Research Notes

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Influence of temperature on blackthorn (*Prunus spinosa* L.) phenophases in spring season

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Abstract

Phenology is the study of periodic biologic changes that plants are going through, under the influence of environmental factors, especially temperature. The present paper evaluates the behaviour of *Prunus spinosa* L. genotypes under the fluctuations of climatic factors by calculating the average time elapsed from bud-breaking to blooming, the average time elapsed between different phenophases, the heat demand and the chilling requirement. The data obtained show that the onset of spring season phenological phases is significantly speeded-up by temperatures increase and provides useful information on temperature influence on phenologic phased triggering and better understanding of temperature influence on phenology in *Prunus spinosa* L. genotypes in Oltenia region of Romania.

Key words: Genotypes, Phenology, *Prunus spinosa* L.

1. Introduction

The possible impact of global climate changes on agricultural ecosystems is the subject of numerous recent studies. One of the indicators used and accepted by many scientists to monitor climate change, is phenology (Rötzer and Chmielewski, 2001). Phenology as the ideal way to demonstrate the effects of global warming on the living world (Sparks and Menzel, 2002). Phenology is the study of periodic biological changes that plants are going through, as influenced by environmental factors, especially temperature. Monthly average air temperatures are often used to predict phenological events (Chmielewski and Rötzer, 2001; Ahas *et al*., 2000). The reproductive potential of plants can be affected by two types of extrinsic and intrinsic factors (Stephenson, 1981). The incidence of extrinsic factors may vary depending on the plant's phenological rhythms, namely the duration of flowering, the duration of fruit-setting, the degree of timing in flowering and fruit-setting (Guitián *et al*., 1993). Long-term systematic surveillance of phenophases provides the opportunity to estimate changes in the onset or the end of phenophases, which enables the assessment of the climate changes' influence on plants (Bauer and Bartošová, 2014). The findings of Zverko *et al*. (2014) indicates that the increase in temperature has a significant influence on triggering of spring season phenophases in some fruit-tree species, including in blackthorn. The change in the onset of phenophases in blackthorn was also observed by Babálová *et al*. (2018). Differences between the accumulation of cold and heat in fruit trees are the result of genetic adaptation to environmental factors (Scorza and Okie, 1990). The time of blooming phenophase depends on cultivars' demands for heat. Climate warming affects both the satisfying of cold demand and satisfying of heat demand (Guo *et al*., 2014). Each tree species has a specific requirement for cold in order to leave dormancy (Cosmulescu and Bîrsanu Ionescu, 2018). The maximum biological effect of one hour of cold is reached between 0° and 7℃. However, this demand for cold hours is very variable, especially from one species to another, but also between cultivars of the same species, with very large differences. When the cold demand is satisfied, the bud-breaking occurs. The model estimated by Amano *et al*. (2010) showed that the time of first flowering for both hawthorn and blackthorn occurred earlier in the most recent 25 years than in any other consecutive 25-year period during the 250- and 125-year observation periods, respectively. Early start-up of vegetation increases the odds of damage caused by late winters or spring season frosts. The available data on time of first flowering, cold demand and heat demand in blackthorn are quite rare. The aim of this paper is to provide information on phenology of blackthorn (*Prunus spinosa* L.) to determine the stability of the order of phenophases and temperature's influence on trends in onset phenology to genotypes of spontaneous flora.

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2. Material and Methods

2.1 Vegetal material

Biologic material is represented by 16 blackthorn genotypes (GV12, GV13, GV14, GV15, GV16, GV17, GV18, GV19, GV20, GV25, GV26, GV28, GV29, GV30, GV33 and GV36) in spontaneous flora, Gura Vaii population (44°12′28″N 23°47′53″E), localized in southern Oltenia region in Romania. Genotypes analysed are situated at altitudes between 81 m and 163 m.

2.2 Meteorological and phenological data

Meteorological data have been used from data recorded by National Meteorological Institute, namely Craiova Meteorological Station. Phenological data have been collected over two successive years (2017 and 2018). In order to record the phenological phases, the reference stages described by Cosmulescu *et al.* (2010) for *Prunus* genus, were used as comparison element.

