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INTERSPECIFIC AND INTERGENERIC HYBRIDS IN HERBAGE GRASSES

IV. *LOLIUM RIGIDUM ET AL.*

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INTRODUCTION

The first lot of seed described as 'Wimmera ryegrass' was received at the Welsh Plant Breeding Station, Aberystwyth, in 1922. It had been grown on the farm of a Mr McDougall at Minyip, Victoria, Australia.

Single plants grown from this seed were studied alongside other *Lolium* plants of various descriptions but mainly *L. perenne*. They proved interesting, particularly in that while they resembled *L. perenne* in certain features, they behaved as strict annuals when sown in the spring. On the other hand, they showed no promise for agricultural purposes in Britain because at their best they were lacking in leaf and in bulk of produce and soon became very excessively stemmy.

From time to time other lots of seed also described as 'Wimmera ryegrass' were again received from Australia, but most of these showed decidedly greater variation in plant type than the original lot, a smaller or greater proportion of them being usually non-annual showing various degrees of capacity to perpetuate themselves vegetatively after producing seed.

In 1932, Dr William Davies (now Director of the Grassland Research Station) collected a seed sample from a ripe seed crop on the farm from which the original seed was derived. Plants grown from this seed (Station Number Ba 2274) exhibited the same features as those from the first seed sample, and some of them were used in the breeding work here described.

Within the type there was some degree of morphological variation, but they all agreed in being strict annuals and in being normally wind-pollinated. In general appearance, the stems and inflorescences rather closely resembled those of *L. perenne*, but not infrequently the inferior palea was either pointed or even slightly awned, while in some plants the stem below the inflorescence was distinctly scabrid. More or less awned types have been reported in *L. perenne*, and in what otherwise appears to be true *L. perenne* a plant has very rarely been found in which the stem is scabrid.

The taxonomic position of this 'Wimmera ryegrass' was an interesting problem because there appeared to be some confusion in the literature. This has been discussed at some length on a previous occasion (Jenkin in Jenkin & Thomas, 1939), and the conclusion was reached that the type is classifiable as *L. rigidum* Gaud.

In the meantime, attempts had been made to assemble a comprehensive range of *Lolium* types at the Welsh Plant Breeding Station. Plants grown from some of the seed samples received differed only in very minor characters from the 'Wimmera ryegrass' plants and were, therefore, readily classifiable as *L. rigidum*, even though they might be described otherwise by those from whom the seed was obtained.

Plants from other lots of seed, although agreeing with the type regarded as *L. rigidum* in being strictly annual and wind-pollinated, failed in other respects to fall easily into this group. They did not altogether agree with one another, although most of them might have been acceptable as *L. multiflorum* Lam. The difficulty of referring them to this species arose from two causes: (1) the ordinary Italian ryegrass, particularly in recent years, has also been referred to as *L. multiflorum* Lam., but Italian ryegrass, as commonly recognized, is a non-annual type; (2) all the types described as '*L. multiflorum*' (with or without authority or qualification) did not in every respect fall easily into *L. multiflorum*, even if this name is applicable only to annual types.

The chief interest of these lots to the present writer was, however, not their exact taxonomic status mainly based upon morphology, but their capacity to produce fertile progeny from intercrossing with one another if or when the opportunity occurred in nature. To study this problem, it would clearly not be enough to collect seed from open pollination, nor even to obtain seed by enclosing the inflorescence of two plants (without prior emasculation) for mutual pollination, because in the latter case it would be quite impossible, supposing either parent to be in any degree self-fertile, to know with certainty that any particular plant would be the result of intercrossing. In all cases, therefore, the inflorescences to be used as a female unit were all emasculated by hand and subsequently pollinated either by hand (in the majority of cases) or by enclosing inflorescences of the pollen parent with them in a pollen-proof container.

All seed samples received at the station are given a 'Station Number' such as the Ba 2274 referred to above for the 'Wimmera ryegrass' sample, and there are certain rules of procedure in applying numbers and symbols so that an exact tally may be kept of all breeding work, but the details of this system are of no special interest in the present connexion.

The somewhat arbitrary subdivision of the present group of annual, normally wind-pollinated types into those referable to *L. rigidum* and those which were not, requires a temporary subgroup reference. The former are then referred to as '*L. rigidum*' and the latter '*L. rigidum* sens. ampl.', and as this latter group consists of lots which do not entirely agree with one another, the interbreeding compatibilities of plants in this sub-division will first be discussed. This will be followed by a discussion of the interbreeding compatibilities of the *L. rigidum* lots, and, finally, that of plants of the two subdivisions when intercrossed.

