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A comparison of methods to estimate seasonal phenological development from BBCH scale recording

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Abstract The BBCH scale is a two-digit key of growth stages in plants that is based on standardised definitions of plant development stages. The extended BBCH scale, used in this paper, enables the coding of the entire development cycle of all mono- and dicotyledonous plants. Using this key, the frequency distribution of phenological stages was recorded which required a less intense sampling frequency. The onset dates of single events were later estimated from the frequency distribution of BBCH codes. The purpose of this study was to present four different methods from which those onset dates can be estimated. Furthermore, the effects of (1) a less detailed observation key and (2) changes in the sampling frequency on estimates of onset dates were assessed. For all analyses, phenological data from the entire development cycle of four grass species were used. Estimates of onset dates determined by Weighted Plant Development (WPD), Pooled pre-/post-Stage Development (PSD), Cumulative Stage Development (CSD) and Ordinal Logistic Regression (OLR) methods can all be used to determine the phenological progression of plants. Moreover, results show that a less detailed observation key still resulted in similar onset dates, unless more than two consecutive stages were omitted. Further results reveal that the simulation of a less intense sampling frequency had only small impacts on estimates of onset dates. Thus,

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H. Petermeier Fachgebiet Biostatistik, Technische Universität München, Maximus-von-Imhof-Forum 2, 85354 Freising, Germany especially in remote areas where an observation interval of a week is not feasible, estimates derived from the frequency distribution of BBCH codes appear to be appropriate.

Keywords Flowering \cdot Grassland \cdot Observation key \cdot Onset dates \cdot Sampling frequency

Abbreviations

ANOVA	Analysis of Variance
BBCH	Biologische Bundesanstalt,
	Bundessortenamt and Chemical Industry
CSD	Cumulative Stage Development
DWD	German meteorological service
IPG	International Phenological Gardens
OLR	Ordinal Logistic Regression
PSD	Pooled pre/post Stage Development
USA-NPN	USA National Phenology Network
WPD	Weighted Plant Development

Introduction

Phenology, the science of recurrent seasonal natural events, may be a harbinger of changes in ecosystems arising from recent global climate change (Menzel 2002). Numerous authors have published articles on the effect of global warming on the timing of important developmental events in plants (e.g. Sparks et al. 2000; Abu Asab et al. 2001; Fitter and Fitter 2002; Menzel et al. 2005, 2006). Most of these studies are based on long-term observation records which focus on key events such as leaf unfolding (Menzel et al. 2006) or first flowering (e.g. Abu Asab et al. 2001; Fitter and Fitter 2002). Datasets are often provided on an international (e.g. Chmielewski and Rötzer 2001; Menzel et al. 2006) or national scale (e.g. Defila and Clot 2001; Menzel et al. 2005) by phenological networks such as the International Phenological Gardens (IPG) or networks of National Meteorological Services. Because these networks are often reliant on volunteers, only a small choice of phenophases can be included in their monitoring programs. Intermediate stages (e.g. 20% of all flowers open) as well as stages marking the end of phenophases (e.g. end of flowering) are often not included. Thus, these datasets just provide information about onsets of key events without the possibility of analyzing the progression of phenophases. It is already known that higher temperatures cause an earlier start of plant flowering in spring and summer (e.g. Menzel and Estrella 2001; Walther et al. 2002; Parmesan and Yohe 2003), an earlier onset of the pollen season in the northern hemisphere (Emberlin et al. 2002; Beggs 2004), and a longer and more intense pollen season for different species (e.g. Spieksma et al. 1995, 2003; Emberlin et al. 2002). However, there are no studies based on other long-term records affirming a longer flowering period due to global warming because the end of flowering is often not monitored within phenological networks and therefore data are rare. Only the newly founded USA National Phenology Network (USA-NPN; www.usanpn.org) includes phenological stages covering the entire development cycle of phenophases (e.g. beginning of flowering, full flowering and end of flowering) and thus switches from event to status monitoring. The USA-NPN base their observation scheme on the extended BBCH scale (Meier 1997). The scale was originally developed jointly by four important chemical companies BASF, Bayer, Ciba-Geigy and Hoechst to standardize descriptions of plant development stages (Bleiholder et al. 1989). Later a slightly changed working group (BBCH: Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) extended this scale to 27 crops and wild plants (Meier et al. 2009). The scale is a detailed growth stage key which includes intermediate stages as well as stages marking the end of phenophases. It allows the observation of the entire development cycle of all mono- and dicotyledonous plants using a decimal coding system. The first numeral of this system ranges from 0 to 9 in ascending order and corresponds to principal growth stages which describe longer-duration development phases such as bud development, leaf development or flowering. The second numeral also ranges from 0 to 9 and corresponds to secondary growth stages which refine the development stages such as the beginning of bud swelling or the end of flowering (Meier 1997).

