

# HETEROSIS IN RED CLOVER

ROLF MANNER

*Agricultural Research Centre, Department of Plant Breeding, Jokioinen*

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Red clover (*Trifolium pratense* L.) is one of the most important fodder crops in northern Europe. The importance of red clover in Finland is emphasized by PAATELA (10) and VALLE (25). Extensive plant breeding work is carried out with red clover in northern Europe, i.e. in Denmark, Finland, Norway and Sweden (4). The characteristics of the red clover material grown in Finland are investigated by PAATELA (11). The plant breeding methods used in Finland for red clover are inter alia described by POHJAKALLIO (13, 14, 15), POHJAKALLIO, MULTAMÄKI & NUORVALA (16) and PAATELA (12).

Red clover is on the whole a cross-fertilizing plant, but several investigators have also found self-fertility in red clover. The self-fertility varies very much and in many cases it seems to be pseudofertility (2, 5, 6, 17, 19, 20, 23, 26, 27, 28). A more or less pronounced heterosis in red clover was noted in certain characters by WILLIAMS (29), WEXELSEN (26), SALAMOV (18) and LIELMANIS (7).

Bulk crossing of red clover for exploitation heterosis has been tried by SALAMOV (18), LIELMANIS (7) and PAATELA (verbal information).

## *Material and methods*

The present author found it of practical interest for plant breeding and plant husbandry to undertake experiments to explore the practical possibilities of making bulk crosses and mixtures between red clover strains, and of investigating the possibilities of obtaining practically usable strains of crosses by a breeding method for combining ability. The present investigation has been carried out at the Plant Breeding Institute Gullåker, Hammenhög, Sweden, in connection with the practical plant breeding work at the station. »Strain» = marketed variety, local strain or number variety.

The following marketed strains are used in the present investigation as parents: the Swedish strains: Merkur, Monark, Essi, Lindby II, Resistentia, Silo, and Bara and the Danish strains Hinderupsgaard, Hjelm, C tofte, and Daeno III. In addition a number of experimental strains in breeding work were also used in crosses. As space does not allow the describing of all the strains used in crosses, the author wishes to state that the material is representative of a broad Swedish and in some part Danish material in red clover. In addition, the author will endeavour to show that this is an average material of diploid red clover cultivated in the south of Sweden. The number of trials (comparisons) and crosses (combinations) is given in the tables. In the tables the yields are given for different harvest years, «n» = number of harvest seasons. Generally speaking it may be said that the number of crosses is rather large, but that the different crosses have undergone trials for only a short time. The latter is due to the fact that the yield experiments in red clover require much time and that unfortunately the time period has been limited.

The trials were carried out at the experimental farm Gullåker in the years 1951—58. The plots have in all cases been 10 m<sup>2</sup> and the number of replications have in most cases been 3—5. The largest difference in flowering time has been 12 days between strains tried in the same experiment (Essi-Merkur) (8), in all other comparisons the differences have been smaller. The question has risen whether the differences in earliness have influenced the possibilities of comparing the earliest and latest developing strains with each other. The above mentioned strains have, however, been compared in a number experimental series (1, 3, 21, 22). In the governmental experimental programme SJF 210 the above strains are also compared in the same experiments. Such an experimental series has also been carried out at Gullåker in the years 1954—58 (21). These trials have taken place in the same experimental fields as the experiments of the present study. The harvest has in the present study been taken in the same time as in the comparable governmental trials at Gullåker and the same methods have been used. The methods including the harvest time determination have consequently been officially approved. The harvest has been carried out when the first flowers have appeared in Merkur i.e. when the earlier strains have had a number of flowers. Reasons for the comparison of strains of different earliness in the same governmental experiments have been inter alia, that the red clover strains differ greatly, i.e., the earliness of the different plants in the same strain varies (24). Further it has been expected that the errors caused by the difference in earliness are smaller than the errors which would arise if the comparisons were undertaken in different experiments, or if different strains in the same experiment were harvested on different days. Further it is expected that the errors will be small or none if two harvests per season are taken. These arguments have also been accepted in the present investigation.

All possible measures were taken to avoid influence from the border effect. All weights refer to fresh green matter weighed immediately after harvest with an accuracy of 100 grams.

