

COMPATIBILITY AND INCOMPATIBILITY IN WITLOOF-CHICORY (*CICHORIUM INTYBUS* L.). 3. GAMETIC COMPETITION AFTER MIXED POLLINATIONS AND DOUBLE POLLINATIONS

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SUMMARY

In witloof-chicory research was carried out on the occurrence of gametic competition after mixed or double pollinations with self- and foreign pollen. In part of the experiments flower heads were emasculated before pollination. Gametic competition was deduced from the percentages of hybrid seeds in the various experiments with flower colour functioning as a marker.

Among self pollen parent (SPP) clones large differences occurred for frequency of germinated pollen grains after self pollination. These differences were positively correlated with the degree of self-compatibility (SC) of the clones. No significant differences for pollen germination rate were found.

Among SPP clones large differences occurred for general hybrid producing ability (GHPA). There was a significant negative correlation between GHPA and SC of the clones. Among foreign pollen parent (FPP) clones, used for pollination of SPP clones, large differences occurred for general hybrid inducing ability (GHIA). Results from removing the stigma and a part of the style at varying intervals after mixed pollination and results from double pollination with a delayed second, FPP, pollination suggest differences in pollen tube growth rate in the apical part of the style and in the basal part and/or in the ovary. Besides general HPA and general HIA also specific HPA (SHPA) and specific HIA (SHIA) occur.

INTRODUCTION

In witloof-chicory (*Cichorium intybus* L.) F₁-hybrid varieties are produced by intercrossing self-compatible (SC) inbred lines (BANNEROT & DE CONINCK, 1970; EENINK, 1981a). For an efficient production of F₁ seeds, either of the two SC parental lines can be used for pollen- and for seed production. However, only part of the SC-lines is suitable for this purpose as often parental combinations result in a high percentage of self-seeds.

Various factors might influence the percentage of hybrid seeds after intercrossing SC lines. In the progamic phase gametic competition may occur, both after intergeneric and interspecific crosses, but also after intraspecific crosses between pollen grains from different plants and between pollen grains from the same hermaphrodite heterozygous plant.

Gametic competition of one of the above types has been found in e.g. *Brassica* (WATTS, 1963), *Coronilla* (BALUCH et al., 1973), *Datura* (SANSOME & PHILP, 1939),

HAPLOPAPPUS (JORDAN, 1975), *Lepus*, *Gossypium*, *Vigna* and *Triticum* (MULCAHY, 1974), *Linum* (BAKER, 1975), *Lycopersicon* (MULCAHY, 1974; ZAMIR et al., 1981), *Oenothera* (HARTE, 1975), *Phlox* (LEVIN, 1975), *Vicia* (ROWLANDS, 1958) and *Zea* (PFAHLER, 1975; SARI GORLA & ROVIDA, 1980). Gametic competition was also observed after using the mentor pollen technique with mixtures of foreign pollen and irradiated self pollen (STETTLER & GURIES, 1976; PANDEY, 1978; DEN NIJS, 1981).

Gametic competition can be influenced by the environment (ZAMIR et al., 1981). According to MULCAHY (1974) and HARTE (1975) competitive ability of pollen grains is under control of both major and minor genes. MULCAHY found an increase in competitive ability of self pollen after repeated selection for this character. Both male and female parent tissues may influence competition, for complicated pollen-stigma-style interactions precede fusion (HOGENBOOM, 1973; HESLOP-HARRISON, 1975; LINSKENS, 1980; FERRARI et al., 1981). In *Petunia* with a gametophytic incompatibility system, rejection or acceptance of pollen tubes by styles depends on a sequence of activities of a number of genes resulting in qualitative and quantitative differences in both RNA and protein synthesis (VAN DER DONK, 1975).

Pollination experiments with mixtures of self and foreign pollen or double pollinations with application of self pollen followed after some interval by foreign pollen were carried out. Emasculated and non-emasculated flower heads were used to investigate the importance of factors for gametic competition and thus for the percentage of hybrid seeds. Results are given in this paper.

