

THE EFFECTS OF GAMMA-RADIATION ON CROSSES BETWEEN *LOLIUM PERENNE* AND *FESTUCA PRATENSIS*

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1. INTRODUCTION

SEED-INCOMPATIBILITY, which relates to failure of normal seed development in consequence of impaired endosperm development (Valentine, 1953), has been reported in a wide variety of interspecific crosses, especially among the Gramineæ. It is due either to chromosomal or to genic differences between the genomes of the species involved in the cross, and has been shown to be in large measure responsible for the reproductive isolation which exists between the two related grass species *Lolium perenne* ($2n = 14$) and *Festuca pratensis* ($2n = 14$) (Reusch, 1959*b*). Thus, where in crosses *L. perenne* is used as the seed parent, endosperm development is normal until approximately 72 hours after pollination, but thereafter precocious cell formation in the tissue markedly retards further nuclear division. In reciprocal crosses, on the other hand, cell formation does not occur, but mitotic irregularities eventually lead to the complete disintegration of the endosperm. In the latter case none of the seeds is viable, but in the former some of the caryopses may germinate, depending upon the amount of endosperm formed before cell formation.

Although with the evidence available at present, the possibility of cryptic structural differences between the chromosomes of *L. perenne* and *F. pratensis* cannot be ruled out, it appears that evolutionary divergence between these two species has been mainly genic. For example, it has been shown that in F_1 hybrids, pairing of the chromosomes is normal, and further, that the chiasma frequency of the hybrids closely approximates that of the parental species (Peto, 1933). Significant differences in seed-compatibility between various genotypic combinations of the two species have also been demonstrated (Reusch, 1959*a*).

It was therefore considered possible that ionising radiations could induce gene changes which might break down the barrier to hybridisation. The results presented here indicate that, in certain instances at least, ionising radiations did reduce the degree of seed-incompatibility.

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2. MATERIALS AND METHODS

Three genotypes each of *L. perenne* and *F. pratensis*, which differed widely in their interspecific seed-compatibility (Reusch, 1959*a*) were used for the crosses, namely :

<i>L. perenne</i>	<i>F. pratensis</i>
1787bA (1)3—L ₁	41 (35)—F ₁
1194bA (1)3—L ₂	324 (15)—F ₂
Ba4952 (16)—L ₃	325 (8)—F ₃

The system of designation is that employed at the Welsh Plant Breeding Station, Aberystwyth, but for convenience the symbols L₁, L₂ and L₃, and F₁, F₂ and F₃ will be used throughout.

As a source of gamma rays Cobalt⁶⁰ was used, and five different doses, all given over a period of one hour, were applied, namely 250r, 500r, 1000r, 2000r, and 3000r.

The inflorescences were treated about 7 days prior to anthesis, care being taken that they were all at approximately the same stage of development.

All units for any particular cross (generally consisting of approximately 300 florets) were emasculated on the same day, and the pollinations were commenced at strictly comparable times so as to eliminate possible environmental effects on endosperm development (Reusch, 1959*c*).

Two criteria of interspecific compatibility were employed, namely :

- (a) Seed set : all ovaries showing definite signs of development were recorded as having set seed.
- (b) Seed viability : all caryopses showing any signs of germination, however weak, were recorded as viable. Since the ability of seeds to germinate is dependent on the presence of at least a small amount of endosperm, the germination data provide an estimate of the seed-compatibility of the various genotypic combinations.

It should be noted that the seed-setting results are based on the total number of florets emasculated, whereas the germination data are expressed as a percentage of the total number of caryopses formed. To a certain degree the two estimates are therefore independent, since it is for example possible to obtain a high germination percentage even when seed-set is low.

3. OBSERVATIONS

(a) *The effect of pollen irradiation on compatibility*

(i) *Seed-set.* Results on the effect of pollen irradiation on seed-set are given in table 1. An analysis of the original data showed that except for the crosses F₃ × L₁ and F₃ × L₃, overall differences between treatments were in all cases significant. However, only in the case of three combinations, namely L₁ × F₂, F₁ × L₂ and F₃ × L₂, did seed-set improve as a result of irradiation.

A general feature of the effect of irradiation was a reduction in seed-set at doses of 250-500r. This was followed by an increase at 1000-2000r, but further increases in dose again resulted in a decline.

