KIWIFRUIT (ACTINIDIA SPP.) PHENOLOGICAL GROWTH STAGES IN SOUTHERN ROMANIAN CLIMATE ACCORDING TO THE BBCH SCALE

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Abstract

The aim of this paper is to present the phenological growth stages of two kiwifruit cultivars (Hayward and Bruno) and some Romanian intra and interspecific Actinidia hybrids. The experimental field was established in 2000. The plants were grown on a T-bar trellis system, under an organic orchard management. The inter row surface was covered with a mixture of perennial grasses and along the row, the soil was kept clean. Drip irrigation and micro spray irrigation system was provided. The phenological growth stages were described in the environmental conditions of Romanian plain (Bucharest area), according to the BBCH General Scale (Meier, 2001) and the nomenclature that has been used for Actinidia deliciosa 'Hayward' (Salinero et al., 2009). Data were recorded during two consecutive growth stages for bud, leaf and shoot development, inflorescence emergence, flowering, fruit development, fruit maturity and plant senescence can improve some horticultural practices and operations on kiwifruit orchard management (such as pruning, girdling, pollination techniques, frost protection, fertilization, irrigation et c.).

Key words: phenology, hybrids, A. arguta, A. chinensis, A. deliciosa.

INTRODUCTION

Domesticated from wild populations located on Yangtze River basin from China, kiwifruit is a recently developed crop, due to its nutritional properties, high vitamin C content, as well as its taste and flavour (Biao et. al., 2018; Litz 2005; Yang, 2010; Young et al., 1995).

Actinidia genus belongs to the family Actinidiaceae and according to the latest revision (Huang et al., 2007) has over 75 species and about 125 known taxa worldwide. Current commercial cultivation is almost entirely based on *A. deliciosa* and *A. chinensis* (Huang, 2016; Zhang et al., 2010). Lesser extent, in colder regions, *A. arguta* commercial potential started to be recognised, in the early 20th century (Ferguson and Huang, 2007).

Kiwifruit is widely distributed in Asia ranging from the tropics (latitude 0°) to cold temperate regions (50°N) (Huang et al., 2007). According with Cui (1993), *Actinidia* species are found from India to Japan, and from Siberia to Indonesia. In different climates and geographical environments, *Actinidia* species exhibit tremendous biological variation (Huang et al., 2007).

The study of periodic biological events was called phenology (Hernández et al., 2014). Throughout the time a large number of studies are reported in the literature concerning descriptions of principal growth stages in different horticultural crop (Aydin et al., 2019; Bratu et al., 2019; Muşat et al., 2019; Panchev et al., 2019; Stănică, 2019a; Stănică, 2019b; Stroe et Cojanu, 2019).

In 1945, using a combination of letters and numbers, Fleckinger defined 'phenological stages' (Fleckinger, 1948). Adopting the same codes, Zadoks et al. (1974) published the first decimal code to standardise the description for the growth stages of different crops. Based upon descriptions of cereals (Zadokset these al.,1974), a uniform decimal code, known as the BBCH - scale (Biologische Bundesanstalt, Bundessortenamt, Chemische Industrie), was proposed by Lancashire et al. (1991) and Bleiholder et al. (1991). Hack et al. (1992) and Hess et al. (1997) proposed a more advanced scale, the extended BBCH and later, the

⁶BBCH-Monograph' (representing a group of 27 crops and weeds) was published (Meier, 1997). BBCH-scale (Meier, 2001) it is used now by many researchers for describing the growth stages of different fruit trees (Hernández et al., 2014).

For kiwifruit, first phenological growth stages according BBCH scale, have been described by Salinero et al. (2009), for Actinidia deliciosa 'Hayward', in Pontevedra region, from northern-vest of Spain. Important contributions to the study of the phenology of Actinidia deliciosa 'Havward' were made also by Brundell (1975a; 1975b). He proposed six stages for bud development after winter dormancy, and six stages for the development of flower buds until full bloom. To describe these stages, he used the initials of a few words that briefly described each stage (for example 'bb' for bud burst). Regarding fruit development, Hopping (1976) established a growth curve divided on three stages (namely I, II and III), based on fruit weight and growth rate. Later, Beever and Hopkirk (1990) revised the characteristics of Hayward fruit development and physiology, but without presenting a phenological scale.

