Dynamics of flowering of male and female inflorescence and pollen germination of hazel in the conditions of the Banja Luka region

P. Ilić[1], G. Đurić[1,2], N. Mićić[2] and H. Flachowsky[3]

[1]Genetic Resources Institute, University of Banja Luka, Banja Luka, Bosnia and Herzegovina; [2]Faculty of Agriculture, University of Banja Luka, Banja Luka, Bosnia and Herzegovina; [3]Julius Kühn-Institut (JKI), Institute for Breeding Research on Fruit Crops, Dresden, Pillnitz, Germany.

Abstract

Studying the hazel flowering was carried out in agro-ecological conditions of the Banja Luka in the period from December to March during 2010/2011 and 2011/2012. The study was conducted on 13 hazel cultivars in two orchards. In the first orchard, the following four cultivars were observed: 'Tankoljuskasti', 'Multiflora', 'Merveille de Bollwiller'and 'Tonda Gentile Romana', whilst the second orchard included the following: 'Hall's Giant', 'Gustav's Zellernuss', 'Istrian Round', 'Avellana', 'Romai', 'Apolda', 'Ludolph's Zellernuss', 'Imperiale de Trebizonde'and 'Jean's'. Phenological observations of the development of flowering phenophase with the aforementioned cultivars were monitored every three days in both years. In 2010/2011 the blooming of male and female inflorescence of the observed hazel cultivars lasted from late December to mid-March in total. The presence and pollen germination was proven to be a limiting factor for a successful pollination and fruit set in 2011. Namely, the pollen of cultivars: 'Hall's Giant', 'Istrian Round', 'Ludolph's Zellernuss', 'Avellana', 'Imperiale de Trebizonde' and 'Romai' did not germinate, and with 'Gustav's Zellernuss', pollen germination was very low (7,22%). In 'Apolda', 'Jean's', 'Merveille de Bollwiller', 'Tonda Gentile Romana', 'Multiflora' and 'Tankoljuskasti' pollen germination ranged between 28,57-81,69%. In 2011/2012 the flowering of male and female inflorescence of the observed hazel cultivars started 30 to 45 days later than the previous year i.e., female inflorescence of all the observed cultivars bloomed in early and mid-March, except in 'Tankoljuskasti', in which female inflorescence bloomed in late January and early February. Male inflorescence of 'Tankoljuskasti', 'Hall's Giant' and 'Gustav's Zellernuss' bloomed in the second half of January and early February, and in all other cultivars in mid-March. Pollen germination in 2012 proved to be a limiting factor for 'Apolda' and 'Romai', as their pollen did not germinate, as well as for 'Hall's Giant' and 'Jean's' where pollen germination was below 5%. For other cultivars, pollen germination was very good and ranged from 37,24 ('Merveille de Bollwiller') to 73,97% ('Multiflora'). Based on the analysis of dynamics of blooming and pollen germination of 13 hazel cultivars in two years, the monitoring of pollen germination and strategy of artificial pollination must be adopted as a basic approach to control hazel fertility in the conditions of the Banja Luka region.

Keywords: phenology, pollen viability, fertility, dynamics of blooming, dynamics pollen germination

INTRODUCTION

Hazel is a monoecious, anemophilous kernel fruit species (Olsen et al., 2000), the fruits of which are widely used in the food industry due to its good taste and high nutritional value. In the area of Bosnia and Herzegovina, hazel, together with raspberry, is a fruit culture which has been grown more frequently in the last period (Advisory Service of the Ministry of Agriculture, Forestry and Water Management of the Republic of Srpska, B. Pašalić, pers. commun., 2016). Although there have not been any studies over the last few decades regarding the cultivation of hazel in agro-ecological conditions of BiH, new hazel cultivars