(ii) *Seed-viability.* The effects of pollen irradiation on seed-viability, although similar to those observed for seed-set, showed greater variability (table 1). If, however, one takes into account the direction of the cross and the normal seed-compatibility of the genotypes, the results still seem to fit into a general pattern.

In the *L. perenne* × *F. pratensis* matings (fig. 1), both the decrease in seed-viability at 250-500r and the recovery at 1000-2000r occurred

TABLE 1

The effect of pollen irradiation on seed-set and seed-viability in crosses between different genotypes of *L. perenne* and *F. pratensis*

Genotype combination	Percentage seed-set and germination at different doses of γ -rays						χ^2 (6)
	Control	250r	500r	1000r	2000r	3000r	
<i>L. perenne</i> (♀) × <i>F. pratensis</i> (♂)							
L1 × F1 Seed-set	77.6	62.8	66.1	59.0	49.0	47.7	65.4 †
Germination	0.0	0.0	0.0	0.0	0.0	0.0	...
L1 × F2 Seed-set	61.8	79.2	81.3	76.2	83.2	83.7	45.7 †
Germination	0.0	0.0	0.0	0.0	0.0	0.0	...
L1 × F3 Seed-set	74.9	73.1	66.7	79.3	80.9	67.2	22.4 †
Germination	0.0	0.0	0.0	0.0	0.0	0.0	...
L2 × F1 Seed-set	81.5	70.5	65.2	81.1	86.3	73.1	46.1 †
Germination	10.8	7.6	8.0	11.9	8.8	6.5	5.1
L2 × F3 Seed-set	85.0	80.3	70.8	84.8	82.9	73.2	26.5 †
Germination	17.6	24.1	6.8	15.1	12.0	10.9	26.7 †
L3 × F1 Seed-set	76.7	73.7	66.0	83.6	78.6	60.8	44.9 †
Germination	46.3	25.6	6.9	17.3	31.6	20.0	90.7 †
L3 × F2 Seed-set	84.4	72.5	58.6	81.1	64.5	49.6	94.4 †
Germination	44.9	40.6	37.9	54.7	38.7	38.1	12.3 *
L3 × F3 Seed-set	76.8	76.3	74.7	76.8	67.4	66.0	15.1 †
Germination	54.1	46.7	42.1	62.0	64.3	62.7	31.3 †
<i>F. pratensis</i> (♀) × <i>L. perenne</i> (♂)							
F1 × L1 Seed-set	71.7	45.6	50.0	61.3	39.8	25.0	174.6 †
Germination	0.9	0.0	2.1	1.8	0.0	14.3	58.0 †
F1 × L2 Seed-set	54.8	73.2	56.6	30.7	43.4	48.0	109.5 †
Germination	0.0	13.4	7.1	4.1	6.4	0.0	35.2 †
F2 × L3 Seed-set	61.2	51.6	50.5	51.5	57.6	46.9	14.0 *
Germination	0.0	0.0	0.0	0.0	0.0	0.0	...
F3 × L1 Seed-set	53.5	52.3	48.7	58.3	54.8	51.4	5.6
Germination	0.0	0.0	0.0	0.0	0.0	0.0	...
F3 × L2 Seed-set	43.8	55.6	62.8	61.0	22.3	13.3	220.0 †
Germination	0.0	0.0	6.1	1.3	0.0	0.0	35.6 †
F3 × L3 Seed-set	60.3	57.0	50.4	56.2	52.6	50.0	8.8
Germination	0.0	0.0	0.0	0.0	0.0	0.0	...

* P = 0.05-0.01.

† P = ≤ 0.01.

to varying degrees in different crosses: in the normally incompatible combinations, namely L1 × F1, L1 × F2 and L1 × F3, and in the cross