In Romania kiwifruit research and culture started in 1993 (Peticilă et al., 2002; Stănică, 2009). The first orchards with *Actinidia deliciosa* were planted at Ostrov (Constanța County), on the border of the Danube River (Stănică & Cepoiu, 1996; Stănică, 2009). The most important studies were conducted in a common Italian-Romanian kiwifruit breeding program, initiated at the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Stănică & Zuccherelli, 2007; Stănică & Zuccherelli, 2009).

Taking into consideration that *Actinidia* is a new fruit specie in Romania, the descriptions of principal growth stages can improve some horticultural practices and operations on kiwifruit orchard management (such as pruning, girdling, pollination techniques, frost protection, fertilization, irrigation or pest control).

In this context, the aim of this research was to define the phenological stages of two kiwifruit varieties ('Hayward' and 'Bruno') and some Romanian intra and interspecific *Actinidia* hybrids, in Bucharest area. This study can improve the cultivation of this new crop in Romania, can contribute in Romanian breeding program and also in zoning of the main *Actinidia* species on our country climatic conditions.

MATERIALS AND METHODS

The study was conducted during two consecutive growing seasons (2018 and 2019), in the Experimental Field at the Faculty of Horticulture, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest, for two kiwifruit varieties and eight Romanian intra and interspecific *Actinidia* hybrids. The plant material is presented in Table 1.

V	ariety/Hybrid	Species				
Hayward		A. deliciosa				
Bruno	© 	A. deliciosa				
R0P10		A. chinensis intraspecific hybrid				
R0P13	- O	A. deliciosa and A. chinensis interspecific hybrid				
R1P8	0	A. deliciosa and A. chinensis interspecific hybrid				
R1P9	0	A. deliciosa and A. chinensis interspecific hybrid				
R1P12		A. deliciosa and A. chinensis interspecific hybrid				
R2P6	e e () ()	A. chinensis and A. arguta interspecific hybrid				
R8P1		A. arguta intraspecific hybrid				
R10P20		A. arguta intraspecific hybrid				

Table 1. Plant material description

The climate in the experimental area is typically temperate-continental, with cold winter and warm, sometimes torrid, summer, with frequent droughts (Asănică, 2010).

The annual mean temperature is between 10.5°C in the peripheral areas, and 12°C in the center, caused by the high concentration of constructions, the street traffic and the industrial activities. The annual precipitation between 550 and 600 mm was recorded, mostly falling between May and July. The dominant air

circulation is east and northeast during the winter and from west to the rest of the year, with the maximum wind speed of 3.5-4 m/sec (Asănică, 2010; Asănică, 2011).

The plants were grown in a preluvosoil, on a T-bar trellis system, under an organic orchard management. The inter row surface was covered with a mixture of perennial grasses and along the row, the soil was kept clean. Drip irrigation and micro spray irrigation system was provided.

The phenological growth stages were described in the environmental conditions of Romanian Plain, Vlăsiei Plain subdivision, between winter dormancy and leaf fall, according to the BBCH Scale (Meier. 2001) General and the nomenclature that has been used for Actinidia deliciosa 'Hayward' (Salinero et al., 2009). The extended BBCH scale considered 10 principal growth stages, numbered from 0 to 9. This study handled 8 of the 10 principal stages - bud, leaf and shoot development. inflorescence emergence, flowering, fruit development, fruit maturity and plant senescence, described in Table 2.

RESULTS AND DISCUSSIONS

The evolution of growth stages, according to BBCH scale (Meier, 2001), provides an accurate description of kiwifruit plants phenology. The scale is based on a two-digit code where the first digit describes the principal growth stages such bud development, leaf development, as flowering etc., while the second digit gives a more precise timing event of the principal stage. primary and Some of the secondary growth stages of kiwifruit phenological according to BBCH scale (described in Table 2), are represented with photographs for the most cultivated Actinidia species - A. deliciosa (Figure 1), A. chinensis (Figure 2) and A. arguta (Figure 3).

During the experimental period, for a better description of the climatic conditions of Bucharest area were noted in Table 3 - the minimum, maximum and mean temperature registered on 2018 and 2019 for every month. Also, was noted the number of days of precipitation and total mm, per every month. These data are very important for correlating the environmental conditions with the phenological growth stage and the main horticultural practices for orchard management.

