, or part of OTC has its own parallel control.

For species with tufted or cushion-like growth forms, entire clones (genets) should be used as monitoring units. In cases where clones are difficult or impossible to discern (as in rhizomatous and mat-forming plants), sample plots should be used instead (0.5 x 0.5 or 1 x 1 m squares). It is often good advice to divide the ground surface covered by an OTC into four quarters, and select the specimen closest to the center of each quarter for monitoring; in that case the control plot beside the OTC should be of the same size and divided in the same way. For woody plants where genets can be difficult to separate or are too large to be handy (e.g., *Cassiope* and *Salix*), branches (ramets) are easy to delimit and should be used as the monitoring units. Note that there is one phenological variable common to all protocols, namely P1: date when the ground is snow free at the sample point.

When chosing sites and plots, try to find those as closely representative of the climate station site as possible. Choose horizontal areas if possible. Avoid areas with extreme aspect. Gentle, north-facing slopes could also be considered; a transect down slope might also be suggested.

Always make detailed map of the sites; use theodolite if available. Please undertake an indepth site description, including location, aspect, materials, drainage, floristic composition, etc. Information on depth and dissappearance of snow at study plots are very valuable, if available (see Snow & Ice). Photographic documentation of sites is extremely valuable, especially in a longer perspective and if photos are adequately filed or published.

Permanent Marking

Each study plant or plot should be labeled with a code number (E1–E20 for experiment plants, C1–C20 for controls). Labeling should be made using metal tags. There are many possibilities, but the most practical ones are (1) soft aluminum write-on tags that you can emboss with a pen, and (2) aluminum DYMO™ bands. More elaborate (and expensive) methods include bird banding rings (for branches of woody plants) and metal signs of the botanical garden type.

For cushion plants and tufted clones ("Sax opp", *Eriophorum vaginatum, Oxyria*, in certain habitats even *Cassiope tetragona* and *Dryas*), use soft steel wire ca. 1 mm diam., 20–30 cm long. Form it into a U-shape, penetrate the soil with one end, some 5 cm from the clone center, and push it down to encircle parts of the root system. Push it out until it surfaces. Attach metal tag, and twist together the ends of the wire.

For tagging ramets of woody plants like *Cassiope tetragona* and *Salix arctica*, just attach the metal label to the stem with wire, making sure it is not too tight. It is good advise to draw a diagrammatic sketch map of each ramet. Annual growth increments are usually easy to delimit; if there are problems seeing where previous growth ceased, consider some kind of marker (e.g., "White Out") to mark ends of branches at the end of the season.

For marking permanent square plots (0.5 or 1 m square), consider corner marks of aluminum profile or stiff, plastic tubing, stuck in the ground to a depth of 0.3–0.5 m with only 2–3 cm visible above ground. Drill holes in the corner marks ca. 1 cm from top and outline the square with 2 mm white polyester rope. An aluminum label with the appropriate code can be attached to one of the corner marks. Again, we recommend drawing a sketch map for each square plot.

If the study site is regularly visited by tourists, don't forget to put up information signs. And if your study area is frequented by grazing animals, it may be necessary to fence the control plots or plants with chicken wire.

Monitoring

All ITEX monitoring should be non-destructive. The response variables are grouped in two main categories, phenological (P) and quantitative (Q), both comprising vegetative as well as reproductive traits. Phenological dates are always recorded as day numbers (Julian dates; see Appendix 1). Plants should be monitored daily, if possible – particularly during periods of rapid change, such as break of dormancy, onset of flowering, and fruit maturation; during periods of slower progress, monitoring every 2–3 days is sufficient. Observations on a particular plant or set of plants should always be made at the same time of day!

For determination of weight of fruits, seeds, leaves, etc., store the plant parts dry in paper bags for two months at room temperature before weighing.

Seed germinability

If possible, make a germination experiment for each species monitored at your site. Pool all seeds after weighing, and divide the entire sample into four weight classes. Subsample by random into four replicate sets of 20 seeds from each class, saw the seeds on filter paper in Petri dishes, soak them with clean water and put the dishes in a randomized block design under 16 hours of light per day at ca. 22°C. Take daily records of the number of germinated seeds per dish and remove the germinated ones instantly. Calculate the mean weight for each weight class. With those results at hand you may now plot seed weight against a fitness parameter (germinability or germination rate); by simple regression calculate the appropriate formula to be applied to your data sheet. Determine the minimum weight for seeds to be germinable at all, and for all sampled seeds above that threshold value, use the formula to convert weight into a more refined estimate of reproductive success (RS). The simplest one is germinability (the percentage of germinable seeds) but a better estimator of RS is relative germination rate (Molau, 1991). Use the formula

$$\text{relGR} = \sum[(S^t - S^{t-1})/(N \times \ln t)]$$

where S^t is the number of seeds germinated until day t, and N is the total number of seeds in the subsample. Replace seed weights in your data matrix with relGR values according to your regression formula. This method was very successfully used for seeds of *Dryas*, *Eriophorum*, and *Ranunculus*, and for *Polygonum* bulbils from Latnjajaure. It is good advice to carry out this experiment once per species at each ITEX site, since seed weight relations with RS estimators may differ among sites within the same species.

