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The Influence of the Degree of Pollination of Black Currant Flowers (*Ribes nigrum* L.) on the Number of Seeds in Fruits and Its Size

Wpływ stopnia zapylenia kwiatów porzeczki czarnej (*Ribes nigrum* L.) na liczbę nasion w owocach i dorodność jagód

INTRODUCTION

For most orchard plants cross-pollination is an essential condition to set fruits, seeds and produce high yield (Free, 1970; McGregor, 1976; Jabłoński and Kołtowski, 1995; Nyéki and Soltész, 1996). Pollination with foreign pollen results in a better shape of fruits, which contain a higher amount of seeds and remain attached to pedicels longer. Most of the cultivated black currant cultivars are to a large extent self-fruitful and weaker pollination does not result in the deformation of fruits, which often occurs in the case of insufficient pollination in a great number of species, e.g. strawberry and raspberry (Szklanowska, 1993; Kołtowski et al. 1997, 1999). However, reduced pollination of black currant causes the falling of berries and negatively influences its quantity, reducing the yield. The yield of black currant fruits delivered from the bush depends on the number of raceme with set berries, number of berries in raceme and average weight of one berry (Szklanowska and Dąbska 1993). Moreover, the weight of berries is positively correlated with the number of set seeds (Clinch and Faulke, 1976; Ugolik and Gawęcka, 1978; Gwozdecki and Smolarz, 1992; Pluta and Żurawicz, 1992; Hofmann, 1995; Szklanowska and Denisow, 1998). There are no reports in the available literature concerning the number of pollen grains needed for the correct fertilization process. Only Lech et al. (1997) estimate that black currant requires more pollen grains per stigma than other orchard taxons.

The degree of pollination, however, may be estimated by the measurement of the number of pollen grains on the stigma (Rodkiewicz, 1994; Behar, 1997).

Accordingly, the main goal of this study was to establish how the varied number of pollen grains, which get to the stigma of flowers in different accessibility of pollinators conditions, influences the efficiency of fertilization, i.e. the number of seeds in fruits.

MATHERIAL AND METHODS

The experiment using the randomised block method in four replications with five plants per plot was conducted at ISiK in Pulawy. The observations were carried out in 1994-1997, on eight cultivars of black currant (Ben Alder, Ben Lomond, Ben Nevis, Ben Tirran, Ceres, Ojebyn, Titania, Triton). The bushes were grown on pseudo-podsolic soil. Three pollination modes were applied: A) free-pollination, B) pollination with own pollen by one bumble bee queen under isolator, C) self- pollination under isolator.

Inflorescences for the analysis of the number of pollen grains per stigma were taken at the initial, medial and late phases of full blooming and were stored in 1:3 glacial acetic acid : ethanol. Prior to examination the styles were stained in Lugol (J + KJ). Then stigma was separated from style and crushed under a coverslip in a drop of lactophenol (Gerlach 1972). The pollen grains were counted under a MB 30S microscope. The efficiency of pollination was estimated indirectly by counting the number of seed sets per fruit. In order to do this mature fruits from a number of randomly chosen branches of each bush were analysed. Berries were classified according to their size into four groups <8 mm, 8-10 mm, 10-12 mm, >12 mm of diameter. Ten berries from each size-class were taken and samples were frozen. Fruits were carefully rubbed and seeds were separated from fleshy and juicy pericarp. Seeds were put on blotting-paper and counted in each sample. Adequate counting allowed to estimate the average number of seeds per one berry of each class. All tests were conducted in four replications for each cultivar and pollination treatment.

The results were worked out statistically by variance analysis and the significance of differences was estimated by Duncan's test at $\alpha = 0.05$.

RESULTS

The number of seeds positively correlated with the degree of pollination and depended on the cultivar (tab. 1). Berries produced from the best pollinated flowers which were accessible to different insects contained the highest number of well-developed seeds. The average number of pollen grains per stigma after free- pollination oscillated from 179 to 309, while the average number of seeds ranged from 17 (Ben Alder) to 34 (Ben Lomond), 26 on average.

It is interesting that a considerable difference in a number of pollen grains (about 100 grains) between stigmas from free-pollinated flowers and those pollinated by only one bumble bee queen did not cause statistically significant differences in the number of seeds set between the hitherto examined mode of pollination.

