<u>Zadanie 75</u>: Badania nad możliwością poszerzenia zmienności genetycznej maliny właściwej (*Rubus idaeus*) pod względem różnej pory dojrzewania i jakości owoców

W roku 2015 realizowano cztery tematy badawcze:

***Temat badawczy 1**.* **Ocena stanu zdrowotnego wybranych form rodzicielskich maliny właściwej, uprawianych w szklarni ogrzewanej przed włączeniem do programu krzyżowań oraz ocena możliwości ich skrzyżowania w oparciu o wykonanie zaplanowanych kombinacji zapyleń.** Cel badań był dwojaki: po pierwsze - potwierdzenie, że rośliny maliny właściwej wytypowane do programu zapyleń są wolne od groźnych chorób wirusowych mogących mieć wpływ wynik krzyżowania, oraz po drugie – potwierdzenie, że w wyniku krzyżowania tych genotypów można uzyskać żywotne nasiona dla otrzymania populacji siewek, charakteryzujących się genetyczną zmiennością pod względem badanych cech. Materiałem badawczym były rośliny 10 genotypów maliny właściwej, rosnące w doniczkach w szklarni. Były to: ‘Canby’, ‘Glen Ample’, ‘Laszka’, ‘Polana’, ‘Polka’, ‘Radziejowa’, ‘Schönemann’, ‘Sokolica’, ‘Veten’ i ‘Willamette’. Przed krzyżowaniem, przy zastosowaniu testu DAS-ELISA i metody RT-PCR, wykonano badania laboratoryjne, które potwierdziły, że rośliny przeznaczone do krzyżowania są wolne od badanych wirusów (wirus krzaczastej karłowatości maliny, wirusa cętkowanej plamistości liści maliny, wirus żółtaczki nerwów liści maliny, wirus chlorozy nerwów liści maliny i wirus plamistości liści maliny) i wirusy te nie mogły mieć wpływu na wynik krzyżowania, które wykonano w miesiącach marzec-kwiecień. Zastosowano dialleliczny układ, uwzględniający krzyżowania wprost (45 kombinacji krzyżowań) i wsobne (10 kombinacji krzyżowań). Najlepsze pod względem wiązania owoców przez zapylone kwiaty były kombinacje krzyżowań: ‘Canby’ x ‘Laszka’, ‘Polana’ x ‘Polana’, ‘Polana’ x ‘Radziejowa’, ‘Polana’ x ‘Sokolica’, ‘Polka’ x ‘Sokolica’, ‘Radziejowa’ x ‘Sokolica’ i ‘Sokolica’ x ‘Sokolica’. W ramach tych krzyżowań wszystkie zapylone kwiaty wydały owoce. Owoce o największej masie uzyskano z kombinacji krzyżowań: ‘Glen Ample’ x ‘Laszka’ (5,72 g), ‘Glen Ample’ x ‘Sokolica’ (4,95 g), ‘Glen Ample’ x ‘Polka’ (4,68 g), a o najmniejszej masie – ‘Canby’ x ‘Canby’ (1,10 g), ‘Polana’ x ‘Polana’ (1,31 g) i ‘Canby’ x ‘Radziejowa’ (1,62). Najwięcej nasion uzyskano z kombinacji zapyleń: ‘Polka’ x ‘Willamette’ (1400 nasion), ‘Polka’ x ‘Sokolica’ (1315 nasion) i ‘Sokolica’ x ‘Sokolica’ (1250 nasion). Dużo mniej nasion uzyskano z kombinacji zapyleń: ‘Glen Ample’ x ‘Polana’ (74 nasion), ‘Glen Ample’ x ‘Glen Ample’ (208 nasion) i ‘Canby’ x Glen Ample’ (387 nasion). Najmniej nasion w owocach było wówczas, gdy owoce powstały w wyniku samozapylenia, co też przekładało się na ich mniejszą masę. Pomimo wykonanego zapylania, nie uzyskano owoców i nasion z następujących kombinacji krzyżowań: ‘Canby’ x ‘Schönemann’, ‘Canby’ x ‘Veten’, ‘Canby’ x Willamette’, ‘Glen Ample’ x ‘Schönemann’, ‘Laszka’ x ‘Schönemann’, ‘Polana’ x ‘Schönemann’, ‘Polka’ x ‘Polka’, ‘Polka’ x ‘Schönemann’, ‘Radziejowa’ x ‘Schönemann’, ‘Radziejowa’ x ‘Veten’, ‘Schönemann’ x ‘Schönemann’, ‘Schönemann’ x ‘Sokolica’, ‘Schönemann’ x ‘Veten’, ‘Schönemann’ x ‘Willamette’ i ‘Willamette’ x ‘Willamette’. Łącznie zapylono 878 kwiatów, z których uzyskano 543 owoców, czyli 76,2% w stosunku do zapylonych kwiatów. Owoce zbierano w fazie dojrzałości konsumpcyjnej (w pełni wybarwione).

