BIOLOGY OF SWEET CHERRY FLOWERING

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ABSTRACT

The aim of the study presented was to evaluate floral biology and fruit set of sweet cherry cultivars 'Van', 'Summit', 'Kordia', 'Hedelfinger' and 'Regina', as dependent on the pollination method. The results showed a rapid growth of pollen tubes in the style. Percentage of germinating pollen was on the same level during the flowering period. The fertilization of ovules already occurred in the second day of flowering. The percentage of fertilized ovules was the greatest on the ninth day of flowering, but covered 30% of the flowers only. The most incompatible cultivars are 'Kordia' and 'Regina'. After self-pollination those cultivars practically did not set fruit. 'Van' and 'Hedelfinger' cultivars were self-incompatible in moderate degree. 'Summit' was a self-compatible cultivar; after self-pollination its fruit set reached 11%. 'Regina' was highly self-incompatible cultivar, after pollination with pollen of 'Kordia' its fruit set was 9.9%.

Key words: pollinator, self-incompatibility, sweet cherry, floral biology

INTRODUCTION

Important element of sweet cherry floral biology is incompatibility. Kobel (1960) hypothesized that income-patibility is controlled by a single gene represented by 7 alleles, consisting of 10 incompatibility groups. However, Thompson (1996) showed that there are 6 alleles consisting of 13 pollen incompatibility groups and one group of universal pollinators. At present, 13 alleles and 22 of pollen income-patibility groups are estimated for sweet cherry (Tobutt et al., 2001; Iezzoni et al., 2005). According to Schmidt et al. (1999), the cultivars which have been growing in Central Europe exhibit pollen incompatibility controlled by 7 alleles and 16 incompatibility groups.

Diploid cultivars have two different alleles. In the case of pollination with own pollen, the transmitting tissue of the style contains the same alleles as the pollen tubes, what leads to self-incompatibility expressed as growth inhibition of the pollen tube. Gametophyte incompatibility in sweet cherry was proven for the first time by Crane and Brown (1937). The inhibition of pollen tube growth occurs upon the reaction between the components of the same alleles of pollen and style. Protein products of S gene, synthesized and localized in cytoplasma and exine of pollen grain, are responsible for incompatibility reaction (Boyes and Nasrallah, 1995; Entani et al., 2003).

Incompatibility (SI) causes the inhibition of pollen tube growth on the stigma or in the style (Bednarska and Lenartowska, 2003). Most of the authors show that the inhibition of pollen tube growth is observed below the stigma (McCubbin and Kao, 2000). According to Anvari and Stösser (1978), the inhibition of pollen tubes in sour cherry occurs in the lower part of the style.

The spontaneous mutation existing in sweet cherry may lead to self-fertile genotypes (Nyéki and Soltész, 1996). The first self-fertile cultivar selected in Canada is ‘Stella’ (Thompson, 1996). The studies concerning the self-fertile sweet cherry cultivars proved that the rate of self-fertility depends on the climate and flower morphology (Brant et al., 1999).

Air temperature may play significant role during the growth of pollen tube; however there is no evidence that high temperature breaks the incomepatibility in sweet cherry (Hedhly et al., 2004). It was reported that the optimal temperature for the proper course of flowering is about 20^{o}C and temperature about 30^{o}C is too high (Eaton, 1959; Beppu and Kataoka, 1999; Hedhly et al., 2004). Some studies proves that sweet cherry needs low temperature during the flowering since the disappearance of embryo sac is slower at 15^{o}C comparing to 25^{o}C (Beppu et al., 2005).

Within the existing incomepatibility groups the selection of the pollinators is of a big importance. The selection may be performed on the basis of laborious cytological studies or on observations of flowering period. The last method is frequently adequate, what is confirmed by the 20-year study concerning the period of flowering of 175 cultivars (Vitrup, 1996). According to this author, the earliest flowering of sweet cherry in Denmark is noted on 17 April, at the latest on 19 May. The duration of flowering period is 17 days in the years with early start of vegetation and 7 days in the years with late time vegetation beginning. Results of many years of observation allow to conclude that the selection of pollinators can be done on the basis of flowering time.