The lowest temperatures were recorded mainly in winter period, but also in late autumn, -12°C in November 2018 and early spring, -22°C in March 2018.

The kiwifruit species stayed in dormancy until March, when the mean temperature registered are higher than 3.5°C.

Higher temperature differences between night and day were recorded in the months February to April and September to November, in every year. When warmer temperatures in late winter encourage early bud break, these young buds are highly frost - susceptible (Debersaques et al., 2019).

The highest temperatures, between 33-36°C, were recorded in the summer and autumn begging. For reduce drought effect, that can cause considerable damage on kiwifruit orchards, it is necessary to improve atmospheric humidity with sprinkler irrigation.

Annual rainfall averaged in 2018 was 661.1 mm, respectively 636.2 mm in 2019. The rainy period was as usual in this area, occurring during end of spring and middle of summer (Table 3).

The optimal amount of precipitation for kiwifruit is between 1200-1500 mm/year, eventually distributed over the growing season (Hennion, 2003; Debersaques et al., 2019). Thus, water supply by irrigation was provided.

Actinidia cultivation requires a wide range of horticultural practices such as pruning, girdling, frost protection, pollination techniques, flower and fruit thinning, fertilization, irrigation or pest control and harvest (Salinero et al., 2009). To properly manage kiwifruit orchards, an accurate tree phenology must be scheduled.

The phenophases from bud development to fruit maturity and senescence were presented in Table 4, for two kiwifruit varieties ('Hayward' and 'Bruno') and eight Romanian intra and interspecific *Actinidia* hybrids (R0P10, R0P13, R1P8, R1P9, R1P12, R2P6, R8P1, R10P20), in two observational years (2018 and 2019). In Table 5 the main operations on kiwifruit orchard management were presented.

BBCH	Growth stage description											
code	Principal growth stage 0: Bud development											
00	Dormant buds grown in the previous crop-year are completely closed. A small ostiole (less than 2 mm ir											
00	diameter) is visible (Figures 1, 2, 3).											
01	Beginning of bud swelling; scales just visible (Figures 1, 2, 3). For A. deliciosa and A. chinensis they are											
	covered by white trichomes.											
03	End of bud swelling; scales protruded through the corky tissue of the stem. For A. deliciosa and A. chinensis											
	scales are densely covered by brown trichomes on their abaxial surface (Figures 1, 2); for A. arguta scales											
07	tips joined apically (Figure 3).											
07	Beginning of bud burst. For <i>A. deliciosa</i> and <i>A. chinensis</i> , leaf and inflorescence buds enclosed by scales covered by brown trichomes (Figures 1, 2); for <i>A. arguta</i> scales tips dispersed along bud axis (Figure 3).											
09	Scales separate and green leaf tips are visible. For A. deliciosa and A. chinensis they are covered by brown											
0)	trichomes (Figures 1, 2); for <i>A. arguta</i> they are covered abaxially by white ones (Figure 3).											
	Principal growth stage 1: Leaf development											
10	The bud develops into an open cluster containing a few visible leaves (Figures 1, 2, 3).											
11	Visible leaves unfolded and start spreading away from the shoot (Figures 1, 2, 3).											
12-18	Two to eight or more leaves unfolded, but not yet at full size (Figures 1, 2, 3).											
19	First leaves completely developed (Figures 1, 2, 3).											
	Principal growth stage 3: Shoot development											
31 32	Shoots reach about 10% of final length. Shoots reach about 20% of final length.											
32 35	Shoots reach about 50% of final length.											
33 39	Shoots reach about 90% of final length.											
0)	Principal growth stage 5: Inflorescence emergence											
51	Inflorescence bud swelling (flowers borne as singlets or triplets in the leaf axils). Buds closed, with n											
	peduncle, greenish sepals visible covered by trichomes (Figures 1, 2, 3).											
53	Flower buds growing, they still closed, reddish peduncles elongating (Figures 1, 2, 3).											
55	Sepals begin to separate. A white-greenish corolla starts to be visible, reddish peduncles continue t											
	elongate (Figures 1, 2, 3).											
56	Sepals continue to separate, and peduncles to elongate and thicken. Corolla clearly visible, longer that											
57	calyx, changes colour from white-greenish to white (Figures 1, 2, 3). Corolla at balloon stage, first flowers with white petals forming a hollow ball. One of the petals separate											
57	from the rest (Figures 1, 2, 3).											
59	Several petals separate, pistils still not visible longer than calyx (Figures 1, 2, 3).											
07	Principal growth stage 6: Flowering											
60	First flowers open, corolla bell-shaped, pistil visible (Figures 1, 2, 3).											
61	Beginning of flowering: 10% of flowers open.											
65	Full flowering: at least 50% of flowers open (Figures 1, 2, 3).											
67	First petals fading or falling. Some pistils still fertile (Figures 1, 2, 3).											
68	Most petals dry or fallen. All pistils dry and no longer functional.											
69	End of flowering, fruit set visible (Figures 1, 2, 3).											
71	Principal growth stage 7: Fruit development Fruit about 10% of final size, showing the typical characteristic of the cultivar (Figures 1, 2, 3).											
71 73	Fruit about 10% of final size, showing the typical characteristic of the cultivar (Figures 1, 2, 5). Fruit about 30% of final size (Figures 1, 2, 3).											
75	Fruit about 50% of final size.											
79	Fruit about 90% of final size: fruit suitable for commercial picking (Figures 1, 2, 3).											
	Principal growth stage 8: Maturity of fruit											
81	Seeds reach their full size, harden and change colour from white to brown, progressing through tan to dar											
	brown.											
85	Fruit ripe for commercial picking, solids content higher than 6.2%. Seed colour becomes black. Fruit a											
0.6	physiological maturity (still not suitable for consumption), begins to soften (Figures 1, 3).											
89	Fruit fully ripe for consumption: fruit has typical taste, flavor and firmness. Soluble solids about 14–16% (Figure 2).											
	Principal growth stage 9: Senescence. Beginning of dormancy											
91	Shoot growth complete; foliage fully dark green.											
93	Beginning of senescence of old leaves; leaves fall (Figures 1, 2).											