are being increasingly used in the production, which biological characteristics have not been studied yet. In the previous period, medium flowering hazel cultivars such as 'Long Istrian', 'Hall's Giant' and 'Romai' have mostly been grown in Bosnia and Herzegovina, which are also grown in the neighbouring Croatia (Vujević et al., 2016) and which exhibit good resistance to low temperatures during flowering (Krpina et al., 1994). Low temperatures, below -15°C, occur every few years in the area of Bosnia and Herzegovina (Republic Hydrometeorological Institute of the Republic of Srpska) in the period of hazel flowering and can be a limiting factor for growing of early flowering and sensitive cultivars. Lack of knowledge about the dynamics of flowering of male and female inflorescence of introduced hazel cultivars, their resistance to low temperatures and the specifics expressed in given agro-ecological conditions can cause a number of problems in terms of mutual pollination and fruit set. In order for hazel to grow in a given area, it is necessary to carry out researches, since it is not possible to transfer the experiences from other regions because there is no universal hazel cultivar and breeding system. The leading countries in the world in the production of hazelnuts have their production regions in which they grow various hazel cultivars (Tombesi and Limongelli, 2002).

The flowering of male and female hazel inflorescence is carried out in the winter period from December to March, depending on the cultivar characteristics and air temperature (Turcu et al., 2001; Bostan, 2009). Dichogamy occurs during the period of flowering. Protandrous (flowering of male inflorescence) occurs in the majority of cultivars and is brought in relation with the temperatures above 10°C which precede the flowering, while protogynous (flowering of female inflorescence) is less frequent and occurs at lower temperatures that precede the flowering (Piskornik et al., 2001). Hazel is an autosterile species (Thompson, 1979) which is characterised by the occurrence of incompatibilities between cultivars, so it is necessary to know mutually compatible cultivars and pollinators in order to avoid problems in the processes of pollination and fruit set (Olsen et al., 2000). Modic (1969) stated in research that certain cultivars are compatible with each other, but that their pollination is not satisfactory. Baldwin (2009) quotes that global warming may cause earlier pollen shedding relative to blooming of female flowers, which can cause problems during pollination of hazelnut. Like other plant species that are pollinated by anemophily, hazel has a high production of pollen in order to meet the needs of pollination of female inflorescence. The ability of hazel to produce large amounts of pollen facilitates the collection of pollen with a view to applying artificial pollination. Hazel pollen germination in most cultivars is satisfactory and is up to 90% (Novara et al., 2017), but under the influence of meteorological factors it can be very low or completely lacking. Uneven flowering of cultivars and pollinators, along with the aforementioned problems pertaining to pollination and fruit setting often affect the reduction of yield in plantings. In order to eliminate the problems, it is necessary to conduct a biological control of hazel fertility. This includes the application of artificial pollination in several stages, including: growing of trees aimed at the production of high quality pollen, collection and storage of pollen, testing of pollen viability, application of pollen by means of air pumps and ventilators at a favourable time. Positive results and elimination of problems that arise due to poor pollination have been obtained in Chile, where there was a yield increase by 37% (Ellena et al., 2014).

The aim of this research was to study phenology of flowering of introduced hazel cultivars in agro-ecological conditions of north-western Bosnia and Herzegovina in order to obtain information relating to the reproductive biology of this species and the justifiability of using artificial pollination as a regular measure in the control of hazel fertility.

MATERIALS AND METHODS

Studies on the biology of flowering of male and female inflorescence and germination of pollen grains in 13 hazel cultivars were carried out from December, 15 to March 31 during 2010/2011 and 2011/2012 in the area of Banja Luka, north western Bosnia and Herzegovina at two locations. The first orchard (Economy of Agricultural Institute of Republic of Srpska, municipality Banja Luka 44°48'25"N 17°12'95"E) included the following cultivars: 'Multiflorum', 'Merveille de Bollwiller', 'Tonda Gentile Romana' and 'Tankoljuskasti',