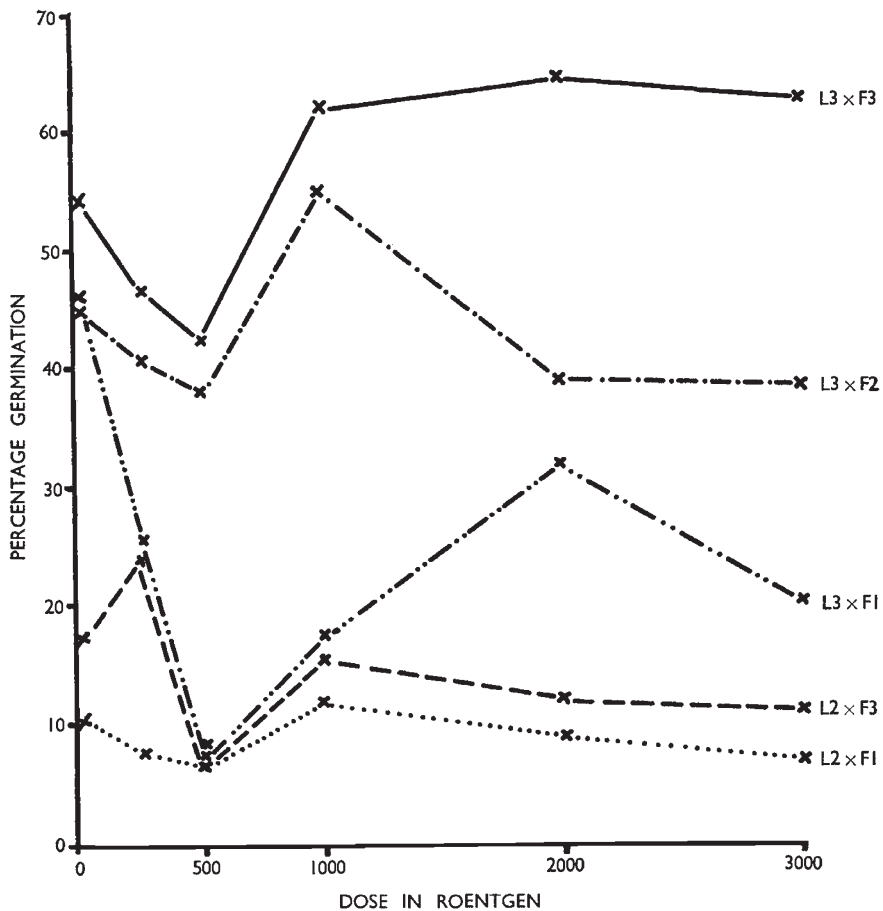


FIG. 1—The effect of pollen irradiation on seed-viability in crosses of *L. perenne* × *F. pratensis*.

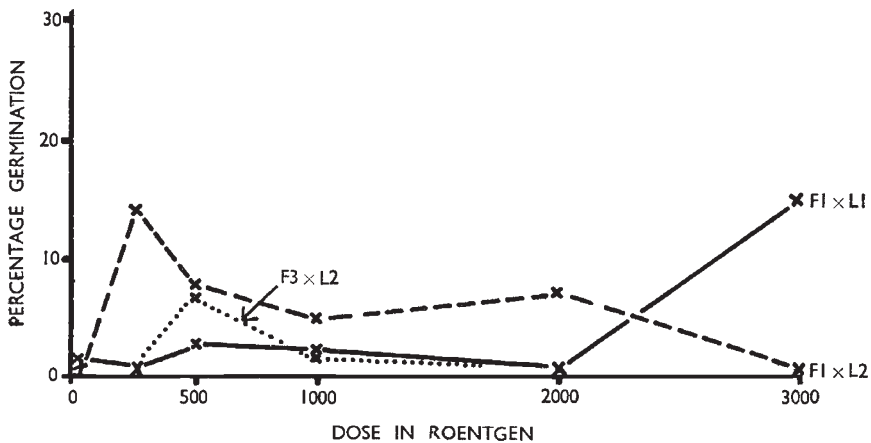


FIG. 2—The effect of pollen irradiation on seed-viability in crosses of *F. pratensis* × *L. perenne*.

of low compatibility ($L_2 \times F_1$), irradiation had no significant effect; in the cross of medium compatibility ($L_2 \times F_3$) and in one of the crosses of high compatibility ($L_3 \times F_1$), a marked reduction in viability occurred at 500r, but the germination percentage at the higher doses did not exceed that observed in controls; in the remaining two crosses of high compatibility ($L_3 \times F_2$ and $L_3 \times F_3$) only a small reduction occurred at 500r. Moreover, in the latter combinations seed-viability at 1000r in the one case, and at doses higher than 1000r in the other, markedly exceeded that of the controls. Genotypic differences in the response to irradiation are therefore evident.