MATERIALS AND METHODS

Flowering clonal plants, directly or indirectly originating from different open pollinated populations, were used in four experiments (Table 1) with 5 plants per clone per experiment. Self pollen parent (SPP) clones were used as female parents but they also delivered pollen for selfpollination. The SPP clones represented genotypes from different I_3 inbred lines of cv. Dubbelblank and differed for degree of SC (Table 1). Foreign pollen parent (FPP) clones were used to pollinate emasculated or non-emasculated flower heads of SPP clones. The FPP clones represented selected plants out of 3 open pollinated populations obtained after crossing cvs Vroege Mechelse, Malina and Meilof.

When clones started flowering they were placed at a constant temperature of 17°C in the phytotron, a temperature favourable for seed production in this crop (EENINK, 1981a). The pollination experiments which were carried out are shown in Table 2. Because pollen tubes in the style could not be made visible with fluorescent staining methods, seed production was used to analyse indirectly gametic competition. To distinguish self-plants from F_1 -hybrid plants flower colour functioned as a marker. The flower heads of the SPP clones were white while the FPP plants had blue flower heads. The blue colour is dominant over white (one gene).

In experiment 1a and 1b stigmas were collected at intervals of 30 and 60 minutes after pollination to investigate rate and frequency of pollen germination after self-pollination as well as after mixed pollination. For this rating the stigmas were stained with lactophenol fuchsin acid. Emasculation was carried out by removing the anthers with a pair of tweezers 30–60 minutes before anthesis. Artificial pollinations started

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Table 1. The clonal plants used in four experiments. SPP = self pollen parent (female parent) FPP = foreign pollen parent. The number of seeds per flower head is based on selfing of 20 flower heads. +: clone used in experiment.

Clone no.	Seeds per flower heads after selfing	Experiment 1		Experiment 2		Experiment 3	Experiment 4
		a	b	a	b		
<i>SPP</i>							
20	1.4	+	+		+	+	+
21	5.2		+		+	+	+
77	0.8				+	+	+
140	-			+			
150	-			+			
160	3.0		+		+	+	+
345	5.0	+	+		+	+	+
429	4.0	+	+		+	+	+
560	0.4	+	+		+	+	+
<i>FPP</i>							
13	-			+			
277	-			+			
364	-	+					
574	0.5	+	+		+	+	+
1044	0.4	+	+		+	+	+
1045	0.7	+	+		+	+	+
1098	2.1	+	+		+	+	+

Table 2. Pollination experiments carried out to investigate gametic selection. SP = self pollen; FP = foreign pollen

Experiment	Year	Character investigated	Emasculation of flower heads	Type of pollination	Cutting off stigma and part of the style
1a	1978	rate and frequency of pollen germination	yes	SP or SP + FP	no
1b	1979		yes	SP or SP + FP	no
2a	1979	pollen tube growth rate	yes	SP + FP	30, 60 etc. minutes after pollination
2b	1980		yes	SP + FP	30, 60 etc. minutes after pollination
3	1979	pollen tube growth rate	yes	first SP and 0, 30, 60 etc. minutes later FP	no
4	1979	pollen tube growth rate	no	first SP and 0, 30, 60 etc. minutes later FP	no

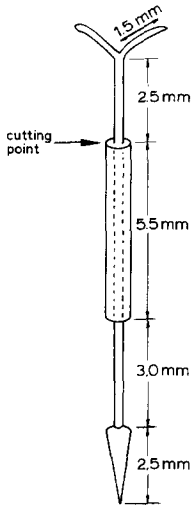


Fig. 1. A floret of witloof-chicory *Cichorium intybus*. The cutting point where the stigma and a part of the style were removed is indicated. Mean lengths are based on 10 flower heads of different clones.

at the time of anthesis (= 0) or later (see Table 2), by rubbing anthers with self or foreign pollen over the stigmas. Mixed pollinations were carried out by using anthers with selfpollen and anthers with foreign pollen at the same time. Anthers of SPP and FPP clones had the same size and contained pollen grains of similar quantity and quality. This procedure was chosen because it is representative of the pollination method used for commercial F_1 -seed production. Collecting pollen from flower heads of SPP and FPP clones followed by mixing *in vitro* is seriously hampered by the small quantity of pollen grains which can be dislodged from a single flower head (approximately 0.2×10^{-3} gram). In experiment 2a and 2b at different times after pollination the stigma and a part of the style were cut off to investigate which percentage of pollen tubes of the SP and FP parent had passed the cutting point at that time. This point is indicated in Figure 1. In experiment 3 and 4 differences in pollen tube growth were investigated by double pollinations carried out at varying intervals.