Miscellaneous

Take detailed notes on all kinds of disturbances (including timing) on the selected plant individuals, e.g., damages by grazing, fungal infestation, seed predation by insect larvae, etc. Also take notes on occasional snow cover during the summer, and how periods of freezing affect the plants. For example, Cassiope flowers may drop at peak anthesis

if exposed to below-freezing temperatures for several days, thereby spoiling most of the sexual reproduction of the season.

Note the general state of the same species in the area: are the monitored plants in synchrony with their neighbors? Take notes on aberrant phenological events in the area, e.g., early flowering by early emergence from snow or on south-facing slopes. Some of the early-flowering species may have a tendency to re-flower in late summer (late August) if the microsite has been unusually warm. Watch out for this phenomenon, especially in the OTCs. It is not uncommon in *Saxifraga oppositifolia* and may appear also in *Cassiope tetragona*. Flowers of such "second waves" represent bud-break one season too early and are rarely perfect; usually the anthers are poorly developed, and the flowers will thus be functionally female and will not set seed.

Permanently marked genets, ramets, and plots can also be used for monitoring of flowering phenology (pattern, velocity, and density). Simply count the number of open flowers per sample plot at even intervals, daily if possible. Take the records at the same time of the day throughout the flowering period. This simple investigation usually yields very useful results, where the shape of the curve for a population is correlated to reproductive strategies of the species. Early-flowering outbreeders show innate, dome-shaped, unskewed curves, where there is no difference in shape among years, but height of peak flowering (absolute maximum momentaneous number) is correlated to climate and performance. Opportunistic and predominantly selfing species, on the other hand, show more ragged curves. Differences between years or species can be tested with a modified *t*-test (see Molau 1993b for further details).

Take notes on observed insect pollination (note pollinator, timing, and activity). Finally, note natural germinantion of the monitored species. Does it occur at all?

Species-Specific Response Variables

Bistorta vivipara

The Alpine Bistort, *Bistorta vivipara* (L.) S.F. Gray *(former: Polygonum viviparum* L.), is common throughout the Arctic as well as in subarctic and temperate alpine areas. It is a late-flowering species thriving in more nutrient-rich habitats, such as alpine meadows. The inflorescences comprise an unusual mixture of bulbils (vegetative diaspores) in the lower half and sexual flowers in the top portion (sometimes missing). The flowers are mostly female, and sexual reproduction does not occur in most populations, although specimens with hermaphrodite flowers and seed production have been found in the Arctic and the Alps (Bauert 1993)

It possesses rhizomes, and clones (genets) are hard to delineate, even though variation in bulbil color may be helpful. The bulbils are vigorous diaspores, and newly germinated ones are a common sight in seepages and along creeks in late summer. Bulbil weight has turned out to be highly sensitive both to temperature and to nutrient manipulation (Molau, unpubl.; Wookey et al. 1994). Bulbil color reflects genetic variation within and among populations (Bauert 1993), and the bulbils are furthermore an excellent material for cloning in common garden experiments and for allozyme electrophoresis. Germinability of bulbis can be tested in the same way as for seeds (see Plant Response Variables)

Randomly select 20 OTC and control plants in your plots, or divide each plot into four quarters, later selecting the reproductive shoot appearing closest to the center of each quarter as the specimens for monitoring. Make a note if they do not develop an inflorescence in a specifik year, which can be used to compare reproductionintensities between years. Randomly select supplementary individuals for all non-reproducing plants each year in order to achieve a total sample of 20+20 plants with inflorescences. This species, particularly the inflorescences, is also very palatable to grazers, and at some sites fencing of controls with chicken wire can be necessary. Note when infloresences or leaves have been lost due to grazing.