I. '*Lolium rigidum* sens. ampl.'

The lots that have been referred to this group and which have been used in breeding work are the following:

Ba 2635. This 'Station Number' refers to seed collected from a single plant growing in the Station's gardens in 1934. This plant was one of a group grown from a seed sample (Bs 1190) received as '*L. rigidum*' but whose ultimate origin is unknown. These Bs 1190 plants were not themselves closely studied because they were not intended primarily for breeding purposes.

The particular plant from which seed was taken was associated in position with other plants of Bs 1190, but it was accessible also to pollen from other *Lolium* plants, and as the inflorescences had not been protected the seed harvested may have been the result of pollination by plants other than those of Bs 1190. This seems to have been the case because the plants grown from this seed, now designated Bs 2635, showed considerable variation, and although a number of them were studied, only one of them is of present interest.

This plant, Ba 2635 no. 14 (Ba 2635 (14)) was self-pollinated and produced seed, 2635 (14) Ba (1), and some plants raised from this seed were used for further breeding—2635 (14) Ba (1) 1, 2 and 3 (Herbarium sheets 140–143).

Plant Ba 2635 (14) behaved as a strict annual, and it may have been the result of either self-fertilization or intercrossing of the Bs 1190 plant with another annual—possibly another plant of the same group. Despite the fact that the Bs 1190 seed sample was described as '*L. rigidum*' the plants grown from it were certainly different from the 'Wimmera ryegrass' type now referred to as *L. rigidum* Gaud., and so also was plant Bs 2635 (14) which would be much more easily classifiable as *L. multiflorum* Lam.

Ba 2967. Seed sample described as '*L. multiflorum* Lam.' from the Jardin Botanico de Madrid. The plants grown from this seed would probably be classifiable as '*L. multiflorum* Lam.', but they were fewer tillered and coarser stemmed than those derived from Ba 2635 (14). The spikelets often consisted of more than ten florets and the awns were usually prominent. The glume was short, so that the inflorescences would be indistinguishable from those of *L. italicum*, but the plants, apart from greater stemminess, differed from the latter in being strict annuals (Herbarium sheets 214–218).

Ba 2993. Seed sample described only as '*L. multiflorum*' received from the Station Centrale de Recherches Agronomiques, Rabat, Morocco. The plants grown from this seed were less tall and less coarse-stemmed than those of Ba 2967, and were, therefore, more similar to those derived from Ba 2635 (14). The flowering stems were rather shorter and the inflorescences more dense than in the latter, and more similar to those of Ba 3121–Ba 3131 (below), except that they were more prominently awned. The glumes were longer than is usual in *L. italicum*, and after flowering they closed up rather tightly giving the inflorescence a constricted appearance (Herbarium sheets 178, 179, 224, 225).

Ba 3013. Seed sample described as '*L. rigidum*' received from the Hortus Botanicus de L'Université de Coimbra, Portugal. In plants of this lot, the flowering stems were very slender and very numerous; glumes sometimes *longer* than the spikelet; spikelets awnless or nearly so. These plants could not easily be referred to either *L. rigidum* Gaud. or to *L. multiflorum* Lam. They were little used in breeding work.

Ba 3116. Seed sample described as '*L. multiflorum*' received from the Instituto Fitotécnico y Semillero Nacional, Estanzuela. In general appearance, the plants were rather similar to *L. italicum*, but they were strict annuals with shorter and more slender flowering stems. The length of the awn varied considerably, the spikelet was spreading (rather than compressed) at maturity and the individual florets closely imbricated; glumes short (Herbarium sheets 180–182, 229).

Ba 3121. Broken inflorescences, including germinable seeds received from the Agricultural Institute, Algiers, and described as '*L. multiflorum* Lamk.'

Ba 3122–*Ba* 3131. Separate individual bags of entire and broken inflorescences (including germinable seed) also received from the Agricultural Institute, Algiers. Apparently, each packet represented a single plant, and for the breeding work, seeds only from a single inflorescence from each bag were used.

It may probably be assumed that each bag represented a different plant, and, while they agreed to a general type, the inflorescences as received differed considerably in density and in number of spikelets. One of these (preserved as a herbarium specimen, sheet 187), representing Ba 3128, consists of forty-nine spikelets.