Temat badawczy 2. **Ocena efektywności programu krzyżowań, wykonanego w układzie diallelicznym, wg IV Metody Griffinga, w oparciu o określenie liczby uzyskanych nasion i ocenę ich żywotności (zdolności do skiełkowania po zabiegu skaryfikacji i stratyfikacji).** Celem badań było potwierdzenie hipotezy, że możliwe jest uzyskanie żywotnych nasion z wykonanego programu zapyleń krzyżowanych genotypów rodzicielskich. Materiałem badawczym były nasiona otrzymane z wykonanego programu krzyżowań. Owoce maliny są pestkowcami, u których nasiona znajdują się wewnątrz bardzo grubej i twardej okrywy nasiennej (endokarp). Dla skiełkowania wymagają specjalnego traktowania, czyli zastosowania zarówno skaryfikacji (sztuczne uszkadzanie okrywy nasiennej w celu ułatwienia wnikania wody i gazów do wnętrza i przyspieszenia kiełkowania nasion), jak i stratyfikacji, przy czym zabieg skaryfikacji poprzedza zabieg stratyfikacji. Z uwagi na nieuzyskanie nasion w niektórych kombinacjach krzyżowań (Temat badawczy 1), skorzystano z nasion z tych samych kombinacji krzyżowań z roku 2014, które zachowane były jako rezerwa nasienna, przechowywana w temperaturze +4°C. Nasiona (500 szt. nasion dla każdej kombinacji zapyleń) sakryfikowano stężonym kwasem siarkowym (95%) w czasie 30 min. Po zakończonej skaryfikacji nasiona stratyfikowano w temperaturze 3°C w ciągu 6 tygodni. Pomimo zastosowanych zabiegów nasiona po wysiewie kiełkowały nierównomiernie i bardzo powoli. Po 70 dniach od wysiewu, przeciętnie dla wszystkich kombinacji krzyżowań, wzeszło tylko 47,4% nasion, przy czym najlepiej kiełkowały nasiona z tych kombinacji krzyżowań, w których formami matecznymi były ‘Laszka’, ‘Radziejowa’ i ‘Sokolica’, a najsłabiej – jeżeli formą mateczną była ‘Polana’.

Temat badawczy 3. **Indywidualna wstępna ocena cech fenotypowych siewek rosnących w szklarni, wszystkich rodzin mieszańców dla określenia potencjału genetycznego form rodzicielskich i ich przydatności do tworzenia nowej zmienności genetycznej.** Celem badań była ocena wigoru wyprodukowanych siewek dla stwierdzenia w jakim stopniu ich rodowód decyduje o sile wzrostu tych siewek we wczesnym stadium rozwoju i przydatności użytych form rodzicielskich do tworzenia nowej zmienności genetycznej u maliny właściwej. Materiałem badawczym były siewki rodzin mieszańców, wyprodukowane w tym temacie. Skiełkowane nasiona (siewki w fazie 2 liścieni z widocznym zaczątkiem liścia młodocianego) pikowano pojedynczo do plastykowych doniczek o pojemności 360 cm^3, które ustawiano na parapecie w ogrzewanej szklarni, przy 16 godz. dniu. W dniu 9.10.2015 r. dokonano oceny siły wzrostu siewek wszystkich rodzin mieszańców, stosując skalę bonitacyjną 1-5, w której 1 przypisano siewkom najsłabiej rosnącym, a 5 siewkom najsilniej rosnącym. Siewki bardzo różniły się wielkością (wysokością i wytworzoną masą liści). Średnia siła wzrostu wszystkich siewek to 2,40 w przyjętej skali oceny. Najwięcej siewek zakwalifikowano do 2 i 3 klasy siły wzrostu (1.659 siewek i 1.128 siewek odpowiednio). Uwidoczniły się tutaj różnice w sile wzrostu spowodowane rodowodem siewek, najwięcej siewek słabo rosnących (ocena bonitacyjna siły wzrostu = 1) było w rodzinach wywodzących się z krzyżówek, w których formą mateczną były ‘Canby’, ‘Glen Ample’, ‘Polana’ i ‘Polka’. Z kolei najwięcej siewek silnie rosnących (ocena bonitacyjna siły wzrostu = 2 i 3) stwierdzono w tych rodzinach, w których formą mateczną była ‘Laszka’, ‘Radziejowa i ‘Sokolica’.

Temat badawczy 4. **Indywidualna ocena cech fenotypowych wszystkich roślin w doświadczeniu polowym - siewek oraz form rodzicielskich.** Celem badań było wykonanie oceny fenotypowej siewek rosnących w doświadczeniu polowym pod względem wybranych cech biologicznych, dla określenia potencjału genetycznego badanych genotypów rodzicielskich maliny właściwej i ich przydatności do tworzenia nowej zmienności genetycznej. Materiałem roślinnym były wszystkie rośliny rosnące w doświadczeniu polowym, czyli siewki wszystkich rodzin mieszańców i rośliny mateczne maliny właściwej, które skrzyżowano w celu uzyskania tych siewek. W drugiej połowie okresu wegetacyjnego (koniec września/początek października) oceniono następujące cechy roślin maliny właściwej, rosnących w doświadczeniu polowym. Były to: stopień przezimowania wszystkich roślin (siewek i roślin matecznych), siła wzrostu roślin (skala bonitacyjna 1-9, w której 1 to najniższa wartość cechy a 9 – najwyższa wartość cechy), liczba wyrastających pędów z karpy krzaka (szt.), wysokość najwyższego pędu w krzaku (cm), typ owocowania (letnie/jesienne), obecność kolców (skala 1-9), ulistnienie krzewów - obfitość, barwa i wielkość ulistnienia oraz stopień porażenia roślin przez choroby i szkodniki (skala 1-9). Obfitość ulistnienia i wielkość liści oceniano przy użyciu skali bonitacyjnej 1-9, natomiast barwę liści oceniano instrumentalnie przy użyciu N-Testera firmy Yara, mierzącego zawartości chlorofilu w liściach (jako średnia z pomiaru 30 liści, położonych w środku długość pędu), oddzielnie dla każdej rośliny. W warunkach polowych siewki i odmiany rodzicielskie, od których się wywodzą, różniły się pod względem wszystkich ocenianych cech. Tylko 332 siewki (7,5% ocenianej populacji) kwitły i owocowały w okresie letnio-jesiennym, najwięcej w rodzinach: ‘Polana’ x ‘Polana’ (47 siewek), ‘Polana’ x ‘Polka’ (36 siewek), ‘Polka’ x ‘Polka’ (33 siewki) i ‘Polka’ x ‘Sokolica’ (24 siewki), a także ‘Polka’ x ‘Veten’ (17 siewek). Były też siewki bezkolcowe - 234 siewek (5,3% ocenianej populacji). Najwięcej siewek bezkolcowych stwierdzono w rodzinach: ‘Glen Ample’ x ‘Glen Ample’ (40 siewek), ‘Glen Ample’ x ‘Polka’ (31 siewek), ‘Glen Ample’ x ‘Sokolica’ (18 siewek), ‘Polka’ x ‘Polka’ (18 siewek), ‘Sokolica’ x ‘Sokolica’(15 siewek) oraz ‘Glen Ample’ x ‘Laszka’, x ‘Laszka’ x ‘Sokolica’ i ‘Polana’ x ‘Polana’ – po 12 siewek. Jednakże tylko 25 siewek (0,57% populacji) to genotypy łączące dwie bardzo ważne cechy użytkowe, czyli bezkolcowość i zdolność do zakwitania i owocowania w okresie letnio-jesiennym. Najwięcej takich siewek było w rodzinach, gdzie formą mateczną była ‘Polka’ (15 siewek), przy czym rodzinie ‘Polka’ x ‘Polka’ takich siewek było aż 11. Najsilniej wyrośnięte siewki wykorzystano do uzupełnienia wypadów w doświadczeniu polowym, założonym jesienią 2014 r. Było to tylko 75 siewek, czyli 2,8% populacji siewek w tym doświadczeniu. Uzupełnienia brakujących roślin w tym doświadczeniu dokonano w dniu w dniu 9.10.2015 r.