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: First leaf unrolls (original set of plants)

P3: Inflorescence appears between sheath (=ochrea; original set of plants)

P4: First flower open (original and supplenentary plants)

P5: First bulbil shed (drops off when touched; original and supplement plants)

P6: First seed dispersal (optional, since rarely observed sexual reproduction)

QUANTITATIVE MEASUREMENTS

Q1: Length of inflorescence stalk (at full flower; from ground to top of raceme, in mm)

Q2: Width of largest leaf (in mm)

Q3: Number of leaves per individual

Q4: Number of bulbils per shoot

Q5: Number of flowers per shoot

Q6: Relative proportion of bulbils (Q4/[Q4+Q5])

Q7: Color of bulbils (make up your own, site-specific color scale)

Q8: Mean bulbil weight (mean ± SD, in µg); optional

Carex stans

The rhizomatous sedge *Carex stans* Drej. (= *C. aquatilis* Wahlenb. subsp. *stans* (Drej.)Hult.) is common in moist tundra throughout the High Arctic. Flowering tillers have 1–2 terminal male spikes and 2–3 female spikes (make

sure that the selected ramets are bisexual!). Clones are normally impossible to delineate; for this reason, please identify and mark individual reproductive culms at least 1 m apart (alternatively small sample squares within OTCs and outside for controls). Note age of monitored shoots; they can be aged by the number of attached dead leaves at the base, and at least three age categories are discernable: new shoot, 1 year old, and 2+ years old. Flowering tends to occur in older shoots, and they die off one season after flowering. Leaf growth can be non-destructively monitored by the same method as used for *Eriophorum vaginatum* (see below).

If *C. stan*s is not present (alpine and Low Arctic sites), ***Carex bigelowii*** Torr., of the 1B list could be used; it grows in drier situations but shares many properties with *C. stans*. In that case, take voucher herbarium specimens, since *C. bigelowii* is a circumpolar taxonomic complex, not fully understood at present.

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: Emergence of first new leaf

P3: First stigmas visible

P4: First anthers exposed

P5: First yellowing of leaves

P6: First seed shed

QUANTITATIVE MEASUREMENTS

Q1: Age class of shoot in flower

Q2: Length of flowering stem to base of terminal spike (at full flower; accuracy 1 cm)

Q3: Number of green leaves (at full flower)

Q4: Length of longest leaf (accuracy 1 mm)

Q5: Total green leaf length per tiller (mm)

Q6: Weight of mature utricles (accuracy 0.1 mg ± SD; optional)

Additional data (optional, no protocol provided): Length of all green leaves (measurements should be made periodically throughout the summer and could be time consuming; G. H. R. Henry, pers. comm.).

Cassiope tetragona

The Arctic White Heather, *Cassiope tetragona* (L.) D. Don, the species of the ITEX logotype, is circumpolar and often dominant in the arctic tundra. Clones are sometimes, but not always, easy to delimited. They may grow extremely old, and several hundred years old clones may attain a ring-like shape in homogeneous substrates. For ITEX purposes, please select main, vigorous branches (ramets), tagged close to the ground. Draw diagrammatic sketch maps of the selected ramets (length of all modules, positions of branchings and old flowers). If clones are not discernable, leave at least 1 m between selected ramets.

The leaves are evergreen and last for many years; annual growth increments are usually easy to delimit, and the species has unique properties as a monitoring tool for climate-related retrospective growth analysis (see Callaghan et al. 1989). The species is moderately early-flowering ("early aestival"), and is uncommon on exposed ridges and in late-thawing snowbeds. The flowers are largely self-pollinated, although pollination by bumblebees has been observed. The capsules split open very late in the season and may even over-winter before dehiscence; please look for this.

Manipulation responses in this species have been extensively studied over several years by Havström et al. (1993) at three sites: one in the High Arctic (Svalbard) and two subarctic-alpine sites (low alpine and high alpine, respectively) near Abisko in N Sweden. In unmanipulated plants there were significant altitudinal and latitudinal gradients in vegetative growth variables. Plants at higher altitudes or latitudes produce shorter shoots (annual growth increments), and fewer but heavier leaves than at low altitudes/latitudes. These gradients probably reflect a lower turn-over rate of green leaves at high altitudes/latitudes, governed by shorter snow-free period and growing season, an adaptive adjustment for maintaining a positive carbon balance. Similar response gradients on a smaller scale are observed along a snow-cover gradient at the ITEX site at Latnjajaure (U. Molau, pers. obs.). This is in accordance with the findings of Kudo (1992), who found that life-spans and weight of leaves increased with decreasing snow-free duration in evergreen species, whereas the opposite trend was evident in deciduous species. Havström et al. (1993) report significant positive responses in leaf number per annual growth increment in temperature enhancement experiments at high altitudes/latitudes, but none at the low-alpine site.

Note any tendency for re-flowering, in tagged ramets as well as in the study population in general. Fungal infestation of leaves (whitish, swollen) is common in late summer; note frequency in study ramets.