and the second one (Jaružani, municipality Laktaši 44°48'20"N 17°20'40"E): 'Ludolph's Zellernuss', 'Avellana, 'Apolda, Hall's Giant', 'Romai', 'Istrian Round', 'Imperial de Trebisonda', 'Gustav's Zellernuss' and 'Jean's'. Planting material originated from Italy and from the Centre for fruit Growing and Viticulture from Čačak (Serbia). Each cultivar was represented by three trees. The flowering was observed every three days and pollen for germination was collected successively during the time of catkin pollination of these cultivars. Germination testing was carried out in glass chambers by a method of hanging drops in sucrose solutions of 12, 14 and 16% with four repetitions for each concentration. Readout of pollen germination and photo recording was performed for 3 h after germination with light microscopes, Swift M 4000-D (Swift Optical Instruments, Schertz, Texas, USA) and Olympus Vanox-T; AH-2 (Olympus, Tokyo, Japan). The data were analysed by general linear modelling in statistical software package SPSS 22 (IBM 2013). Post-hoc analysis of variance (ANOVA) in cases of statistically significant differences ($p<0.05$) were carried out by Tukey's test ($p<0.05$). All data were presented as mean ± SE (standard error).

RESULTS AND DISCUSSION

The dynamics of flowering of male and female inflorescence in 13 hazel cultivars conducted in the period of December 2010 to March 2011 and December 2011 to March 2012 is shown in Figure 1.

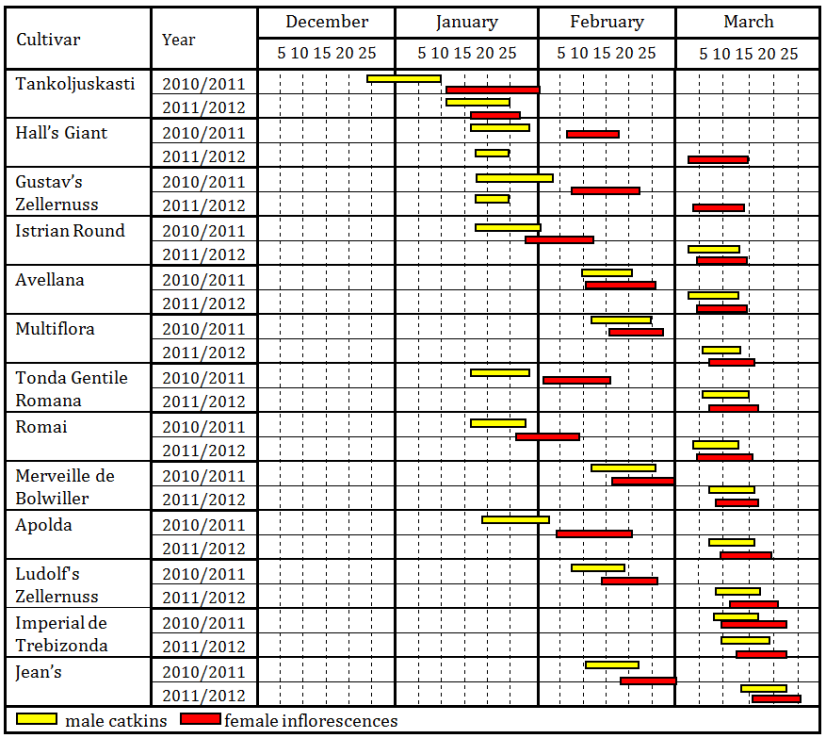

Figure 1. Dynamics of flowering of male and female inflorescence during 2010/2011 and 2011/2012.

Hazel flowering during the two years of research lasted from late December to late March. With most cultivars, there was a protandrous flowering, expressed with more or less the same amount, except for 'Avellana' where the flowering of male and female inflorescence was almost even.

According to the time of flowering, it can be concluded that large variations in the flowering of male and female inflorescences are evident in two years in all the observed cultivars of hazelnut except for the 'Imperial de Trebizonda'. Only in this cultivar the dynamic of flowering of male and female inflorescences in both years were approximate and in both

years the blooming was in March.