Zał. 1 – Abstrakt

Effect of Postharvest Treatment of Red Raspberry (*Rubus idaeus* L.) Seeds on the Germination and Growth Vigor of Young Seedlings

Edward Żurawicz

Department of Breeding of Horticultural Crops, Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland (Edward.Zurawicz@inhort.pl)

One of the issues in red raspberry (*Rubus idaeus* L.) breeding is poor seed germination. This is associated with the thickness of the seed coat and deep physiological seed dormancy caused by the presence of germination inhibitors in the endocarp, seed coat, endosperm, as well as in the embryo itself. Even long stratification does not guarantee good germination of red raspberry seeds. Therefore, pre-germination treatments, involving both scarification and stratification, are required to achieve fast and even germination. This helps to speed up the red raspberry breeding process. In 2014, research aimed at increasing the efficiency of germination of red raspberry seeds and the growth vigor of the obtained seedlings was conducted at the Research Institute of Horticulture in Skierniewice, Poland. It was based on different treatments of red raspberry seeds with concentrated sulfuric acid during the scarification process. The seeds had been obtained from crosses (a half-diallel mating design, Griffing's method II) among ten parental forms, producing 55 hybrid families (45 from cross-pollination and 10 from self-pollination of maternal cultivars). During scarification, the seeds were subjected to concentrated sulfuric acid for 20, 30 and 40 minutes. Assessment of the germinated seeds (seedlings), performed 2.5 months after the sowing date, revealed different effects of the applied treatments. The best germination was recorded for the hybrid family where 'Laszka' was the maternal form, and the poorest in the cross-combinations where 'Polana' was used as the maternal cultivar. The highest percentage of germinated seeds, on average for all the hybrid families, was obtained when the seeds had been treated with concentrated H_2SO_4 for 30 minutes (45.3% of germinated seeds), and the lowest when the seeds had been treated with concentrated H_2SO_4 for 40 minutes (35.6% of germinated seeds). Seeds that germinated earlier also produced larger seedlings compared with those germinating later.

Keywords: raspberry cultivars, seed scarification, mating design, cross-pollination, self-pollination

EFFECT OF POSTHARVEST TREATMENT OF RED RASPBERRY (*RUBUS IDAEUS* L.) SEEDS ON THE GERMINATION AND GROWTH VIGOR OF YOUNG SEEDLINGS

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Edward ŻURAWICZ
Department of Breeding of Horticultural Crops, Research Institute of Horticulture
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland (Edward.Zurawicz@inhort.pl)

INTRODUCTION

One of the issues in red raspberry (*Rubus idaeus* L.) breeding is poor seed germination. This is associated with the thickness of the seed coat and deep physiological seed dormancy caused by the presence of germination inhibitors in the endocarp, seed coat, endosperm, as well as in the embryo itself. Even long stratification does not guarantee good germination of red raspberry seeds. Therefore, pre-germination treatments, involving both scarification and stratification, are required to achieve fast and even germination of these seeds.

The objective of this study was to compare the effect of three postharvest treatments of red raspberry seeds on their germination and growth vigor of young seedlings.

MATERIAL AND METHODS

Research was performed at the Research Institute of Horticulture in Skierniewice, Poland. It was based on different treatments of red raspberry seeds with concentrated sulfuric acid during the scarification process. The seeds had been obtained in winter/spring 2014 from crosses (a half-diallel mating design, Griffing's method II) among ten parental forms, producing 55 hybrid families (45 from controlled cross-pollination and 10 from self-pollination of maternal cultivars) (Table 1). During scarification process, the seeds were subjected to concentrated sulfuric acid for 20, 30 and 40 minutes (250 seeds per each treatment). After scarification seeds were stratified at 3°C for 6 weeks and sown in the pots placed on growing benches in a heated glasshouse (20-22 during daytime, 14-16°C at night, with a 16-hr day). Assessment of the germinated seeds (seedlings), was performed every 7 days starting 18 days after sowing date. Seedlings with two seed leaves were planted separately in small pots which were placed on the growing benches in the same glasshouse. 2.5 months (75 days) after the sowing date, the growth vigor of seedlings (using the ranking scale 1-5) was assessed.