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: First coloring of flower buds (whitish-yellow, protruding)

P3: First elongation of pedicels

P4: First open flower

P5: First corolla drop

P6: First capsule splits open – if possible

QUANTITATIVE MEASUREMENTS

Q1: Total number of flowers per ramet

Q2: Total number of developing capsules per ramet

Q3: Fruit:flower ratio (Q2/Q1)

Q4: Length of annual growth increment (main shoot, accuracy 1 mm)

Dryas

The White Dryas or Arctic Avens, *Dryas integrifolia* Vahl, and its close relative *D. octopetala* L. (Mountain Avens) are characteristic of drier tundra sites throughout the Arctic. Both species are woody chamaephytes (dwarf shrubs), and as they agree in most ecological traits, either one can be used for ITEX monitoring. *Dryas integrifolia* is basically Nearctic, whereas *D. octopetala* (here treated in the broad sense, including *D. punctata* Juz.) is almost circumpolar. The plants form tussocks or mats; clones may attain high ages and are often difficult to delineate in the Low Arctic. The leaves may be summer-green or evergreen (take notes!), the margins are entire in *D. integrifolia* and crenate in *D. octopetala*. In interior Alaska there are two distinct ecotypes of *D. octopetala*, often growing almost side by side: besides of the circumpolar small-leafed and deciduous subsp. *octopetala* in fellfield sites, there is a large-leafed evergreen form (subsp. *alaskensis* Hult.) in snowbed sites (see McGraw and Antonovics 1983).

The showy white flowers are heliotropic and mainly pollinated by flies. They are usually perfect and comprise numerous bright yellow stamens and green pistils. The flowers are usually weakly protandrous (Philipp et al. 1990) and self-compatible, even though seed set is highly reduced when selfed (U. Molau, unpubl. data). *Dryas integrifolia* is usually andromonoecious (i.e., most genets produce some male (or female-sterile) flowers in addition to the perfect ones). Purely male flowers are rare in *D. octopetala*, but this species appears in gynodioecious populations in some areas (i.e., populations with a certain fraction of male-sterile (functionally female) genets); such populations are not uncommon in subarctic Fennoscandia (U. Molau, pers. obs.). The plumed seeds (achenes) are dispersed by wind. The plumes of fruiting styles twist together at first, but untwist at maturity.

For ITEX monitoring, choose 20 clones (tufts) for temperature enhancement experiments and 20 for controls; if the growth form is matted, select 20 + 20 plots (0.5 m square), or use at laest 5 OTCs + 5 equal-sized control plots beside the OTC, divide their ground surface into four quarters, and select the plants closest to the centers of each quarter for monitoring.

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: First leaf erected

P3: Appearance of first color (white tip) of flower bud (= bud break)

P4: First open flower

P5: Last petal shed (pull gently if needed)

P6: First twisting of maturing seeds (or observation of no twist at all)

P7: First seed dispersal (pull the elongated, barbed styles gently)

P8: First yellowing or browning of leaves (summer-green forms)

QUANTITATIVE MEASUREMENTS

Q1: Dimension (area) of clone (accuracy 0.1 m^2); if square plots are used, give plot size instead

Q2: Total number of flowers per clone or plot

Q3: Length of longest new leaf (at the time of petal shed; petiole not included; accuracy 1 mm)

Q4: Length of pedicel (from axil to base of flower; at the time of petal shed; accuracy 1 mm); if entire clones are monitored, give mean length ± SD of all pedicels.

Q5: Number of seeds per flower (optional)

Q6: Mean seed weight (± SD) in µg (optional)

Q7: Seed yield per flower (Q5 x Q6; optional)

Q8: No. of flowers (of total) destroyed by caterpilars

Note any insect predation on leaves, flowers, or seeds, since this can interrupt normal development. Be especially aware of the presence of the aphid *Mysus polaris* on the roots (may be dificult to detect) and caterpillars of the moth *Sympistis zetterstedtii* on the sexual parts of the flowers, and of the seed bug *Nysius groenlandicus* on the seeds (Achenes) Also take notes on the floral structure of the population (gynodioecious, andromonoecious) and whether plants are ever- or summer-green.

WARNING: Avoid male-sterile clones in gynodioecious populations of *D. octopetala*; these have flowers with the androecium reduced to a ring of 1–2 mm high, brownish staminoides.

Eriophorum vaginatum

The Sheathed Cottongrass, *Eriophorum vaginatum* L., forms compact upstanding tussocks in marshy and peaty areas, often in permafrost areas with a thin active layer. The clones (genets) are easy to tell apart, and tussocks should be used as monitoring units for ITEX. The culms are normally 20–40 cm tall, and the heads are solitary and not subtended by leafy bracts. The species is relatively early-flowering throughout its range, and has been subject to intense investigation in Alaska (e.g., Chester and Shaver

1982, Fetcher and Shaver 1983, Lariguadrie and Kummerow 1991, Mark et al. 1985, McGraw 1993, Murray and Miller 1982, Shaver et al. 1986, Tissue and Oechel 1987). Prior to an experiment, cut all old heads away (wool may persist for years after poor summers and cause confusion) in order to create a blank base-line.