In our climatic zone the selection of pollinators for new cultivars were performed by Schmidt et al. (1999) and Tobutt et al. (2001). The new cultivars recommended for cultivation, also in Poland, were taken into consideration. According to the authors cited above, the following cultivars belong to the second group of incompatibility with S_1S_3 alleles: 'Regina', 'Cristalina', 'Van', 'Venus', 'Erica', 'Merton Bounty', 'M. Crane', 'M. Favorite', 'Octavia', 'Sodus', 'Valeska', 'Windsor', 'Gil Peck', 'Karesova'. Thus, those cultivars are inter-sterile. At present, there is no information about incompatibility group for 'Kordia' (in the USA known as 'Attica').

In the case of sweet cherry the pollen tube growth from the stigma to base of the style takes 2-3 days, while the fertilization takes place 6-8 days after pollination (Kron and Husband, 2006; Ughini and Roversi, 1996). According to Stösser and Anvari (1983), the time of effective pollination takes 4-5 days. Roversi and Ughini (1998) proved that sometimes the time of effective pollination might last up to 13 days. Self-fertility of sweet cherry cultivars is a sought after but a rare trait (Dirlevander et al., 2007; Hauck et al., 2002; Godni et al., 1998). In the case of open pollination self-fertile cultivars set 10-15% more fruit comparing to pollination with own pollen (Blazková, 1996). This process depends on growth of mentor pollen tube, in other words, the growth of own pollen tube is promoted by the growth of the tube of foreign pollen (Visser, 1984). Self-fertile cultivars are good pollen donors for other cultivars (Békefi and Brózik, 2005).

The aim of the study presented was to evaluate flowering and fertilization process of several sweet cherry cultivars and qualification of fruit setting depending on pollination method. The efforts were undertaken to find the pollinators for the most important sweet cherry cultivars.

MATERIAL AND METHDOS

The studies were carried out during 2003-2008 on sweet cherry cultivars 'Van', 'Summit', 'Kordia', 'Hedelfińska', 'Regina' grown in Garlica Murowana Experimental Sta-tion near Cracov, Southern Poland. In 2005 there was no cropping because flowers were damaged by spring frosts (on April 22nd and 23rd the temperature dropped to –4.3°C). Also in 2006 the trees did not bear fruit because the temperature during the winter was very low (–21°C on January 22nd and –27° C on February 9th, followed by a warmer period with –11.1°C, at the end of February and beginning of March). In 2007 the flowering proceeded correctly, but on 1st of May temperature dropped to –3.4°C, the flowers were injured and there was no cropping as well.

The flowers were either open-pollinated, self-pollinated or cross-pollinated. The flowers for open-pollination were open to the wind and bees. The flowers for self-pollination treatment were pollinated with their own pollen and then bagged. The flowers in cross-pollinated combination were pollinated with pollen collected from selected cultivars and bagged as well. Each treatment was performed in five replications. Cross pollination was performed without emasculation because that process proceeds that way in nature. Moreover, the self-pollination was performed in the same way. Comparison of the two combinations enabled to eliminate the emasculation. Remaining of the pistils during the cross-pollination enabled partial pollination with own pollen and co-action of own and foreign pollen. The observations of fruit setting were performed in four replications. One hundred flowers collected from each treatment-/replication were prepared as specimens for observation under luminescence microscope. The observation of pollination, pollen tube growth through the style and fertilization of the ovules were performed in the successive days of flowering. The average number of pollen grains on the stigma, pollen tubes growing through the regions of the style and the number of fertilized ovules were evaluated. The fruit setting was calculated on the basis of the fruit number estimated at the time of ripening.

The results of microscopic observation and of fruit set were statistically analysed by calculating mean values, quantile interval and median. Quantile interval equals the difference between the third and the first quantile. Since 50% of all observations are between these intervals, the bigger is quantile distance the greater is differentiation. The median (the second quantile) is the middle value of a distribution: half the scores are above the median and half are below the median (Woźniak, 1994). The confidence intervals for microscopic observations were statistically evaluated at $p = 0.05$.

RESULTS AND DISCUSSION

The sweet cherry crop was obtained in three years only during the six years of the investigation (Tab. 1). This confirms the great risk of sweet cherry cultivation in Poland. The temperature during the flowering time in the investigated years is presented in Table 2.