All the Ba 3122–Ba 3131 lots were described as '*L. multiflorum* L. var.', but when plants were grown from them they did not differ very appreciably in general type from those of Ba 3121 nor from one another. They did differ somewhat in awn development, but none was prominently awned. They also differed somewhat in density of the inflorescences, but they agreed generally in that the stems were relatively short and stout, and in the type of inflorescence produced.

It is not uncommon to find in *L. italicum* that at flowering time a section of the inflorescence is rather widely open and spread, and it may sometimes be seen in *L. rigidum*, but in the present lots this feature was greatly exaggerated. The glumes, which were relatively very long, made a very wide angle with the rachis, and at the same time the spikelets themselves loosened up over practically the entire length of the inflorescence so that the whole group came to be dubbed the 'herring-bone' type. The nearest approach to these lots was Ba 2993.

After flowering, the spikelets closed up, and as the glumes were usually long and the florets not very closely imbricated in the spikelets, the inflorescences became very constricted over their entire length but yet not to the extreme found in *L. loliaceum*, where the rachis is stouter and more deeply concaved for the protection of the spikelets (Herbarium sheets 180–203).

(*a*) *Intragroup crossing*

Except in the case of the three plants derived from Ba 2635 (14) by self-pollination, it is not known that the plants grown from these lots were related except also that plants grown from lots Ba 3122–Ba 3131 would within each 'Station Number' have a common mother plant.

Group Ba 2635 (14). The plants derived from Ba 2635 (14) by selfing undoubtedly suffered quite severely from inbreeding. They were, therefore, by no means ideal breeding material, and when plants 2 and 3 were intercrossed they failed to give any progeny plants when no. 3 was used as the pistillate parent, and in the reciprocal cross only in 29 % of the florets were caryopses developed.* These seeds gave a germination of 67 %, and all the seedlings survived, constituting family 636 bE (1).

One attempt to intercross two plants of family 636 bE (1) failed to give surviving seedlings. These plants were now very weak, but three of them were successfully used in outcrosses.

Group Ba 2967. Eight crosses (involving 2352 emasculated florets) were made between plants of Ba 2967 and their direct derivatives, and a maximum of 89 % seed-setting was reached. Some of the plants proved to be heterozygous for a deformed type of seedling and/or a chlorophyll-deficient type, but normal green seedlings developed into mature plants. Germination: up to 97 %.

* It should be borne in mind that in hand-crossing the grasses, a high seed-setting requires a combination of favourable factors so that low seed-setting is not necessarily an indication of cross-incompatibility. The flowering of an emasculated unit takes several days to be completed, and the pollen available may not be sufficient to provide a full pollination.

Group Ba 2993. Only two crosses were made within this group. Seed-setting, up to 84 %; germination, up to 97 %; seedlings normal.

Group Ba 3013. The inflorescences of two sister plants derived from a plant of Ba 3013 by self-pollination were enclosed together for mutual intercrossing, but seed-setting was low at 38 and 6 % respectively. Germination reached 100 %, but some of the seedlings were chlorophyll-deficient.

Group Ba 3116. Two sister plants derived from plant Ba 3116 no. 2 by self-pollination were intercrossed. Seed setting, 79 %; germination, 93 %; seedlings normal.

When one of these two plants was intercrossed with a plant derived from Ba 3116 no. 1 by self-pollination, the latter plant, 3116 (1) Ba (1) 1, being used as the pistillate parent, no caryopses were produced. This might suggest the presence of sterility factors within the line, but it may be significant that plant 3116 (1) Ba (1) 1 also failed to set seed when outcrossed to a *L. rigidum* plant. It may, therefore, be highly female sterile.

Group Ba 3121–*Ba* 3131. Although not originating from a single seed sample the plants derived from these seed lots have shown a close resemblance both in morphology and in behaviour.

In the eleven crosses made (involving 5654 emasculated florets), the average seed-setting was 58 % with a range from 14 to 83 %. Seed germination ranged from 63 to 95 %, and the seedlings developed and matured normally.

Brief discussion

These results show that although cases may be met with where fertilization fails even within a group of plants of similar origin, possibly owing to genetic factors, and other low seed-setting results may be obtained either for a similar reason or because of unfavourable conditions, seed-setting and seed germination have often reached high figures. Evidently, however, neither low seed-setting nor a relatively low seed germination can alone be regarded as indicative of anything approaching species differentiation, particularly where the seedlings, as in these cases, develop and mature normally.