According to our experiences from Latnjajaure (Molau, unpubl. data), the inflorescences are close to the ground when in flower, an the subsequent elongation of the shafts reflects the proportion of fertilization of the ovules. OTCs may restrict pollen-flow in this wind-pollinated species, and we got a smaller elongation of shafts in the OTCs than in the control plants. Thus, the length of inflorescence shafts cannot be used as a response variable related to experimental warming, but is informative with regard to reproductive success.

Try to select tussocks of the same size for experiment and control; we have indications that growth (leaf length) and tussock size are positively correlated.

Leaf growth per tiller can be monitored non-destructively following a method elaborated at Toolik Lake in Alaska by Gus Shaver and collaborators; this method has also been successfully used at Latnjajare. Select a tiller in the central part of the tussock; try to find a tiller that is clearly delimited from neighboring ones and shows no sign of being close to flowering (tillers live for about 4 years after which they produce a torpedo-shaped leaf sheeth in the center containing the inflorescence bud in late summer; after flowering the subsequent season that tiller will die and be replaced by doughter tillers). Normally a vegetative tiller of average size comprises a number of dead persistent leaves around a few (usually 2–4) live ones (mean life span of individual leaves is a little more than 1 year). Now gently cut all the dead leaves at a similar height close to the tiller base. This will leave you with a "stub" that can be used as a base-line on which a mm-scale ruler can rest when the length of each live leaf is measured. The first census should be made at thawing time, and the progress monitored at even intervals throughout the growing season. We have used 10d intervals, but even 30d intervals would give decent results. Mark the tiller so that you can easily spot it – we use colored plastic paper clips with the central portion removed, put around the tiller base and fastened in the tussock with a piece of thin steel wire. Number the live leaves (i.e., leaves with some green portion) starting from the oldest live leaf (the one with least green). It is recommended that you measure green and dead portions of all leaves, as you then easily will recognize every individual leaf the next time. Give numbers to new leaves as they appear. Add constantly to your base-line by cutting leaves as they die. With this data set, you can calculate the total annual leaf growth in the tiller. You will also be able to calculate senescence rate, live leaf number, average life spans of individual leaves, and turnover.

Seed weight gives nice results in this species, and the seeds are easy to handle. Remove the pappus (wool) before weighing.

Eriophorum vaginatum is essentially circumpolar, but rare in the semi-arid Arctic, e.g., Ellesmere Island. Where *E. vaginatum* is absent, *E. triste* (Th. Fr.) Hadac & Löve (= *E. angustifolium* Honck. subsp. *triste* (Th. Fr.) Hult.) can be used as an alternative species; note that this taxon is rhizomatous, and sample plots, not tussocks, have to be selected as monitoring units.

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: Appearance of first inflorescence bud

P3: First open flower (= first anthers exposed)

P4: First seed shed

QUANTITATIVE MEASUREMENTS

Q1: Diameter of tussock (average, horizontal, to tips of leaves); accuracy 1 cm

Q2: Number of flowering stalks per tussock

Q3: Mean length of 10 longest leaves (from tip of sheath to apex) ± SD; accuracy 1mm

Q4: Tiller growth (total annual leaf production per tiller in mm; optional)

Q5: Seed : Ovule ratio (optional)

Q6: Seed weight (mean ± SD; accuracy 0.01 mg; optional)

WARNING: In some areas, *E. vaginatum* is reported to be gynodioecious, where male-sterile plants have vestigial stamens about 1 mm long including the filaments (Stevens and Blackstock 1993). For ITEX purposes, select clones with normal anthers only.

Oxyria digyna

The Mountain Sorrel, *Oxyria digyna* (L.)Hill, is common throughout the Arctic and also widespread in subarctic and temperate alpine areas. It grows in wide variety of adverse or disturbed habitats, often in damp situations, and is the only typically late-flowering species among the ITEX 1A plants. For further details on the ecology of the species, see Humlum (1981).

It has short fleshy yellow rhizomes, and clones (genets) may sometimes be hard to delineate, especially in late snowbeds. Each clone produces one or several compound paniculate racemes, but some plants do not flower every year. The tiny flowers are open when the bushy red stigma is visible. The fruit is a flattened nut with a winged pericarp.