Due to the advantages of a standardized observation system like this, which allows comparing homologous growth stages of different species, more and more phenological stages of existing datasets have been assigned to the BBCH scale as, for example, within the framework of COST action 725 (http://www.cost725.org), 'Establishing a European phenological data platform for climatological applications' (Menzel et al. 2006). Furthermore, several European phenological networks also modified their phenological guidelines according to the BBCH scale to provide higher compatibility between networks (Bruns et al. 2003).

Phenological networks such as the one of the German meteorological service (DWD; http://www.dwd.de) recommend making highly regular observations every 2-3 days and noting down the dates of occurrence of single phenological events. Where circumstances, such as remote research plots, difficult accessibility and limited financial resources, only allow a less frequent inspection, it seems more appropriate to refine the code by including intermediate stages, and to observe the phenological status of populations instead of single individuals. Thus, on each sampling date, the frequency distribution of phenological stages of a certain number of individuals could be recorded. The classical onset dates of key events can then be interpolated from these data and it is not necessary to be present at the exact start of the stage. This approach requires a less frequent observation intensity, e.g. once a week, in contrast to most other studies where observations are conducted every 2-3 days or even daily (e.g. Cleland et al. 2006; Yuan et al. 2007).

Despite these advantages, there have so far been no studies using the BBCH scale to observe the entire development cycle of wild plants. Most recent studies utilizing the BBCH scale either present further descriptions of phenological stages of certain species (e.g. Salazar et al. 2006; Finn et al. 2007; Saska and Kuzovkina 2010) or deal with topics related to agricultural crop research (Bazok et al. 2009; Janusauskaite 2009; Kraska et al. 2009; Rodriguez-Rajo et al. 2010) where single phenological events and not the entire development cycle of plants are considered. Consequently, there is no accepted methodology to analyze the frequency distribution of phenological stages.

Therefore, the purpose of this paper is to present and compare four different methods to analyze phenological data of populations recorded on a refined BBCH scale in order to estimate onset dates for each phenological stage. Calculations were based on phenological data of the entire development cycle of four grass species in the Freisinger Moos, Germany, in 2009.

Furthermore, it was tested whether a detailed observation key is necessary to estimate onset dates or if there are stages which are redundant. Moreover, the elimination of phenological stages from the observation key should help to answer the following questions: (1) What effect does the omission of a stage have on onset dates of other stages? (2) Is it possible to estimate onset dates of stages if that stage is not specifically recorded? (3) Are all methods consistent in this approach? Finally, a simulated increased interval between consecutive recordings should help to answer the following questions: (1) Does an increased sampling interval of two or even three weeks reveal similar estimates of onset dates? (2) Are all methods consistent in this approach?

Materials and methods

Phenological observations

Data were recorded from a cultivated meadow at a peatland site in the Freisinger Moos, Germany (48°22'N, 11°41'E). Records were taken separately from three 0.75 m x 0.75 m plots and one 0.75 m x 0.40 m plot. Differences in plot size were due to space restrictions on the site. All plots were contiguous. Four grass species, Alopecurus pratensis L. (meadow foxtail), Dactvlis glomerata L. (cocksfoot), Festuca pratensis Huds. (meadow fescue) and Poa trivialis L. (rough-stalked meadow grass) were observed once a week from the beginning of April to the end of September 2009. In each plot, 12 individuals of A. pratensis, D. glomerata and F. pratensis were randomly chosen every week to be observed. The number of individuals was considered large enough for further statistical analyses and small enough to make all observations feasible in a single day. Only 6 individuals were observed of P. trivialis because of the small number of individuals in each plot. D. glomerata could only be monitored in three plots because the species did not exist in the remaining plot. As single individuals of each species were not marked, different individual plants were likely observed on consecutive sampling dates.

Phenological observations were conducted according to the BBCH scale for cereals (Meier 1997), which was slightly modified for wild grasses. In general, wild grasses grow closer together, often in combination with other species, and are much smaller than cereals. Therefore, the distinction of secondary growth stages is more difficult and time-consuming than for cereals. Thus, some principal and secondary growth stages of the BBCH scale could not be transferred to the new key or needed to be redefined (Table 1).

