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THE CORRELATION OF THE ACCUMULATION OF COLD UNITS WITH BIOCHEMICAL PROCESSES IN THE FLORAL BUDS OF APRICOTS (ARMENIA VULGARIS) FROM ROMANIA

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ABSTRACT. Knowledge of the physiological and biochemical mechanisms which form the fundamental for temperate climate cultures of fruit-bearing trees' resistance to cold is an important aspect of horticultural practice. This study aims to determine the relationship between the content in phenolic compounds, proteins and peroxidase in floral buds of 9 varieties of apricot (Rares, CR24-12, CR2-63, Carmela, Viorica, Sirena, Saturn, Mamaia and Comandor) grown in North-West of Romania and the initiation of ectodormancy, endodormancy and ecodormancy stages. The phenolic compound content showed a seasonal dynamic in all the studied varieties. The peak quantity of phenolic compounds was recorded in all cultivars at the end of January, the Carmela and Rares varieties standing out (0.015 ± 0.17 mg GAE/g fresh weight, respectively 0.012 ± 0.039 mg GAE/g fresh weight), followed by a decrease in February. Peroxidase activity showed the same pattern in all cultivars. Protein content showed a pattern similar to those of peroxidase and phenolic compounds, reaching a peak at the end of January, followed by a dramatic decrease starting in February. The studies of biochemical parameters showed a characteristic seasonal dynamic for each cultivar, influenced by the accumulation of cold units that are needed for some phenological stages to occur.

Keywords: Armenia vulgaris, phenolic compounds, peroxidase, flower buds, chill units

INTRODUCTION

Fruit bearing trees' buds go into a state of latency as a response to decreasing temperature and daylight during autumn, a state which makes them more resistant to low temperatures. Depending on the time of the year when this latency starts taking hold, one refers to a state of ectodormancy or forced repose, endodormancy or standard repose and ecodormancy or secundary repose. The first stage of repose is determined by unfavorable environmental factors occurring in autumn, which cause the initiation of the repose state before its characteristic time. Endodormancy is caused by internal factors and it is subject to genetic determinism (Samish, 1954). Ecodormancy is determined by the action of unfavorable factors which occur after vegetative reawakening, such as cold or draught-induces stress, which can induce critical signals which in turn stop bud growth (Horvath et al., 2003; Lang, 1987; Lang, 1996).

The deep repose state is characteristic for woody varieties in the temperate climate. It is not a sudden mechanism put in motion by the plants but rather a progressive process which evolves until the deep rest state is achieved (Lang, 1987), requireing the manifestation of genes which stabilize cellular membranes under the effects of low temperatures (Faurobert & Gomez, 2006).

Initiation of the repose state begins before the onset of negative temperatures and the escalation of endodormancy takes place once negative temperatures have stabilized as such (Cook & Jacobs, 2000). Recent studies have shown that the photo-exposure period and temperature interaction has an important part to play in repose status control concerning the varieties in the Prunus type (Heide, 2008).

There is a limited ammount of information concerning the seasonal evolution of repose in apricot trees (*Prunus armeniaca* L.). Presently, in order to appreciate the depth and progress of bud repose (because of the lack of visual morphological modifications of said buds during repose and/or because of the lack of available endogenous markers) scientists calculate the accumulation of cold units in order to pinpoint the specific stages of repose (Arora et al., 2003). Even with recent significant progress, there are still major unknowns concerning the physiological and biochemical mechanisms which cause the inception, duration and end of the repose state of flowering buds (Arora, et al., 2003).

The evaluation of the initial moment of repose in different varieties of apricot has been done using both phisical and physiological parameters (the weight of the flowering buds and phenological stages) (Guerriero et al., 2006; Ruiz et al., 2007), histology for the flowering buds and their weight after the forced entry into a repose state (Julian, 2008).

A series of changer which occur in the biochemistry of the buds seems to indicate the shift from the endodormancy stage to the ecodormancy stage (Szecskó1 et al., 2002; Zahra et al., 2009). Crabbe, (1994) suggests that certain biochemical markers could indicate the relative level of repose in organs, tissues and even cells.