Table 1. Crossing design for the red raspberry cultivars (Skierniewice, 2014)

♀ \ ♂	Canby	Glen Ample	Laszka	Polana	Polka	Radziejowa	Schönemann	Sokolica	Veten	Willamette
Canby	xx	x	x	x	x	x	x	x	x	x
Glen Ample		xx	x	x	x	x	x	x	x	x
Laszka			xx	x	x	x	x	x	x	x
Polana				xx	x	x	x	x	x	x
Polka					xx	x	x	x	x	x
Radziejowa						xx	x	x	x	x
Schönema							xx	x	x	x
Sokolica								xx	x	x
Veten									xx	x
Willamette										xx

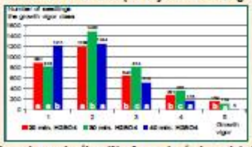
Explanation: ♀ - mother, ♂ - father; x – controlled cross-pollination, xx – self-pollination

Crossings of cultivars, production and scarification of seeds

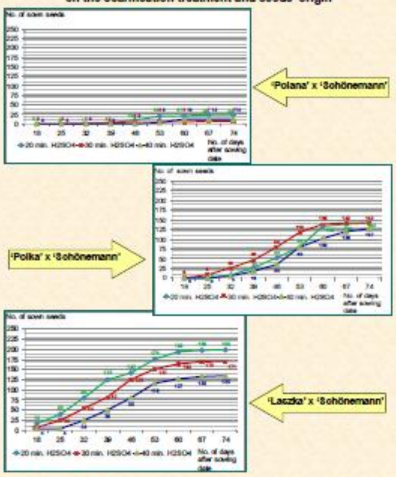
RESULTS

The studies revealed different effects of the applied treatments. The highest percentage of germinated seeds, on average for all the hybrid families, was obtained when the seeds had been treated with concentrated H_2SO_4 for 30 minutes (45.3% of germinated seeds), lower for seeds treated for 20 minutes (39.3% of germinated seeds) and the lowest when the seeds had been treated with concentrated H_2SO_4 for 40 minutes (35.6% of germinated seeds). Seeds that germinated earlier also produced larger seedlings compared with those germinating later. The best germination was recorded for the hybrid family where 'Laszka' was the maternal form, and the poorest in the cross-combinations where 'Polana' was used as the maternal cultivar.

Growth vigor of red raspberry seedlings depending on the scarification treatment (75 days after sowing seeds)



Dynamics of seed germination (% of germinated seeds) depending on the scarification treatment and seeds' origin



CONCLUSIONS

1. Red raspberry for even seed germination and fast growth of young seedlings require scarification and stratification.
2. Of three tested scarification methods the best germination possess seeds which are subjected to concentrated sulfuric acid for 30 minutes. Seedlings obtained from these seeds have also the best growth vigor.
3. Germination ability of red raspberry seeds depends also from the genotypes of the crossed parental forms.

REFERENCES

Dale A. and B. C. Jarvis. 1983. Studies on germination in raspberry (*Rubus idaeus* L.). Crop Res. (Hort. Res.) 23: 73-81.
Jennings D. L. and B. M. M. Tulloch. 1965. Studies on factors which promote germination of raspberry seeds. J. Exp. Bot. 16: 329-340.
Jennings D. L. and B. M. M. Tulloch. 1965. Studies on factors which promote germination of raspberry seeds. J. Exp. Bot. 16: 329-340.
Wada S. and B. M. Reed. 2011a. Optimized scarification protocols improve germination of diverse *Rubus* germplasm. Sci. Hort. 130: 660-664.
Wada S. and B. M. Reed. 2011b. Standardizing germination protocols for diverse raspberry and blackberry species. Sci. Hort. 132: 42-49.

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Cross-Pollination Increases the Number of Drupelets in the Fruits of Red Raspberry (*Rubus idaeus* L.)

E. Żurawicz
Research Institute of Horticulture
Konstytucji 3 Maja 1/3
96-100 Skierniewice
Poland

Keywords: raspberry cultivars, raspberry fruit, self-pollination, mating design, metaxenia

Abstract

The study was conducted in 2014 at the Research Institute of Horticulture in Skierniewice, Poland. Its aim was to assess the effects of self-pollination (self-fertilization) and cross-pollination (cross-fertilization) on the size of the fruit of ten red raspberry cultivars, determined as the number of drupelets in one fruit. The investigated cultivars were: 'Canby', 'Glen Ample', 'Laszka', 'Polana', 'Polka', 'Radziejowa', 'Schöneman', 'Veten' and 'Willamette'. They were self- and controlled cross-pollinated in a greenhouse in the spring of 2014. The pollination program was based on a half-diallel mating design (Griffing's method II) among these cultivars, producing 55 hybrid families (45 from controlled cross-pollination and 10 from self-pollination of maternal cultivars), which allowed to study the influence of the pollen cultivar on the number of drupelets in a single raspberry fruit. The study revealed an overwhelming advantage of cross-pollination over self-pollination. The average number of drupelets in the fruits from cross-pollinated flowers ranged from 48.3 in 'Glen Ample' (76.9% more drupelets than in self-pollinated 'Glen Ample') to 92.9 in 'Radziejowa' (96.8% more drupelets than in self-pollinated 'Radziejowa').