(According with Meier, 2001; Salinero et al., 2009; Labeke et al., 2015)



Figure 1. Some phenological growth stages of 'Bruno' variety (A. deliciosa), according to BBCH scale

The different stages of bud development mostly took place in the beginning to mid-March, for all three studied species, when the mean temperature registered was higher than 3.5°C.

Leaf development and emergence/development of the inflorescences occurred from the last weeks of March till beginning April.

Full flowering ensued from early May for *A. chinensis* and *A. arguta* through middle of May, for *A. deliciosa*, when the mean temperature registered was higher than 15° C.

Phenological stage and temperature monitoring are very important, because cultivars that have a

lower basal temperature for bud and flower development might be more susceptible to spring frost especially if no frost protection is present (Labeke et al., 2015).

According Spano et al. (1997), temperature initiates all biological processes that result in the occurrence of a certain phenological stage and also, temperature affects the morphological and quality characteristics of fruits.

Fruit maturity has been reached in the first weeks of September for *A. arguta*, at the last weeks of October for *A. chinensis* and at the beginning of November for *A. deliciosa*.



Figure 2. Some phenological growth stages of R0P10 intraspecific hybrid (A. chinensis), according to BBCH scale

Studying the hybrids of the different species of *Actinidia*, it is clear that differences between the developments of the phenological stages exist.

The growing season for kiwifruit is long: up to 240 days. This begins with vine pruning in winter, which follows the previous year's harvest (Baker et al., 2018). During the winter months (December to February) the vines lay dormant, allowing growers the opportunity to remove last season's fruiting canes and to select and tie down new canes which form the

foundations for new growth (Baker et al., 2018).

According to Costa et al. (1996), in established kiwifruit vines, pruning and girdling practices are carried out to renew fruiting wood, to achieve a good balance between vegetative growth and fruit production, and to improve light interception and air penetration through the canopy. Each year the winter pruning is initiated at the end of autumn, when all leaves are fallen (BBCH stage 97), and is continued meanwhile vines remain dormant (stage 00).



Figure 3. Some phenological growth stages of R8P1 intraspecific hybrid (A. arguta), according to BBCH scale

Springtime (March to May) sees the kiwifruit vines begin to grow again. New shoots appear on the canes along with the first flower buds.

Especially important for kiwifruit production is the recognition of phenological stages during the development of the floral bud, because they are key for flower and fruit thinning and to increase the success of pollination (Salinero et al., 2009).

