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Examination of reasons for poor fruit set in the sour cherry cultivar 'Stevnsbär' by means of fluorescence microscopy

Undersøgelse af årsager til ringe frugtsætning i 'Stevnsbær' ved hjælp af fluorescensmikroskopi

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Summary

Fruit set may theoretically be 100 per cent. »Sources« that decrease fruit set in the sour cherry cultivar 'Stevnsbär' were examined with a fluorescence microscope. Fertilization was 27 per cent in 1985 and 16-17 per cent in 1986.

The main reason for poor fruit set in 'Stevnsbär' was found to be a lack of pollen tube growth through the style. In 15-42 per cent of the hand-pollinated flowers no pollen tubes at the base of the style were found.

Other less important reasons were found to be 1) Lack of pollen transfer. No pollen grains were found in up to 5 per cent of the stigmas. 2) Lack of pollen germination. Fruit setting was reduced by up to 10 per cent due to lack of pollen germination. No reasons were found for 29-57 per cent of the »lacking« fruit setting.

Key words: 'Stevnsbär', pollen transfer, pollen germination, receptive period of stigma, pollen tubes growth, fertilization.

Resumé

Frugtsætningen kan teoretisk være 100 pct. Forskellige »kilder« i befrugtningsprocessen, der kan reducere denne procent i 'Stevnsbær', er undersøgt ved hjælp af fluorescensmikroskopi.

Befrugtningsprocenter var 27 i 1985 og 16-17 i 1986.

Den væsentligste årsag til den mangelfulde befrugtningsprocent var, at pollenrørene i 15-42 pct. af blomsterne stoppede væksten undervejs i griflerne.

Andre mindre væsentlige årsager var 1) en svigtende pollenoverførsel, i 0-5 pct. af de bestøvede blomster, og 2) en svigtende pollenspiring, i 1-10 pct. af de bestøvede blomster. Der kunne ikke ved de foretagne undersøgelser findes nogen årsager til 29-57 pct. af de »manglende« frugtsætninger.

Nøgleord: 'Stevnsbær', pollenoverførsel, pollenspiring, støvfangsmodtagelighed, pollenrørsvækst, befrugtning.

Introduction

Poor fruit set is usually seen in 'Stevnsbär'. Normally 80-85 per cent of the flowers will drop of the tree before harvest. Some of these flowers are unfertilized. There may be several reasons for unsuccesful fertilization. Limited ovule longevity

appeares to be an important reason for poor fruit set in som *Prunus*-species (2, 5, 6, 8, 10, 16, 17).

The technique of fluorescens enables one to follow the process of fertilization step by step. Thereby obtaining knowledge about where the fertilization is failing.

The report deals with fluorescensmicroscopic examinations of 'Stevnsbär' flowers.

Materials and methods

The utilized 'Stevnsbär' tree was planted in 1979 and was in good condition.

1985 and 1986

Flowers to be pollinated were emasculated at the balloon stage (day 0). Pollination was done with flowers of 'Stevnsbär' and of 'Fanal'. These flowers were picked the day before pollination at the balloon stage and stored at about 20°C without petals and sepals until pollination. The pollinated flowers were enclosed in paperbags. From each treatment 20 flowers per day were collected over the preceding 10 days (day 1-10).

1986 (In addition)

Flowers were emasculated on day 0. 20 flowers were self-pollinated on each of the following 10 days. Pollination was carried out with flowers picked the day before pollination at balloon stage.

The flowers were collected the day after pollination. Free pollinated flowers were collected on day 5.

The material was fixed in FAA (1 formaldehyde 40 per cent: 1 acetic acid 100 per cent: 8 ethanol 80 per cent) for 15-17 hours. Maceration was done with 8N NaOH for 15-17 hours. Staining with 0.1 per cent anilin in 0.1 N K_3 PO_4 for 15-17 hours.

Styles were detached from ovaries. Ovaries were opened and ovules taken out. Squash-preparations were made of styles and ovules.

Microscopic examination was done with a Reichert-Jung Micro Star 110 fluorescence microscope. In UV-light pollen grains, pollen tubes and nonviable ovules will show an intensive fluorescence.

The following notes were taken: Number of pollen grains on stigma, number of pollen tubes in the style at 1/3, 2/3 lenght and at the base, fertilization: Pollen tube growth through the micropyle.

Results

Weather

The average temperature in the experimental period 1985 was 14.8°C and in 1986 12.2°C.

Pollen transfer

Generally more pollen grains were observed on the stigmas in 1985 than in 1986. Cross-pollination resulted in 49 per cent more pollen grains per flower than self-pollination in 1985 and in 124 per cent more pollen grains per flower in 1986, Table 1.

Hand-pollination did not succeed in every attempt. However only between 1 to 5 per cent of the hand-pollinated flowers had no pollen grains on stigmas, Table 1.