The BBCH principal growth stages 0, 2 and 7 were excluded completely from the new observation key. The principal growth stage 1 was not modified; stage 3 was included but rarely observed. The principal growth stages 4–6 were modified slightly for wild grasses. Principal growth stages 8 and 9 were redefined, because flowers of wild grasses are too small to test the development status of fruits, as it is done in the original BBCH scale for cereals, without destroying parts of the spike or panicle. The principal growth stages 5 and 9 were extended by one secondary growth stage because the resolution of the original key was not sufficient to describe development. The new stages were coded with the two-digit code of the stage they belonged to and with an additional index (59a, 95a; see Table 1). Secondary growth stage 59a is only applicable for grass species with a panicle rather than a spike.

Secondary growth stages 15–19 could not be detected in 2009 because the species did not have more than four leaves simultaneously. Secondary growth stages 31–36 were also not detected because these stages proceed in parallel with more advanced growth stages which were recorded in preference. Secondary growth stage 97 was not observed until the end of September. All development stages occurring after secondary growth stage 95a were summarized as stage 100, indicating the end of development, which needed to be introduced for mathematical reasons to allow estimation of earlier growth stages. This stage was not considered further in analyses. The description of each secondary growth stage was further supported by digital photographs (Fig.1).

Data analysis

Weekly raw data (absolute frequencies of specimens within certain stages) were first converted into percentages. To estimate onset dates of secondary growth stages, four different methods were developed and compared (Fig. 2). Calculations to determine onset dates were automated in MATLAB (R2009b; The MathWorks, Natick, MA, USA, 2009) except for the ordinal logistic regression method which was run in R (R2.10.1; R Development Core Team, Vienna, Austria, 2006). All observed dates were converted to day of year (1 January = 1, etc.). Calculations were done for each species and plot separately.

Weighted Plant Development (WPD)

The basis of the method of weighted plant development is a linear interpolation between two consecutive sampling dates to determine onset dates of secondary growth stages. For this, a phenological mean value (development mean; DM) per sampling date (*t*) was calculated from the relative frequency distribution data. First, the ordinal scaled, two-digit decimal code was converted to ranks (*i*) starting with 1. Each value on the new scale was then weighted by the corresponding relative frequency (x_i) between 0 and 1. The weighted values were finally summed over all secondary growth stages for each sampling date (Eq. 1).

$$DM(t) = \sum_{i=1}^{p} (x_i(t) \cdot i)$$
(1)

where P is the total number of phenological stages and i the corresponding phenological code. The dates of phenological stages were then estimated from linear interpolation between sampling date means.

Table 1	Description of the	phenological growth	stages of wild	grasses according to	the BBCH code for cere	eals (Meier 1997)
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BBCH code	Description	Alopecurus pratensis L.	Dactylis glomerata L.	<i>Festuca</i> pratensis Huds.	Poa trivialis L
Principal grow	th stage 1: Leaf development				
11	First leaf unfolded	х	х	х	х
12	2 leaves unfolded	х	х	х	х
13	3 leaves unfolded	х	х	х	х
14	4 leaves unfolded		х		х
Principal grow	th stage 4: Booting				
43	Mid boot stage: sheath just visibly swollen	х	х	х	х
45	Late boot stage: flag leaf sheath swollen	х	х	х	х
47	Flag leaf sheath opening	х	х	х	х
Principal grow	th stage 5: Inflorescence emergence, heading				
51	Beginning of heading: tip of inflorescence emerged from sheath, first spikelet just visible	х	х	х	Х
55	Middle of heading: half inflorescence emerged	х	х	х	х
59	End of heading: inflorescence fully emerged	х	х	х	х
59a	Panicle unfolded (in panicle forms only)		х	х	х
Principal grow	th stage 6: Flowering, anthesis				
61	Beginning of flowering: first anthers visible	х	х	х	х
65	Full flowering: 50% of anthers mature	х	х	х	х
69	End of flowering: all spikelet have completed flowering but some dehydrated anthers may remain	х	х	х	Х
Principal grow	th stage 8: Ripening				
85	Inflorescence starts yellowing	х	х	х	х
89	Inflorescence yellow	х	х	х	х
Principal grow	rth stage 9: Senescence				
93	First seeds fallen	х	х	Х	х
95	50% or more seeds fallen	х	х	х	х
95a	All seeds are fallen	х	х	Х	х
100	Reproductive period finished	х	х	х	х

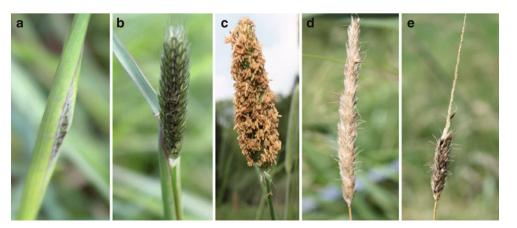
Crosses indicate secondary growth stages which were observed in the field in the Freisinger Moos in 2009. Stages not observed are omitted. Principal growth stages with the index a (=after) were added because the resolution of the original key was not sufficient to describe the development precisely

Pooled pre/post Stage Development (PSD)

The method of pooled pre/post stage development is also based on linear interpolation between sampling dates.