INTRODUCTION

In the cultivation of raspberry, complete pollination of flowers is important. This role is performed by various pollinating insects, among which the honeybee (*Apis mellifera* L.) plays the largest part (Couston, 1963; Shanks, 1969; McGregor, 1976; Wieniarska, 1987; Szklanowska and Wieniarska, 1992, 1993). An important role in the pollination of raspberry flowers is also played by the bumblebee (*Bombus* spp.), the bee *Osmia aglaia*, and other wild insects visiting the flowers of raspberry plants (Willmer et al., 1994; Cane, 2005). The pollen deposited on stigmas can come from the same flower, from other flowers of the same bush, from the flowers of neighbouring bushes of the same variety, or from the flowers of other varieties. The condition necessary for the setting of raspberry fruit is not only complete pollination of flowers, but also the fertilization of ovules located in ovaries in the lower part of pistils. Many studies have been conducted on the pollination of raspberry flowers (Hardy, 1931; Couston, 1963; Zych, 1965; Keep, 1968; Daubeny, 1969, 1971; Jennings and Topham, 1971; Lawrence, 1976; Redalen, 1976; Kühn, 1987; Colbert and de Oliveira, 1990; Szklanowska and Wieniarska, 1992; Touhimetsa et al., 2014). Raspberry fruits are composed of aggregated drupelets seated on a conical receptacle of the flower; the more of them there are, the larger and more fully developed the fruits are, and the higher the fruit yield (Lawrence, 1976; Redalen, 1976; Colbert and de Oliveira, 1990; Changon et al., 1991; Couston, 1991; Milivojević et al., 2011).

Wild red raspberries (*Rubus idaeus* L.) are diploid, sexual and self-incompatible, but their cultivated derivatives are all self-fertile, and thus self-compatible (Keep, 1968). This means that the cultivated varieties should be very productive when pollinated with their own pollen. Support for this opinion can be found in an earlier report by Hardy (Hardy, 1931), according to which there is no evidence that the yield of raspberries is increased by cross-pollination. However, other studies on the pollination of raspberry flowers (Jennings, 1967; Daubeny, 1969, 1971; Virdi, 1971) have shown that among the varieties of raspberry there are cases of self-sterility (partial self-incompatibility) as well as partial inter-incompatibility (cross-incompatibility with some cultivars), which leads to poor setting of fruit and lower yield performance of the plants. According to these authors there is a range of variation in self-fertility between raspberry cultivars.

Confirmation of the existence of partial self-sterility in raspberry is provided by numerous reports indicating a considerable advantage of open pollination over self-pollination. This was proven by comparing the performance of raspberry plants covered with isolators, which prevented access by pollinators during flowering, with those to which pollinators had free access. It was demonstrated that without free access by the pollinating insects, the resulting fruits were smaller and sometimes malformed; the number of drupelets, and thus the berry weight, was reduced (Couston, 1963, 1991; Redalen, 1976; Free, 1993; McGregor, 1976; Chagnon at al., 1991; Szklanowska and Wieniarska, 1992,1993), which rendered the fresh fruit unmarketable. However, in open pollination in those studies, the flowers were pollinated by insects with pollen from different varieties, i.e. with a mixture of pollen of different genotypes. Interesting results on the pollination in raspberry were also obtained by Virdi (1971), Redalen (1976), Kühn (1987) as well as Colbert and de Oliveira (1990). The first of these researchers found that the raspberry selection Ore-US was partially self-incompatible and cross-incompatible with ‘Malling Jewel’, and very compatible when open pollinated. Redalen (1976) studied the effect of pollinating the cultivars ‘Norna’ and ‘Asker’, and the clone II-2LGP with pollen of several other cultivars, which he called pollinators. In those studies, ‘Norna’ gave a low set of berries and drupelets after selfing compared to controlled cross-pollination. The best pollinators for ‘Norna’ were ‘Preussen’, ‘Malling Jewel’ and II-2LGP, and for ‘Asker’ – ‘Glen Clova’, ‘Malling Jewel’, and ‘Preussen’. In a study by Kühn (1987), ‘Willamette’ proved to be highly fertile and there were no differences between open-, self- and controlled-pollination; however, the largest fruits were obtained with control-pollination by ‘Glen Clova’. Colbert and de Oliveira (1990) studied the influence of pollen variety on red raspberry fruit development and found that a metaxenic phenomenon does occur in raspberries. In their studies, the freely cross-pollinated ‘Festival’ had an average drupelet weight greater than that of its self-pollinated control. It was also shown that the cultivar ‘Muskoka’ benefited more from cross-pollination than ‘Festival’. Lack of good pollination and fertilization of raspberry flowers can be a problem in protected cultivation in high plastic tunnels and glasshouses, where usually only one cultivar is grown, and can be the reason for low yield performance of cultivars.

The aim of the study, conducted under greenhouse conditions, was to determine the effect of various pollinators (pollen sources) on the number of seeds (drupelets) produced by the fruits of selected red raspberry cultivars as a result of controlled cross-pollination and forced artificial self-pollination.

MATERIALS AND METHODS

Plant material. The plant material consisted of selected cultivars of red raspberry (Table 1). Certified plants of all the cultivars (50 plants of each) were purchased in late autumn of 2013 from a specialized nursery farm. They were ‘short cane’ type of plants, about 50 cm in length. Prior to planting they were kept in a cold store (temp. +2°C, humidity 95%).

The plants were planted in 3.8 litre pots on two dates – 27 January 2014 and 18 February 2014, each time using 25 plants of each cultivar. After planting, the pots with the plants were placed on growing benches in a heated greenhouse (20-22°C during daytime, 14-16°C at night, with a 16-hr day). The plants were watered if necessary. No pesticides were used against diseases and pests, but sticky traps were densely arranged throughout the greenhouse to capture any possible insects. All of the plants were tested to make sure that they were not infected with serious viral diseases (Raspberry bushy dwarf virus, Raspberry leaf mottle virus, Raspberry vein chlorosis virus, and Raspberry leaf blotch virus).