(b) *Intergroup crossing*

It is a convenience to refer to the groups indicated above by means of letter symbols, because the use of the Station Numbers of the plants used in intergroup crossing would be cumbersome. For present purposes, the following symbol letters are used:

A = Group Ba 2635 (14)	E = Group Ba 3116
B = Group Ba 2967	F = Group Ba 3121–Ba 3131
C = Group Ba 2993	

Plants of group Ba 3013 were not used in intergroup crossing.

In all cases, seedling development and survival was quite normal, but seed-setting was low in some cases and germination was also sometimes relatively poor.*

In the cross resulting in family 702 bE (1) both seed-setting and germination were low, but the female parent in this case was highly inbred and flowered very poorly. The caryopses were poorly developed and the seedlings weak, so that in this particular family only about 50 % of the seedlings survived.

On the other hand, within the same group of crosses, two F_1 sister plants, when intercrossed, gave a seed-setting of 94 %, a seed germination of 91 %, and seedling survival

* The results are tabulated overleaf.

of about 100%, so that evidently there is no necessary incompatibility between plants of the two groups.

One relatively poor result was also obtained when plants representing Ba 2967 and Ba 2993 were intercrossed, but the seedlings produced were strong. In one case where two sister plants derived from a cross between representatives of these two groups were intercrossed, the high figures of 93% seed-setting and 98% seed germination was obtained.

Derivative family	Cross ♀		Cross ♂	Percentage seed-setting	Percentage germination
638 bE (1)	*B*	×	*A*	52	90
702 bE (1)	(*A* × *A*)	×	(*B* × *A*)	7	35
711 bE (1)	(*A* × *A*)	×	(*B* × *A*)	38	63
705 bE (1)	(*B* × *B*)	×	(*B* × *A*)	55	88
718 bE (1)	(*B* × *A*)	×	(*B* × *A*)	94	91
912 bE (1)	[(*B* × *A*) × (*B* × *A*)]	×	[(*B* × *A*) × (*B* × *A*)]	62	71
913 bE (1)	[(*B* × *A*) × (*B* × *A*)]	×	[(*B* × *A*) × (*B* × *A*)]	27	37
888 bE (1)	[(*A* × *A*) × (*B* × *A*)]	×	[(*B* × *A*) × (*B* × *A*)]	56	92
728 bE (1)	*B*	×	*C*	26	67
941 bE (1)	(*B* × *C*)	×	(*B* × *C*)	72	99
938 bE (1)	(*B* × *C*)	×	(*B* × *C*)	93	98
939 bE (1)	(*B* × *C*)	×	(*B* × *C*)	86	95
940 bE (1)	(*B* × *C*)	×	(*B* × *C*)	76	87
994 bE (1)	[(*B* × *C*) × (*B* × *B*)]	×	[(*B* × *B*) × (*B* × *B*)]	84	*
995 bE (1)	[(*B* × *B*) × (*B* × *B*)]	×	[(*B* × *C*) × (*B* × *B*)]	75	*
996 bE (1)	[(*B* × *B*) × (*B* × *B*)]	×	[(*B* × *C*) × (*B* × *B*)]	88	*
1002 bE (1)	[(*B* × *C*) × (*B* × *C*)]	×	[(*C* × *C*) × (*C* × *C*)]	79	*
706 bE (1)	(*A* × *A*)	×	*F*	13	76
997 bE (1)	[(*B* × *B*) × (*B* × *B*)]	×	*E*	89	*
737 bE (1)	*F*	×	*C*	58	87
928 bE (1)	(*F* × *F*)	×	(*C* × *C*)	63	93
930 bE (1)	(*C* × *C*)	×	(*F* × *F*)	72	93
1003 bE (1)	[(*F* × *F*) × (*F* × *F*)]	×	[(*C* × *C*) × (*C* × *C*)]	27	*
1005 bE (1)	[(*F* × *F*) × (*F* × *F*)]	×	[(*F* × *F*) × (*C* × *C*)]	56	*
745 bE (1)	*F*	×	*E*	21	60
764 bE (1)	*E*	×	*F*	71	97

* Seed germination not tested.

In fact, there is nothing in these results to indicate that the relationship between plants of the different groups was appreciably different from that existing between plants within the different groups as shown by the intragroup results.

It will be noted also that plants derived from crosses between representatives of different groups were often highly effective as pollen parents so that pollen production in such plants was at least usually adequate.

II. *Lolium rigidum*

Plants classifiable as *L. rigidum* Gaud. from the following seed samples were used in crosses discussed below.