RESULTS

Pollen germination and hybrid seed production pooled per clone. For each experiment, except for experiment 2a, the mean pollen germination or hybrid seed production was calculated for each SPP and/or FPP clone. Pollen germination was rather erratic, which implies that the results should be regarded with some reservations. In any case there were large consistent differences between SPP clones for mean number of germinated pollen grains per stigma after selfing. For instance both in 1978 and 1979 clone 560 exhibited the lowest frequency of germinated grains while the pollen germination of clones no 345 and 429 was always highest in these years. After mixed pollination the differences between clones for germination frequency were smaller than after self pollination; they were not consistent and also showed no relationship with clonal differences for pollen germination after selfing.

Both among SPP clones and among FPP clones large and consistent differences were found for the percentage of hybrid seeds in three experiments (Table 3). Clones

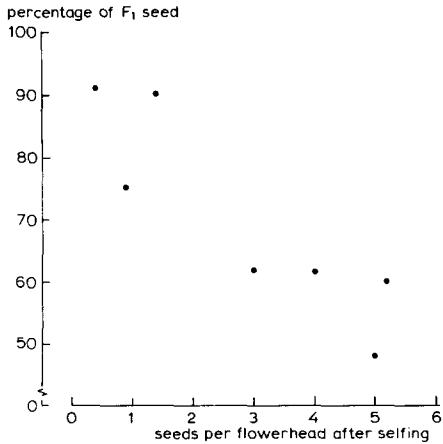


Fig. 2. Relationship between the number of seeds per flower head after selfing and the overall mean percentage of F₁-hybrid seeds after mixed and double pollinations in three experiments (2b, 3, 4) for seven SPP clones.

20 and 560 always produced the highest percentage of hybrid seeds while this percentage for clone 345 was always low. Clone 1045 and 1098 induced the highest percentage of hybrids while clone 574 had the lowest inducing ability. Apparently both the SP parent and the FP parent influence the percentage of hybrid seed formation and thus the gametic competition. This was also found in earlier experiments (EENINK, 1980). Thus the clones can be assigned a hybrid producing ability (HPA) and a hybrid inducing ability (HIA).

There is a close negative linear relationship ($r = 0.89^{**}$) between general (G)HPA and the degree of SC expressed in number of seeds per flower head after selfing. Fig. 2 shows that the percentage of hybrid seeds on a female parent is higher with lower degrees of SC and vice versa. Preliminary experiments (EENINK, 1981a) showed a positive correlation between the degree of SC and the percentage of pollen germination after selfing. Here a similar relation was found. This implies that there must also be a relationship between the frequency of pollen germination after selfing and the percentage of hybrid seeds after mixed pollinations. Indeed such a relationship seems to exist for the SPP clones in experiments 2b-4. For instance clone 560 showed a limited pollen germination after selfing and the highest percentage of hybrid seeds, while the clones 160 and 429, with a high frequency of germinated pollen grains after selfing, produced a low percentage of F₁ hybrids. However, as pollen germination was only investigated for six SPP clones these results should be verified by further investigations.

Rate and frequency of pollen germination. As mentioned before, behaviour of pollen germination on stigmas of separate clones was rather erratic. Nevertheless a general yearly trend could be observed for most clones. Fig. 3 shows the numbers of germinated pollen grains per stigma after self-pollination and after mixed pollination per year within 30 to 180 minutes after pollination, and pooled for four or six SPP clones.

In 1978 there was a slight increase of pollen germination between 30 and 120 minutes after selfing. After mixed pollination germination increased significantly within this period. At 60 or 120 minutes after pollination far more pollen grains had germinated after mixed pollination than after selfing.