Flower and fruit thinning are done to reduce excessive fruit load, thus diminishing the competence among fruits for carbohydrates and obtaining a final higher fruit size, and to eliminate lateral and misshapen fruits (Salinero et al., 2009). Considering the BBCH scale, flower thinning should be done from stage 55 to stage 60, whereas fruit thinning is advised from stage 71 to stage 73 (Salinero et al., 2009). These operations that increase fruits quality are achieved especially for *A. deliciosa* and *A. chinensis*.

During the spring–summer period, fertirrigation is commonly applied at orchards to provide necessary nutrients, macro and microelements like N, P, K, Ca, Mg etc. (Salinero et al., 2007; Salinero et al., 2009).

	2018						2019					
Month	Temperature (°C)			Total precipitation		Temperature (°C)			Total precipitation			
	Max.	Min.	Mean	mm	days	Max.	Min.	Mean	mm	days		
Jan	14	-11	1.01±6.21*	46.6	10	9	-16	$-1.69\pm5.30^{*}$	57.3	16		
Feb	17	-13	$1.08{\pm}6.18^{*}$	94.3	19	19	-8	$3.42{\pm}7.26^{*}$	7.6	4		
Mar	24	-22	$3.59{\pm}8.56^{*}$	68.8	15	25	-4	$8.74{\pm}8.70^{*}$	33.3	5		
Apr	30	0	15.43±8.83*	4.9	4	26	-2	$10.96 \pm 7.35^*$	75.3	12		
May	31	6	$18.62 \pm 8.01^*$	7.1	8	28	4	$16.59 \pm 7.32^*$	153.3	16		
Jun	34	9	21.91±7.55*	166.5	16	33	12 22.75±7.22*		72.9	10		
Jul	32	11	22.53±6.75*	90.2	17	36	10	22.10±8.11*	70.7	9		
Aug	35	12	$23.62 \pm 8.30^{*}$	8.7	2	36	10	23.38±9.13*	24.1	5		
Sep	34	-1	$18.68 \pm 9.10^{*}$	33.6	5	34	2	$18.80 \pm 9.35^*$	8.2	4		
Oct	28	0	13.45±8.39*	15	4	30	1	13.32±8.33*	44.1	10		
Nov	20	-12	$4.83{\pm}6.83^*$	65.6	11	25	-2	9.61±7.14*	76.7	15		
Dec	10	-13	$-0.51 \pm 5.10^{*}$	59.8	4	17	-7	$3.71{\pm}6.22^{*}$	12.7	10		
	Temperature average 12.07 °C			Total precipitation 661.1 mm/year		Temperature average 12.69 °C			Total precipitation 636.2 mm/year			

Table 3. Monthly maximum, minimum and mean air temperatures (°C); total precipitation (mm)
and days per month, for 2018 and 2019 growing seasons

* Standard deviation

 Table 4. Comparison of phenological stages between A. deliciosa, A. chinensis and A. arguta Romanian hybrids, in two growing seasons (2018-2019)

BBCH	Bud	Leaf	Inflorescence	Flowering	Fruit	Fruit	Senescence			
CODE	development	development	emergence		development	maturity				
	(01)	(11)	(51)	(61)	(71)	(85)	(93)			
Variety			2018							
Actinidia chinensis										
R0P10	06.03	17.03	27.03	03.05	22.05	24.10	19.11			
Actinidia deliciosa										
Hayward	08.03	24.03	07.04	18.05	04.06	07.11	29.11			
Bruno	06.03	21.03	05.04	13.05	28.05	07.11	25.11			
			is and A. delicios	a interspecifi	ic hybrid					
R0P13	08.03	24.03	07.04	13.05	28.05	07.11	25.11			
R1P8	08.03	24.03	07.04	15.05	04.06	07.11	29.11			
R1P9	08.03	24.03	07.04	15.05	04.06	07.11	29.11			
R1P12	08.03	24.03	07.04	15.05	04.06	02.11	29.11			
		A. chinen.	sis and A. arguta	<i>interspecific</i>	hybrid					
R2P6	06.03	17.03	01.04	13.05	25.05	24.10	19.11			
			Actinidia a	rguta						
R8P1	04.03	20.03	01.04	08.05	19.05	18.09	12.11			
R10P20	04.03	20.03	01.04	08.05	19.05	18.09	12.11			
Variety 2019										
			Actinidia ch	inensis						
R0P10	04.03	22.03	01.04	09.05	28.05	25.10	21.11			
			Actinidia de	liciosa						
Hayward	06.03	28.03	12.04	25.05	10.06	08.11	03.12			
Bruno			09.04	20.05	05.06	08.11	30.11			
		A. chinens	is and A. delicios	a interspecifi	ic hybrid					
R0P13	06.03	28.03	12.04	20.05	05.06	05.11	30.11			
R1P8	06.03	28.03	12.04	23.05	10.06	05.11	03.12			
R1P9	06.03	28.03	12.04	23.05	10.06	05.11	03.12			
R1P12	06.03	28.03	12.04	23.05	10.06	05.11	03.12			
		A. chinen	sis and A. arguta	interspecific	hybrid					
R2P6	04.03	22.03	05.04	20.05	01.06	25.10	21.11			
			Actinidia a	rguta						
R8P1	02.03	25.03	05.04	16.05	25.05	17.09	21.11			
R10P20	02.03	25.03	05.04	16.05	25.05	17.09	21.11			
-										