Table 1. The degree of pollination of flowers of 8 cultivars of black currant, number of seeds in fruits and weight of 1000 seeds depending on manner of pollination (an average from 4 years)

Cultivar	Number of pollen grains per stigma			Number of seeds per one berry			Weight of 1000 seeds in g		
	Manner of pollination								
	A	B	C	A	B	C	A	B	C
Ben Alder	$240,9_{g\text{-}h}$	$110,0_{bc}$	$71,5_{abc}$	$16,8_{abc}$	$14,4_{ab}$	$12,6_{a}$	$1,28_{h\text{-}i}$	$1,31_{ij}$	$1,38_{j}$
Ben Lomond	$282,0_{i}$	$128,9_{e}$	$71,9_{abc}$	$33,9_{d}$	$27,1_{a\text{-}d}$	$23,3_{a\text{-}d}$	$0,99_{a}$	$1,01_{ab}$	$1,12_{c\text{-}f}$
Ben Nevis	$198,0_{f}$	$132,9_{e}$	$76,4_{a\text{-}d}$	$30,6_{cd}$	$24,9_{a\text{-}d}$	$18,9_{abc}$	$1,05_{abc}$	$1,16_{def}$	$1,28_{gfi}$
Ben Tirran	$268,9_{hi}$	$113,0_{c\text{-}e}$	$58,2_{a}$	$24,2_{a\text{-}d}$	$19,3_{abc}$	$19,7_{abc}$	$1,08_{a\text{-}d}$	$1,21_{fgh}$	$1,20_{fgh}$
Ceres	$217,2_{fg}$	$95,5_{a\text{-}e}$	$60,8_{a}$	$25,1_{a\text{-}d}$	$21,8_{a\text{-}d}$	$15,8_{abc}$	$1,04_{abc}$	$1,09_{bcd}$	$1,36_{ij}$
Ojebyn	$179,1_{f}$	$99,0_{a\text{-}e}$	$62,3_{a}$	$28,3_{bcd}$	$25,7_{a\text{-}d}$	$23,8_{a\text{-}d}$	$1,10_{b\text{-}e}$	$1,07_{a\text{-}d}$	$1,09_{bcd}$
Titania	$308,6_{i}$	$107,3_{b\text{-}e}$	$67,5_{ab}$	$20,6_{a\text{-}d}$	$17,9_{abc}$	$16,9_{abc}$	$1,11_{c\text{-}f}$	$1,15_{def}$	$1,19_{efg}$
Triton	$215,6_{fg}$	$118,4_{de}$	$80,4_{a\text{-}d}$	$26,6_{a\text{-}d}$	$24,7_{a\text{-}d}$	$22,6_{a\text{-}d}$	$1,12_{c\text{-}f}$	$1,11_{c\text{-}f}$	$1,17_{def}$
average	$238,8_{C}$	$113,1_{B}$	$68,9_{A}$	$25,8_{B}$	$22,0_{AB}$	$19,2_{A}$	$1,09_{A}$	$1,13_{B}$	$1,22_{C}$

Means within columns referring to the same feature marked by the same index are not significantly different from each other at $\alpha = 0.05$

A-free-pollination, B-pollination with own pollen by one bumblebee queen, C-self-pollination

Fruits produced from flowers pollinated with own pollen by bumble bee under net isolator contained an average of 22 seeds per one berry, that is only 4 less than in berries set after free-pollination. The smallest number of seeds (14) was present in Ben Alder fruits, and the highest in Ben Lomond (27) – the same as in the previously considered pollination treatment. Stigmas from self-pollinated flowers received the smallest number of pollen grains. This number, depending on the cultivar, ranged from 58 to 80. The fruits set after autogamy contained only 19 seeds on average. The average number of seeds in the examined cultivars oscillated from 12 (Ben Alder) to 23 (Ben Lomond) and 24 (Ojebyn).

Statistical analysis showed that stigmas in self-pollinated flowers received significantly weaker pollination in comparison with other pollination modes. Fruits produced in complete isolation contained a significantly smaller number of seeds than those produced after free-pollination.

The cultivars under examination revealed small but significant influence of the degree of pollination, that is the number of delivered pollen per stigma, on the weight of 1000 seeds. 1000 seeds set by fertilization of embryos in weakly self-pollinated flowers completely isolated from pollinating insects had the largest weight. This is a well-known regularity since a smaller number of seeds per fruit results in better nutrition conditions. The weight of 1000 seeds set from flowers foraged by one bumble-bee was 1.13 g and 1.09 g in free pollination condition.

