ENDURE

European Network for Durable Exploitation of crop protection strategies

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Protocols for studying weed biology:

Phenology of annual weed species

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Dissemination Level	
PU Public	**PU**
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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Glossary

ENDURE	European Network for Durable Exploitation of crop protection strategies
WeedML	A computer language for weed modelling
WTDB	Weed Traits Database
BBCH	a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species.
Phenology	study of the seasonal timing of life cycle events

Summary

Objectives

The objective of this deliverable is the development of a phenological protocol. The purpose of this protocol is to enable researchers to monitor the phenological development of annual weedy species during the arable growth season. This monitoring results in the amount of time (expressed in day degrees) until the onset of a specific phenological stage. The protocol is designed to compare phenological development of a single species between regions within Europe.
This type of phenological information can be used to translate observations on weed traits, such as the timing of the onset of flowering, seed maturation and scenescense in one region to another. Knowledge that is often required for modeling weed growth, but can also be highly valuable to determine the best timing of weed control, the effects of climate change, the relationship between weeds and plant parasitic organisms such as insects, fungi and nematodes, and the competition between weeds and crops.

Rationale and work process during the work for this deliverable:

The activities within this deliverable are linked to the other deliverables within the subactivity RA 4.5 “Weed Biology and Management”. To help us understand weed ecology, this subactivity aimed at the development of three products:

- WeedML: a language for weed modeling
- Universal Simulator: software to run WeedML models
- WTDB: the weed traits database.

For more information on these activities we refer to www.weedml.org.

The WTDB resides on the internet and is open for all to read. This database contains data on plant traits of weed species commonly found in Europe. The development of the WTDB started with an inventory of the most commonly found species and the parameters commonly required for modeling purposes. The list of species is as follows:

1. *Abutilon theophrasti*
2. *Amaranthus spp.*
3. *Ambrosia artemisiifolia*
4. *Beta vulgaris*
5. *Brassica napus*
6. *Centaurea cyanus*
7. *Chenopodium album*
8. *Datura stramonium*
9. *Galinsoga parviflora*
10. *Galium aparine*

11. *Papaver rhoeas*
12. *Polygonum spp.*
13. *Raphanus raphanistrum*
14. *Sinapis arvensis*
15. *Solanum nigrum*
16. *Stellaria media*
17. *Tripleurospermum inodorum*
18. *Alopecurus myosuroides*
19. *Apera spica-venti*
20. *Avena fatua*
21. *Bromus sterilis*
22. *Echinochloa crus-galli*
23. *Poa annua*
24. *Cirsium arvense*
25. *Elymus repens)*
26. *Rumex obtusifolius*
27. *Tussilago farfara*

It was decided to include several parameters on phenology in the WTDB, such as the average duration until flowering, the average duration of the juvenile stage, and the average duration until seed maturity.
For several species, data on these parameters could not be found in scientific literature, or was highly variable, especially between regions.
As a result, a discussion on the relevance of geographical origin of the plant material and the relevance to our understanding of weed phenology between scientists involved in these activities was started.
The work described in this deliverable is the result of the outcomes of that discussion. It was decided to develop a research protocol to study annual weed species phenology and test this protocol in several regions of Europe in a joint experiment.

Teams involved:

JKI (Germany), INRA (France), AU (Denmark), IHAR (Poland), CNR (Italy), SSSUP (Italy), PRI (The Netherlands) and RRES (UK).

Geographical areas covered:

The protocol has been tested in Germany, France, Denmark, Poland, Italy (North and South), The Netherlands and the UK. The protocol is however, applicable outside this test area as well.

State of the art

The definition of a phenological study is the study of the seasonal timing of life cycle events. In the case of weedy annual plant species, important phenological events are the timing of emergence, duration of the juvenile stage and duration until seed maturity. During the above-mentioned discussion on phenology and regional differences, the bioclimatic law (Hopkins, 1918) was discussed. This law states that the variation in the occurrence of phenological events between two or more geographical positions in the United States bears the same proportion to the distance between them: 4 days of time equals:

- 1 degree of latitude,
- 400 feet of altitude,
- 5 degrees of longitude

(other things being equal)
This law is valid for the whole North American Continent.
According to more recent work (Chmielewski &Rötzer, 2001) the timing of phenological phases in Europe is influenced mainly by air temperature, the temperature in spring decreases from south to north Europe and with increasing altitude. They hypothesize that the altitude, longitude and latitude provide a possibility to map the beginning of phenological phases across Europe:

pp(x,y,z)=c + ax*x + ay*y + az*z, with
pp(x,y,z): starting date of a phenological phase at altitude z, longitude x and latitude y.
c: constant
ax, ay, az: regression coefficients

These laws are known to be valid for trees and bushes, but no information is available on their applicability to weedy species. However, the existence of such a law for weedy species, would make the translation of the timing of control measures for which the development stage of the weed is important (such as stale seedbed techniques) from one geographical region in Europe to another possible. The first requirement to test the existence of such a law is a method to determine and compare the phonological development of weedy species between different geographical origin within Europe.