Select dense and distinct clones for ITEX purposes; do not use situations with many small flowering individuals in dense stands. Look for old inflorescences or stalks to find reproductive clones. *Oxyria* plants, particularly the inflorescences, are very palatable to grazers, and at some sites fencing of controls with chicken wire can be neces-

sary. Do not abandon a vigorous clone if it should not be flowering for a year or two, also 0 inflorescences is a quantitative measurement of interest to ITEX. Note when infloresences or leaves have been lost due to grazing.

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: First leaf unrolls

P3: First visible inflorescence (at ground level between petioles)

P4: First flower open

P5: First seed dispersal (i.e., when fruits fall off easily by touching the plant)

QUANTITATIVE MEASUREMENTS

Q1: Number of inflorescences per clone (0, 1, 2, etc.)

Q2: Length of inflorescence stalk (at full flower; from ground to base of raceme, in mm)

Q3: Width of largest leaf (in mm)

Q4: Number of mature fruits per plant (harvest in paper bags, one per clone)

Q5: Mean fruit weight (weigh all fruits from a clone, dried at room temperature, as a single batch, calculate mean fruit weight; accuracy 0.1 mg).

Ranunculus nivalis

The Snow Buttercup, *Ranunculus nivalis* L., is almost circumpolar, often abundant in moist tundra, flowering near the edge of the melting snow. Prefloration time (i.e., period from thawing to flowering) is rather short, 5–15 days depending on weather conditions. Individual plants (clones) may be hard to delineate; in those cases select square plots and mark individual flowering stalks at least 0.5 m apart for monitoring.

The flowers are born singly on erect pedicels. The flowers are robust, open, and easy-to-handle. Flies are the main pollinators, and despite some self-compatibility, insect visits are required for seed set (U. Molau, unpubl. data). The fruits are one-seeded nutlets; usually there are 30–70 nutlets per flower. For further details, see Philipp et al. (1990).

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: Flower open (attaining bowl shape)

P3: Last petal shed

P4: First seed dispersal (NB! Start harvesting nutlets at this point)

P5: First yellowing of leaves

QUANTITATIVE MEASUREMENTS

Q1: Height of flowering shoot from ground to base of flower (in mm)

Q2: Width of largest basal leaf (in mm)

Q3: Number of nutlets per flower (harvest in seed bags)

Q4: Mean weight of nutlets (± SD, in µg; optional)

Q5: Seed yield (Q3 x Q4)

Q6: Seed : Ovule Ratio

WARNING: Do not confuse with other co-occurring *Ranunculus* species; at Latnjajaure *R. acris* L. starts flowering in the same square plots about two weeks after the last *R. nivalis* petal has been shed.

Salix

No arctic willow is even close to being circumpolar in distribution. Since they constitute such an important counterpart of the tundra vegetation, four different dwarf-shrub species with roughly the same ecological properties have been selected: *Salix arctica* Pall., *S. herbacea* L., *S. polaris* Wahlenb., and *S. reticulat*a L. Together they cover the entire Arctic, and one or two, sometimes three, of these species will be present at each ITEX field site. We recommend that each site undertake monitoring of at least one *Salix* species.

All willows are perfectly dioecious, but the frequency distribution of the sexes may vary among populations (see Crawford and Balfour 1983) and year (M.A. Lohiluoma pers. obs.). As in all arctic plants with spatial gender separation, they are generally early-flowering. Nevertheless, plants of *S. herbacea* (the species having the widest ecological amplitude of the four) are commonly found even in late-thawing snowbeds – but with highly reduced reproductive success. High abortion rates are common, since all four species obviously need insect vectors for a decent seed set.

The flowers are borne in catkins, the size and density of which vary strongly among the four species. Female catkins of *S. arctica* and *S. reticulata* are dense, many-flowered, and ± cylindrical; in *S. herbacea* and *S. polaris* they are loose, few-flowered (3–10 flowers per catkin), and rounded with the capsules spreading. Because of the differences in female catkin morphology, the response variables listed below have been differentiated with regard to species: length of mature catkin (measured from top to the axil of the closest subtending leaf [i.e., including catkin shaft]) in *S. arctica* and *S. reticulata*, number of flowers (capsules) in the other two species.

Capsular dehiscence is easily recorded: they will start to split open from the apex, and as the two valves separate and recurve, the white wool of the seeds becomes visible. Investigating reproductive success, in terms of counting mature seeds and aborted seeds and ovules, needs some further guidelines. Because seed wool will obscure observation in dry stage, capsules should be dissected in

water with some detergent added. The best way is to investigate capsule contents in a stereo lens with the sample (in water in a Petri dish) illuminated from beneath. It is also possible to count the seeds in a few drops 50% alcohol solution with light from above. Normaly, three classes of ovules/seeds are easy to identify: (1) large, filled seeds (1–2 mm long), (2) ± empty seeds of the same size (late embryo abortions), and (3) small unfilled ones (less than 0.5 mm long) representing unfertilized ovules (U. Molau, pers. obs.). Investigate 10 capsules per ramet if available. Capsules with more than 10 seeds are rare. Note any signs of seed predation.