Even though there are less studies on the refrigeration

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requirements of different cultivars in apricot (compared to other species grown in a temperate climate), the available data shows that for many apricot varieties, the cold requirement ranges from 800 and 1.400 Cold Units (CU) in the case of varieties which require high ammounts of CU (Bailey et al., 1978; Erez, et al., 1979; Bailey, et al., 1982; Guerriero et al., 2006; Ruiz et al., 2007). The cold requirements is not considered constant for the same cultivare, as it is influenced both by its genetic determinism and a series of environmental factors, such as latitude and longitude, the degree of readyness for winter, temperature dynamics, etc. (Lang, 1989).

In superior plants the antioxidant metabolism undergoes modifications tied to the annual cycles. Protection against free radicals is acchieved via the antioxidant action of some enzimes (catalaze, peroxidaze, superoxiddismutaze, etc.). At the end of the endodormancy stage, large increases in antioxidating activity have been observed in apricot varieties with low cold requirements. Results suggest that minimal threshholds in antioxidating activity may be decisive in the elimination of free radicals formed during the winter (Tamassy & Zayan, 1983; Bartolini et al.,1997; Bartolini et al., 2006). Along with the destructive effect which free oxygen radicals have on plants, it seems they have a critical role in transduction signals. Considering these facts, maintaining the balance between formed free radicals and removed/annihilated free radicals is of great importance. It is thought that plants which exhibit resistance to oxidizing stress generated by oxygen free radicals are also resistant to cold (Iba, 2002).

Studies concerning the activity of sulphydryl compounds (SH), of reduced glutathione (GHS) and the activity of catalaze during the development cycle of flowering buds have been performed on apricot varieties with different production capabilities. The catalase, SH-containing compounds and reduced glutathione changes according to a three stage model, from November to February. In the first two stages a gradual increase of catalaze activity and GHS can be observed, in parallel with a decrease of total concentration in SH-containing compounds. In the third phase (at the beginning of February), catalase activity tends to decrease along with an increase in SH-containing compounds and GHS (Viti & Bartolini, 1998). Multiple studies report the fact that the phenolic content of buds differes, varying according to the repose stage and the variety whithin the same species. As such, peach-tree flowering buds exhibit an increase in phenolic compound content at the beginning of repose, followed by gradual decrease and eventual total elimination during actual flowering (Szalay et al., 2005). In the case of pistachio (*Pistacia vera* L.), (Zahra et al., 2009) large variations in poliphenols content can be observed in certain varieties in the November-March interval.

A tight relationship between the protein content and the accumulation of cold units has been determined by Yamane et al. (2006) concerning Japanese apricot (*Prunus mume* Seibold & Zucc.) and by Faurobert & Gomey, (2006) concerning apricot trees. In another study, Tamura et al., (1998) have suggested that the 19 kDa proteine can be an adequate marker for the measurement of the degree of repost in Japanese pear trees (*Pyrus pyrifolia* Nakai). Studies concerning the seasonal variation in protein content and their role in physicological defense mechanisms in plants at low temperatures have been undertaken by Thomashow (1999) and Iba (2002).

This study has had two objectives: i). to study the dynamic of phenolic compounds, protein and peroxidase content in flowering buds in nine apricot cultivars (Rares, CR24-12, CR2-63, Carmela, Viorica, Sirena, Saturn, Mamaia si Comandor) grown in NorthWestern Romania during vegetative repose and ii). the determination of cold unit requirements for the completion of certain phenological stages.

MATERIALS AND METHODS

Biological material

Flowering buds were collected starting in the month of January, at 12-14 day intervals, from 5-year old apricot trees from S.C.P.P. Oradea (Fruit Research Station and Manufacturing Oradea), grafted on sour cherry (*Prunus cerasifera*), planted in 4 x 3 m intervals, of the following varieties: CR 24/12, CR 2/63, Rareş, Carmela,Viorica, Sirena, Saturn, Mamaia and Comandor.

Temperature determination

Measurements were taken every hour, using the HANNA 143 HI logger. The calculation of cold units was done using the UTAH Model (Richardson et al., 1974), via the Wizard Java Script Version 1 software. The UTAH model allows for the fact that different temperatures have varied effects on the accumulation of cold units. Temeperature between 2.5 and 9.1^{o}C are considered as having the most significant contribution in the accumulation of cold units and every hour spent in this temperature interval has produced one cold unit. Lower or higher temperatures can have a negative effect on the accumulation of cold units and they have been attributed values accordingly.