However, a development index (DI) per sampling date (t) and secondary growth stage (i) rather than a phenological mean was calculated. For each sampling date, the relative frequency distribution was divided into three groups in

Fig. 1 Examples of the observation key of *Alopecurus pratensis* L., which is based on the BBCH code for cereals. a BBCH code 47: Flag leaf sheath opening, b BBCH code 55: Middle of heading: half inflorescence emerged, c BBCH code 69: End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain, d BBCH code 89: Inflorescence yellow, e BBCH code 95: 50% or more seeds fallen



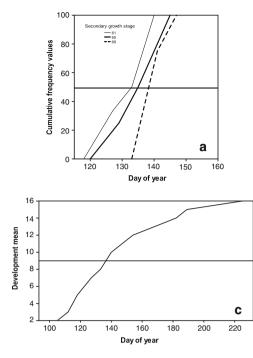
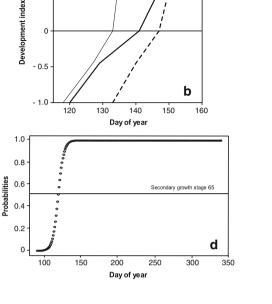


Fig. 2 Illustration of the different mathematical approaches of the four presented methods to estimate onset dates of secondary growth stages. a, b Progression curves of single secondary growth stages 61-69 calculated by methods of Cumulative Stage Development (CSD) and Pooled pre-/ post-Stage Development (PSD). Horizontal lines indicate thresholds used to define onset dates of corresponding growth stages. c Progression curve of the entire plant development calculated by the method of Weighted

respect to the specific single secondary growth stage of interest (k). The first group was the percentage of secondary growth stages occurring before the stage of interest (1...k-1), the second group was the percentage occurring after the stage of interest (k + 1...p). The third group was the percentage of observations exactly at the stage of interest. To calculate the development index all groups were weighted with a factor f in relation to their influence on the estimates of onset dates (Eq. 2). The first group was weighted with -1 because individuals in this group still needed to pass through the stage of interest. The bigger this group the later the onset date of the stage of interest. The second group was weighted with +1 because development of individuals was further advanced than the stage of interest. The bigger the influence of this group the earlier was the onset date of the stage of interest. The third group was weighted by 0 because these individuals are exactly at the stage of interest. The bigger this group the greater the probability that the date of onset equalled the sampling dates.

$$DI(t,k) = \sum_{i=1}^{p} x_i(t) \cdot f(k)$$
⁽²⁾

Where
$$f = \begin{cases} -1, if(i < k) \\ 0, if(i = k) \\ 1, if(i > k) \end{cases}$$



1.0

0.5

С

Plant Development (WPD). The horizontal line indicates the threshold to define the onset date of secondary growth stage 65. Development mean values on the y-axis indicate converted BBCH codes to ranks. d Progression curve of secondary growth stage 65 modelled by the method of Ordinal Logistic Regression (OLR). The horizontal line indicates the threshold used to define the onset date of secondary growth stage 65. Thresholds can be changed for CSD and OLR methods

and $x_i(t)$ is the relative frequency of secondary growth stage *i* at sampling date *t*.

The resulting development indices were then linearly interpolated to estimate the onset date of each secondary growth stage as the date when the development index equalled zero. If more than one development index per secondary growth stage equalled zero, the date of the first index was defined as the onset date.

Cumulative Stage Development (CSD)

The method of cumulative stage development is analogous to the procedure of Brügger (1998) which, in turn, is based on the work of Schirone et al. (1990). Both studies used the method to determine the progression of phenophase development in tree crowns.

Onset dates of each secondary growth stage were determined with the help of summation curves. At each sampling date (t) relative frequency values from 0 to 1 at stage *i* ($x_i(t)$) were added to the relative frequency values at the following stages (x(t, k)) (Eq. 3).

$$CF_i(t) = x_i(t) + \sum_{k=i+1}^{p} x(t,k)$$
 (3)

The resulting cumulative frequency values (CF) per sampling date and secondary growth stage were then linearly interpolated over time. Onset dates were defined as the point in time when 50% of all individuals were at the stage of interest. If this threshold was crossed twice because of later developing individuals observed at subsequent visits, the first date of crossing was defined as the onset date. If more than one cumulative frequency value per secondary growth stage equalled 50%, the date of the first value was defined as onset date.