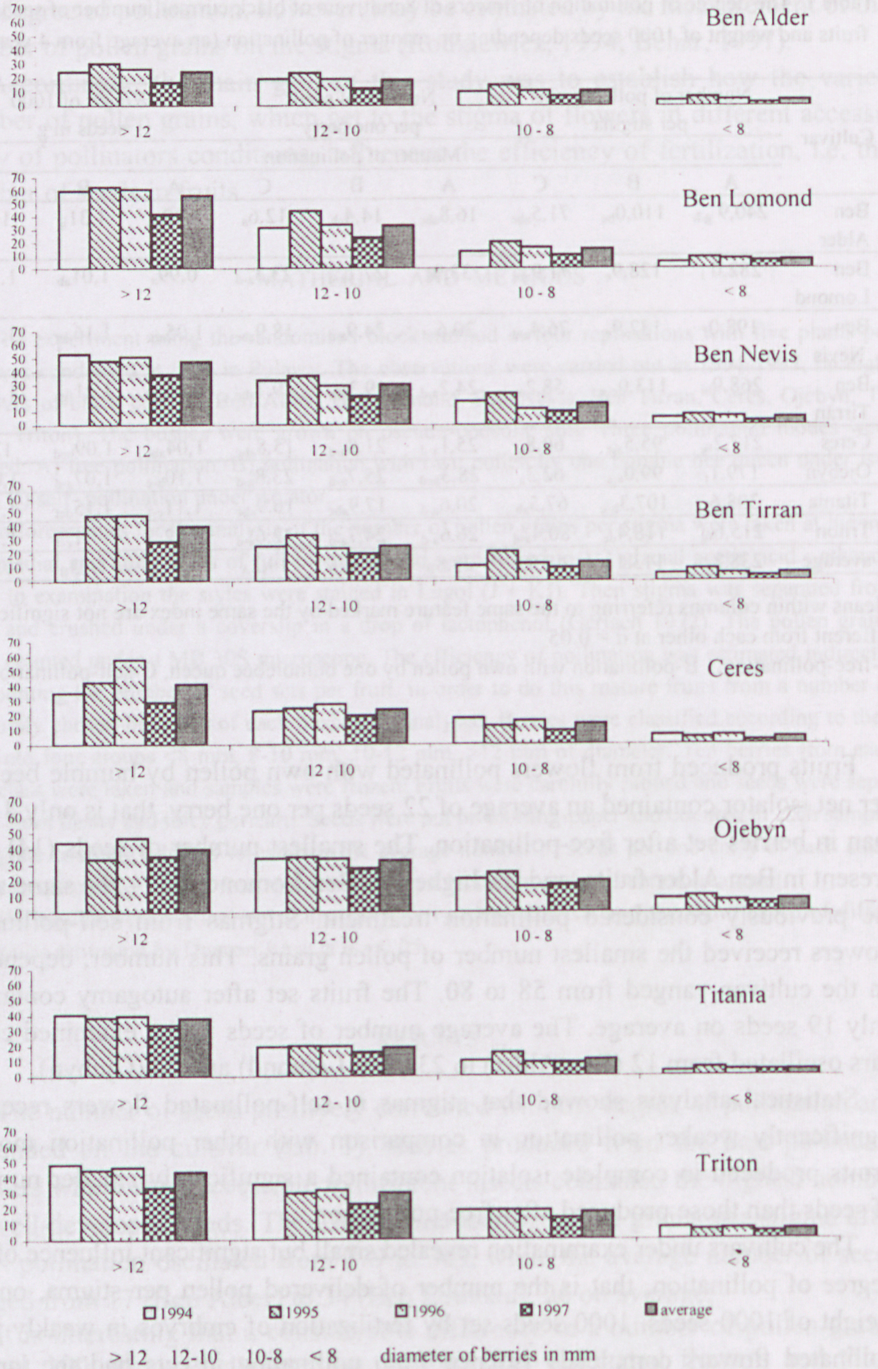

Fig. 1. The number of seeds per one berry in each size-class of eight cultivars of black curant irrespective of the manner of pollination in 1994-1997

The number of seeds per fruit influenced its diameter and therefore its mass (Fig. 1). Always, irrespective of the cultivar and the manner of pollination, the highest number of seeds was present in fruits >12 mm-41 on average. The berries in the second size-class (diameter 10-12 mm) contained an average of 27 seeds, while in the third class (8-10 mm) 15 seeds. The smallest number of seeds were present in the smallest berries (<8 mm) an average of 5. Therefore, the size of black currant berries correlated with the number of seeds which, in turn, depended on the degree of pollination of flowers. It is generally known that the developing seeds discharge hormones which positively affect the processes of cell-division of the fruit parenchyma and ultimately their size.