The seeds used for the experiments were received, in the case of the marketed strains, from the breeders. The crosses were produced by growing the strains in alternating rows or alternating plots. The seed was then harvested separately for

the different crosses. The number of replications varied from 3 to about 20 in the seed cultivations, being in most cases 3—5. As the parents had good normal fertility crosses between the strains grown together as well as within the strains occurred. The »hybrid seed» production was consequently not based on male sterile material. Two different types of »hybrid seed» were produced, namely: 1. *Separate bulk crosses* i.e. two or more strains are crossed together, but only the seed for the mother plots is included. For example, if the strains A and B are crossed together the seed harvested is a mixture of about 50 % A × B and 50 % A × A, and if three strains A, B and C are crossed together the harvested seed will be a mixture of about 33.3 % A × B, 33.3 % A × C and 33.3 % A × A. 2. *Reciprocal bulk crosses* i.e. two or more strains are sown together either in different rows or the seed is mixed before sowing the seed cultivation. According to the above mentioned examples the seed harvested will be about 50 % A × B, 25 % A × A and 25 % B × B, if two strains are crossed together. If three strains are crossed together, the seed will be about 22.2 % A × B, 22.2 % A × C, 22.2 % B × C, 11.1 % A × A, 11.1 % B × B and 11.1 % C × C.

Of the two forms of bulk crosses three different types of crosses have been investigated, namely, with 1. two strains involved, 2. three strains involved and 3. more than three strains involved.

The percentage of crosses obtained is discussed in an article on beets (9). In cases where two strains are grown in alternating rows the percentage of crosses will be  $\pm 50$ , if no better fertility is to be found between different strains than within the strain and the earliness of the strains being about the same.

In many cases in the present investigation selection for persistence in the generation for seed cultivation was performed. This selection was a natural selection for persistence, performed in such a way that seed was taken in two — or three — year old seed cultivations established and treated in the same manner as earlier described, or plants were chosen from old plantations.

The mixtures of seed were made in the laboratory in such a manner that equal quantities of germinative seeds were mixed and the seed was sown in seed cultivations; the harvested seed from the seed cultivation was then used for green matter trials.

To obtain the largest possible opportunities for comparison, all values in the calculation were performed as  $\pm$  values in comparison with the check. The present paper does not give the names of the best crosses nor the names of the parents used in them, as these crosses may possibly be of commercial interest to my former employer Otto J. Olson & Sons Ltd.

#### *Comparison between mother strains and progeny*

The first point considered in planning the present investigation was whether the bulk crosses have on an average better or worse yields than the parental strains? The parental strains are the best average strains grown in the area in which the experiments were conducted.

In the present material a total of 36 crosses have been tested. On an average the bulk crosses gave larger green matter yields in all the four harvests investigated. Since the different bulk crosses are not very much tested it has not been possible to draw conclusions as to the performance of individual crosses.

Table 1. Comparisons between mother strains and progeny

	Number of combi- nations	First harvest year			Second harvest year			
		n	First harvest	Second harvest	Total yield	n	First harvest	Second harvest
<b>All bulk crosses</b>								
<i>Mothers</i>		69			44			
Kilograms per hectare			28322	16016	44338	20451	13429	33880
Relative value			100	100	100	100	100	100
<i>Progeny</i>	36	69			44			
Kilograms per hectare			28886	17998	46884	20747	13917	34664
Relative value			102	112	106	101	104	102

Table 2. Crossings in which two strains are crossed together

	Number of combi- nations	First harvest year			Second harvest year			
		n	First harvest	Second harvest	Total yield	n	First harvest	Second harvest
<b>All bulk crosses</b>								
<i>Parents</i>		14			14			
Kilograms per hectare			28947	17428	46375	22291	11622	33913
Relative value			100	100	100	100	100	100
<i>Progeny</i>	8	14			14			
Kilograms per hectare			30231	17677	47908	21995	12127	34122
Relative value			104	101	103	99	104	101
<b>Separate bulk crosses</b>								
<i>Parents</i>		10			10			
Kilograms per hectare			27351	17545	44896	22880	12748	35628
Relative value			100	100	100	100	100	100
<i>Mothers</i>		10			10			
Kilograms per hectare			28403	16069	44472	22005	13418	35423
Relative value			100	100	100	100	100	100
<i>Progeny</i>	4	10			10			
Kilograms per hectare			29939	17675	47614	23155	13622	36777
Relative value (parental mean)			109	101	106	101	107	103
Relative value (mothers)			105	110	107	105	102	104
<b>Reciprocal bulk crosses</b>								
<i>Parents</i>		4			4			
Kilograms per hectare			30439	19636	50075	20816	11743	32559
Relative value			100	100	100	100	100	100
<i>Progeny</i>	4	4			4			
Kilograms per hectare			30961	17683	48644	19096	11185	30281
Relative value			102	90	97	92	95	93