2.3 Calculating the cold hours

In order to calculate the cold hours (CH) the model used by other researchers was used (Cosmulescu and Bîrsanu Ionescu, 2018; Luedeling *et al*., 2009; Ruiz *et al*., 2007; Crepinsek *et al*., 2012). This model calculates the number of hours (H) when the temperature (T) is lower than 7℃, without considering the negative temperatures. The number of cold hours (CH) at a given time (t) after a fixed starting time, was calculated using the formula: $CH_t = \Sigma_{i=0}{}^{t}H$, if 0℃ < T < 7℃ then add 1; otherwise 0. The date of November 1st was taken as a benchmark to calculate the cold hours because before that time the temperatures are generally too high to make a particular contribution to the accumulation of cold. The cold hours has been calculated by the end of March, which is corresponding to the date of bud-breaking, with 15 day periods of time, steps that are being used also by Cosmulescu and Bîrsanu Ionescu (2018), Ruiz *et al*. (2007), Crepinsek *et al*. (2012).

2.4 Determining growing degree day

The heat demand for the growth of blackthorn genotypes was obtained by adding up daily average temperatures (Tmean), after subtracting the baseline temperature (Tbase, biological 0, 8℃) (Baciu, 2005) using the method used by other authors (Cosmulescu and Bîrsanu Ionescu, 2018; Crepinsek *et al*., 2012): GDD = ∑Tmean-Tbase; if Tmean < Tbase; then add 0. Growing degree day was calculated from the date of bud-breaking, considered to be stage B until the flowering phenophase, i.e. the F stage.

2.5 Calculation of average time from bud-breaking until flowering

The average time from bud-breaking to flowering was calculated by summing up the days from bud-breaking, i.e. B stage until the onset of flowering, namely F stage.

2.6 Statistical analysis

The data obtained from the observations were statistically processed using the descriptive statistics and the ANOVA program to detect differences between experimental group.

3. Results

Phenological characteristics of blackthorn (*Prunus spinosa* L.) are described in Table 1. The start of bud-breaking (B) over the two analyzed years occurs in the second week of March, except for the GV33 genotype that in 2018 had the bud-breaking occurred on the date of March the 28th. The end of bud-breaking (D) occurs in the last week of March of the year 2017, and the beginning of April in 2018. The start of flowering phenophase occurred no earlier than March 26 in 2017 (GV12, GV13, GV14, GV15, GV16, GV17, GV18, GV19, GV20 genotypes) and on April 7 at the latest in 2018 (GV25, GV26, GV28, GV29, GV30, GV33, GV35 genotypes), ie earlier than genotypes analyzed by Zverko *et al*. (2014) in Slovakia. Table 2 presents information on duration of various phenophases in blackthorn genus genotypes (*Prunus spinosa* L.) in Gura Văii population in southern Oltenia, Romania. Monthly average temperatures over period January-April in the years 2017 and 2018 in Craiova, Romania are presented in Table 3. The heat demand in blackthorn bud-breaking or blossoming (Table 4), based on the observations made, has varied between 32−36℃ in 2017, and between 29−46℃ in 2018. In 2017, the blackthorn genotypes needed 1344−1475 hours of cold in order to get out of dormancy state, while in 2018 they needed 1872−1896 hours of cold (Table 4). For blackthorn genotypes, the cold hours was calculated over two calendar years between November 15 and April 30, and the results are shown in Table 5.

Table 1. Reference stages of fruit-setting phenophases in blackthorn genotypes (*Prunus spinosa* L.) in Gura Vaii population.

Year	Reference stages of fruit-setting phenophases*											
	B	C	D	E	F1	F2	F3	G	H	I	J	K
2017	March 8−14	March 18−19	March 20−22	March 24−25	March 26−28	March 28−31	March 30.03−April 01	March 31.03−April 06.04	April 04−09	April 08−13	April 20−21	October 09−15
2018	March 14−28	March 26−29	April 01−03	April 03−05	April 05−07	April 06−09	April 07−10	April 09−12	April 13−15	April 16−18	April 23−26	October 13−16

*B = bud breaking; C = blossom occurrence; D = blossom separation; E = stamina occurrence; F1-F3 = flowering (F1-beginning of flowering, F2-75% of the flowers are open; F3-end of flowering); G = petal falling; H = fruit setting; I = green fruits with dyingsepal crown; J = young fruit; K = mature fruit

Table 2. Duration of different fruit-setting phenophases in blackthorn genotypes (*Prunus spinosa* L.) in Gura Vaii population.