Ba 2274. Reference was made in the Introduction to this lot of seed which was described as 'Wimmera ryegrass' and which was collected from a seed crop grown on McDougall's farm at Minyip, Victoria (Herbarium sheets 109–111, 116–118).

Ba 2990. Seed received from the Director of Agriculture and Forests, Jerusalem; stated to have been collected near Huleh; described as '*L. subulatum* Vis.'. The plants raised from this seed closely resembled those of Ba 2274, but the stems were rather less tall and less bent at the nodes (Herbarium sheets 112, 113, 119–123, 139).

Ba 3120. Seed described as '*L. rigidum* Gaud.', received from the Ministry of Agriculture, Giza, Egypt; stated to have been collected on light soil on fields near the Giza pyramids. The plants closely resembled those of Ba 2990.

Ba 3138. Seed received from the Council for Scientific and Industrial Research, Canberra, Australia, and described as '*L. strictum*. Source: Rabat, Morocco'.

Ba 3139. Seed received from the Director of Agriculture, Nicosia, Cyprus; described as '*Lolium strictum* Presl.' (Herbarium sheets 114, 115, 132, 133).

The plants raised from these lots of seed agreed very closely in morphological features and generally also in growth type and behaviour, so that they could easily be grouped together as a rather distinct class. On the other hand, they differed quite markedly in growth type, leaf type and stem type from those designated '*L. rigidum* sens. ampl.'.

Intra- and intercrossing within the general group

As the crosses between plants of the same origin did not show any significant differences from those in which plants from different sources were intercrossed, it seems unnecessary to discuss the results separately or even to give the detailed results for all crosses made. It is convenient, however, to refer to the lots under letter symbols:

G = Ba 2274 K = Ba 3138
H = Ba 2990 L = Ba 3139
J = Ba 3120

Altogether, forty-two hand crosses (involving over 13,000 emasculated florets) were made between plants within this general group. Some of the plants used were derived immediately from the original seed; others from intercrossing such plants or even their direct derivatives, so that some of the crosses made were rather complex within the limits of the general group.

The highest seed-setting and the highest germination were obtained in a cross of the type

$$[(H \times H) \times (H \times H)] \times [(G \times H) \times L].$$

In this particular cross, 91 % of the florets emasculated set seed, and this seed gave a germination of 100 %. A seed setting of over 70 % was common, as also was a germination of over 90 % while seedling survival closely approached 100 %.

There were, however, a few exceptional results which should be discussed.

(1) When the two sister plants, 641bE(1)1 and 641bE(1)2 (641bE = $H \times G$) were intercrossed, 221 emasculated florets only gave one seed and this failed to germinate.

The cause of failure is not immediately apparent because the conditions for seed-setting seemed to be approximately normal.

In *L. perenne* I have found instances where closely related plants have failed to produce seed when the cross is made in either direction, while in other cases only in one direction does the cross consistently fail. The cause of such failures has not been investigated in detail, but the suggestion seems to be that failure is due to the operation of 'sterility' factors.

In the present case, the cross was made only in one direction, so that it is not known whether the reciprocal cross would have been effective.

Original plants of lots Ba 2274 and Ba 2990 intercrossed quite readily. In the cross which produced family 641bE(1), seed-setting was 81 % and germination 83 %, so that no incompatibility was indicated. In two other crosses both seed-setting at 76 and 86% respectively and germination at 63 and 67% respectively were lower but by no means very low.

A plant from one of these crosses, plant 640bE(1)2, was used as the pollen parent in a cross with an ($H \times H$) plant. This cross gave a seed-setting of 81% and a germination of 92%. It was also used as the pistillate parent with a plant of Ba3139 resulting in a seed-setting of 70% and a germination of 88%.

It is, therefore, evident that there was no general breeding incompatibility between plants of the two groups H and G, but that sterility factors may come into play when related plants are intercrossed.

(2) A second case may be related to the first. Again two sister plants, 741bE(1)3 and 741bE(1)6, were involved. They had been bred within the Ba2990 group. The cross that had produced family 741bE(1) was of the type ($H \times H$) × ($H \times H$) and the antecedents of the present cross are:

♀		♂	Family	Percentage seed-setting	Percentage seed germination
Ba2990(2)	×	Ba2990(6)	1391bA(1)	87	97
Ba2990(3)	×	Ba2990(2)	1390bA(1)	78	97
1391bA(1)1	×	1390bA(1)2	741bE(1)	77	91
741bE(1)6	×	741bE(1)3	916bE(1)	33	98
916bE(1)	×	916bE(1)	1007bE(1)	0	—

It is, therefore, quite possible that the relatively low seed-setting was entirely accidental, or else it may indicate partial incompatibility, but in any case it could not be due to incompatibility between plants of different groups as breeding in this case was entirely within the Palestine group.