Preparation of the vegetal extract

Annual branches yielded 20 floral buds each, which were detached, weighed, processed with the mortar and pestle in a cold environment, in the presence of a phosphate buffer with pH 7.2, passed into eppendorf tubes and centrifuged la 10.000 rpm at 4^{o}C for 20 minutes. The resulting supernatant was used to determine protein, peroxidase and phenolic compound quantities. The harvesting of the buds was performed in the

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following stages: B (the growth of the buds), F (first open flower) and G (first scattered petals), according to Fleckinger, (1945). The ratio between the bud weight and the phosphate buffer solution was 1:20 (g/v).

Peroxidase determination

Enzymatic activity of peroxidase was measured with a spetrophotometer, the principle of the method being the oxidation of p-phenylenediamine by the peroxidase enzymes present in a vegetal extract, as a result of which a violet-colored solution is obtained.

There is a direct proportion between the color intensity of the solution, measured with the spectophotometer at 483 nm wavelenght (UV-VIS Spetrophotometer UVmini-1240, Shimadzu) and the activity of the peroxidase.

Determination of total soluble protein

The protein concentration from bud extract was determined as described by Bradford (1976), using bovine serum albumin (BSA) as standard.

The supernatant was used for the spectrophotometric determination of protein, at wavelength 595 nm (Shimadzu UV-Visible mini-1240).

Determination of polyphenolic compounds

Total phenolic content was determined by the Folin-Ciocalteu method. This method combined 100 µl bud extract, 1700 µl distillated water and 200 µl Folin-Ciocalteu (diluted 1:10) reagent; then mixed well using a Vortex. The mixture was allowed to react for 3 minutes, and then 1 ml of 15% Na_2CO_3 (Arnous et al., 2001) solution was then added and mixed well. The samples were incubated at room temperature, in the dark for 2 hours. The absorbance was taken at 750 nm using a spectrophotometer (UV-VIS Spetrophotometer UVmini-1240, Shimadzu). The standard curve was linear, between 0.1-0.5 mg/ml gallic acid. The results were expressed in gallic acid equivalents (GAE; mg/g fresh weight). Adequate dilution was needed if the absorbance value measured was over the linear range of the standard curve.

RESULTS AND DISCUSSIONS

Correlation of cold units with physiological processed and latency intervals

Calculation of the accumulation of cold units CU (chill units) according to the UTAH model, within a six year interval (2005-2010) has shown the fact that in the studied geographic area (47°09′13″N 21°55′18″E) the anual accumulation of CU varied between 900 CU in 2005-2006 and 1380 CU in 2007-2008 (Fig 1.).

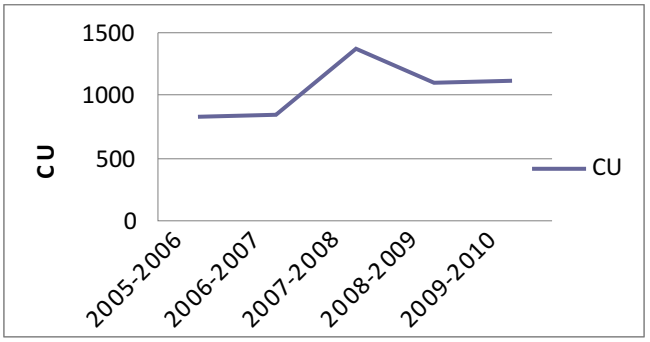

Fig. 1 Accumulation of chilll units (CU) over a period of 6 years (2005 - 2010)

The analisys of the accumulation of cold units on a monthly basis (October – April) between 2005 and 2010 shows there are two accumulation peaks – one in November and the other one in February or March (Fig. 2). The accumulation of chill units in this interval can be considered an important element in the process of characterizing an area and in the appreciation of the stress exhibited by the areal, via temperature, on grown species.