Table 5. Kiwifruit growth stages and the main orchard management practices on a growing cycle

	Winter			Spring			Summer				Autum		
E	DEC	JAN	FEB	MAR	APRIL	MAY	JUNE	JULY	AUG	SEPT	ОСТ	NOV	
Sease	Dorm	Dormant		Budbreak Flowering		Fruit set Fruit growth			Leaf fall				
	Winter pruning		Budbreak sprays Pollination		Male pruning Canopy mat thinning and								

During flowering, in May, in commercial orchards natural (introduction of bee hives) and/or artificial (hand and machine pollination) systems of pollination can be apply (Salinero et al., 2009). The bee hives must be moved into the orchard when 10–20% of flowers are open (Clinch, 1990). The introduction of bee hives should be done at BBCH stage 61, and hand and machine pollination from BBCH stage 65 to stage 67 (Salinero et al., 2009).

As summer starts, kiwifruit vines undergo tremendous growth and growers frequently prune the vines to direct growth and manage the canopy (the canes can sometimes reach up to 5-6 meters in length during the growing process) (Baker et al., 2018). The fruit grow quickly, and crop volume can be estimated. Growers selectively thin kiwifruit to optimize fruit size and taste (generally the less there are, the larger and tastier they grow) (Baker et al., 2018).

Summer pruning is carried out during the growing season in the spring-summer period, and should be done at BBCH stages 18 and 19 in spring, to remove upright growing suckers, and during the summer, starting immediately after fruit set (stage 69) until stage 73, to cut growing ends of fruiting canes, what will result in larger fruit. The summer pruning sometimes is done until few days before harvest (stage 85), to cut growing ends, to prevent tangling, and twisted and tangled ends of all shoots (Salinero et al., 2009).

Girdling is performed only in some kiwifruit orchards. It must be done on 1-year-old wood (parent canes), supporting seasons floral shoot, and is usually carried out after fruit set until 2–4 weeks afterwards (therefore starting after stage 69 until 73) (Salinero et al., 2009).

In last weeks of summer and beginning of autumn, the kiwiberry, respectively kiwifruit, are tested for ripeness. When they pass a certain criterion for quality (BBCH stage 85), the fruits are carefully picked.

CONCLUSIONS

Kiwifruit has certain requirements as well as all fruit species, regarding temperature, humidity, wind, soil etc. Besides of these, commercial crops require significant management practices to be productive. Vine training, pruning, pollination, shelter from the wind, pest and disease control among other things all have a significant impact on the profitability and productivity of the crop. These horticultural practices impact the size and the dry matter of fruit and also the market acceptance.

The phenological enlargement of kiwifruit could improve the quality of fruits by providing information about evolution of the varieties and local hybrids under the environmental conditions of Southern Romanian. An accurate understanding of kiwifruit plant phenological stages it is essential for an appropriate orchard management.

In conclusion, this study can improve the cultivation of this new crop in Romania, can contribute in Romanian breeding program and also in zoning of the main *Actinidia* species on our country climatic conditions.

Further observation needs to be done, because the effect of climate and especially the temperature on seasonal variation requires longer observation periods than presented in the present study. To obtain more accurate results, continuing research is proposed for more years.

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