Ordinal Logistic Regression (OLR)

This method to determine onset dates of secondary growth stages is based on an ordinal logistic regression (Agresti 2007). First, the two-digit BBCH codes were ranked into ascending positive integers i as it was done for WPD. Frequency distribution data were then used in the following model (Eq. 4):

$$\log\left(\frac{P}{1-P}\right) = \alpha + \sum \beta_i \cdot x_i \tag{4}$$

where $P=P(Y_i=1|x_i)$ is the probability and Y is the ordinal response variable, α is the intercept parameter, β_i the slope parameters and X_i the explanatory variable, in this case time.

The resulting intercept values for each secondary growth stage were put into a logistic regression function. Onset dates were defined as the date when the probability of a given individual to be in the considered stage is 50%, i.e. the number of expected successes "observed stage" equals half of a population; the rest of the population stays in earlier or later stages. In comparison to the other methods, OLR is based on the frequency distribution over time. WPD, PSD and CSD, however, are based on the frequency distribution over phenological stages at a fixed sampling date.

The effects of degraded observation keys and reduced sampling frequency

To test the effects of a less detailed observation key on estimates of onset dates of secondary growth stages, the key was degraded by sequentially leaving out a single secondary growth stage from the original observation data. Records of each omitted stage were redistributed to the previous and subsequent stages according to the proportion originally occurring in these stages. Percentages were reassigned in this way because field experience indicated that the developmental status of an individual would not always be allocated to the previous growth stage. Consequently, secondary growth stage 14 was the first stage from which observations could be properly redistributed. New, degraded data were then used to estimate onset dates. Furthermore, it was tested how similar onset dates are if two secondary growth stages were eliminated. For this, data were reassigned as described before but the percentages of two omitted stages were redistributed.

The influence of a reduced sampling frequency on onset dates of each secondary growth stage was tested by modifying the original data so that only observations of every second (both even and odd weeks) or third week were considered. These data were then used to estimate onset dates.

Analyses regarding the effects of degraded observation keys were only conducted on WPD and PSD methods because analyses showed that onset dates of stages can only be estimated with OLR and CSD if field data were collected. Consequently, effects regarding less intense sampling frequencies were also conducted only on these methods.

Statistical analysis

An ideal method to assess accurate onset dates should deliver matching onset dates for the different plots in the same managed peatland meadow, thus produce repeatable results. Furthermore, the authors suppose that the ranking of the onset dates derived by the four methods should at least be constant across the four grassland species observed. To test differences between methods and plots on the estimated dates over all secondary growth stages and of each single secondary growth stage two-way analysis of variance (ANOVA) was used. Further two-way ANOVAs were conducted to test differences between methods and plots on estimated dates if a degraded observation key or a reduced sampling frequency was considered. All statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA, 2008).

Results

Comparing onset dates by different methods

For all species, ANOVA revealed no significant differences in estimated dates between plots (Table 2) which had been anticipated because they were all treated similarly. Estimated dates differed significantly between methods for *F. pratensis* and *P. trivialis* but not for *A. pratensis* or *D. glomerata* (Table 2).

Figure 2 shows phenological progression curves of onset dates estimated by the four methods for all species. Notably, for all species, onset dates of secondary growth stages showed minor differences (≤ 1 day) between WPD and PSD as well as between CSD and OLR. However, onset dates of secondary growth stages, especially from stage 51 on, were earlier if estimated by CSD or OLR compared to WPD and PSD (3–5 days on average depending on species). Differences in onset dates estimated by WPD/PSD and CSD/OLR were larger for stages at the very

 Table 2
 Results of a two-way ANOVA showing effects of different methods and plots on the phenological progression of the four species

	Methods	Plots
Alopecurus pratensis L.	0.262	0.824
Dactylis glomerata L.	0.199	0.080
Festuca pratensis Huds.	0.001	0.075
Poa trivialis L.	< 0.001	0.124

Values shown are *p*-values. Values in italics are significant (p < 0.05)

beginning (secondary growth stage 13) and later on in the development cycle, with differences between onset dates of more than 1 week. However, highly significant (p<0.001) differences between methods, as determined by two-way ANOVA, were rarely detected for phenological stages towards the end of the development cycle (Fig. 3). Differences were mostly found for stages of principal growth stages 4 and 5.