By free pollination between 0–290 pollen grains per stigma were supplied, on average 70 pollen grains per stigma were found. 5 per cent flowers without pollen grains on stigmas were found under natural conditions.

Pollen germination

In 1985 pollen germination was 20 per cent for both 'Stevnsbär' and 'Fanal'. In 1986 pollen germination was 23 per cent for 'Fanal' and 24 per cent for 'Stevnsbär'. Pollen germination failed in

Table 1. Pollen transfer, number of pollen grains.

Treatment	Number of pollen grains per stigma Avg. (min.-max.)		Per cent flowers without pollen grains on stigmas	
	1985	1986	1985	1986
Self-pollination	99a(20–217)	45a(6–103)	1	3
Cross-pollination	148b(55–282)	101b(9–277)	1	5

1 per cent of the flowers in 1985 and in 9–10 per cent of the flowers in 1986. The difference was significant.

Pollen germination was on an average 31 per cent by free-pollination, and no flowers were found without pollen germination.

Table 2. Pollen germination, per cent, and number of pollen grains after self-pollination from day 1 to 10, 1986.

Day of pollination	Pollen germination on stigma, per cent	Number of pollen grains per stigma
1	23	44
2	35	42
3	32	90
4	24	157
5	12	40
6	18	106
7	7	7
8	7	4
9	4	2
10	11	19
LSD	10	28

The receptive periods of stigma

Reduction in pollen germination is seen from day 5, Table 2, indicating that the receptivity of the stigma starts to fail from this day. From day 7 pollen germination and the number of pollen grains per stigma were reduced considerably. Pollinated as well as unpollinated flowers started to shed the styles from day 10 in 1986. Style-shedding began in 1985 on day 7.

Growth of pollen tubes

Growth of pollen tubes was slower in 1986 than in 1985, Table 3. 'Fanal'-pollen tubes apparently grew faster than 'Stevnsbär'-pollen tubes, Table 3.

Only a small per cent of the germinated pollen grains had a fast growth through the styles. It took at least 6 days before the maximum number of pollen tubes could be observed at the base of the style, Table 3. A higher per cent 'Fanal'-pollen tubes were observed in the styles than 'Stevnsbär'-pollen tubes.

Generally the number of pollen tubes decreased from stigma to base at style.

Table 3. Growth-rate of pollen tubes. Number of days from pollination to observation of pollen tubes at base of style and pollen tubes in per cent of pollen grains.

Treatment	Number of days to first observation of pollen tubes at base of style				Number of days to observation of max. per cent pollen tubes at base of style			
	1985		1986		1985		1986	
	No.	pct.	No.	pct.	No.	pct.	No.	pct.
Self-pollination	2	9	4	4	6	38	10	47
Cross-pollination	2	10	3	13	6	58	8	66

Tabel 4. Avg. per cent flowers with no pollen tubes at base of style.

Treatment	Per cent flowers		
	1985 (Avg. day 2–10)	1986 (Selfp. avg. day 3–10 crossp. avg. day 4–10)	Avg.
Self-pollination	42	38	40
Cross-pollination	15	21	18
LSD			16

Some pollen tubes stopped their growth some where in the style and did not succeed in reaching the base. On average 40 per cent of the self-pollinated flowers had no pollen tubes at the base of the style, Table 4. In cross-pollinated flowers only 18 per cent had no pollen tubes at base of style.

Fertilization

Pollen tubes entering the micropyles were seen for the first time 4 days after pollination, Fig. 1. After cross-pollination in 1986 it took 6 days from pollination to fertilization, table 5.

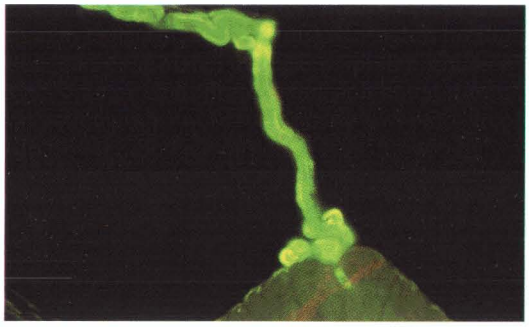

Fig. 1. Fertilization. Pollen tube entering a micropyle.

Table 5. Per cent fertilized flowers.

Day	Self pollination		Cross pollination	
	1985	1986	1985	1986
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	30	10	26	0
5	11	15	28	0
6	19	1	47	24
7	47	21	25	1
8	20	42	20	26
9	30	16	25	5
10	40	15	20	25
Avg.	(day 4–10) 27		(day 4–10) 27	
	(day 4–10) 17		(day 6–10) 16	

There was no significant difference in fertilization per cent between self- and cross-pollination. The fertilization per cent was 11 per cent better in 1985 than in 1986. The difference was significant (LSD=8).