Pollination of flowers. Pollination was performed according to Griffing's method II, which involved controlled cross-pollination and self-pollination (Table 2). A total of 55 pollination combinations were performed, of which 45 represented controlled cross-pollination, and 10 self-pollination (auto-pollination). To carry out the pollination program, the plants were maintained until they had formed well-developed lateral shoots with inflorescences and flower buds developing on them (3-4 lateral shoots about 50 cm long). Pollination of maternal forms was performed from 22 March to 8 April 2014. For this purpose, use was made of the first fully developed flower buds (4-5 buds) growing on the first 4-5 inflorescences of the mother plants, after prior removal of the sepals and stamens. In order to obtain pollen for pollination, the first, earliest to grow, fully developed buds on paternal plants were collected. The extracted anthers were dried at room temperature for 24 hours. Viability of the pollen used to pollinate the flowers was not tested; pollination was performed once, immediately after emasculation; the pollen was applied with a fine paint brush. After this treatment, the mother plants were covered with isolators. In the combinations with self-pollination, the flowers were not emasculated, but the plants were protected with isolators against uncontrolled pollination. Pollination of those flowers consisted in transferring the pollen with a fine paint brush onto the pistils within the same flower at full bloom.

RESULTS AND DISCUSSION

The maternal and paternal plants used in this study showed no evidence of the presence of any of the viruses tested for; thus viral diseases had no effect on the setting of fruit by the pollinated flowers. The results of the completed pollination program – the pollinated flowers and the fruits and seeds obtained, are shown in Table 3. Within the tested pollination combinations, from 14 to 46 flowers had been pollinated. Altogether, 1,588 flowers had been pollinated, from which 1,294 fruits (81.5% relative to the number of pollinated flowers) were obtained. The best in this regard were the combinations: 'Canby' x 'Canby', 'Canby' x 'Polana', 'Glen Ample' x 'Laszka', 'Glen Ample' x 'Schönemann', 'Laszka x Laszka', 'Polka x Radziejowa', 'Polka' x 'Veten', 'Polka' x 'Willamette', 'Radziejowa' x 'Schönemann', 'Schönemann' x 'Sokolica' and 'Veten' x 'Veten', in which all of the pollinated flowers produced fruit. By contrast, the worst setting of fruit was found in the pollination combinations: 'Canby' x 'Sokolica', 'Canby' x 'Schönemann' and 'Polana' x Schönemann', in which only about 50% of the pollinated flowers produced fruits. A total of 83,975 seeds were obtained from the harvested fruits, at an average of 64.9 seeds per fruit.

The number of seeds obtained in the different pollination combinations varied widely and depended on the parental forms crossed. The lowest number of seeds was obtained following self-pollination (auto-pollination, selfing). This was the case with all the maternal cultivars, although the differences between them were considerable. The largest number of seeds resulting from self-pollination was produced by ‘Laszka’, an average of 54 seeds per fruit. The cultivars in which self-pollination resulted in 40 to 50 seeds per fruit were ‘Canby’, ‘Polana’, ‘Radziejowa’, and ‘Sokolica’. Fruits of the other self-pollinated cultivars contained far fewer than 40 seeds per fruit, with the fruits of ‘Glen Ample’ containing the fewest seeds – only 27.3 seeds. The problem associated with a lower production of fruit in raspberry when flowers are pollinated with their own pollen (selfing) than when they are cross-pollinated, manifesting itself in a smaller number of drupelets (seeds) in the fruit, had also been reported previously by Jennings (1967), Daubeny (1969, 1971), Virdi (1971) and Radalen (1976). These researchers attributed this problem to partial self-incompatibility of the varieties tested. The results presented in this paper are consistent with their reports and do not agree with other reports suggesting that all cultivated varieties of raspberry are self-fertile, and thus self-compatible (Hardy, 1931; Keep, 1968), nor with the more recent reports claiming that self- and cross-pollination of raspberries has no effect on yield and fruit quality (Touhimetsa et al., 2014).

A much larger number of seeds was obtained from the fruits produced as a result of pollinating maternal cultivars with the pollen of paternal cultivars, used as pollinators. These pollinators, however, were of different value to the maternal cultivars tested. In each group of paternal forms there were pollinators that were better than others at contributing to the production of seeds in the fruits of maternal cultivars. Thus the fruits of ‘Canby’ contained the largest number of seeds when the pollinator of its flowers was ‘Willamette’ (69.9% increase in the number of seeds relative to self-pollination). For the other cultivars, the best crossing combinations of the parental forms were (percentage increase in brackets): ‘Glen Ample’ x ‘Willamette’ (141.1%), ‘Laszka’ x ‘Sokolica’ (96.5%), ‘Polana x ‘Sokolica’ (83.6%), ‘Polka’ x ‘Veten’ (151.3%), ‘Radziejowa’ x ‘Veten’ (124.2%), ‘Schönemann’ x ‘Willamette’ (131.6%), ‘Sokolica’ x ‘Willamette’ (51.6%) and ‘Veten’ x ‘Willamette’ (111.4%).

The varied effect of pollinators on the production of seeds in raspberry fruits, and thus on the size of those fruits, is a result of different interactions between maternal and paternal genotypes. This interaction can mean partial self-incompatibility in the case of auto-pollination, through partial cross-incompatibility to good cross-compatibility with the genotypes that are the source of pollen for the pollinated cultivars. Such relationships in the pollination of red raspberry flowers had previously been reported by Jennings (1967), Daubeny (1969, 1971), Virdi (1971), Radalen (1976) and Colbert and de Oliveira (1990). The good response of raspberry cultivars to pollination by pollen of other cultivars, manifesting itself in the production of a larger number of drupelets and larger fruits, was called by the latter investigators a metaxenic phenomenon.