In the first year of observation, the male inflorescences of seven cultivars practically finished flowering before the female inflorescences began ('Tankoljuskasti', 'Hall's Giant', 'Gustav's Zellernuss', 'Istrian Round', 'Tonda Gentile Romana', 'Romai', 'Apolda). The male inflorescences in six of them flowered in January and female in February, while in 'Tankoljuskasti' cultivar blooming of male inflorescences was in December and female inflorescences in January. In 'Avellana', 'Multiflora', Merveille de Bollwiller', 'Ludolf's Zellernuss' and 'Jean's' flowering of male and female inflorescences roughly coincided in February.

In the second year of observation, both male and female inflorescences of nine cultivars were flourishing in March ('Istrian Round', 'Avellana', 'Multiflora', 'Tonda Gentile Romana', 'Romai', Bolvieri, 'Apolda', 'Ludolf's Zellernuss'and 'Jean's'). In 'Hall's Giant' and 'Gustav's Zellernuss' male inflorescences were flourishing in January and female in March. In 'Tankoljuskasti' both male and female inflorescences flourished in January.

Based on this data it can be concluded that there is a huge difference in the flowering of observed hazel cultivars between the first and the second year of observation, as well as between the cultivars within the observed years. The occurrence of these differences was probably affected by the temperature fluctuations that led to the initiation and cessation of the process of flowering (Table 1), the occurrence of differences between male and female inflorescences, freezing of male inflorescence and accelerated flowering period. In the second year of observation the flowering started in mid-January, which was followed by colder weather, snow and temperatures below -20°C lasting until the end of February (Table 1). The second wave of flowering occurred in March in this year.

Table 1. Climate data during the phenophase of flowering of hazel cultivars (Meteorological station Banja Luka).

	2010	2011			2012			
	Month							
	XII	I	II	III	XII	I	II	III
Average monthly temperature (°C)	1.5	1.9	1.7	7.1	3,9	2.0	-2.8	9.3
Absolute maximal temperature (°C)	20.7	19.6	21.2	25.2	20.3	15.8	19.2	25.0
Date	8	9	7	25	3	4	24	17
Absolute minimal temperature (°C)	-13.8	-10.4	-8.7	-7.6	-10.6	-10.8	-21.5	-7.0
Date	19	25	25	9	21	31	9	8
Sum of monthly rainfall (mm)	87.6	51.6	29.3	34.2	120.7	68.2	68.4	5,0
Medium relative humidity (%)	81	85	80	68	82	78	78	58
Number of ice days	5	6	5	1	2	3	13	0

Table 2 shows the highest average germination of hazel pollen during 2010/2011 and 2011/2012.

Based on the data from Table 2 it can be seen that the pollen germination as an indicator of its viability is not uniform and regular in most of the studied cultivars. Thus, the occurrence of a phenomenon of alternative pollen germination was recorded in eight cultivars, whilst 'Hall's Giant' showed a low pollen germination during both years. A regular and high germination during both years was only present in 'Multiflora', while mean germination was registered with 'Tonda Gentile Romana', 'Merveille de Bollwiller' and' Tankoljuskasti'.

Table 2. The highest average hazel pollen germination during 2010/2011 and 2011/2012.

Cultivar	Year	Pollen viability (%±SE)
Tankoljuskasti	2011	28.57±2.60
	2012	45.52±1.44
Hall's Giant	2011	-
	2012	5.83±1.15
Gustav's Zellernuss	2011	7.22±0.91
	2012	70.44±2.67
Istrian Round	2011	-
	2012	58.89±2.79
Avellana	2011	-
	2012	59.87±2.78
Multiflora	2011	81.69±3.24
	2012	73.97±2.23
Tonda Gentile Romana	2011	49.46±2.52
	2012	62.03±2.97
Romai	2011	-
	2012	70.97±2.72
Merveille de Bollwiller	2011	53.24±2.03
	2012	37.24±2.83
Apolda	2011	48.65±2.47
	2012	1.21±0.04
Ludolph Zellernuss	2011	-
	2012	55.54±3.07
Imperial de Trebizonda	2011	-
	2012	46.96±4.03
Jean's	2011	47.60±2.82
	2012	6.49±1.62

CONCLUSION

The results of the researches on the dynamics of flowering and pollen germination show that there are problems related to pollination and fruit set in the observed cultivars of hazel. Flowering of male and female inflorescence of compatible pollinators and the occurrence of alternative formation of viable pollen hinder good pollination of stigmatic peduncles of female inflorescence and regular fruiting of hazel in the observed plantations. Based on the above it can be concluded that the solving of the above problems and achieving of a high production potential in the agro-ecological conditions of the Banja Luka region are only possible by applying artificial pollination as a regular horticultural practice to improve hazel productivity.