Onset dates could not be estimated for secondary growth stage 12 because observations in 2009 started too late to survey the entire progression of this stage.

Effects of degraded observation keys and reduced sampling frequency

Two-way ANOVA revealed no significant differences between WPD and PSD methods to estimate onset dates

for all four species using degraded observation keys (p= 0.360 for *A. pratensis*, p=0.906 for *D. glomerata*, p=0.331 for *F. pratensis*, and p=0.750 for *P. trivialis*).

Omitting the recording of single secondary growth stages affected onset dates of up to eight stages if WPD was used for estimation (Fig. 4). The number and type of affected stages differed depending on which secondary growth stage was omitted. Using PSD, onset dates of up to three secondary growth stages were affected. The onset dates of the omitted and adjacent stages were always affected (Fig. 4). Averaged over all plots and secondary growth stages, onset dates differed about 0.2-0.6 days for WPD and 0.9-1.2 days for PSD depending on species. Based on mean absolute deviations (averaged over plots) onset dates were affected by 2-9 days for WPD and 3-7 days for PSD depending on species. An effect of more than 2 days was noted if secondary growth stages 59a, 69, 93 and 95a were omitted. For PSD, the omission of secondary growth stages 43, 59, 61, 89 and 95 also affected onset dates by more than 2 days (Fig. 4).

When omitting the recording of two secondary growth stages, an estimation of onset dates with PSD was no longer possible. Using WPD for estimation, onset dates were affected by up to 14 days (depending on stage and species) if two stages were eliminated.

ANOVA showed no significant effect of methods (WPD and PSD) on onset dates of all four species (p=0.856 for *A. pratensis*, p=0.521 for *D. glomerata*, p=0.650 for *F. pratensis*

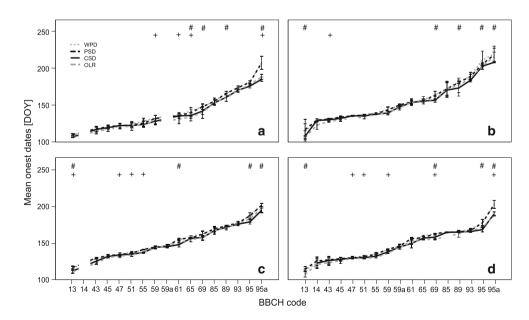


Fig. 3 Phenological progression curves of the four species in 2009 showing onset dates estimated by Weighted Plant Development (WPD), Pooled pre-/post-Stage Development (PSD), Cumulative Stage Development (CSD) and Ordinal Logistic Regression (OLR). *Gaps* in *lines* are due to different observation keys used for different species. *Pluses* indicate stages where onset dates were highly

significantly (*p*<0.001) different between methods. *Hashes* indicate stages where onset dates estimated by CSD and OLR differed by more than 1 week. *Error bars* indicate the standard error within the different plots. **a** *Alopecurus pratensis* L., **b** *Dactylis glomerata* L., **c** *Festuca pratensis* Huds., **d** *Poa trivialis* L.

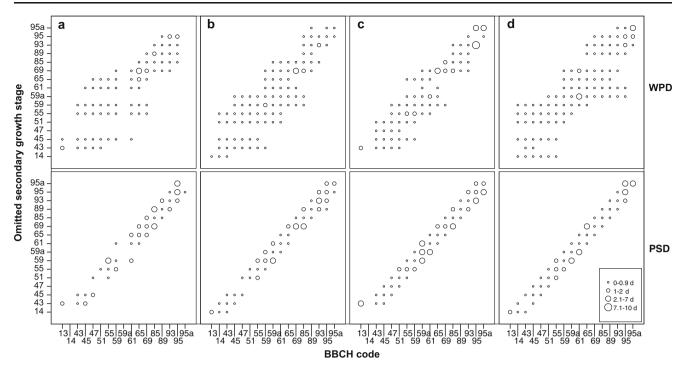


Fig. 4 Effects of a degraded observation key on onset dates of secondary growth stages estimated either by WPD (Weighted Plant Development) or PSD (Pooled pre-/post-Stage Development). *Circle sizes* indicate mean absolute deviations from original onset dates in days. *Horizontal gaps* indicate no change in onset dates even though