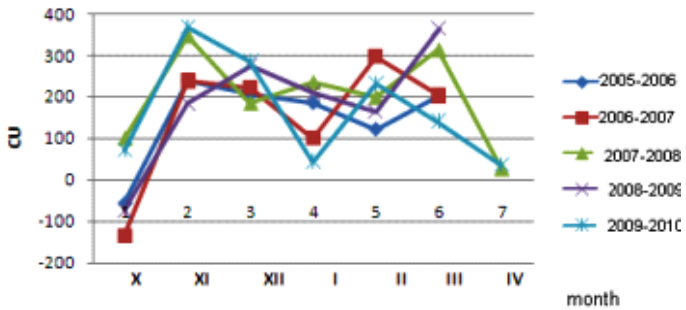

Fig. 2 The Accumulation of chilll units (CU) between the months of october – april, between 2005 – 2010

Within the studied area and the environmental conditions in 2009, leaf loss and inception of ectodormancy occured at the beginning of November, when successive negative temperatures of -3.2, -3.7, -3.5 were recorded in the first three days of the month. The constant accumulation of chill units CU began on the 3rd of November (Fig. 3). The beginning of forced repose is linked both to the leaves falling (Fuchigami & Nee, 1987; Guerriero et al., 2005; Naor et al., 2003) and the constant accumulation of chill units (Erez et al., 1979; Richardson et al., 1974; Ruiz et al., 2007).

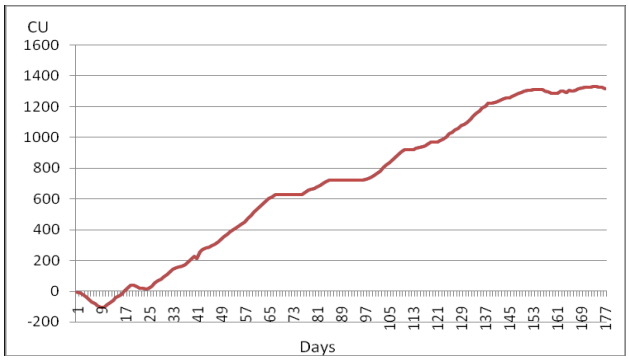

Fig. 3 Chilll units (CU) accumulation dynamic in the 01.10.2009-14.04.2010 interval

The bud swelling phenophase (Fig. 4) (considered from the moment when 15% of the buds are swollen) beggins on 07th March in CR 24/12 and Rareş cultivars, after a total accumulation of 912 CU. The last two cultivars which enter this phenophase are Mamaia and Comandor, once they've accumulated 992.6 CU.

The difference between the first two varieties (CR 24-12, Rareş) and the last variety which entered the floral bud swelling phenophase (Comandor) is 6 days and the difference between accumulated CU units in this interval is 80.6 CU.

The beginning of the flowering period (10% of the flowers open, acording to Adato (1990) occurs on the 28th of March (Fig. 4) in CR 24/12 and Rareş varieties, after an accumulation of 1181 CU. The smallest number of chill units accumulated between the bud swell phenophase and the inception of flowering was recorded in the Comandor variety (188.4 CU) and the largest was recorded in the CR 24/12 and Rareş varieties (230.8 CU).

The phenophase associated with the end of flowering (Fig. 4) (when only 10% of flowers are unscattered, according to Adato (1990) begins in the CR24/12, CR 2-63, Rareş and Carmela varieties with 1181 CU, lasts for 4 days, ending with the Mamaia and Comandor varieties (1187.2 CU). Within this time interval 6.2 CU were accumulated.