Harmonization of material and methods among the Network

For the development of this protocol a prototype protocol was developed and tested by the following partners JKI (Germany), INRA (France), AU (Denmark), IHAR (Poland), CNR (Italy), SSSUP (Italy), PRI (The Netherlands) and RRES (UK). Two annual weeds were chosen to test this prototype: *Polygonum persicaria* (polpe) and *Echinochloa crus-galli* (echcg). These species were chosen because of their abundance in almost all the test regions, and the lack of data on their phenology in literature, and thereby lack of data on these species in the WTDB. Seeds of these species were collected from two geographical locations and provided to all the partners. Together with local seeds (for every geographical location local seeds were collected as well) these seeds were used to test the prototype of the protocol in the spring of 2009.

Specific research questions were formulated prior to testing the prototype of the protocol:

1. Is the timing of phenological events different between countries for polpe/echcg?
2. Is the timing and duration of phenological events at one location (country) different for seeds originating from different origins (countries)?

Based on the results of this test, the protocol was adapted and finalized into the final phenological protocol as described in the following paragraphs.

Protocol to study the phenology of weedy annual species

Objective

The objective of this phenology protocol is to enable researchers to monitor the phenological development of annual weedy species during the arable growth season. This monitoring results in the amount of time (expressed in day degrees) until the onset of a specific phenological stage. The protocol was developed to answer research questions such as the following:

- Is the timing of phenological events different between countries for individual species?

- Is the timing and duration of phenological events at one location (country) different for seeds originating from different origins (countries)?

Methodology

Materials

The materials needed to do a phenology study are:

- Seeds of the species of interest (from different geographical origins)
- Field Location
- Weather station to record soil and/or air temperature and precipitation during the observation period
- BBCH growth stage description (Hess et al., 1997)

Seeds

Depending on the specific research questions, mature seeds from one species should be collected from either one or more geographical locations.

(The latter is the case if one is interested in the differences in phenological development of a species originating from different climatic conditions. Climatic conditions (environments) experienced by local weeds are predictable across weed generations. As a result, local weed plants adjusted the phenotype of their offspring to enhance its success in the environment it is likely to encounter (adaptive maternal effects). If seeds are dispersed into a different environment (country) the expression of traits (such as the timing and duration of phenological events) may be miscued for that new habitat and differ with the offspring of local seeds. However, the differences in trait expression between local and introduced seeds of the same species will be reduced (or will even disappear) after one generation due to adaptive maternal effects (Galloway, 2005).)

After collection of the mature seeds, seeds should be stored dry and dark at 4 °C in paper bags during the winter (for some species storage at slightly higher temperatures or at room temperature will increase germination in the following season).
Depending on the species, stratification (chemical or cold treatments) needs to be applied to ensure proper germination in the summer period. Several stratification protocols are available and the choice depends on the seed conditions and species. Prior to seeding, germination of the seeds should be tested in petri dishes on moist filter papers. This germination percentage can give an indication on how many seeds need to be sown on a site to obtain the required plant density. Excess seedlings can be removed after emergence and before any competition between neighbouring plants will occur.

Field and sowing

The field should be free of the species on which you want to make the phenological observations.
Seeds are sown in rows on an arable field at several (e.g. 3) sowing dates. Optimal distance between rows and plants within a row are species dependent. For the species used to test the protocol (polpe and echcg) the following distances and row lengths were used: Rows of 1 m long and distance between rows of 0.20 cm. (See figure below for more details). The sowing date will depend on the species. For the two species used in the trials to test the prototype protocol the seeds were sown after the first frost period, as soon as a seed bed preparation is possible. Seeds can be sown at several sowing dates, the timing of the second and third sowing date at a certain number of degreedays after the first sowing date. Five

replications per each sowing date are used. After emergence the number of plants must be thinned to obtain the desired density.