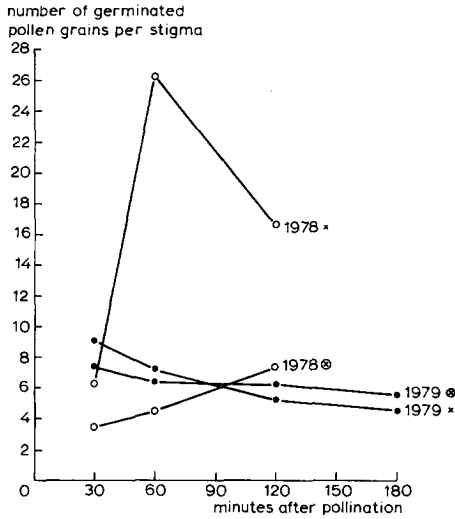


Fig. 3. Pollen germination at different periods after self (○) or mixed (×) pollination. Pooled data for 4 SPP clones in 1978 and for 6 SPP clones in 1979. Means are based on 16–32 stigmas in 1978 and on 24–48 stigmas in 1979.

In 1979 the germination behaviour on the same SPP clones and using the same FPP clones differed considerably. Both after selfing and after mixed pollinations there was no increase of pollen germination between 30 and 180 minutes after pollination; nor was there a significant difference between self pollinations and mixed pollinations for germination frequency.

The reasons for the discrepancy between the results of 1978 and 1979 are not known. In both years the same plant material and the same temperature were used; there might only have been differences in solar radiation. So far the above results gave no clear indication for consistent differences in germination rate between clones as a factor influencing gametic competition and thus percentage of hybrid seeds.

Percentage of hybrid seeds after mixed pollinations. Figure 4 shows the percentage of hybrid seeds after cutting off the stigma and a part of the style for three parental combinations of experiment 2a. The results for the parental combinations 140 × 13 and 150 × 13 are similar, though they differ for the basal percentage of hybrid seeds (BPH) which is the percentage after mixed pollinations without cutting the style. Here the difference for BPH was caused by the SP parent; BPH for the combination of clone 140 × clone 13 being about 75% and for the combination of clone 150 × clone 13 only 25%.

For the above mentioned mixed pollinations the removing of the stigma and a part of the style at 60 minutes after pollination results in an increase of the percentage of hybrid seeds up to 100, indicating that the FP tubes had already passed the cutting point at that time while the SP tubes had not. However, 30 to 60 minutes later 'sufficient' SP tubes had also passed as both hybrid seeds and self seeds developed and the percentages of hybrid and self-plants did not change significantly anymore and BPH was reached. Apparently the advantage of the foreign pollen, gained during the first 60 minutes after pollination, was lost. The behaviour of the combination of clone 140 × clone 277 differed significantly from that of the other combinations. Cutting-off the style resulted in a decrease instead of an increase in the percentage of hybrid seeds

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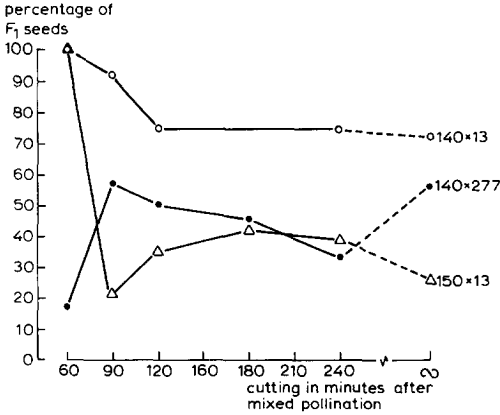


Fig. 4. The percentage of hybrid seeds after cutting off the stigma and a part of the style for three parental combinations of experiment 2a. The percentages per parental combination are based on 20-40 seeds.

compared with BPH which was about 55%. This decrease must have been caused by FPP clone 277 whose pollen tubes during the first 60 minutes after pollination seem to grow slower than the tubes of pollen from the SPP clone 140.

In Figures 5a and 5b the effects of cutting off the stigma and a part of the style are shown for experiment 2b. Most curves in Fig. 5a with results per SP parent pooled across FPP clones are rather similar in behaviour and no considerable increase or decrease of the percentage of hybrids occurred. SPP clone 429 demonstrated an increase of hybrid seeds from 65%, when cutting took place 30 minutes after mixed pollination, till 90% when cutting was delayed till 240 minutes after pollination. Apparently with this clone the SP tubes grew more rapidly, shortly after pollination, than the FP tubes. Hybrid seed formation of SPP clone 77, not indicated in Fig. 5a, behaved in a similar way.