In 2003 the temperature during sweet cherry flowering was optimal. Mean maximal temperature within the flowering period reached 23°C, while in 2004 the maximal temperature was lower by a half. Also the minimal temperature in 2003 was higher than in 2004. In 2003 total mean daily temperature equalled to 147°C, and in 2004 to 106.6°C. Thus, in 2003 the sweet cherry flowers got 40% more heat than in 2004.

The optimal flowering conditions occurred also in 2007, when the mean daily maximal temperature reached 18.2°C. However, the sudden fall of temperature at the end of flowering resulted in a total loss of the crop in that year (Tab. 1). In 2008 different temperature conditions were noted during flowering time. Mean maximal daily temperature was 13.7°C, and since the minimal temperature was low (3.7°C), the flowering period was prolonged.

Table 1. Period of sweet cherry flowering and frost injury in the years of study

Year	Beginning – end of flowering
2003	1 May – 7 May
2004	23 April. – 3 May.
2005	Frost injury of buds 22/23 April (temperature – 4.3°C)
2006	In the years 2005/2006 in Malopolska region the flower buds of all sweet cherry cultivars were injured by winter and spring frost (22 January –27°C, 9 February –21°C, warming at the end of February, 5 March – 11.1°C).
2007	20 April – 1 May Frost injury of flower buds at the end of the flowering – 1 May
2008	15 April – 30 April.

Table 2. Total maximal, minimal and mean daily temperature during flowering period in the years 2003, 2004, 2007 and 2008 (°C)

Temperature	2003		2004		2007		2008	
	total	mean daily	total	mean daily	total	mean daily	total	mean daily
Maximal	206.8	23.0	146.7	14.6	218.3	18.2	218.9	13.7
Minimal	84.1	9.3	69.3	6.9	70.3	5.8	59.8	3.7
Mean daily	147.0	16.3	106.6	10.6	153.7	12.8	143.4	9.0

The microscopic observations showed the rapid growth of pollen tubes in the style (Tab. 3). Percentage of germinating pollen in all combinations was similar within the flowering period without significant differences. The fertilization of ovules already occurred in the second day of flowering. So far, occurrence of fertilization at the beginning of flowering was not reported (Kron and Husband 2006, Ughini and Roversi 1996). The percentage of fertilized ovules was the greatest on the ninth day of flowering, but covered 30% of the flowers. Only after open-pollination the pollen tubes entered the ovary.

Thus, despite the optimal condition during flowering and the presence of pollinators and bees in the orchard, a higher percentage of fertilization was not achieved. This shows that any disturbances in the flowering process results in a rapid yield decrease. The microscopic observation on two sweet cherry cultivars: ‘Van’ and ‘Hedelfinger’ shoved which part of the style inhibits the pollen tube growth at the highest rate (Tab. 4).

T a b l e 3. The growth of pollen tubes through the parts of the style and the fertilized ovules after open pollination (mean of three years for 'Van', 'Hedelfińska', 'Kordia', 'Regina')

Days of flowering	Stigma, style zone and fertilized ovules					
	percentage of germinating pollen tubes	pollen tubes reaching 1/3 of the style [%]	pollen tubes reaching ½ of the style [%]	pollen tubes at the base of the style [%]	pollen tubes growing into ovary [%]	fertilized ovules [%]
1	77.0 a*	38.0 a	26.2 a	5.6 a	0.0 a	0.0 a
2	90.8 a	57.8 bc	40.3 ab	18.2 ab	10.9 abc	7.1 abc
3	92.4 a	51.3 ab	43.4 ab	15.2 ab	4.9 ab	3.5 ab
4	81.1 a	49.8 ab	43.2 ab	27.1 ab	16.4 abc	8.7 abc
5	93.0 a	60.7 bc	44.9 abc	22.6 ab	14.5 abc	9.7 abc
6	95.4 a	64.0 bc	50.1 abc	23.0 ab	14.6 abc	7.3 abc
7	97.8 a	83.2 bc	77.2 bc	38.5ab	35.6 c	13.9 abc
8	98.7 a	90.4 c	67.6 abc	49.3 b	30.9 bc	24.8 bc
9	86.9 a	76.0 bc	58.2 abc	44.4 b	38.9 c	27.4 c
10	99.2 a	93.3 bc	67.9 abc	45.3 b	32.4 bc	20.1 abc
11	97.0 a	70.0 bc	43.1 ab	35.2 ab	19.6abc	8.6 abc
12	98.0 a	95.6 c	91.3 c	35.0 ab	13.6 abc	8.4 abc
Confidence interval	87.4-96.3	59.9-75.1	46.5-62.2	23.3-35.0	15.5-25.7	7.92-15.6
Median	91.9	73.0	57.3	20.0	16.6	11.8