DISCUSSION

The comparison of the number of pollen grains per stigma with the number of seeds per berry showed a clear dependence. The better the degree of pollination of stigmas in flowers, the more seeds were contained in a berry and the larger its diameter and thus its weight. The berries set from the best pollinated flowers always contained more seeds than the isolated ones. The observed dependence entirely confirms the opinion of other authors who worked on the pollination and confirms fructification of black currant (Szklanowska, 1993; Nyèki and Soltèsz, 1996; Kołtowski et al. 1997, 1999). However, the data presented in the literature do not include the degree of pollination of flowers. The number of pollen grains which get to the stigma in the pollinating process is (apart from the biological value of pollen) essential for the correct estimation of the efficiency of the process of pollination. The data obtained in my experiment confirm the observations of Lech et al. 1997, to the effect that black currant is a species requiring a lot of pollen grains per stigma for adequate fertilization. As a result, the seeds were set most effectively when there were more then 200 pollen grains per stigma. However, the reduction in pollination by approx. 50% (an average of 113 pollen grains per stigma) in flowers pollinated by only one bumble-bee did not significantly influence the number of seeds set. It must be presumed, then, that there is an upper limit above which an increase in the quantity of pollen per stigma is less effective for the fertilization process. When the number of pollen grains per stigma during self-pollination was 70 on average, seed setting was considerably worse. The data presented in this paper point to a substantial and important role of pollinators in pollen transfer to stigmas among cultivars of black currant. This supports the findings of many authors concerning the significant influence of insect pollinators on black currant yield (Clinch and Faulke, 1976; Ugolik and Gawęcka, 1978; Szklanowska, 1993; Hofmann, 1995; Kołtowski et al. 1997, 1999).

The number of seeds per one berry significantly correlated its diameter, which is in total agreement with the data collected by other authors (Kołtowski et al. 1997; Szklanowska and Denisow, 1998), who always found more seeds in larger berries.

CONCLUSIONS

1. The number of pollen grains per stigma of the examined cultivars of black currant depended on pollination treatment and was the highest (average 200) when different pollinators took part in the process.

2. The number of pollen grains per stigma was positively correlated with the number of seeds set per berry.

3. The number of seeds set per fruit, that is the degree of ovules fertilized, was always positively correlated with the size of berry, irrespective of the pollination mode. The biggest berries contained 26-42 seeds, the smallest 5-16.

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SUMMARY

The experiment using the randomised block method was conducted in 1994-1997 in Pulawy. Eight cultivars of black currant: Ben Alder, Ben Lomond, Ben Nevis, Ben Tirran, Ceres, Ojebyn, Titania and Triton were examined. The influence of the degree of pollination measured by the number of pollen grains per stigma on the number of seeds in fruits and its size were examined. The pollen grains were counted in crushed slides in lactophenol. The degree of pollination positively correlated with the number of seeds in berries. The best free-pollinated flowers with over 200 pollen grains per stigma set an average of 26 seeds. However, an almost 50% decrease in pollination made by only one bumble bee queen did not cause a significant decrease of seed setting (an average of 22 per one berry). This suggests the existence of an upper limit above which an increase in the quantity of pollen per stigma does not result in an increase of efficiency during fertilization. As expected, the smallest number of seeds developed from ovules fertilized in worse self-pollinated flowers –only 19. The number of seeds per bèrry positively correlated with size-class of berry, irrespective of the manner of pollination. The smallest number of seeds was present in <8 mm berries (approx. 5 seeds), while in 10-12 and >12 mm (26-42) seeds were present. The number of seeds per berry revealed close dependence on the cultivar as well, with the smallest number in Ben Alder and the highest in Ben Lomond.

STRESZCZENIE

Doświadczenie metodą bloków losowych prowadzono w latach 1994-1997 na terenie ISiK w Puławach. Uwzględniono następujące odmiany porzeczki czarnej: 'Ben Alder', 'Ben Lomond', 'Ben Nevis', 'Ben Tirran', 'Ceres', 'Ojebyn', 'Titania', 'Trition'. Badano wpływ stopnia zapylenia kwiatów, mierzony liczbą ziaren. pyłku na znamieniu słupka, na liczbę nasion w owocach i

dorodność jagód. Ziarna pyłku liczono w preparatach wykonanych w laktofenolu. Ze stopniem zapylenia kwiatów dodatnio korelowała liczba wykształconych w owocach nasion. Najlepiej zapylone kwiaty, które na jednym znamieniu miały średnio powyżej 200 ziaren pyłku wiązały owoce zawierające przeciętnie 26 nasion. Jednak słabsze o około 50% zapylenie kwiatów przy udziale matki trzmielej nie spowodowało istotnego ograniczenia wiązania nasion – było ich średnio 22 w jednej jagodzie. Może to wskazywać na istnienie granicy, powyżej której dalsze dopylenie jest już mniej efektywne. Najmniej nasion stwierdzano w owocach powstałych z najsłabiej zapylonych autogamicznie kwiatów – tylko 19. Z liczbą wykształconych nasion w owocach korelowała bez względu na sposób zapylania dorodność jagód. Najmniej nasion (ok. 5) stwierdzano w owocach o średnicy <8 mm, najwięcej (26-42) w jagodach z najwyższych klas wielkości. Liczba nasion w owocach okazała się też cechą odmianową. Najmniej nasion stwierdzano w owocach odmiany ,Ben Alder', najwięcej ,Ben Lomond'.