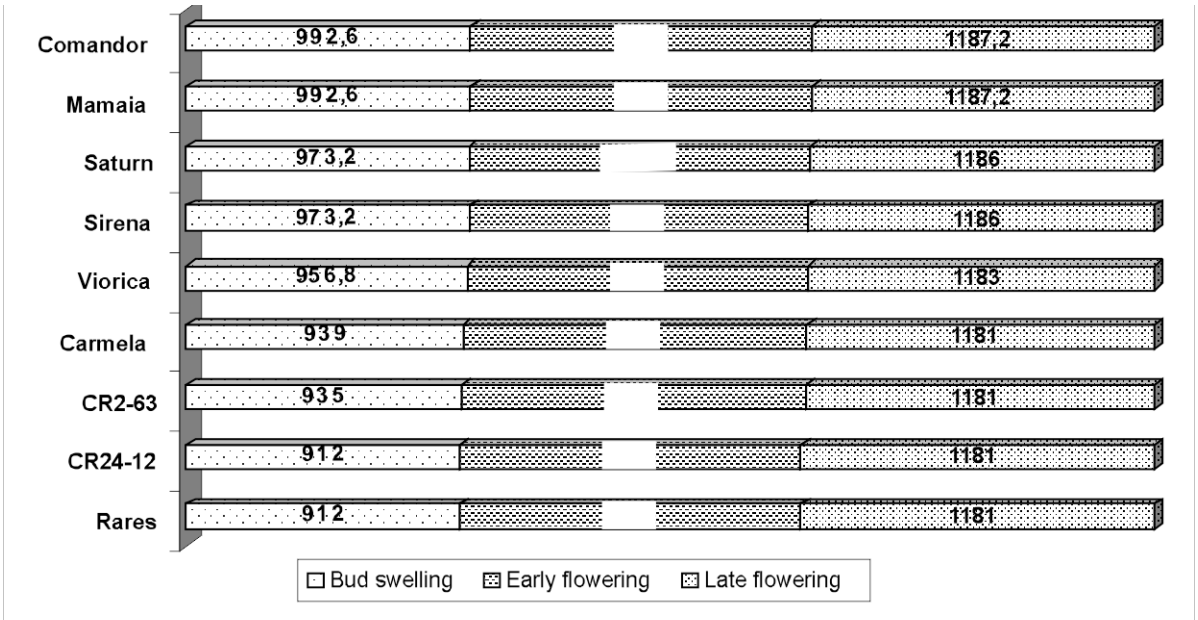

Fig. 4 Sequence of phenophases in relation to the accumulation of chill units (CU) in the studied nine apricot cultivars

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Modifications of the biochemical processes in relation to the accumulation of chill units

The phenolic compound content (Fig. 5A) showed a seasonal dynamic in all studied varieties. In relation to the analyzed intervals, the peak quantity of phenolic compounds was recorded at the end of January in all cultivars, the Carmela and Rareş varieties standing out with 0.015 ± 0.17 mgGAE/g frees weight, respectively 0.012± 0.039 mg GAE/g fresh weight, followed by a significant drop in phenolic compounds in February, with a minimum of 0.0009± 0.02 mg GAE/g fresh weight at the end of March (29.03.2010). The highest quantity of phenolic compounds was determined in the CR 2-63 cultivar 0.0045± 0.023 mg GAE/g fresh weight, followed by the CR 24-12 cultivar (0.00027± 0.026 mg GAE/g fresh weight). During the studied stages, the smallest value of phenolic compounds was recorded in April, in a time associated with the end of flowering.

This data is in concordance with the results obtained by Zahra et al., (2005), who have recorded the lowest values of phenolic compounds in the interval succeeding flowering in their four cultivars of Pistacia vera L. In our recent article, (Vicas & Laslo, 2011) was determined the dynamic of the phenolic compounds in the flower buds for 5 nectarine cultivations (Cora, Ark 165, Delta, Romamer II, Crimson Gold) in N-W Romania, during the stage of vegetative rest. Our results shown that the content of phenolic compounds has had a seasonal dynamic for all varieties which have been studied. In relation to the period analysed, the maximum quantity of phenolic compounds was recorded for all cultivations at the end of January, two cultivations stood out, Delta and Romamer II.

Peroxidase activity (Fig. 5B) showed the same pattern in all cultivars. It increased, reaching a peak at the beginning of February in the Viorica cultivar (0.17 ± 0.00 U/mg protein). It then deacreased, reaching a minimum at the beginning of March (08.03.2010) in the Sirena cultivar (0.027 ± 0.0014U/mg protein). What followed was another increase of peroxidase activity at the end of March and April. For example in 29.03.2010 the highest peroxidase activity was recorded in the CR23-12 cultivar (0.077 ± 0.0007 U/mg protein), whereas in the month of April the highest enzyme activity was recorded in the Comandor cultivar (0.110 ± 0.0007U/mg protein).

The minimum quantity of phenolic compounds and peroxidase activity (08.03.2010), followed by the increase of peroxidase activity indicates the end of the endodormancy interval for plants and the beginning of the ecodormancy stage. Negative temperature days between the 6th and 16th of March (Fig. 6) determined a slight intensification of peroxidase activity, followed by a decrease of activity once pozitive temperatures became stable during the second half of March and April. Our observations are in accordance with previously published results (Szecskó1, 2002; Zahra, et al., 2009), which confirm the protective role phenolic compounds and peroxidase have concerning fruit-bearing tree varieties' resistance to cold.