In Fig. 5b 4 separate parental combinations are shown. The curves for the combinations 21 x 1098 and 21 x 574 demonstrate the same behaviour and there is no influ-

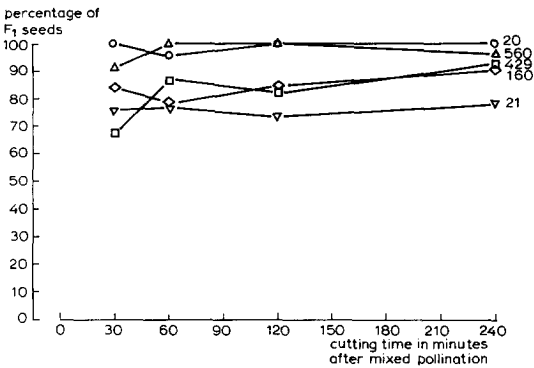


Fig. 5a. The percentage of hybrid seeds after cutting off the stigma and a part of the style for 5 SPP clones pooled across 4 FPP clones after mixed pollinations. The percentages are based on 80-160 seeds.

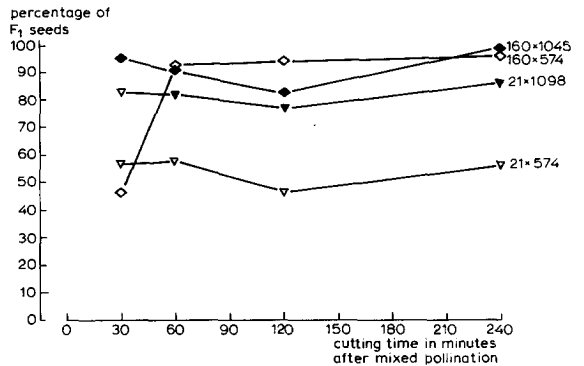


Fig. 5b. The percentage of hybrid seeds after cutting off the stigma and a part of the style for 4 separate parental combinations after mixed pollinations. The percentages are based on 20-40 seeds.

Table 3. Results per SPP clone and per FPP clone for percentage of hybrid seeds after mixed or double pollinations in three experiments. The ranking order of clones per experiment is indicated between brackets.

Experiment	Mean percentage of hybrid seeds										
	SPP clone							FPP clone			
	20	21	77	160	345	429	560	574	1044	1045	1098
2b	99(1)	77(6)	86(3)	85(4)	56(7)	82(5)	97(2)	73(4)	81(3)	88(2)	91(1)
3	78(2)	41(5)	53(3)	32(7)	43(4)	39(6)	82(1)	34(4)	46(3)	59(2)	63(1)
4	94(2)	62(6)	87(3)	71(4)	44(7)	66(5)	97(1)	64(4)	66(3)	84(1)	78(2)
Overall mean	90(2)	60(6)	75(3)	62(4)	47(7)	62(5)	92(1)	57(4)	64(3)	77(2)	77(1)

The means per SPP clone are based on 60–200 flower heads and 320–960 seeds.

The means per FPP clone are based on 100–340 flower heads and 560–1680 seeds.

ence of the time of cutting on the percentage of hybrids. This suggests that 30 minutes after pollination the pollen tubes of both parents had already passed the cutting point in their ultimate ratio. In the first combination BPH is higher, which can only be ascribed to the influence of FPP clone 1098. This agrees with the rather high hybrid inducing ability of this clone (Table 3) and the low ability of clone 574. Between the combinations 160×1045 and 160×574 there is a large difference in curve path. The former is hardly influenced by the time of cutting while the latter suggests that at 30 minutes after pollination about equal numbers of SPP and of FPP tubes had passed the cutting point but at 60 or more minutes after pollination a preponderance of FPP tubes has passed, resulting in a higher percentage of hybrid seeds.

Percentage of hybrid seeds after double pollinations. Figures 6a and 6b show the percentage of hybrid seeds after double pollinations of emasculated flower heads. Pollen of the SP parent was applied at time 0 (near anthesis) and foreign pollen at the same time or 30 to 240 minutes later. Figure 6a, with the means per SPP clone, pooled across the FPP influences, shows large differences among SPP clones for percentage of hybrid seeds as determined by the time of the second, FPP, pollination.