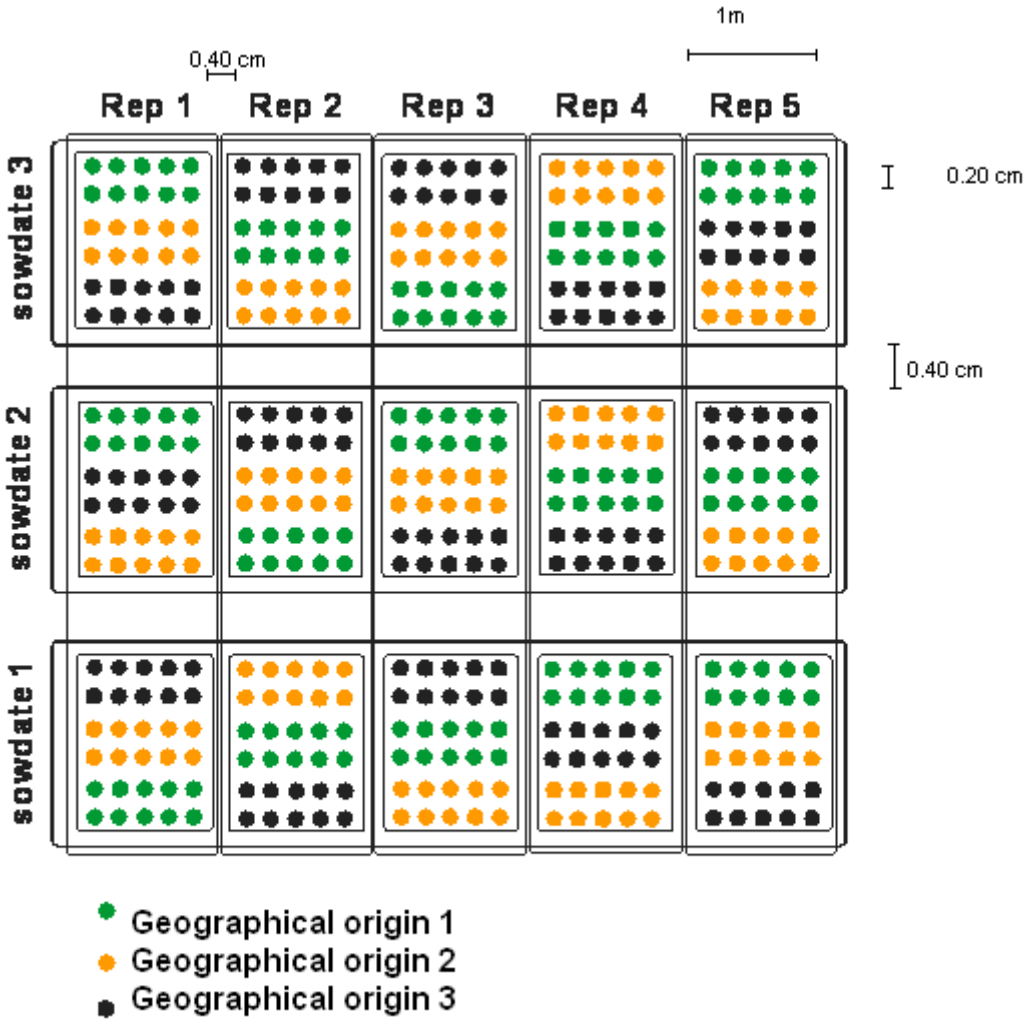

*Figure 1. Description of a proposed experimental lay out to study the differences between geographical origins in the phenological development of annual weedy species. Distances depicted in this figure between rows and repetitions are based on requirements for Echinogloa crus-galli and Polygonum persicaria***.**

Observations: BBCH Growth stage description(Hess et al., 1997)

The extended BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species. It resulted from teamwork between the German Federal Biological Research Centre for Agriculture and Forestry (BBA), the German Federal Office of Plant Varieties (BSA), the German Agrochemical Association (IVA) and the Institute for Vegetables and Ornamentals in Grossbeeren/Erfurt, Germany (IGZ).

The basic principles of the scale:

- The general scale forms the framework within which the individual scales are developed. It can also be used for those plant species for which no special scale is currently available.
- Similar phenological stages of each plant species are given the same code.
- For each code, a description is given, and for some important stages, drawings are included.
- For the description of the phenological development stages, clear and easily recognised (external) morphological characteristics are used.
- Except where stated otherwise, only the development of the main stem (dicots) or the number of tillers (monocots) is taken into consideration,
- Each plant is given a value for the primary phenological stage (see Table 1) and the secondary growth stages.
- The secondary growth stages 0 to 8 correspond to the respective ordinal numbers or percentage values. For example stage 3 could represent: 3rd true leaf, 3rd tiller, 3rd node or 30% of the final length or size typical of the species or 30% of the flowers open.
- Post harvest or storage treatment is coded 99.
- Seed treatment before planting is coded 00.