Year	Statistical analysis	Number of days						
		November 1 -B	B-F1	F1-F3	F1-H	F1-J	F1-K	J-K
2017	Mean ± SD	131.8 ± 1.44	15.80 ± 1.82	5.13 ± 1.02	12.13 ± 1.02	26.25 ± 0.77	201.00 ± 2.03	175.75 ± 1.44
	Variation range	128−134	13−20	4−7	11−14	25−27	197−204	173−178
	Variation coefficient	1.09	11.54	19.99	8.45	2.95	1.01	0.82
2018	Mean ± SD	135.63 ± 3.48	22.69 ± 3.34	3.56 ± 0.51	8.56 ± 0.96	18.38 ± 1.02	191.75 ± 1.44	174.00 ± 1.21
	Variation range	134−148	11−25	3−4	7−10	17−20	190−195	172−176
	Variation coefficient	2.57	14.73	14.38	11.26	5.58	0.75	0.70

*B = bud breaking; F1 = beginning of flowering; F3 = end of flowering; H = fruit setting; J = young fruit; K = mature fruit

Table 3. Monthly average temperatures over period January-April in the years 2017 and 2018 in Craiova, Romania.

Year	January	February	March	April	Annual mean (January−April)
2017	−5.20	2.10	9.80	11.00	4.40
2018	1.42	1.07	4.03	16.20	5.68
Difference 2017−2018	−6.62	1.03	5.77	−5.20	−1.28
Mean	−1.91	1.57	6.92	13.60	5.05

Table 4. Cold accumulation for bud-breaking and growing degree day from bud-breaking until flowering of *Prunus spinosa* L. genotypes in Gura Vaii population.

Descriptive statistics	CH (hours of cold)		GDD (℃)*	
	2017	2018	2017	2018
Mean	1385.75	1873.5	32.25	36.81
Standard deviation	54.29	6.0	1.0	5.64
Minimum	1344	1872	32.0	29.0
Maximum	1475	1896	36.0	46.0
CV%	3.92	0.32	3.10	15.31

*GDD=growing degree day, CH=cold accumulation

Table 5. Cold hours (CH) from November 1 to fixed dates in Craiova, Romania, during the 2017-2018 period, according to the 0−7 ℃ Model, with a summary of ANOVA.

Year /date	November 15	November 30	December 15	December 31	January 15	January 31	February 15	February 28	March 15	March 31	April 15	April 30
Cold demand (0−7 ℃ Model, 1 h between 0 and 7 ℃=1 chill unit)												
2017	72	408	696	960	1008	1008	1128	1296	1416	1440	1440	1536
2018	48	384	648	936	1224	1392	1656	1776	1872	1944	1944	1944
Mean	60	396	672	948	1116	1200	1392	1536	1644	1692	1692	1740
Standard Deviation	16.97	16.97	33.94	16.97	152.74	271.53	373.35	339.41	322.44	356.38	356.38	288.50
CV	28.28	4.29	5.05	1.79	13.69	22.63	26.82	22.1	19.61	21.06	21.06	16.58

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6819744	11	619976.7	9.35	0.00026	2.71
Within Groups	794880	12	66240			
Total	7614624	23				

4. Discussion

The results regarding phenological characteristics of blackthorn (Table 1) are in line with the literature that mentions the onset of flowering phenophase and fruit setting in species of *Prunus* genus in March, after vegetative pause and the end in late April (Cosmulescu *et al*., 2010, 2015; Cosmulescu and Gruia, 2016). Negru *et al*. (2005) claims that blackthorn blooms slightly later than the Asian plum trees, but earlier than domestic plum-tree. Blackthorn bloomed from the beginning of March until mid-March, continuing until mid-April in Wytham, Oxfordshire (Gyan and Woodell, 1987). Bud-breaking, the start and end of the flowering phenophase and fruit binding are varying from one year to the next. This is expected due to climatic and environmental conditions (Buljko, 1977; Gunes *et al*., 2000). The development time of flowering phenophase, from the beginning of flowering (F1) until the end of flowering (F3), was different, between March 26 and April 1 in 2017, and between April 5−10 in 2018 (Table 1). Differences recorded in flowering date are due to differences between active temperatures and they are not determined by the need for cold. According to the results obtained regarding duration of various phenophases in blackthorn (Table 2) it is found that over both years, the period between the young fruit phenophase and mature fruit phenophase (J – K) is longer than the period when bud-breaking occurs (November 1 – B), which is in line with the results obtained by Gunes (2003). The average monthly temperatures recorded in February and March 2017 (Table 3), were higher by 1.03℃ and, respectively, 5.77℃, than the average monthly temperatures of February and March 2018. That difference caused the bud-breaking occurring 6 days earlier; while blooming occurred 10 days earlier. Palesova and Snopkova (2010) in Central