TABLE 2

The effect of ovule irradiation on seed-set and seed-viability in crosses between different genotypes of L. perenne and F. pratensis

Genotype combination	Percentage seed-set and germination at different doses of γ -rays						$\chi^2_{(5)}$
	Control	250r	500r	1000r	2000r	3000r	
<i>L. perenne</i> (♀) \times <i>F. pratensis</i> (♂)							
$L_2 \times F_1$ Seed-set .	78.4	73.6	70.3	68.1	49.7	44.3	54.0 †
Germination .	4.0	2.5	3.8	7.5	2.1	1.0	11.7 *
$L_3 \times F_3$ Seed-set .	78.5	66.4	65.5	51.0	37.8	34.2	141.3 †
Germination .	59.4	48.0	51.7	27.1	18.1	16.0	97.0 †
<i>F. pratensis</i> (♀) \times <i>L. perenne</i> (♂)							
$F_1 \times L_2$ Seed-set .	57.0	55.9	53.3	46.0	47.0	42.5	24.7 †
Germination .	1.3	6.3	7.0	3.2	1.0	4.7	16.1 †
$F_3 \times L_3$ Seed-set .	62.5	46.5	42.7	40.5	33.9	27.3	77.2 †
Germination .	0.0	0.0	0.0	0.0	0.0	0.0	...

* $P = 0.05-0.01$.† $P = 0.01$.

In the crosses with *F. pratensis* as seed parent (fig. 2), two factors partially conceal the effects of irradiation. Firstly, crosses showing a high degree of seed-compatibility where *L. perenne* is used as seed parent are not the most successful combinations in reciprocal matings. Secondly, the reduction in seed-compatibility at 250-500r, if it does occur, cannot be observed, since no viable seeds are produced when untreated pollen is used. Nevertheless, there is still some evidence for genotypic differences in the response to irradiation, in that the greater the genetic difference between genotypes, the greater was the dose required for optimum viability. For example, L_1 and F_1 are, on the basis of *L. perenne* \times *F. pratensis* crosses, more incompatible, and hence probably more divergent genetically, than L_2 and F_1 . In the former, the greatest viability was obtained at 3000r, in the latter at 250-500r.

(b) *The effect of ovule irradiation on compatibility*

Table 2 and fig. 3 show results on the effect of ovule irradiation for two series of reciprocal crosses. In both *L. perenne* × *F. pratensis* and *F. pratensis* × *L. perenne* crosses, seed-set decreased with dose. A similar overall response was observed for seed-viability in the *L. perenne* × *F. pratensis* combination $L_3 \times F_3$, the slight increase at 500r being non-significant. In $L_2 \times F_1$, on the other hand, viability at