The length of annual growth increments is usually easy to measure since the shots system is sympodial. Use main shoots and take measurements late in the season when growth has ceased (distance from last sprouting point to end of terminal wintering leaf bud). Samples for leaf weight should be taken just when they start to become yellow; sample entire leaves (with petioles intact) in paper bags and store dry at room temperature two months before weighing.

When undertaking ITEX monitoring of willows, select branches (ramets) as monitoring units. Select 20 branches (different individuals) of each sex per species for temperature enhancement experimentation, and the same amount for controls. Since flower formations occurs the year before flowering it is possible to determine the sex by dissect one single leaf bud Two standard protocols are provided in the Appendices to this Manual: one for females and one for males. Some species-specific variables are not included in the standard protocols; take additional notes on back sides!

In prostrate willows it is often difficult to cope with postulated sample sizes, since sex expression does not come as one of the first traits of the season. When you start up your sampling of willows, over-sampling is good advice. Sex determination is a problem in early stages, and we would encourage any enlargements by 50–100 %.

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free (plant or plot)

P2: First leaf bud burst (for the first year sex determination may be done later)

P3: First pollen shed (of all males) / First stigmas visible (females)

P4: All pollen shed (males) / Onset of seed dispersal (females; capsules split open at top, white wool visible)

P5: First yellowing of leaves

P6: Last green leaf turning yellow

P7: All leaves shed (optional)

P8: Onset of seed dispersal (capsules split open, wool visible)

Make note of the activity of wooly-bear caterpillars on a seperate sheet

QUANTITATIVE MEASUREMENTS

Q1: Total number of flowering catkin per monitored branch

Q2: Annual growth increment (accuracy 1 cm in *S. arctica*, otherwise 1 mm)

Q3: Length of longest leaf (petiole included; accuracy 1 mm)

Q4: Weight of largest leaf (with petiole; accuracy 0.1 mg)

(Q5–Q8: females only)

Q5: Total number of mature catkins per branch

Q6: *S. arctica/reticulata*: length of mature catkins from axil of subtending leaf (mean ± SD; accuracy 1 mm)

S. herbacea/polaris: number of capsules per catkin (mean ± SD)

Q7: Fruit:flower ratio of catkins (number of mature fruits divided by original number of flowers, given as mean ratio ± SD per branch). Alternatively use whole catkins instead of flowers, i.e. mature:flowering ratio of catkins.

Q8: Seed : Ovule ratio (mean ± SD; optional)

Additional records: *Salix arctica*: (1) measure maximum diameter of entire plant (between opposite branch tips); (2) diameter of branch at base (use calipers; accuracy 0.1 mm). For all species, note insect predation and damage (rolled leaves, holes, seed predation, egg deposits, larval grazing of leaf margins) and fungal growth (calculate percentage of infested leaves). On a separate sheet, make two additional columns for females (Q9–10), accomodating the number of flowering catkins (Q9) and the ratio flowers per catkin (Q10).

Woolly-bear caterpillars (*Gynaephora groenlandicasee pg. 30*) are important predators on the leaf buds and young catkins early in the season. For the reproductive succes as well as for the vegetative growth the number and activity of the caterpillars is of great importance. When they are present in the *Salix* plots, notes should be taken of the woolly-bear caterpillar on a seperate sheet.

Saxifraga oppositifolia

The Purple Saxifrage, *Saxifraga oppositifolia* L., is perhaps the best-known of all circumpolar arctic and alpine plants. Nevertheless, investigations on its ecology and reproductive biology are surprisingly sparse. Recently, flowering phenology, mating system, and reproductive success of *S. oppositifolia* were studied in a north Swedish population at the Latnjajaure ITEX site (Stenström and Molau 1992). The flowers normally have five purple

petals (color varies among genets), ten stamens, and a bi-lobed gynoecium with two styles. The leaves are small, evergreen, and densely packed on the shoots, making quantitative vegetative measurements difficult. Individual clumps (clones, genets) are normally easy to delimit, and should be used as sampling units in ITEX. In moist habitats the growth form is often more matted, and genets may be hard to separate; avoid such sites.

Saxifraga oppositifolia is one of the earliest flowering plants of the Arctic; depending on weather conditions and latitude, records of prefloration time range from 5 to 15 days. The flower buds are developed during the preceding season (August) and normally over-winter in a highly developed stage, with colored petals and differentiated ovules, but with pollen at the PMC stage (Sørensen 1941). Flower opening can be a lengthy process, and the opening buds may (depending on weather conditions, especially radiation climate) remain at a cylindrical stage for days. Mikael Stenström (pers. comm.) suggests that flowers should be regarded as open when they are accesible for pollinators, i.e., when petals start to spread distally and stigmas become visible.