The results of this study show that the source of pollen plays an important role in the pollination of flowers of raspberry cultivars. It determines the number of drupelets in the fruits, and thereby their size, and the size of the resulting crop. This is of great importance, especially in protected cultivation of raspberries, where only one cultivar is usually grown in a plastic tunnel or a greenhouse. Such cultivar should be characterized by a high degree of self-fertility, or it should be planted together with one that will serve as a good pollinator.

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Literature Cited

Cane, J.H. 2005. Pollination potential of the bee Osmia aglaia for cultivated red raspberries and blackberries (Rubus: Rosaceae). HortScience 40(6): 1705-1708.

Chagnon, M., Gingras, J. and De Oliveira D. 1991. Honey bee (Hymenoptera: Apidae) foraging behavior and raspberry pollination. J. Econ. Entomol. 84(2): 457-460.

Colbert, S. and de Oliveira, D. De. 1990. Influence of pollen variety on raspberry (*Rubus idaeus* L.) development. J. Heredity 81 (6): 434-437.

Couston, R. 1963. The influence of insect pollination on raspberries. Scott. Beekeep. 40: 196-197.

Couston, R. 1991. Insect pollination of soft fruits and associated problems. Acta Hort. 288: 249-254.

Dale, A. 2014. Protected cultivation of raspberries. *Acta Hort. 946:* 349-354.

Daubeny, H.A. 1969. Some variations in self-fertility in the red raspberry. Can. J. Plant Sci. 49: 511-512.

Daubeny, H.A. 1971. Self-fertility in red-raspberry cultivars and selections. J. Amer. Soc. Hort. Sci. 96(5): 588-591.

Free, J.B. 1993. Insect pollination of crops, 2nd ed. Academic Press, New York.

Hardy, M.B. 1931. Self and cross fertility of red raspberries. Proc. Amer. Soc. Hort. Sci. 28: 118-121.

Jennings, D.L. 1967. Observations on some instances of partial sterility in red raspberry cultivars. Hort. Res. 7: 116-122.

Jennings, D.L. and Topham, P.B. 1971. Some consequences of raspberry pollen dilution for its germination and for fruit development. New. Phytol. 70(2): 371-380.

Keep, E. 1968. Incompatibility in *Rubus* with special reference to *Rubus idaeus* L. Can. J. Genet. Cytol. 10: 253-262.

Kühn, B.F. 1987. Pollination of the raspberry cultivar Willamette. Tidsskrift for Planteavl (*Ărslev*) 91 (1): 85-88.

Lawrence, F. J. 1976. Sterility in *Rubus*. Fruit Var. J. 30(1): 22.

McGregor, S.E. 1976. Insect pollination of cultivated crop plants. Agricultural handbook 496. USDA, Washington DC: 318 pp.

Milivojević, J.M., Nikolić, M.D. Dragisić-Maksimović, J.J. and Radivojević, D.D. 2011. Generative and fruit quality characteristics of primocane fruiting red raspberry cultivars. Turkish J. Agric. And Forestry 35 (3): 289-296.

Redalen, G. 1976. Pollination and fruit set in raspberries. Acta Hort. 60: 169-173.

Shanks, C.H. 1969. Pollination of raspberries by honeybees. *J. Apicul. Res.* 8:19-21.

Szklanowska, K. and Wieniarska, J. 1992. Wpływ owadów zapylających na plonowanie malin (*Rubus idaeus* L.). Skierniewice, Seria C, Biul. Inf. 3-4 (115-116): 105-107.

Szklanowska, K. and Wieniarska, J. 1993. The effect on field by the bees visits on raspberry flowers (*Rubus idaeus* L.) the successive ramifications in inflorescence. Acta Hort. 352: 231-233.

Touhimetsa, S., Hietaranta, T., Uosukainen, M., Kukkonen, S. and Karhu, S. 2014. Fruit development in artificially self- and cross-pollinated strawberries (*Fragaria* × *ananassa*) and raspberries (*Rubus idaeus*). Acta Agric. Scandinavica Section B-Soil and Plant Sci. 64: 408-415.

Virdi, B.V. 1971. Reduced fertility in red raspberry (*Rubus idaeus* L.). M.S. Thesis, University of British Columbia.

Willmer, P.G., Bataw, A.A.M. and Hughes, J.P. 1994. The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecological Entomology* 19: 271-284.

Wieniarska, J. 1987. The role of pollinating insects in fruiting of some red raspberry cultivars. Fruit Sci. Rep. (Skierniewice) 14: 65-70.

Zych, C. C. 1965. Incompatibility in crosses of red, black and purple raspberries. Proc. Amer. Soc. Hort. Sci. 86: 307-312.

Tables

Table 1. List and short description of the red raspberry cultivars tested (Skierniewice, 2014).

Cultivar	Origin	Pedigree	Ripening time	Yield	Fruit size	Fruit color	RBDV sensitivity
Canby*	USA	Viking x Lloyd George	early	high	medium	intense red	sensitive
Glen Ample*	UK	Complex with G. Prosen and Meeker	medium early	high	large	bright red	low sensitive
Laszka*	POL	80408 x 80192	early	high	very large	bright red	sensitive
Polana**	POL	Heritage x Eva Herbsternte	half of August	medium high	medium	intense red	low sensitive
Polka**	POL	Autumn Bliss + L. George + *R. rataeifolius*	beginning of July	high	medium and large	intense red	very sensitive
Radziejowa*	POL	92271 x 96221	medium early	medium high	large	intense red	sensitive
Schönemann*	GER	Lloyd George x Preussen	late	high	large	dark red	low sensitive
Sokolica*	POL	96131 x 96221	medium early	high	large	bright red	sensitive
Veten*	NOR	Asker x Lloyd George	early	medium to high	medium to large	red to dark red	low sensitive
Willamette*	USA	Lloyd George x Newburgh	late	high	large	intense red	tolerant

Explanation: * - Floricane raspberry, ** - Primocane raspberry

Table 2. Crossing design for the red raspberry cultivars tested (Skierniewice, 2014).