Organisation of the scale:

The entire developmental cycle of the plants is subdivided into ten clearly recognizable and distinguishable longer-lasting developmental phases. These principal growth stages are described using numbers from 0 to 9 in ascending order (see Figures 1a and b). The principal growth stages are described in Table 1. Owing to the very many different plant species there may be shifts in the course of the development or certain stages may even be omitted. The principal growth stages need not proceed in the strict sequence defined by the ascending order of the figures, but can occasionally also proceed in parallel.

Table 1: Principal growth stages, Stage Description

0 Germination / sprouting / bud development
1 Leaf development (main shoot)
2 Formation of side shoots / tillering
3 Stem elongation or rosette growth / shoot development (main shoot)
4 Development of harvestable vegetative plant parts or vegetatively propagated organs / booting (main shoot)
5 Inflorescence emergence (main shoot) / heading
6 Flowering (main shoot)
7 Development of fruit
8 Ripening or maturity of fruit and seed
9 Senescence, beginning of dormancy

If two or more principal growth stages proceed in parallel, both can be indicated by using a diagonal stroke (example 16/22). If only one stage is to be indicated, either the more advanced growth stage must be chosen or the growth stage of particular interest, depending upon the plant species. The principal growth stages alone are not sufficient to define exactly application or evaluation dates, since they always describe time spans in the course of the

development of a plant. Secondary stages are used if points of time or steps in the plant development must be indicated precisely. In contrast to the principal growth stages they are defined as short developmental steps characteristic of the respective plant species. They are also coded by using the figures 0 to 9.
The combination of figures for the principal and the secondary stages, results in the two-digit code. The two-digit code is a scale which offers the possibility of precisely defining all phenological growth stages for the majority of plant species. For a detailed description of the two-digit code we refer to Hess et al (1997).
The BBCH-scales allow the comparison of individual codes only within one principal growth stage: an arithmetically greater code indicates a plant at a later growth stage. Sorting codes into numerical order therefore allows a listing in order of the stage of plant development.

Timing of observations

After sowing the seeds, the fields are monitored *daily for emergence*. A stick is placed next to each plant at every emergence date. The result is that plants are marked according to their emergence day. For each sowing date, the development of the first four emerging plants per row is monitored. When plant densities become high and competition can be expected, each row is thinned to those first four plants per row.
After emergence of the first four plants, observations can be reduced to *three times a week*.

Data analysis

First, the temperature sum should be calculated as:

$$\sum GDD = \frac{(T_{\max} + T_{\min})}{2} - T_b \text{, where if } \frac{(T_{\max} + T_{\min})}{2} \le T_b \text{ then } \frac{(T_{\max} + T_{\min})}{2} = T_b$$

The temperature sum is calculated using the emergence dates of the plants as starting point for calculations. The base temperature (T_b) is species dependent, when the base temperature is undetermined, a base temperature of 0 °C can be chosen.

Second, the amount of GDD for each phenological stage should be calculated for each species, geographical origin and sowing date.

Degree of validation and operability of findings:

Several difficulties were encountered during the test phase of the prototype protocol. The most important or noticeable of these difficulties are described in this paragraph.

The origin of the seeds appeared to be very important for the requirement of a seed stratification period, independent of sowing location. For instance, both *Polygonum persicaria* and *Echinogloa crus-galli* seeds from the Netherland were able to germinate and produce the required densities without a stratification treatment, independent of their sowing location (e.g. The Netherlands, Denmark, UK). The seeds of these species of Italian origin however, showed very low field emergence, and would probably have benefited a lot from a stratification procedure. It was not possible to investigate the mechanism behind this difference.

The identification of maturation of seeds, without permanently damaging the seed heads turned out to be very difficult, if not impossible. It was concluded that instead of working with individual heads, the total seed production per plant should be estimated.

Figure 2. Impression of the experiment with Echinogloa crus-galli to test the prototype protocol.

Conclusions

The phenology protocol was developed and tested by eight partners in seven countries participating in the network. The joint experiment gave insight in the practical difficulties that needed to be solved and required some adjustment of the prototype protocol.
This led to a harmonization of the approach to study phenological development of annual weeds among partners. The current protocol can be used to study the phenological development of annual weedy species. The protocol uses the extended BBCH scale (Hess et al 1997) to code phenologically similar growth stages of mono- and dicotyledonous plant species. Together with temperature records from a local weather station, this information can be used to calculate the average heat sum required to reach the onset of a specific stage for each species and geographical origin. The temperature sum is calculated using the emergence dates of the plants as starting point for calculations. The base temperature (T_b) is species dependent, when the base temperature is undetermined, a base temperature of 0 °C can be chosen.
Conclusions on geographical influence in relation to phenology can be drawn by comparing the differences in temperature sum between treatments.

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