Saxifraga oppositifolia possesses exceptional intrinsic properties for experimentation and monitoring. The stigmas are purplish, but the pollen is bright orange. The plants are strongly protogynous, and the gynoecium will be receptive for 3–4 days before the anthers dehisce and the orange pollen is exposed. At that time, stylar receptivity is rapidly declining, and no further seed set will result. Since flowering in *Saxifraga oppositifolia* clones is almost synchronous, the entire clones will be functionally entirely unisexual: female at first for 3–4 days, then males. Self-pollination is thus extremely rare under natural conditions. Bumblebees are the main pollinators, but flies seem to be important as well.

The stamens retain a purplish color until the anthers start to dehisce and the bright orange pollen mass is exposed. Since also the stigmas are light purple-colored, pollination events are easy to trace, and the deposited orange pollen grains might even be countable with a good hand lens; at least, presence of orange grains on stigmatic surfaces imply that pollination has taken place.

Capsule dehiscence is easy to monitor in *Saxifraga oppositifolia* if you know where to look. Capsules start to open at the end of the common part, between the two divergent stylar beaks. Collect half of the capsules (at least 5–10) dry in seed bags for subsequent weighing of the seeds; pickle the remaining fruits in 70% alcohol for later determination of seed number and number of aborted embryos and ovules (hyaline).

Make notes of the presense of the seed bug *Nysius groenlandicus*

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: First flower open

P3: First pollination (first orange pollen on stigma)

P4: First anther dehiscence (orange pollen exposed)

P5: First petal fading (wrinkled or devoid of color)

P6: Last petal fading

P7: First capsule open (splits at top between apical beaks)

QUANTITATIVE MEASUREMENTS

Q1: Vegetative growth (5 shoots per genet; mean ± SD, accuracy 1 mm)

Q2: Total number of flower buds (at beginning of season)

Q3: Total number of flowers per individual

Q4: Number of pollinated flowers in clone at the time first anther opens

Q5: Number of mature fruits (presence of seeds in a capsule is easily detected by sqeezing the capsule gently between two fingers)

Q6: Number of seeds per capsule (mean ± SD; optional)

Q7: Total number of flower per capsule (mean ± SD; optional)

Additional records: Take notes on eventual events of re-flowering later in the season (date of first occurrence, number of flowers, degree of perfectness of flowers [functionally female?]).

Silene acaulis

The Moss Campion, *Silene acaulis* L., is common throughout the Arctic as well as in subarctic and temperate alpine areas. It is a relatively early-flowering species, mainly pollinated by bumblebees, although butterflies (*Colias* and *Erebia* species) may be locally important . The seed bug (*Nysius groenlandicus*) may be detrimental to the seed set within annual fluctuations of importance for plant reproduction

The species normally forms dense tussocks (clones), easily delineated. However, the species is gynodioecious, and all populations are made up of a mixture of female and hermaphrodite clones; sometimes even purely male cones may appear (Alatalo & Molau, unpubl.). Seed set is usually much reduced in hermaphrodite clones. If you undertake ITEX monitoring and experimentation of this species, please make a good assessment of sex ratio in your population. Also, since the species is essentially gynodioecious, it is good advice to extend the sample size to 20+20 clones (20 females, 20 hermaphrodites); use additional copies of the standardized protocol for this purpose.

For monitoring of flowering (optional), make an extra protocol and count the numbers of open flowers per clone every second day.

PHENOLOGICAL DATES (day numbers)

P1: Date snow-free

P2: First open flower

P3: First open anther

P4: First stigma receptive

P5: First capsule cracks open (at top)

QUANTITATIVE MEASUREMENTS

Q1: Size of cushion (acuracy 1 cm)

Q2: Number of flowers

Q3: Number of capsules

Q4: Fruit : Flower Ratio (Q3/Q2)

Q5: Number of seeds per capsule (mean ± SD)

Q6: Seed:ovule ratio (mean ratio per clone ± SD; optional)

Q7: Flowers female (F) or hermaphrodite (H), or proportions thereof

Q8: No. of seed bug (*Nysius groenlandicus*) present on the cushion (optional)

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VOUCHER SPECIMENS OF ITEX PLANTS

Remember to ship your ITEX plants to the VOUCHER COLLECTION !

As agreed at the 6. ITEX workshop voucher specimens of all ITEX plants from all ITEX sites should be send to the Herbarium in Fairbanks, where they will be kept as a reference for the validity of the experimenters taxa.

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