The reason for a larger number of comparisons in the first harvest year (Table 1) lies partly in the fact that a number of second year experiments were not harvested by the time the results were collected in to tables and partly in the fact that a number of second year experiments could not be harvested because the plants were killed by clover rot (*Sclerotinia trifoliorum* Eriks.).

*Dependence on number of strains crossed together*

In the present investigation an effort has been made to cross two, three or more strains together. The mean results are given in Table 2 (two strains crossed together), Table 3 (three strains crossed together) and Table 4 (several strains crossed together). The results show that no big differences are to be found between crosses in which 2 or 3 strains are crossed together if a comparison is made with the

Table 3. Crossings in which three strains are crossed together

	Number of combinations	First harvest year			Second harvest year				
		n	First harvest	Second harvest	Total yield	n	First harvest	Second harvest	Total yield
<i>Parents</i>	12				9				
Kilograms per hectare			29321	17772	47093		24538	13444	37982
Relative value			100	100	100		100	100	100
<i>Mothers</i>	12				9				
Kilograms per hectare			29406	17987	47393		25182	13421	38603
Relative value			100	100	100		100	100	100
<i>Progeny</i>	4	12				9			
Kilograms per hectare			30733	18749	49482		26103	13359	39462
Relative value (parental mean)			105	106	105		106	99	104
Relative value (mothers)			105	104	104		104	100	102

Table 4. Crossings in which many strains are crossed together

	Number of combinations	First harvest year			Second harvest year				
		n	First harvest	Second harvest	Total yield	n	First harvest	Second harvest	Total yield
<i>Parents</i>	8				6				
Kilograms per hectare			27654	15427	43081		19619	13462	33081
Relative value			100	100	100		100	100	100
<i>Mothers</i>	8				6				
Kilograms per hectare			27420	14908	42328		19255	12828	32083
Relative value			100	100	100		100	100	100
<i>Progeny</i>	4	8				6			
Kilograms per hectare			26483	15092	41575		14346	12560	26906
Relative value (parental mean)			96	98	97		73	93	81
Relative value (mothers)			97	101	98		75	98	84

parental mean, but if a comparison is made with the mothers, the bulk crosses, based on three strains, have shown results that are not as good as the bulk crosses based on only two strains. The result of this last mentioned comparison is furthermore in agreement with the results obtained with bulk crosses in which many strains were crossed together (Table 4). This table shows that the yields of the bulk crosses based on many strains have been considerably smaller in comparison with those of the parents than those of bulk crosses, which are based on only a few strains.

The yields in the second harvest year are much smaller — relatively — for the bulk crosses based on many strains than those based on only two or three strains.

#### *Comparisons between separate and reciprocal bulk crosses*

In Table 5 a comparison is given between separate and reciprocal bulk crosses. The number of combinations and trials was much larger for the separate bulk crosses. The separate bulk crosses have on an average given higher green matter yields than the means of the parents. The corresponding comparison for the reciprocal bulk crosses shows the average green matter yields for both harvest years to be smaller than for the parents.