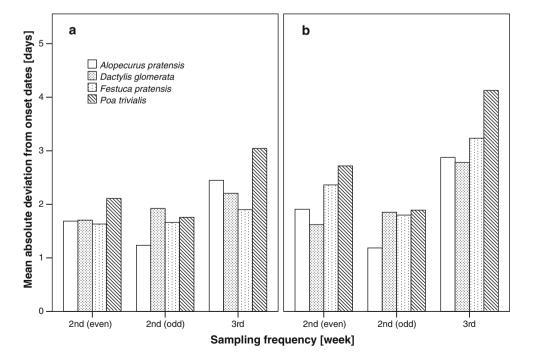
one secondary growth stage was left out. *Vertical gaps* are due to different observation keys used for the four species. **a** Alopecurus pratensis L., **b** Dactylis glomerata L., **c** Festuca pratensis Huds., **d** Poa trivialis L.

and p=0.734 for *P. trivialis*) if sampling frequency was reduced.

Simulating observations every second week instead of every week changed estimates of onset dates by an average of 1-

2 days for WPD and 1–3 days for PSD depending on species (Fig. 5). Observations every 3 weeks affected estimates of onset dates by 2–4 days on average for both methods. Based on mean absolute deviations of each secondary growth stage,

Fig. 5 Mean absolute deviations from original onset dates if sampling frequency is reduced to every second (even or odd) or third week. Onset dates were either estimated by Weighted Plant Development (a) or Pooled pre-/post-Stage Development (b) for all four species



larger differences in onset dates of 5–9 days, depending on species, occurred when only every third week was considered. Observations conducted every second week caused a shift of 2–8 days depending on species. Onset dates for secondary growth stages could either be earlier or later in relation to onset dates estimated with the original data set.

Discussion

Advantages and disadvantages of methods

All four methods described in this paper can be used to determine the phenological progression of plants by estimating onset dates of secondary growth stages from recorded frequencies of BBCH codes. However, methods sometimes differed substantially in calculation effort and results.

The basic difference of WPD from all other methods lies in the fact that only one value per sampling date is calculated. This provides information only about the development of plants in general but no information about the progression of each secondary growth stage (Fig. 2). However, this information allows the estimation of the beginning, the speed of passage and the end of single stages, which helps to understand the development of plants in more detail.

Furthermore, WPD is based on an interval-scaled observation key which presumes that all stages are of equal length. However, this contradicts reality because plants pass through some developmental stages faster than through others. This was quantified in a study by ÖKL (2006) where 2,749 phenological records of flowering stages of six important grass species in Austria in 2006 were analyzed. It was shown that D. glomerata, for example, needed 12 days from secondary growth stage 59 to 61 but just 5 days from growth stage 61 to 65 (ÖKL 2006). However, intervalscaled observation keys are often used in phenological approaches (e.g. Price and Waser 1998; Vitasse et al. 2009), so this method was also included in this study. The OLR method, in contrast, treats the observation key as ordinal scaled. Consequently, stages of different length are already considered in this approach. CSD and PSD are based on different calculation procedures. A switch to an intervalscaled observation key is not necessary.

CSD, in contrast to PSD, provides fewer data points to interpolate onset dates due to cumulative calculation procedures which often lead to steeper slopes resulting in earlier onset dates (Fig. 2). Phenological progression is therefore often advanced if onset dates were calculated with CSD in comparison to PSD. A similar progression also occurs if onset dates were estimated by OLR. Onset dates between WPD/PSD and CSD/OLR differed most in stages at the very beginning and end of the development cycle of plants. Differences in onset dates of more than 1 week could be mostly detected for secondary growth stages 13, 69, 95 and 95a. A linear regression between the number of weeks a secondary growth stage lasts and the standard deviations of mean onset dates (averaged over methods and species) confirmed a strong positive relationship (R^2 = 0.765, p < 0.001) between length of stage and difference in onset dates. Equally, the secondary growth stage 59 is also long lasting which explains the highly significant differences in onset dates for two out of four species. All other highly significant differences between onset dates, as well as differences of more than 1 week, appear to be species specific and not connected to the duration of stages. Significant differences in onset dates between methods for F. pratensis and P. trivialis, as shown by ANOVA (Table 2), are also due to differences between onset dates estimated by WPD/PSD and CSD/OLR, as post-hoc tests indicated (data not shown).

Both CSD and OLR methods have in common that different thresholds can be determined to define onset dates. In this study, a threshold of 50% is chosen because it is often used in other studies (e.g. Vitasse et al. 2009) and recommended for observations based on the BBCH code (Meier 1997). However, it is important to note that due to the sigmoidal nature of the progression of each secondary growth stage an interpolation error can occur, which is greatest at the beginning and the end of the curve. Thresholds defining onset dates of secondary growth stages should therefore be within the linear part of its course of the progression (Brügger 1998).