The percentage of hybrid seeds obtained on SPP clones 20 and 560, with a high GHPA, was moderately influenced by the time of the second, FPP, pollination. Especially for SPP clone 20, FPP pollination delayed for 60 to 240 minutes hardly further influenced the percentage of hybrid seeds. This contrasts with the percentage of hybrids after delayed pollination of several other clones, such as 429, 21 and 160, all with a low GHPA. When the second, FPP pollination was delayed for only 30 minutes, the percentage of hybrid seeds decreased drastically and almost immediately reached a rather stable but low level of 15–20%.

Fig. 6b shows the percentages of hybrid seeds after double pollinations for 6 separate parental combinations which are representative of the others. Delayed pollination of SPP clone 560 with FPP clone 1045 hardly influenced the percentage of hybrid seeds negatively and this makes this parental combination ideal for commercial F_1 hybrid seed production.

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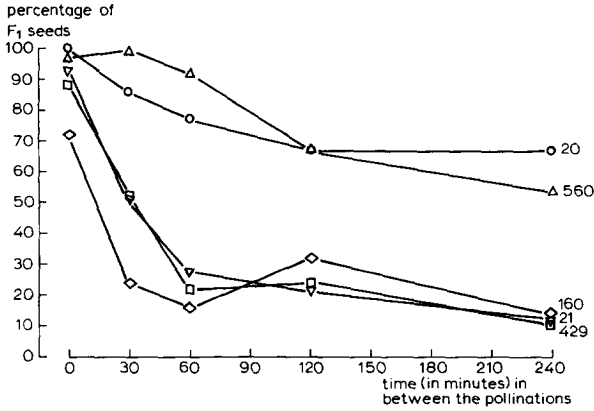


Fig. 6a. The percentage of hybrid seeds after double pollinations of five emasculated SPP clones with self pollen followed by foreign pollen pooled across 4 FPP clones per SPP clone. The percentages are based on 80–160 seeds.

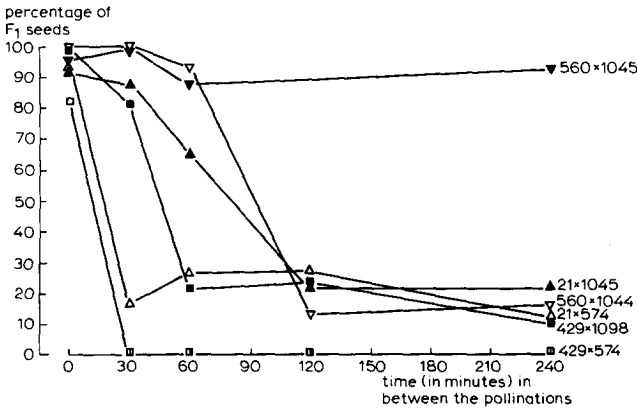


Fig. 6b. The percentage of hybrid seeds after double pollinations of emasculated SPP clones with self pollen followed by foreign pollen for 6 separate parental combinations. The percentages are based on 20–40 seeds.

Delayed pollination of the same clone 560 with FPP clone 1044 resulted in low percentages of hybrid seeds when pollination was delayed for 120 minutes or more. The hybrid seed percentage curves of the combinations 429 × 1098 and 429 × 574 were rather similar. The former combination produced rather more hybrid seeds than the latter: when pollination of SPP clone 429 with FPP 574 was delayed for 30 minutes or more, hybrid seeds were no longer produced. Curves of the combinations 21 × 1045 and 21 × 574 also suggest a difference in tolerance for delayed pollination.

Figures 7a and 7b show the curves of experiment 4 for percentages of hybrid seeds after double pollinations of non-emascuated flower heads. At varying times after anthesis foreign pollen was applied while the self pollen was already present at that time resulting from spontaneous self pollination.

Figure 7a presents the average results per SPP clone, pooled across the FPP clones.