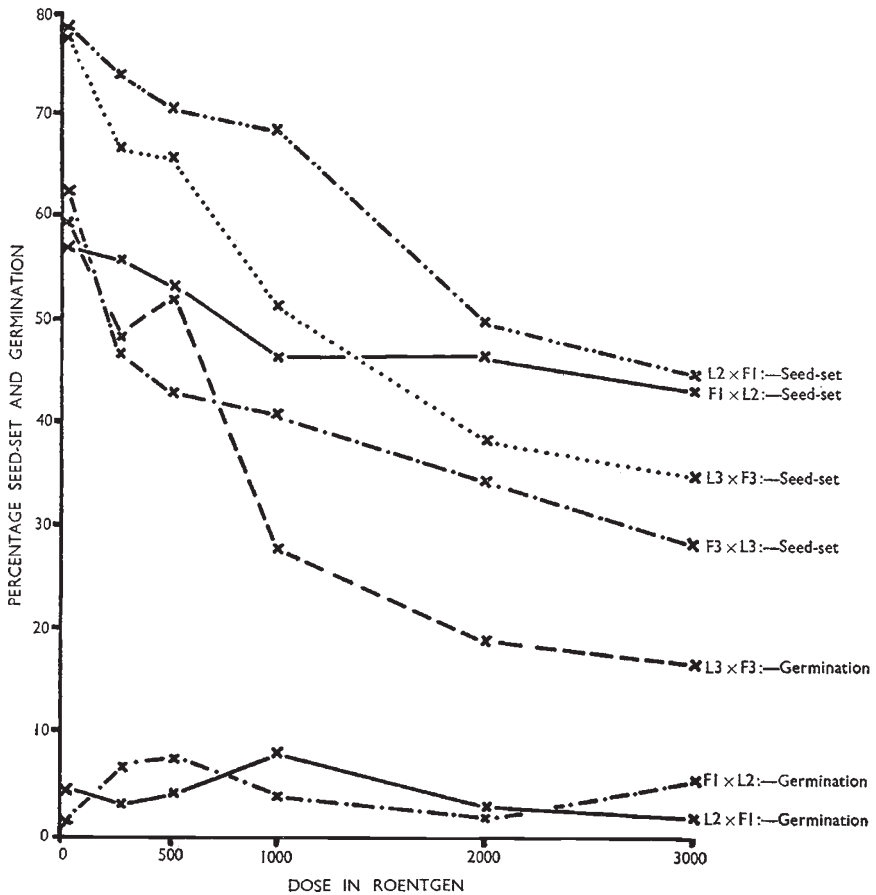


FIG. 3—The effect of ovule irradiation on seed-set and seed-viability in crosses between *L. perenne* and *F. pratensis*.

1000r was significantly higher than at 250r, but not significantly higher than in the control. It should be noted that normal viability in the latter cross was low compared with the former.

In one of the *F. pratensis* × *L. perenne* crosses analysed, namely $F_3 \times L_3$, ovule irradiation had no effect. In the other ($F_1 \times L_2$) a significant increase in viability occurred up to 500r, after which there was a decrease. The slight increase at 3000r was not significant.

(c) *Endosperm development following irradiation*

In order to determine the nature of the effect of irradiation on seed-compatibility, a single cross, namely $L_3 \times F_3$, was examined embryologically following the use of pollen treated with 3000r. In this combination, as shown above, seed-viability at the higher doses of irradiation was greater than that where untreated pollen was used. Table 3 gives the mean number of endosperm nuclei at fixed intervals after pollination. Results following the use of untreated F_3 pollen and untreated intraspecific pollen are also given.

Up to 48 hours after pollination the rate of endosperm development in the two crosses did not differ from that in the control. By 72 hours, however, a stage at which precocious cell formation takes place in the endosperm of hybrid seeds (Reusch, 1959*b*) the number of endosperm

TABLE 3

Mean number of endosperm nuclei in the L. perenne genotype L_3 , and in the cross $L_3 \times F_3$ following pollination with both treated and untreated pollen

Control and crosses	Mean number of endosperm nuclei at different times after pollination					
	12 hours	24 hours	48 hours	72 hours	96 hours	120 hours
L_3 —intraspecific control	2.0	7.5	99.0	640.0	1414.0	2545.0
$L_3 \times F_3$ —untreated pollen	2.0	8.5	101.0	454.0	809.0	1527.0
$L_3 \times F_3$ —pollen treated with 3000r	2.0	8.3	105.0	453.0	890.0	1775.0

nuclei in the crosses was lower than that in the control. It is significant that at 96 hours and later, this retardation in endosperm growth rate was less marked when using irradiated pollen.

4. DISCUSSION

The purpose of this investigation was primarily to determine the effect of irradiation on seed-compatibility, as measured by viability, but the results have shown clearly that seed-set is also markedly influenced and in a broadly similar manner. On the data available it is difficult to find any single satisfactory explanation for the observed responses. However, the almost regular reduction in seed-viability at the lower doses, followed by an increase, in those crosses where viable caryopses are produced, seem to indicate two separate effects of irradiation. The first effect causes a deterioration of seed-compatibility, while the second brings about an improvement. What is the nature of these effects? Since in crosses between *L. perenne* and *F. pratensis*, seed-incompatibility results from an impaired development