Onset dates estimated by PSD are sometimes identical for two consecutive secondary growth stages. This may happen because plants were not individually marked in the plots. Thus, due to recording of different individual plants, species can appear further advanced at one sampling date than at the following, which can result in the same onset dates. Equal onset dates as well as multiple crossing points could have been avoided if individuals had been marked. However, searching for marked individuals in a cultivated meadow, where plants grow close together and reach a height of almost a metre was regarded as too time-consuming. This was also the reason to limit the number of individuals to 12, even though it is known that larger sample sizes improve statistical estimates. Furthermore, data analyzed by CSD and OLR also result in the same onset dates if the first (for CSD) or second (for OLR) of two consecutive secondary growth stages was not observed in the field because these methods need field data for each secondary growth stage as a basis for estimating onset dates.

Observation key and sampling frequency

For WPD and PSD methods, onset dates of single secondary growth stages can be estimated even though no

field data were collected. Using WPD compared to PSD, more stages are influenced if one secondary growth stage is left out but the error is smaller. For WPD, omission of most stages affects onset dates by less than 2 days. In the study by ÖKL (2006), the most rapid progression between secondary growth stages was observed for Bromus erectus Huds. which passed through secondary growth stage 61 in just 2 days. Thus, our simulated shift in onset dates of less than 2 days is still within the minimum range of a short duration stage. For PSD, a slightly bigger influence on onset dates was observed. More than half of all stages affected onset dates by more than 2 days if left out. However, deviations were still small. In only two cases was the shift bigger than 5 days, which is within the time-span of most secondary growth stages in the ÖKL (2006) study. Those stages, whose onset dates differed most between estimates by WPD/PSD and CSD/OLR, were also identified as inducing complex shifts in the assessment of plant development when omitted from observation. Thus, long duration stages, if left out, influence changes of onset dates more than others. Estimates of onset dates determined by both methods are similar to the original estimates if a single secondary growth stage was left out. However, if more than one secondary growth stage was eliminated, onset dates of those stages could not be estimated by PSD. Using WPD for estimation, results are more promising; however, deviations were larger (up to 14 days; data not shown). Thus, no further analyses regarding the elimination of more stages were conducted.

Simulating an increased observation interval of every 2 weeks caused a shift in onset dates of 2 days or less for almost all species and both methods. In comparison to the $\ddot{O}KL$ (2006) study, these shifts indicate that the onset dates are still within the range of a short duration stage. Sampling even less frequently (every third week) affected onset dates slightly more (≤ 5 days), which is again within the range of most secondary growth stages in the $\ddot{O}KL$ (2006) study.

The DWD recommends a regular observation intensity of 2–3 days in peak season (Deutscher Wetterdienst 1991). BBCH intensity, used for field observations in this study, is already a factor of three lower. Even recording intervals of every third week allow determination of onset dates although absolute error might be up to 1 week or slightly more. However, especially in remote areas where an observation intensity greater than once a week is not feasible, an observation method based on BBCH codes as described here seems to be appropriate.

Conclusion

Even though the BBCH scale has attracted attention of scientists outside agricultural plant science, there have

been, so far, no studies using the BBCH code to investigate the entire development cycle of wild plants. Due to the lack of studies, there is no common method to determine onset dates for each secondary growth stage from frequency distributions of BBCH codes. In this study, four different methods were compared. WPD is fast and simple in calculation and onset dates can be estimated even though field data were not collected for each secondary growth stage. However, WPD provides no information about the progression of single secondary growth stages and the threshold for estimating onset dates is fixed. The progression of single secondary growth stages can be determined using PSD, CSD and OLR methods. PSD, in contrast to CSD and OLR, can estimate onset dates for each secondary growth stage even though no field data were collected. Both CSD and OLR methods allow different thresholds to define onset dates; PSD, however, does not.

WPD and PSD methods allowed determination of onset dates if a degraded observation key or a less frequent sampling intensity was used. However, deviations of onset dates estimated by PSD were often slightly bigger than those of onset dates estimated with WPD. Results showed that the observation key can be less detailed but neither methods can estimate onset dates if two or more consecutive stages are left out. In particular, the omission of long duration stages influenced estimates of onset dates more than others. Thus, an observation key should preferably include long duration stages to avoid large errors in onset dates. Simulating a less intense sampling frequency leads to onset dates diverging only slightly from original estimates. For remote areas, in particular, recording a frequency distribution of BBCH codes with an interval of more than 1 week appears feasible to generate valid phenological observations on a less frequent but more intensive basis.

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