A correlation between the intensity of peroxidase and phenolic compounds activity and the early blooming or late blooming nature of the cultivars could not be established concerning neither peroxidase nor phenolic compunds.

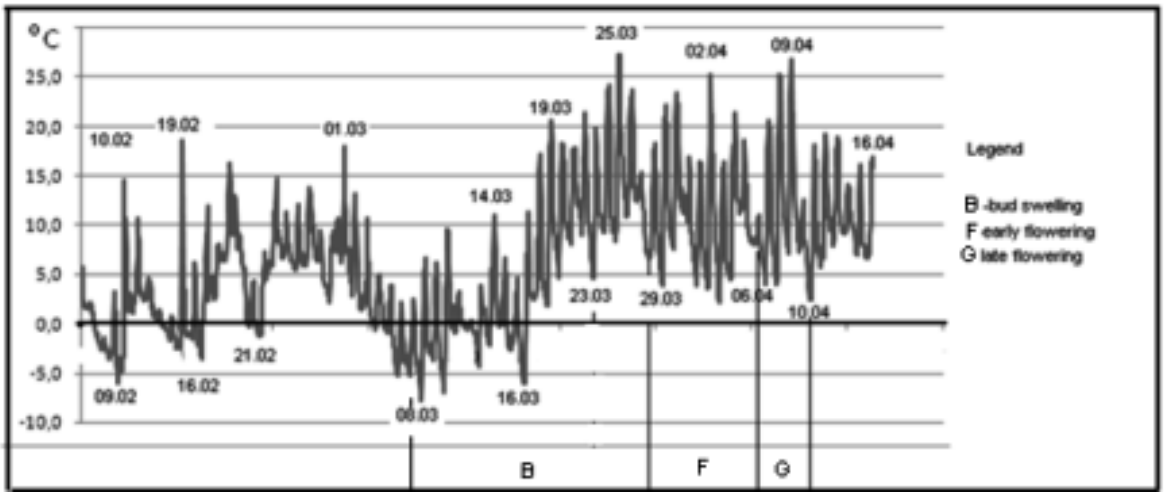

Fig. 6 Representation of daily dynamic of temperatures in the 07.02 2010-16.04.2010 interval and of the duration of the three phenophases

When the activity of peroxidase was determined in the flower buds for 5 nectarine cultivations (Cora, Ark 165, Delta, Romamer II, Crimson Gold) from N-W Romania (Vicas &Laslo, 2011) the greatest enzyme activity was registered during the month of April with values between 0.1889 ± 0.001 U/mg protein, for the cultivation Cora and 0.1015± 0.002 U/mg protein for the cultivation Romamer II. The smallest enzyme activity was registered during the month of January.

The protein content (Fig. 5C) exhibits similar dynamics to those of peroxidase and phenolic compound, reaching a peak at the end of February, followed by a dramatic drop during February, with a minimum at the beginning of April. Numerous studies (Tamassy & Zayan, 1983; Thomashow, 1999; Iba, 2002) have focused on the definition of plant proteins' role in the increase of cold resistance. Results have shown that a high soluble protein count and a low level of free aminoacids can be associated with an increase in cold resistance. The highest quantity of protein was recorded in January, in the Rareş cultivar (0.25 ± 0.011mg BSA/mg fresh weight). The minimum soluble protein content was recorded during April (13.04.2010), in the Mamaia cultivar (0.0096±0.0007mg BSA/mg fresh weight). In the month of March comparatively low protein content values were recorded, in relation to those recorded at the end of the month. The same situation was recorded in the case of peroxidase activity. The large variations in temperatures during the month of March (-7 - +250C) (Fig. 6) determined the large fluctuations in peroxidase activity and protein content.