of the endosperm (Reusch, 1959*b*), it is evident that they must be concerned with the development of the endosperm and the following are suggested: (a) Up to 5000 chromosomally damaged pollen grains are not affected in their functional ability so that they are able to participate in fertilisation and triple fusion. However, as a result of chromosomal abnormalities, degeneration of the embryo and/or endosperm of a proportion of the caryopses sets in soon after fertilisation in a manner similar to that reported in *Lilium formosum* (Cave and Brown, 1954). In consequence, many of the seeds will not be viable. This degeneration presumably accounts for the reduction in viability in crosses of *L. perenne* by *F. pratensis* following pollen treatment with doses of 250-5000, and is therefore not genic. With doses higher than 5000, the functional ability of the pollen grains is presumably affected, so that selective elimination of the more severely damaged ones will occur. In consequence there will be a tendency towards a levelling-off of the deleterious effect. In the case of ovules, selective elimination cannot occur, so that their functional ability (measured in terms of the number of ovules which set seed) should decrease with an increase of dose. This did occur. (b) The recovery in seed-compatibility at doses higher than 5000 is due to a second influence of irradiation, which allows those caryopses, in which chromosomal damage does not lead to a cessation of growth, to develop more endosperm than seeds set following the use of untreated pollen. Since this beneficial effect of irradiation markedly varies with different genotypic combinations, it would appear that gene changes are involved, so that superimposed upon the initial non-genic deleterious effect there is a beneficial genic effect, the nature of which could be explained as follows:

If one accepts that two genotypes, which under normal conditions of interspecific cross-pollination give rise to only a few viable seeds, are genetically more diverse than two which yield a large proportion of germinable caryopses, then, on the basis of a threshold of gene changes for improvement of endosperm development, one would expect that a larger number of gene changes and hence larger dosages of irradiation would be required to bring about an improvement in seed-compatibility in the former than in the latter. That this is the case is clear from the *L. perenne* × *F. pratensis* crosses (see earlier). On this basis it is also to be expected that in crosses where initial differences are too great, irradiation-induced gene changes will be ineffective in reducing the genetic disparity between the genomes in the endosperm. This would then be the case in those combinations where irradiation had no effect on seed-compatibility.

Since the crosses of *F. pratensis* × *L. perenne* all produced no viable seeds under normal conditions, it is evident that the deleterious effects of irradiation discussed above cannot be detected. The beneficial effect, on the other hand, was clearly observable, in that a number of the combinations yielded viable seeds following irradiation of both pollen and ovules.

Following on the above, one further point needs consideration, namely the manner in which endosperm development is influenced. From the *L. perenne* × *F. pratensis* cross analysed, it does appear that the degree of suppression of nuclear division, following precocious cell formation, is mainly affected. In consequence a larger amount of endosperm tissue is formed following the use of treated pollen, than in cases where untreated pollen is used. Similar observations have been made on low × high chromosome species crosses of *Avena* and *Triticum*, where better-filled caryopses were obtained with X-irradiated pollen than with untreated pollen (Nishiyama and Iizuka, 1952).

For crosses of *F. pratensis* × *L. perenne* one must assume that the intensity of abnormal divisions within the endosperm are reduced.

5. SUMMARY AND CONCLUSIONS

1. Results are presented on the effect of gamma irradiation on seed-set and seed-viability in reciprocal crosses between *L. perenne* and *F. pratensis*.

2. Following irradiation of the pollen, a reduction occurred in hybrid seed set at doses of 250-500r. This was followed by an increase at 1000-2000r, but further increases in dose generally again resulted in a decline. Irradiation of ovules led to a progressive decrease in seed set with increase of dose.

3. In crosses of *L. perenne* × *F. pratensis*, pollen irradiation, in general, did not raise the germination capacity of the caryopses above that for crosses in which untreated pollen was used. All combinations which gave rise to viable seeds under normal conditions, showed some decrease after irradiation at 500r, followed by an increase at the higher doses. Both the degree of reduction at 500r and the increase at higher doses varied with the genotypes involved in the cross.

4. Where *F. pratensis* was the seed parent, no viable seeds were obtained after pollination with untreated pollen. In three combinations out of six, however, viable seeds were produced as a result of pollen irradiation. Here, genotypic differences in the response to irradiation were also apparent.

5. It is suggested that the initial reduction in seed-viability is a consequence of endosperm degeneration in a proportion of ovules due to fertilisation by chromosomally damaged pollen grains. The increase at the higher doses is considered to be due to gene changes which reduce genetic unbalance within the endosperm, thus allowing for an improved development of the tissue.

6. It may be concluded that through the agency of ionising radiations, it is possible, in certain instances at least, to reduce the degree of seed-incompatibility. The improvement in viability may, however, be mainly limited to crosses made in one direction. Thus in *L. perenne* × *F. pratensis* crosses, the germination capacity of the caryopses was generally not improved, and in incompatible combinations irradiation

had no effect. In reciprocal matings, on the other hand, viable seeds were obtained from combinations which after normal pollinations gave no germinable caryopses.

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