♀ \ ♂	Canby	Glen Ample	Laszka	Polana	Polka	Radziejowa	Schönemann	Sokolica	Veten	Willamette
Canby	xx	x	x	x	x	x	x	x	x	x
Glen Ample		xx	x	x	x	x	x	x	x	x
Laszka			xx	x	x	x	x	x	x	x
Polana				xx	x	x	x	x	x	x
Polka					xx	x	x	x	x	x
Radziejowa						xx	x	x	x	x
Schönemann							xx	x	x	x
Sokolica								xx	x	x
Veten									xx	x
Willamette										xx

Explanation: ♀ - mother, ♂ - father; x – controlled cross-pollination, xx – self-pollination

Table 3. Results of crossings of the red raspberry cultivars (Skierniewice, 2014).

No.	Cultivars crossed	Features evaluated				
		Flowers pollinated	Fruits harvested	Seeds collected	Mean number of seeds/fruit	Increase in seeds (self-pollination = 100%)
1	Canby x Canby	26	26	1 218	46.8	100.0
2	x Glen Ample	26	25	1 278	51.1	109.2
3	x Laszka	32	27	1 974	73.1	156.2
4	x Polana	23	23	1 105	48.0	102.6
5	x Polka	25	14	849	60.6	129.5
6	x Radziejowa	27	19	1 315	69.2	147.9
7	x Schönemann	30	16	1 186	74.1	158.3
8	x Sokolica	29	15	1 778	71.1	151.9
9	x Veten	14	14	1 106	79.0	168.8
10	x Willamette	37	21	1 668	79.4	169.7
	Mean for cross-pollination		*174*	*12 259*	*70.5*	
11	Glen Ample x G.Ample	34	26	710	27.3	100.0
12	x Laszka	23	23	814	35.4	129.7
13	x Polana	30	23	1 301	56.6	207.3
14	x Polka	23	16	759	47.4	173.6
15	x Radziejowa	27	18	743	41.3	151.3
16	x Schönemann	30	30	1 220	40.7	149.1
17	x Sokolica	21	18	1 047	58.2	213.2
18	x Veten	24	17	746	43.9	160.8
19	x Willamette	26	21	1 386	66.0	241.8
	Mean for cross-pollination		*166*	*8 016*	*48.3*	
20	Laszka x Laszka	24	24	1 295	54.0	100.0

21	x Polana	22	16	1 225	76.6	141.9
22	x Polka	29	21	1 067	50.8	94.1
23	x Radziejowa	24	18	1 132	62.9	116.5
24	x Schönemann	29	16	999	62.4	115.6
25	x Sokolica	27	20	2 123	106.1	196.5
26	x Veten	29	22	1 690	76.8	142.2
27	x Willamette	22	19	1 957	103.0	190.7
	Mean for cross-pollination		*132*	*10 193*	*77.2*	
28	Polana x Polana	27	25	1 067	42.7	100.0
29	x Polka	20	14	870	62.1	145.4
30	x Radziejowa	33	27	1 890	70.0	163.9
31	x Schönemann	27	14	788	56.3	131.9
32	x Sokolica	37	32	2 510	78.4	183.6
33	x Veten	35	21	1 489	70.9	166.0
34	x Willamette	22	17	1 185	69.7	163.2
	Mean for cross-pollination		*125*	*8 732*	*69.9*	
35	Polka x Polka	29	27	1 021	37.8	100.0
36	x Radziejowa	30	30	2 628	87.6	231.7
37	x Schönemann	26	23	1 794	78.0	206.3
38	x Sokolica	34	32	2 602	81.3	215.1
39	x Veten	41	41	3 897	95.0	251.3
40	x Willamette	29	29	2 312	79.7	210.8
	Mean for cross-pollination		*155*	*13 233*	*85.4*	
41	Radziejowa x Radziejowa	39	36	1 699	47.2	100.0
42	x Schönemann	21	21	1 977	94.1	199.4
43	x Sokolica	30	21	1 744	83.0	175.8
44	x Veten	29	26	2 751	105.8	224.2
45	x Willamette	23	15	1 239	82.6	175.0

	Mean for cross-pollination		*83*	*7 711*	*92.9*	
46	Schönemann x Schönemann	32	26	847	32.6	100.0
47	x Sokolica	30	30	1 870	62.3	191.1
48	x Veten	35	34	2 314	68.0	208.6
49	x Willamette	46	40	3 019	75.5	231.6
	Mean for cross-pollination		*104*	*7 203*	*69.3*	
50	Sokolica x Sokolica	38	29	1 428	49.2	100.0
51	x Veten	37	34	2 356	69.3	140.9
52	x Willamette	25	15	1 119	74.6	151.6
	Mean for cross-pollination		*49*	*3 475*	*70.9*	
53	Veten x Veten	29	29	1 068	36.8	100.0
54	x Willamette	25	20	1 556	77.8	211.4
	Mean for cross-pollination		*20*	*1 556*	*77.8*	-
55	Willamette x Willamette	46	38	1 244	32.7	
	Total	1 588	1 294	83 975		
	Mean number of seeds / fruit for self-pollination				40.5	
	Mean number of seeds / fruit for controlled cross-pollination				71.8	
	Mean number of seeds /fruit for all hybrid families				64.9	