(3) The third case is more extreme, but the plants concerned happen to be related to those of the second case, plant 741bE(1)6 again being involved. Only three seeds were obtained when plants 917bE(1)2♀ was intercrossed with plant 917bE(1)1♂, and it is known that plant 917bE(1)1 was not male-sterile. The antecedents of this cross are:

♀		♂	Family	Percentage seed-setting	Percentage seed germination
Ba2274(51)	×	Ba2990(2)	640bE(1)	76	63
1391bA(1)1	×	1390bA(1)2	741bE(1)	77	91
640bE(1)2	×	Ba3139(1)	786bE(1)	70	88
741bE(1)6	×	786bE(1)3	917bE(1)	91	100
917bE(1)2	×	917bE(1)1	1009bE(1)	1	?

Three of the main groups are, therefore, involved, and the antecedent crosses all gave high results except that in one case seed germination was rather low.

Plant 917bE(1)2, when used as the pistillate parent with another rather distantly related plant, gave a seed setting of 53%, so that there is a strong suggestion that the incompatibility was in the present case between the two individual closely related plants 917bE(1)1 and 917bE(1)2, and did not in any way involve incompatibility between the groups as such.

(4) The fourth case involves two original plants, Ba3120(2) (Egypt) and Ba3139(3) (Cyprus), with the former as the female parent. Seed-setting in this case was reasonably good at 51%, but the seed germinated poorly at 23%.

When plant Ba3120(2) was used as the pollen parent and another Ba3139 plant as the female, seed-setting reached 90% with a seed germination of 70%, but when it was again used as the pistillate parent in a third cross (with a plant of Ba 2990 as the male) seed-setting was 46% and germination 53%.

In *L. perenne* cases have frequently been met with where a plant may consistently give poor results when used as the pistillate parent, and in the course of breeding work many such plants have been rejected because poor germination might be genetically selective. The present case of a plant giving seed of low germinating capacity is therefore not by any means unusual.

(5) The two inbred sister plants 2274(52)Ba(2)1/1♀ and 2274(52)Ba(2)1/2♂ when intercrossed gave a seed-setting of only 6% with a germination of 74%.

The parent of these two plants was 2274(52)Ba(1), which had itself been produced by the self-pollination of plant Ba 2274(52). This original plant was rather extensively used and appeared to be entirely normal.

Plant 2274(52)Ba(1) was one of those investigated cytologically by Dr P. T. Thomas in another connexion, who found that it produced both normal and giant pollen grains (Jenkin & Thomas, 1938, 1939). It has been suggested that such giant pollen grains might result in the production of triploids in the next generation, but the present plants were not cytologically examined.

It was pointed out on another occasion (Jenkin & Thomas, 1939) that even the first generation from selfing plant Ba 2274(52) showed loss of vigour, and this was still more pronounced in the second generation to which the present plants belong. Such loss of vigour is expressed in relatively poor growth but it would seem that it can also extend to actual flowering so that a plant may fail to flower normally. The anthers and stigmas may be poorly exserted, so that in the present case the stigmas in the pistillate parent might be poorly presented and the pollen might be poorly liberated by the male parent giving a poor seed-setting result, due to defective action on both sides or on either side.

In any case, the poor result obtained in this case has no bearing upon the question of the breeding compatibility of plants from the different groups because the plants here involved were inbred within a group.

Brief discussion

In general, the breeding compatibility of plants referred to the genus *L. rigidum* Gaud. has been found to be high, and the few relatively poor results recorded seem to have no bearing upon the general question, even although they may be interesting in relation to individual plants and even to lines of descent.

It can, therefore, be concluded that although the groups of plants used represented rather widely separated geographical areas there was no indication of inter-incompatibility.

III. *LOLIUM RIGIDUM* AND *LOLIUM RIGIDUM* SENS. AMPL. INTERCROSSED

The annual, normally wind-pollinated *Lolium* types were separated into these two groups because of morphological and other features in which they differed.

For convenience of reference, the subgroups have been given symbol letters:

Lolium rigidum sens. ampl.: *A*, *B*, *C*, *D*, *E*, *F*.