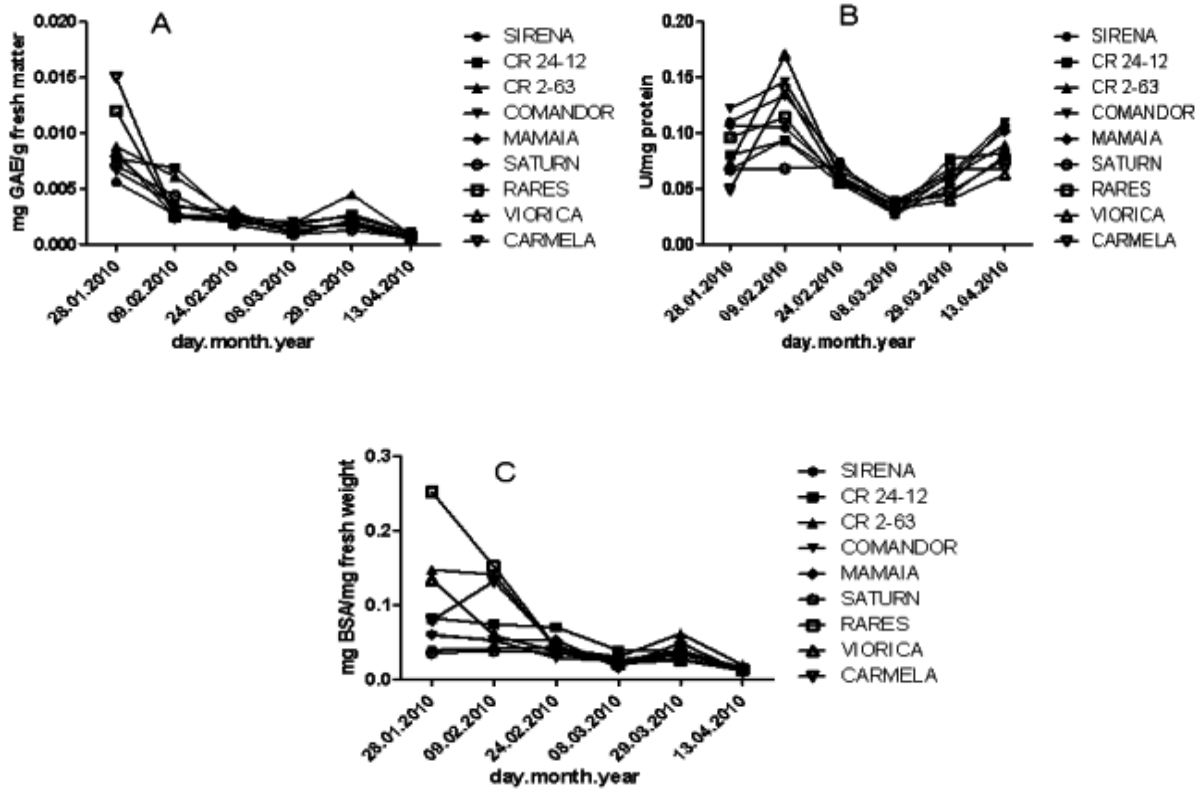

Fig. 5 A. The quantity of phenolic compounds (mgGAE/g fresh weight);
B. The enzyme activity of peroxidase (U/mg protein);
C. The protein content (mg BSA/mg fresh weight) at 9 cultivations of apricots during an interval between 26 February 2010 and 13 April 2010 B.

CONCLUSIONS

The effective quantity of chill units measured from the inception of forced repose to the beginning of the bud swelling phenophase varies between 1078 CU (CR 24-12, Rareş cultivars) and 1156 CU (Comandor cultivar).

The phenolic compound content showed a seasonal dynamic in every studied variety. In relation to the analyzed interval, the maximum quantity of phenolic compounds was recorded in all cultivars the end of January. The Carmela and Rareş cultivars stood out, exhibiting 0.015± 0.17 mg GAE/g fresh weight, respectively 0.012± 0.039 mg GAE/g fresh weight, followed by a decrease during February.

Peroxidase activity showed the same pattern in all

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cultivars. It increased, reaching a peak at the beginning of February in the Viorica cultivar (Viorica 0.17± 0.00 U/mg protein), followed by a decrease, reaching a minimum at the beginning of March (08.03.2010), in the Sirena cultivar (0.027± 0.00 14U/mg protein).

Protein content showed a similar dynamic to those of peroxidase and phenolic compounds, with a maximum at the end of January, when trees are in deep repose, followed by a dramatic decrease starting in February.

The achieved minimum quantity of phenolic compounds and peroxidase ectivity occurred on 08.03.2010, followed by an increase in peroxidase activity, indicating the end of the endodormancy stage and the inception of ecodormancy.

A correlation between the intensity of peroxidase and phenolic compounds activity and the early blooming or late blooming nature of the cultivars could not be established concerning neither peroxidase nor phenolic compunds.

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