Slovakia have also confirmed that temperatures increased in recent decades have a significant influence on the development of spring season phenophases. Thus, the higher air temperatures in the spring season months do trigger the earlier start of spring season phenological phases. The same trend was also observed in Suffolk, UK, where the first date of flowering was observed on May 11 over the period 1930–1940, while over 1998-2005 the flowering occurred on April 28 (Sparks *et al.*, 2006). The same trend is observed in mainland Europe too. Ahas *et al*. (2002) indicates a significant speed-up in the spring season phenological phases across whole Europe over the past fifty years. The same situation was also reported for spring season phenophases in the European hazelnut (Defila and Clot, 2001), plum tree and peach tree (Cosmulescu *et al*., 2010, 2015; Cosmulescu and Gruia, 2016). Although in 2018 blooming occurred 10 days later than in 2017, the flowering period was shorter than in 2017, as the April 2018 average temperature was 5.20℃ higher than in April 2017. In general, duration of flowering phenophase is directly proportional to temperature increase, so that during blooming there are intervals with lower temperatures, the blooming is prolonged, and if temperatures are increasing, the succession rate of the phenological phases increases too. Results based on just two years of continuous measurements and observations are not enough to be used in discussing the influence of climate changes rather than very carefully, but the same trends are observed across whole Europe too. Therefore, the authors consider that if the temperature increase continues in the area, the early occurrence of phenophases compared to the previous records will be a fact. Vegetation phenophases are influenced and triggered when a certain amount of temperature is accumulated. The time of blooming phenophase depends on varieties' demands for heat. Richardson *et al*. (1974), Citadin *et al.* (2001) consider that the heat demand is another factor that causes the bud-breaking and bloom of species in temperate climate. It has also been reported that an increase in temperature above the required values resulted in a reduction of the number of buds that open (Scalabrelli and Couvillon 1986; Citadin *et al*., 2001; Harrington *et al*., 2009). Variations for heat demand in blackthorn bud-breaking or blossoming can be caused either by genotype or by location, whichever is specific for each genotype. From year to year the heat demand of the analyzed genotypes varied (Table 4), the differences are caused by the sum of temperatures above the biological threshold that were accumulated prior to bud-breaking. Also, the intermittent periods of heat and cold during winter can cause longer durations of cold need than continuous cold temperatures. Fan *et al*. (2010) considers that genetic components play a limited role in determining the heat demand of each genotype. Each fruit-tree species has a specific demand for cold that refers to the hours accumulated below the chilling temperature threshold, hours that are important for leaving the dormancy state. The need for cold is the result of long-term climatic adaptation of genotypes of tree species in different regions. Instead, it limits the climatic distribution of fruit-tree genotypes within temperate zones (Sherman and Beckman, 2003). The need for cold is the main factor determining the flowering time (Egea *et al*., 2003; Ruiz *et al*., 2007; Alburquerque *et al*., 2008), an important agronomic feature for fruit trees in temperate zones. The available data on chill and heat demand in blackthorn (*Prunus spinosa* L.) are quite rare. Fan *et al*. (2010) are reporting for the *Prunus* genus a need for cold hours ranging from 320 to 1049 in 2008, and between 294 and 970 hours in 2009. The analysed blackthorn genotypes have differences in cold hours (CH) in two years (Table 4). The differences are caused by the evolution of temperatures until bud-breaking. Sahli *et al.* (2012) confirm that the winter conditions in the period after chilling accumulation are highly correlated with yearly differences in flowering date. Release from dormancy requires a specific minimum cold temperature requirement be met for growth to resume when temperatures warm in spring. The effects of insufficient chill are significant and result in delayed and prolonged bud burst. After the chilling requirement is fulfilled, in ecodormancy phase, the tree no longer accumulates chilling, and bud break occur after the respective forcing or heat requirements for these stages have been fulfilled (Luedeling *et al.*, 2009). The length of ecodormancy phase reflects the time between the end of endodormancy and the fulfillment of heat requirement. In analysing the data in Table 5, it is noted that there are significant differences in cold hours between the two analyzed years. The bud breaks take place before March 15, over the two years under review, 2017 and 2018, except for the GV33 genotype, which in order to get out of dormancy state by the end of March. The intermittent periods of heat and cold during winter season can lead to longer periods of cold demand than continuous cold temperatures, and this could be one of the explanations of data recorded.

In conclusion, the results confirm that the increase in temperature has a significant impact on the earlier occurrence of spring season phenophases in blackthorn. The authors understand that their climatic and phenological observations have been made only over two years, which limits their findings as a significant sign of the climates change impact. Nevertheless, the study shows that the temperature increase is significantly speeding-up the start of spring season phenophases. An anticipated increase in air temperature indicates that the ecosystems in the study area will be likely facing this problem in the future. The results show reactions of spring season phenophases to changed environmental conditions in southwestern area of Romania; thus blackthorn could be used as a potential indicator of climate changes. Climatic and phenological observations will be continuing in the future as well.

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