Lolium rigidum: *G*, *H*, *J*, *K*, *L*.

Fifteen crosses, involving 3838 emasculated florets, were made between plants representing these two divisions.

As some, at least, of the plants represented in the '*L. rigidum* sens. ampl.' division might be classified as *L. multiflorum* Lam., while the plants of the other division have been referred to as *L. rigidum* Gaud., it might perhaps be expected that plants representing the two divisions could not readily be intercrossed. The results are, therefore, given below in some detail:

Family	Derivation ♀		♂	Florets emasculated	Caryopses obtained	Percentage seed-setting	Percentage germination
639 bE (1)	*A*	×	*G*	132	65	49	22
637 bE (1)	*H*	×	*A*	232	177	76	100
723 bE (1)	*L*	×	(*A* × *A*)	132	89	67	67
663 bE (1)	*G*	×	*B*	339	118	35	73
658 bE (1)	*H*	×	*B*	175	135	77	100
661 bE (1)	*B*	×	*H*	211	194	92	77
664 bE (1)	*C*	×	*G*	123	110	89	85
662 bE (1)	*H*	×	*C*	147	111	76	83
734 bE (1)	*G*	×	*F*	316	211	67	90
735 bE (1)	*F*	×	*G*	319	157	49	80
752 bE (1)	*K*	×	*F*	211	176	83	100
754 bE (1)	*F*	×	*K*	318	265	83	95
796 bE (1)	*L*	×	*F*	201	138	79	68
955 bE (1)	[(*G* × *H*) × *L*]	×	*E*	539	443	82	75
957 bE (1)	(*H* × *H*)	×	*E*	443	7	2	29

Only one outstandingly poor result was obtained in these crosses, the parent plants involved being 741 bE (1) 9♀ and 3116 (1) Ba (1) 1♂. It is not known with certainty if plant 3116 (1) Ba (1) 1 was male fertile, because the cross was made by the automatic pollination method, the emasculated inflorescences of the female parent being enclosed for flowering with the inflorescences of the pollen parent.

It may be recalled that when this particular plant was used as the *pistillate* parent with another plant of its own group it failed to produce seed, so that it is possible that it was highly sterile whether used as a pollen or as a pistillate parent.

Any doubt that might be created by this cross if it stood alone is dissipated by the fact that in the parallel cross resulting in family 955 bE (1), a seed-setting of 82 % was obtained and 75 % of the seed germinated.

Relatively poor results were obtained in one other cross (family 639 bE (1)). No satisfactory explanation of the low germination of the seeds can be offered, but it will be recalled that occasionally similar results were obtained from intercrossing plants within the *L. rigidum* and the *L. rigidum* sens. ampl. divisions.

Apart from these two instances, the results can be considered to be quite normal for intraspecific crosses. The hazards of hand-crossing in these grasses are such that low seed-setting is inevitable in a proportion of the crosses made, but using closely inbred plants or intercrossing closely related plants seems to have a further depressing effect. Even the poor results sometimes obtained in the present crosses do not indicate that the groups or divisions to which the plants belong are necessarily incompatible.

Male fertility in derivative plants

In the study of the two divisions, *L. rigidum* and *L. rigidum* sens. ampl., derivative plants were often used as pollen parents, usually with such satisfactory results as to show that they produced pollen in adequate quantities.

In the present crosses, such derivative plants were not extensively used, so that little direct evidence of effective pollen fertility in the 'hybrids' is available.

Other evidence is, however, available for plants in some of the derivative families—mainly plants that were grown in pots:

637 bE (1). Two plants of this family were self-pollinated and respectively gave 2·5 and 60 seeds per 100 spikelets. The same two plants were successfully used as pollen parents, giving up to 87 % seed-setting.

639 bE (1). One plant gave 59 seeds per 100 spikelets from self-pollination. It was also successfully used as a pollen parent in a cross with a full sister plant giving a seed-setting of 71 %.

658 bE (1). Plants of this family were only used as pistillate parents. They set seed up to 85 % of the florets emasculated.

661 bE (1). Two plants self-pollinated; self-fertility low at 9 and 14 seeds per 100 spikelets. As pollen parents they gave up to 85 % seed-setting.

662 bE (1). It was noted that in one plant of this family the anthers dehisced and liberated pollen.

663 bE (1). Two plants self-pollinated proved to be of very low self-fertility yielding respectively only 6 and 2 seeds per 100 spikelets. Used as pollen parents in crosses these two plants gave up to 84 % seed-setting.