*Table 5. Comparison between separate and reciprocal bulk crosses*

	Number of combinations	First harvest year			Second harvest year				
		n	First harvest	Second harvest	Total yield	n	First harvest	Second harvest	Total yield
<i>Separate bulk crosses</i>									
<i>Parents</i>		62				39			
Kilograms per hectare			28256	16034	44290	20592	13525	34117	
Relative value			100	100	100	100	100	100	
<i>Progeny</i>	36	62				39			
Kilograms per hectare			28969	18252	47221	21473	13985	35458	
Relative value			103	114	107	104	103	104	
<i>Reciprocal bulk crosses</i>									
<i>Parents</i>		7				5			
Kilograms per hectare			28905	15852	44757	19354	12682	32036	
Relative value			100	100	100	100	100	100	
<i>Progeny</i>	3	7				5			
Kilograms per hectare			28151	15746	43897	15090	13385	28475	
Relative value			97	99	98	78	106	89	

The difference between the reciprocal and the separate bulk crosses is that the reciprocal bulk crosses are  $(P_1 + P_2) + (P_1 \rightleftharpoons P_2)$ , while the separate bulk crosses are  $P_1 + (P_1 \times P_2)$ . The present author found at an early stage an indication that the separate bulk crosses would give the best practical results if the best strain — the strain with the most desirable characteristics was used as mother strain. In this connection it must however be pointed out that the separate bulk crosses, not only



in comparison with the parental mean (Table 5) but also in comparison with the mothers, have given higher green matter yields (Table 2).

### *The influence of selection on bulk crosses*

From the results discussed above it appears that the bulk crosses have on an average given larger green matter yields than both the mothers and, on an average, the parents, if compared in every individual case. It is further indicated by the data given previously that the average performance is not as good in the second harvest year as in the first. The present author consequently came at an early stage to be interested in trying a selection for persistence parallel with the production of the

Table 6. Comparisons between parents and progeny at bulk crossing and selection

	Number of combi- nations	First harvest year			Second harvest year				
		n	First harvest	Second harvest	Total yield	n	First harvest	Second harvest	Total yield
<i>Parents</i>		13				10			
Kilograms per hectare			26958	14812	41770	18468	13607	32075	
Relative value			100	100	100	100	100	100	
<i>Mothers</i>		13				10			
Kilograms per hectare			27246	15125	42371	18168	14190	32358	
Relative value			100	100	100	100	100	100	
<i>Progeny</i>	5	13				10			
Kilograms per hectare			28349	18386	46735	22350	14869	37219	
Relative value (parental mean)			105	124	112	121	109	116	
Relative value (mothers)			104	122	110	123	105	115	

seed of bulk crosses. The selection was carried out either in plantations or in sown stands. In the latter case three-year old sown stands were harvested for seed after natural selection. In planted stands selection was performed in the second or third year.

In Table 6 are given the average results of separate bulk crosses performed with selected plants of different strains.

### *Summary*

The possibilities of utilizing heterosis in reciprocal and separate bulk crosses and through selection parallel with bulk crossing in red clover are discussed. The reciprocal bulk crosses have not reached the average green matter performance of the pure strains in the crosses. The performance in the second harvest year was on an average not as good as in the first harvest year. On an average, the separate bulk crosses have given higher green matter yields than both the mothers and the parental

means. The yield performance was better — relatively — in the first harvest year than in the second harvest year in comparison with the mothers and with the parental means. If the bulk crosses are performed on plants selected for persistence the green matter yields have on an average been — relatively — higher than in other bulk crosses. The yields in the second harvest year have been comparatively better than in the first harvest year.

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## SELOSTUS:

## HETEROOSI PUNA-APILASSA

ROLF MANNER

*Maatalouden tutkimuskeskus, Kasvinjalostuslaitos, Jokioinen*

Vuosina 1950—56 on Gulläkerin kasvinjalostuslaitoksella suoritettu puna-apilakantojen välisiä risteytyksiä. Täten saatuja risteytymiä on kokeiltu kantakokeissa mainitun kasvinjalostuslaitoksen koe-kentillä vuosina 1951—58. Näitä risteytymiä on verrattu vanhempiinsa. Yhteensä on kokeiltu 36 eri risteytysyhdistelmää. Risteytymät ovat keskimäärin antaneet suurempia tuorehusatvoja kuin vanhempansa. Parhaimmiksi näyttävät tuorehusatdot muodostuneen silloin, kun risteytymien siemen on otettu valioyksilöistä tai vanhasta nurmesta. Viimeksi mainitussa tapauksessa apilan kestävyyskin näyttää parantuneen huomattavasti. Se on nimittäin tällöin pystynyt antamaan suhteellisesti suu-rempia tuorehusatvoja toisena kuin ensimmäisenä satovuotena.