ACKNOWLEDGEMENT

The work was supported by the Ministry of Science and Technology of the Republic of Srpska through the project entitled "Genotypic specificity of hazel (*Corylus avellana* L.) fertilization in ecological conditions of the Banja Luka region" (grant No. 19/6-020/964-92/10).

Literature cited

Baldwin, B. (2009). Field and controlled temperature studies on the flowering of hazelnuts in Australia. Acta Hortic. *845*, 213–218 https://doi.org/10.17660/ActaHortic.2009.845.29.

Bostan, S.Z. (2009). Phenological traits of important hazelnut cultivars in Ordu, Turkey. Acta Hortic. *845*, 207–212 https://doi.org/10.17660/ActaHortic.2009.845.28.

Ellena, M., Sandoval, P., Gonzalez, A., Galdames, R., Jequier, J., Contreras, M., and Azocar, G. (2014). Preliminary results of supplementary pollination on hazelnut in South Chile. Acta Hortic. *1052*, 121–127 https://doi.org/10.17660/ActaHortic.2014.1052.15.

Krpina, I., Cvrlje, M., and Vujevic, P. (1994). Influence of extremely low winter temperature on some hazelnut cultivars. Acta Hortic. *351*, 329–334 https://doi.org/10.17660/ActaHortic.1994.351.36.

Modic, D. (1969). Prilog poznavanju cvatnje i oplodnje nekih sorti lijeske. Doctoral thesis (Zagreb, Croatia: Poljoprivredni fakultet, Sveučilišta u Zagrebu).

Novara, C., Ascari, L., La Morgia, V., Reale, L., Genre, A., and Siniscalco, C. (2017). Viability and germinability in long term storage of *Corylus avellana* pollen. Sci. Hortic. (Amsterdam) *214*, 295–303 https://doi.org/10.1016/j.scienta.2016.11.042.

Olsen, J.L., Mehlenbacher, S.A., and Azarenko, A.N. (2000). Hazelnut pollination. Horttechnology *10* (*1*), 113–115.

Piskornik, Z., Wyzgolik, G.M., and Piskornik, M. (2001). Flowering of hazelnut cultivars from different regions under the climatic conditions of southern Poland. Acta Hortic. *556*, 529–536 https://doi.org/10.17660/ActaHortic.2001.556.77.

Republic Hydrometeorological Institute of the Republic of Srpska (Bosnia and Herzegovina). http://rhmzrs.com/.

Thompson, M.M. (1979). Genetics of incompatibility in*Corylus avellana* L. Theor. Appl. Genet. *54* (*3*), 113–116 https://doi.org/10.1007/BF01159464. PubMed

Tombesi, A., and Limongelli, F. (2002). Hazelnut varieties and genetic improvement. Paper presented at: 2nd Convegno Nazionale sul Nocciolo (Giffoni V.P.).

Turcu, E., Turcu, I., and Botu, M. (2001). Flowering of hazelnut cultivars in Oltenia, Romania. Acta Hortic. *556*, 365–370 https://doi.org/10.17660/ActaHortic.2001.556.54.

Vujević, P., Milinović, B., Jelačić, T., Halapija, K., Čiček, D., and Biško, A. (2016). Uvođenje novih sorti lijeske u proizvodnju i značaj utvrđivanja autentičnosti. Zbornik sažetaka 11. znanstveno – stručno savjetovanje hrvatskih voćara s međunarodnim sudjelovanjem (Zagreb: Hrvatska voćarska zajednica). http://beta.bib.irb.hr/804601.