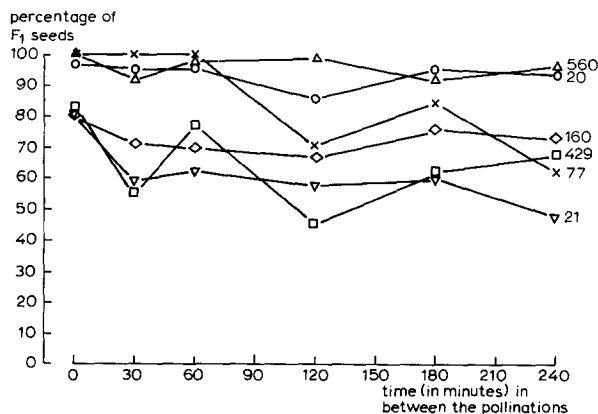


Fig. 7a. The percentage of hybrid seeds after double pollinations of 6 unemasculated SPP clones with self pollen followed by foreign pollen pooled across 4 FPP clones per SPP clone. The percentages are based on 80–160 seeds.

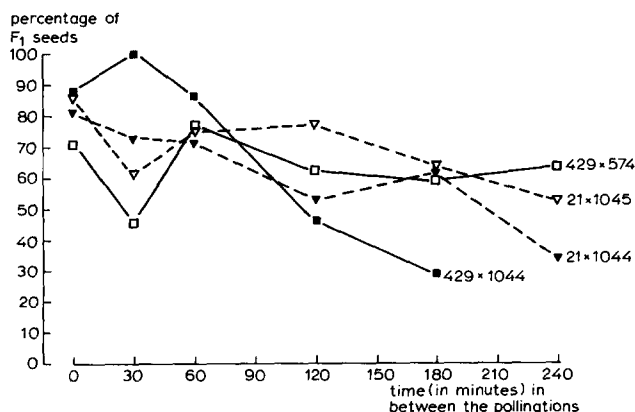


Fig. 7b. The percentage of hybrid seeds after double pollinations of unemasculated SPP clones with self pollen followed by foreign pollen for 4 separate parental combinations. The percentages are based on 20–40 seeds.

Curve paths of the SPP clones for hybrid seed percentage are comparable to those for experiment 3 with emasculated flower heads. Here too clones 20 and 560 are very tolerant to delayed pollination as far as the hybrid seed percentage is concerned. The 4 other clones are more sensitive to delayed pollinations. The percentage of hybrid seeds decreased considerably when the FPP pollination was delayed for 120 minutes or more. It is remarkable, however, that the fall of hybrid seed percentage after delayed FP pollination is far less in this experiment without emasculating, than in the experiment with emasculating.

Fig. 7b shows curves for 4 separate parental combinations of which some appeared most sensitive to delayed pollination. Delayed pollination of SPP clone 429 with clone 1044 gave a much greater decrease of hybrid seed percentage than after pollination with clone 574. Smaller differences were observed between the parental combinations 21 × 1045 and 21 × 1044.

DISCUSSION AND CONCLUSIONS

The present research on pollen germination rate and frequency revealed rather large differences between SPP clones for number of germinated pollen grains per stigma after self pollination. There was correspondence between this *in vivo* pollen germination and the degree of SC as measured in seed set, which agrees with earlier results (EENINK, 1981a).

A rather weak negative correlation was found between *in vivo* pollen germination after selfing and the percentage of hybrid seeds after mixed or double pollinations. No consistent differences between SPP clones were found for germination rate. This suggests that in witloof chicory, with a sporophytic incompatibility system (EENINK, 1981b) and trinucleate pollen grains which usually germinate very rapidly (HOEKSTRA, 1979), gametic competition after mixed or double pollinations will not be caused by germination rate differences as was found for *Phlox* (LEVIN, 1975).

Examination of pooled results per SPP clone and per FPP clone for hybrid seed percentage after mixed or double pollinations revealed large differences between such clones for general hybrid producing ability (GHPA) and general hybrid inducing ability (GHIA). Therefore genotypes can be selected for a high GHPA and for a high GHIA. Selection for a high GHPA is facilitated by the fact that there is a significant negative correlation between the degree of SC of an SPP clone, expressed in seeds per flower head after selfing, and GHPA, expressed in percentage of hybrid seeds after mixed or double pollination of the SP parent by a series of FP parents.