Unfortunately it could not be determined whether ovules were viable or nonviable.

Discussion

The flowering period was shorter, shed of style earlier and pollen tube growth faster in the year with the highest average temperature (14, 20).

Below is shown how fruit set in 'Stevnsbär' was reduced by different causes.

The observed number of pollen grains on the stigmas were for both the hand-pollinations and for the free-pollinations in accordance with the numbers found on cherry stigmas by others (1, 4). Significantly more pollen grains were transferred to stigmas from flowers of 'Fanal' than from flowers of 'Stevnsbär'. This can be due to a higher production and/or a higher release of pollen grains by 'Fanal'.

Generally pollen transfer was better in 1985 than in 1986. This could be due to a higher production and/or a higher release of pollen grains in 1985 than in 1986. In the hand-pollinated flowers no pollen grains were found in up to 5 per cent of the flowers. So lack of pollen transfer was only a small item in the total reduction of fruit set.

In literature pollen germination is said to vary between 15 and 74 per cent dependent on variety and year (1, 9, 13, 21). In this examination pollen germination for both 'Stevnsbär'- and 'Fanal'-pollen was only 20–24 per cent. This could be a result of different methods. Pollen germination was estimated on stigmas while others generally germinate pollen in special germination media.

It is remarkable that pollen germination by free-pollination was about 30 per cent higher than by artificial pollination.

Fruit setting was reduced by up to 10 per cent due to lack of pollen germination. Most flowers without pollen germination were found in 1986, possible due to reduced pollen quality in this year.

Even though the stigmatic surface degenerates during the flowering period (18, 19) pollen germination and pollen tube growth will still occur (3, 17). Therefore the lenght of the receptive period of stigmas did not limit fruit setting. But pollen germination was reduced considerably later in the flowering period. This was probably not only caused by a reduced receptivity of stigmas. But it also indicates that late opening flowers have a

Table 6. Reasons to poor fruit set in 'Stevnsbär'.

	Self pollination		Cross pollination	
	1985, p.c.	1986, p.c.	1985, p.c.	1986, p.c.
Theoretical fruit set	100	100	100	100
– Lack of pollen transfer	1	3	0	5
Remainder	99	97	100	95
– Lack of pollen germination	1	10	1	9
Remainder	98	87	99	86
– Lack of pollen tube growth	42	38	15	21
Remainder	56	49	84	65
– Fertilization	27	17	27	16
Other reasons	29	32	57	49

poorer quality than early opening flowers in accordance with *Wociór* (21).

The growth of pollen tubes through the style takes 3–4 days in apples (12) and 2–3 days in cherries (15, 17). In 'Stevnsbär' growth of pollen tubes through the style took minimum two days in 1985 and minimum three days in 1986. Growth of pollen tubes were generally relatively slowly, thus it took 6–10 days before the maxium number of pollen tubes could be observed. Slow growth of pollen tubes may to some extend have reduced fruit setting.

The main reason for poor fruit set in 'Stevnsbär' was found to be a lack of pollen tube growth through the style. In 15–42 per cent of the hand-pollinated flowers no pollen tubes at the base of the styles were found.

Apparently 'Fanal'-pollen grew better through the styles than 'Stevnsbär'-pollen, indicating that cross-pollination is favourable. Selfincompatability seen by swollen termination of pollen tubes in the first third of the style (1) was not observed, and the difference in pollen tube growth between 'Fanal' and 'Stevnsbär' was equalized by the growth through the ovaries. Finally no difference in fertilization per cent was found between self- and cross-pollination.

Growth of pollen tubes through the ovaries took at least 1–3 days (Table 3 and Table 5). Fertilization: Growth of a pollen tube through the micropyle was noted first 4–6 days after pollination. The per cent fertilization obtained was low compared to an initial fruit set of 46 per cent obtained by *Hansen* (7) and a final fruit set of 22 per cent found by *Redalen* (11).

There is an unexplained remainder of 29–59 per cent in 1985 and 32–57 per cent in 1986. Probably some of this remainder is a post called »short ovule longevity«.

Several investigations have shown that the limiting factor for fertilization and fruit set in cherries is ovule longevity (2, 6, 8, 10, 16, 17). Ovule degeneration has been demonstrated to start before pollen tubes have had time to reach the micropyle.

Conclusion

Microscopic examinations of 'Stevnsbär' flowers have shown that in 15–42 per cent of the hand pollinated flowers pollen tube growth stopped somewhere in the style and thereby excluded fertilization. Other less important reasons for reduced fruit setting in 'Stevnsbär' were shown to be a lack in pollen transfer (in 0–5 per cent of the pollinated flowers) and a lack in pollen germination (in 1–10 per cent of the pollinated flowers). It was not possible to find an explanation for 29–57 per cent of the »lacking« fruit set.

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