664 bE (1). Two plants self-pollinated also showed very low self-fertility, each yielding only 2 seeds per 100 spikelets. These plants were used as pollen parents in crosses and seed-setting ranged from 41 to 73 %.

723 bE (1). Self-fertility was again low in two plants tested. In all three plants that were available in pots, the anthers were fully dehiscent and used as pollen parents, seed-setting ranged from 17 (conditions poor) to 85 %.

734 bE (1). It was noted that the anthers were fully dehiscent in each of the two plants available in pots. One plant was self-pollinated and gave four seeds per 100 spikelets. This plant used as a pollen parent resulted in low setting in each of two crosses—11 and 38 %.

735 bE (1). Anthers fully dehiscent in a plant available in a pot, but seed-setting only reached 16 % in a cross for which it was used as the male parent.

752 bE (1). Anthers dehiscent in two plants available in pots; one plant self-pollinated yielded 66 seeds per 100 spikelets. Both plants were used as pollen parents in crosses. One result was low at 18 % seed-setting, and the other high at 79 %.

754 bE (1). Anthers dehiscent in a plant available in a pot; one plant used as pollen parent resulted in a seed-setting of 45 %.

These results can only be regarded as samples taken at random, but as far as they go, they show that although poor seed-setting has sometimes resulted, no instance of pollen sterility has been recorded, and a number of results show a seed-setting of over 80 % when plants derived from crosses between plants of *L. rigidum* and *L. rigidum* sens. ampl. were used as male parents.

General discussion and conclusions

The work here described was not designed nor intended for the determination of the taxonomic position of the annual, normally wind-pollinated *Lolium* types designated for convenience '*L. rigidum et al.*', but if or when an attempt is made to trace the phylogeny of the different branches of the genus the results may be found to have some importance, although it is realized that further evidence might have been obtained had it been possible to carry out cytological investigations parallel with the breeding work. In such cytological work, however, it would be necessary to bear in mind that inbreeding and close breeding might have important disturbing effects either through the lowering of the flowering energy of the plants concerned or by producing the homozygous condition of certain factors which themselves can cause a lack of co-ordination during meiosis.

The problem of maternal influence upon seed-setting and seed germination, even apart from incompatibility, is also involved, because in the present work, and in breeding work with *L. perenne* in particular, individual plants have been met with which have proved to be poor pistillate parents even when the conditions for intercrossing have not been unfavourable.

In considering the present results, therefore, it seems reasonable to regard those crosses that have been the most successful as being of greater significance than those which have given poor results, especially as both good and a few poor results have been obtained in all three types of intercrossing that have been described.

On this basis, it can only be concluded that all the types here tested, whether morphologically they fall within the limits of a fairly well-defined group or not, have been found to be of normal intraspecific compatibility. This does not mean that for purposes of classification they cannot yet be divided into groups and possibly into subgroups on purely morphological characters, even though such characters may be genetically very simple.

It is evident, in any case, from these results that any of these *Lolium* types which have been described could intercross to produce fully fertile progeny in nature if the opportunity occurred unless they failed to coincide in time of flowering.

Summary

1. The present account is concerned with the breeding interactions of annual, normally wind-pollinated types of *Lolium*.

2. The plants used were derived from widely separated geographical areas, and the seed samples which they represented were variously named by the senders.

3. On morphological characteristics the lots represented in the breeding work now described were eventually separated into two main groups:

(1) Lots which could be classified as representing *L. rigidum* Gaud.

(2) Lots which could not easily be referred to this species owing to morphological divergences but which still varied considerably one lot from another.

4. Some exceptional results were recorded, but these do not seem to be significant in relation to the intercompatibility of the *Lolium* types and groups involved.

5. The breeding interactions of plants within group (2) above (referred to in the paper as *L. rigidum* sens. ampl.) despite their morphological differences gave results consistent with those to be expected in intraspecific breeding.

6. Similar results were obtained by interbreeding plants of diverse sources of origin but classifiable as *L. rigidum* Gaud.

7. Finally, when plants of the two main divisions were intercrossed the results were again consistent with those obtainable in intraspecific breeding.

8. It is therefore concluded that despite geographical separation and morphological divergences there is no evidence of species differentiation as judged on the basis of interfertility in the normally wind-pollinated annual *Lolium* types.

9. Provided therefore the opportunity occurred and all the conditions were suitable in nature, these various types could interbreed freely.

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