The above relationship suggests that a major cause of gametic competition lies in differences between SPP clones for degree of SC. This was already suggested by DESPREZ & BANNEROT (1980). The degree of SC coincides at least partly with differences for pollen germination frequency after selfing. Earlier results (EENINK, 1981a) already suggested that the barriers which prevent selffertilization were not only located in or on the stigma, but also in the style and/or in the ovary. Both a major gene (the S-allele) and modifier genes were responsible for the barrier(s) (EENINK, 1981b). Results from mixed and double pollinations support this hypothesis, for during the first 30 or 60 minutes following a mixed pollination with self and foreign pollen there were differences in pollen tube growth rate. This was established after removing the stigma and a part of the style at different intervals after pollination. When the cutting was delayed for more than 60 minutes in all parental combinations again a certain basal percentage of hybrids was obtained. Apparently the advantage of either pollen, self or foreign, gained during the first 60 minutes after pollination, was lost. This may be caused by several phenomena such as an acceleration of growth rate of the slower pollen tubes in the basal part of the style, a retardation of growth of the rapid tubes or both, or differences in penetration capacities of the ovary, the ovule or the embryo-sac. Such different selective barriers have also been suggested in *Coronilla* (BALUCH et al., 1973). Maybe the self pollen and/or the foreign pollen send a signal downwards to basal parts of the style or the ovary to block or retard the passage of the first arriving pollen tubes (often from the self pollen) enabling the later arriving pollen tubes (often from the foreign pollen) to overcome the time handicap and to fuse with the egg-cell. Information transmittance, after pollination, through the style to various parts of flowers long before the male nuclei reach the egg apparatus, has been found by FULLER

& LEOPOLD (1975), GILISSEN (1978), DEURENBERG (1976) and SPANJERS (1978). In some combinations the first arriving pollen tubes, pioneer pollen, might also pave the way for the later arriving tubes, partly at its own cost, as has been suggested for apple by VISSER & VERHAEGH (1980).

Results from mixed pollinations and cutting off of stigma and a part of the style are supported by those of the experiments with double pollinations, in which the foreign pollen was applied 0–240 minutes later than the self pollen. For instance both in the experiment with mixed pollinations (Exp. 2b) and in the experiments with double pollinations (Exp. 3, Exp. 4) SPP clones 77 and 429 apparently have a high pollen tube growth rate, especially shortly after pollination, resulting in many self seeds if the stigma and a part of the style were cut off too soon after mixed pollination or if the second, FPP, pollination was delayed too much. In several other parental combinations even a very much delayed second pollination hardly had a negative effect on the percentage of hybrid seeds. In related combinations, however, with just one parent replaced by another, delayed pollination resulted in a dramatic decrease of the hybrid seed percentage. This implies that the mechanism, like the possible selective barrier somewhere down in the pistil, responsible for gametic competition and selection can be significantly altered if one of the two parents of a combination is replaced by another.

In the present study often the basal percentage of hybrids can be predicted on the basis of the level of GHPA and GHIA of the parents. For instance SPP clone 560 has a high GHPA and FPP clone 1045 has a high GHIA which results in a high percentage of hybrid seeds after mixed or double pollination. GHPA of SPP clone 21 and GHIA of FPP clone 574 are leading to a low percentage of hybrid seeds after mixed or double pollination. However, the basal percentage of hybrid seeds in certain combinations, like 160×574 and 429×1044 in experiment 4, suggests that specific combining abilities for the percentage of hybrid seed production (= SHPA and SHIA) occur also.

There is a large difference for percentage of hybrid seeds between experiment 3 and 4, which were carried out at the same time under the same conditions but which differed for emasculation. When SPP flowers were emasculated, this percentage was 30–40% lower than with unemasculated flower heads except when the crosspollination was not delayed. Apparently the abundance of self-pollen in non-emasculated flower heads prior to artificial pollination with foreign pollen had no negative effect on the hybrid seed percentage. Maybe castration disturbed normal metabolic processes, thus influencing the interaction between female and male parts of the flower resulting in a weakening of the incompatibility barrier, as has been found in *Petunia* (LINSKENS, 1964). Such weakening of the incompatibility barrier for self pollen may be responsible for the lower percentage of hybrid seeds compared with that percentage after delayed pollinations of non-emasculated flowers.

The high percentage of hybrids for certain parental combinations in experiment 4 without emasculation and a surplus of self pollen on the stigma opens up good prospects, because this procedure is representative of commercial F_1 -hybrid seed production. Further investigations are underway to unravel the phenomena and selective barriers located in basal parts of the style or in the ovary responsible for high or low percentages of hybrid seeds after mixed or double pollinations.

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