*mean followed by the same letter do not differ at p = 0.05

Table 4. Critical values for confidence intervals calculated for pollination,. pollen tubes growth through the zone of the style and fertilization of ovules estimated after open pollination for two sweet cherry cultivars (three year mean percentage)

Stigma and style	Van			Hedelfinger		
	mean	confidence interval	median	mean	confidence interval	median
Pollen grains on the stigma	87	78-95	89	87	76-96	96
Germinating pollen tubes	82	70.7-91.5	86	82	70-95	94
Pollen tubes in $^{1}/_{3}$ of the style	68	56-80	68	68	47-88	77
Pollen tubes in half of the style	52	42.5-62.6	50	68	53-82	68
Pollen tubes at the base of the style	25	18.2-32	28	36	24-48	31.7
Pollen tubes in the ovary	16	9.2-23.5	20	23	12.5-33.7	14.5
Fertilization	15	7.6-21.6	12	15	6.4-24.2	8.6

Analysis of pollen tubes growth through the style tissue was based on the statistical calculation at $p = 0.05$. After open pollination the self and foreign pollen is present on the stigma. The most pollen tubes reached one third of the style, what confirms the opinion that in this zone of the style there is no strong rejection of the tubes. However, nearly 75% of pollen tubes did not reach the base of the style. This observation suggests that those tubes might have originated from the self pollen. It might be concluded that the reaction of incompatibility causes the inhibition of incompatible pollen tube growth below the stigma zone and at the base of the style. Thus, the findings of other authors that the inhibition of pollen tubes occurs below the stigma (McCubbin and Kao, 2000) and the incompatible tubes are arrested in the lower part of the style (Anvari and Stösser 1978) seems to be correct.

Sweet cherry 'Van', 'Hedelfinger', 'Kordia' and 'Regina' are considered to have different rates of self-sterility. The most self income-patible cultivars are 'Kordia' and 'Regina'. After self-pollination those cultivars practically do not set fruit (Tab. 5).

T a b l e 5 . Mean values, quantile intervals and median calculated for fertilization and fruit-set of sweet cherry cultivars – ‘Van’. ‘Hedelfinger’. ‘Summit’. ‘Kordia’. ‘Regina’ (based on three years’ study)

Treatment	Mean value [%]	Quantile interval [%]	Median [%]
Fertilization after open pollination of investigated cultivars	34	13-40	33
Fruit set after open pollination of investigated cultivars (end of May)	52	39-64	50
Fruit set of investigated cultivars (before harvest)	20	10-26	17
Van x Hedelfinger – fertilization	38	16.7-73	27
Van x Hedelfinger – fruit set (end of May)	30	12-43	36
Van x Hedelfinger – fruit set (before harvest)	15.6	9.8-27	10
Hedelfinger x Van – fertilization	28	10-47	28.7
Hedelfinger x Van – fruit set (end of May)	46	30-62	46
Hedelfinger x Van – fruit set (before harvest)	25	20-30	25
Regina x Kordia – fertilization	23	5-40	23
Regina x Kordia – fruit set (end of May)	44	23-63	44
Regina x Kordia – fruit set (before harvest)	9.9	5.7-14.2	10
Summit – fertilization after self- pollination	12	4-16.5	14
Summit – fruit set after self-pollination (end of May)	46	20-76	49
Summit – fruit set after self-pollination (before harvest)	11	3.2-20.7	12
Hedelfinger – fertilization after self-pollination	7.5	8-9	8.5
Hedelfinger – fruit set after self-pollination (end of May)	29.5	28-30	29.5
Hedelfinger – fruit set after self-pollination (before harvest)	7.9	2.4-8.5	7.9
Van – fertilization after self- pollination .	3.1	005.5	4
Van – fruit set after self-pollination (end of May)	13	0-23	16
Van – fruit set after self-pollination (before harvest)	2.2	0-5	1.6
Regina – fertilization after self- pollination	2	0-5	1
Regina – fruit set after self-pollination (end of May)	36	0-80	29
Regina – fruit set after self-pollination (before harvest)	0.0013	0-0.4	0
Kordia – fertilization after self- pollination	2.5	0-5	2.5
Kordia – fruit set after self-pollination (end of May)	37.5	0-75	37
Kordia – fruit set after self-pollination (before harvest)	0.004	0-0.008	0.004

'Van' and 'Hedelfinger' are self-incompatible in moderate degree. After self-pollination fruit set in 'Van' was 2% and 7.9% in 'Hedelfinger'. Air temperature did not break the gametophyte incompatibility. In 2003, despite the fact that during flowering period the mean maximal temperature was 23°C, minimal daily temperature 9.3°C and mean daily temperature 16.3°C, the suppression of incompatibility did not occur. Those results confirm the observations of Hedhly et al. (2004), Eaton (1959) and Anvari and Stösser (1978) who reported about unfavourable effect of high temperature on fruit set in sweet cherry. During the flowering period lower temperature is advan-tageous since it prolongs both the stigma receptivity and the period of effective fertilization. During flowering in 2004 the optimal temperature conditions occurred (mean maximal temperature was 14.6°C, mean minimal temperature 6.9°C and mean daily temperature 10.6°C). Similar conditions during flowering period occurred in the year 2008. In both the years the fruit set was the highest.

'Summit' is a self-compatible cultivar – after self-pollination its fruit set reaches 11%. This does not confirm Thompson's (1996) observation. On the contrary, Blazková (1996) proved that open pollination of self-compatible cultivar resulted in a higher fruit set. In our study, 'Summit' fruit set was 20.7% after self-pollination and up to 38.2 % after open–pollination.

After cross-pollination of 'Van'×'Hedelfinger', the fruit set reached 15.6%, and after opposite crossing 'Hedelfinger'×'Van' the fruit set was 25%. These results are in agreement with the study of Tobutt et al. (2001).

'Regina' is highly self-incompatible cultivar, after pollination with pollen of 'Kordia' the fruit set was 9.9%. There are no reports concerning such a compatibility between these cultivars. Crossing 'Kordia'×'Regina' resulted in the fruit set at the level of 15.3% (one year result). 'Kordia' starts flowering two to three days earlier than 'Regina' and produces less pollen grains conglomerated in big aggregates.

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BIOLOGIA KWITNIENIA CZEREŚNI

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STRESZCZENIE

Celem pracy był opis przebiegu zapylenia i zapłodnienia kwiatów kilku odmian czereśni oraz określenie zawiązania owoców w zależności od sposobu zapylania. Uwzględniono następujące odmiany: 'Van', 'Summit', 'Kordia', 'Hedelfińska', 'Regina'. Zamieszczone wyniki wskazują na, obejmujący prawie wszystkie kwiaty, bardzo szybki przebieg wzrostu łagiewek pyłkowych w szyjce słupka. Procent kiełkujących łagiewek pozostawał na niezmienionym poziomie przez cały okres kwitnienia (brak różnic statystycznych). Zapłodnienie zalążków następowało już w drugim dniu kwitnienia. W dziewiątym dniu kwitnienia zapłodnienie osiągnęło najwyższy poziom, ale objęło zaledwie 30% kwiatów. Najbardziej samoniezgodne są odmiany 'Kordia' i 'Regina'. W przypadku zapylenia własnym pyłkiem odmiany te praktycznie nie zawiązują owoców. Odmiany 'Van' i 'Hedefińska' są w umiarkowanym stopniu samoniezgodne. Odmiana 'Summit' należy do odmian samopłodnych w przy-padku zapylenia własnym pyłkiem zawiązanie wynosi 11%. 'Regina' jest odmianą o wysokim stopniu samoniezgodności, przy zapyleniu pyłkiem odmiany 'Kordia' zawiązanie wynosi 9,9%.

Słowa kluczowe: zapylacz, samoniezgodność, odmiany czereśni