A PARTIALLY FERTILE TRIPLE SPECIES HYBRID IN NICOTIANA

F. A. McCRAY

Harvard University, Forest Hills, Massachusetts

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INTRODUCTION

Crosses between three distinct species in Nicotiana are comparatively rare, only nine having appeared in the literature on this genus. The one here reported upon, (rustica × paniculata) × Langsdorfi, as well as (rustica × Tabacum) × paniculata, and (rustica × paniculata) × glutinosa, was produced in Europe many years ago (East 1928) but has not been studied much since. In 1930 Kostoff also described such a triple hybrid combination, as well as Tabacum var. sanguinea × (Langsdorfi × Sanderae), and listed five others which he had secured, as follows: (rustica × paniculata) × Sanderae, Tabacum × (sylvestris × Rusbyi), Tabacum × (Rusbyi × tomentosa), (glaucus × Langsdorfi) × Sanderae, and (glaucus × Langsdorfi) × alata. His plants of the (rustica × paniculata) × Langsdorfi combination, however, were sterile and evidently differed fundamentally in chromosome constitution from mine.

It may be noted that in three of the hybrids just listed, two of the alata-Langsdorfi-Sanderae group, which cross freely together giving fully fertile hybrids with perfect pairing of chromosomes, are combined with a third species. These three, then, might reasonably be expected to be comparatively easy to secure. In two other cases two of the 12-chromosome Tabacum-like species, tomentosa, sylvestris and Rusbyi, are combined with Tabacum. In only one other case, (rustica × paniculata) × Sanderae, besides the one here described, do three species having different chromosome numbers enter into the hybrid combination. This combination is very similar to mine, Sanderae having the same number (9) of chromosomes as Langsdorfi.

MATERIALS AND METHODS

All the plants and seeds used in these investigations were obtained at Bussey Institution, Harvard University, where the work was done. Most of the species used had been under observation there for several years and have been described in numerous papers. Authority for all these species is given by East (1928). All the plants used were grown in a screened greenhouse, and the usual precautions taken to prevent accidental
crossing. All the pollinations were made by hand except as otherwise noted.

Seeds were germinated in sterilized soil in the seed bench, and the seedlings allowed to grow there until large enough to transplant.

Practically all the material used for cytological studies except root tips was fixed in Allen's modification of Bouin's fluid, B-15, and stained in Haidenhain's haematoxylin. Pollen mother cells were usually examined in Belling's iron aceto-carmine (Belling 1926) before the buds were fixed in Bouin for the permanent preparations, and in a few instances, which will be mentioned, the observations reported were made from these smears. Some of the root tips were fixed in Flemming's strong solution, and some in Navashin's fixative. A few of these preparations were stained with gentian violet, but usually the haematoxylin was used.

EXPERIMENTS AND RESULTS

In the beginning of the writer's work on this problem he made numerous attempts to combine three species, most of which were fruitless in spite of the fact that in some instances many pollinations were made. The combinations tried, the number of pollinations made, the number of capsules produced, and the viability, if any, in each case are shown in table 1.

In 1928 Doctor D. Kostoff gave me two plants of the cross *rustica* var. *humilis* × *paniculata*, presumably the same plants which he had already crossed with *Langsdorfi* to produce his triple hybrids. Upon these two plants I made 12 pollinations with *Langsdorfi* pollen. Five capsules were obtained, containing a small amount of seed. Of 30 seeds planted, 20 germinated. Some of the seedlings were killed by damping off, but 4 (numbers 3, 4, 5 and 6) were raised to maturity. Of a later planting of 10 seeds, only one germinated and this seedling died early.

The four triple hybrid plants, especially 3 and 6 which were more vigorous, were backcrossed to all three parent species, and also selfed. Some capsules were secured from all these combinations. Seeds from these 4 plants backcrossed to *rustica* were planted in October 1930. Of these, 14 germinated and 6 plants were grown, 5 of which were offspring of number 6. These plants were designated 131, 231, 331, 431, and 531. The seeds secured by selfing the triple hybrids failed to germinate when planted at the same time.

The hybrids of *rustica* × *paniculata* have been used in a number of investigations and repeatedly described (East 1921, Goodspeed, Clausen, and Chipman 1926, Christoff 1928) as have, of course, all the pure species concerned. Much further description seems unnecessary. Briefly, the F₁ is
Summary of attempts to produce triple species hybrids in Nicotiana.

<table>
<thead>
<tr>
<th>F₁ hybrid ♀</th>
<th>♂</th>
<th>NUMBER OF POLLINATIONS</th>
<th>NUMBER OF CAPSULES</th>
<th>VIABILITY OF HYBRIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>suaveolens × longiflora</td>
<td>plumbaginifolia rustica Langsdorffii Tabacum var. macropylla alata</td>
<td>20 6 136 12</td>
<td>4</td>
<td>...</td>
</tr>
<tr>
<td>plumbaginifolia × Langsdorffii suaveolens × glutinosa</td>
<td>paniculata nudicaulis Langsdorffii Tabacum var. glutinosa Tabacum</td>
<td>5 8 111 39 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>paniculata × glutinosa</td>
<td>Tabacum var. macropylla nudicaulis Langsdorffii gauca</td>
<td>39 14 147 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>suaveolens × plumbaginifolia</td>
<td>Langsdorffii Tabacum var. macropylla</td>
<td>24 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>paniculata × Sanderae</td>
<td>alata Langsdorffii Tabacum macropylla glutinosa gauca</td>
<td>15 29 2 5 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nudicaulis × trigonophylla</td>
<td>rustica tomentosa Palmeri</td>
<td>10 10 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rustica × paniculata</td>
<td>tomentosa Palmeri acuminata trigonophylla Rustbyi caudigera</td>
<td>12 9 2 7 6 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tabacum angustifolia glutinosa Tabacum macropylla caudigera Tabacum purpurea glauca Langsdorffii</td>
<td>7 13 23 6 9 15 12</td>
<td>1 2 1 1 12</td>
<td>No germination No germination No germination No germination 3 seeds germinated, all died early Some matured, partly fertile</td>
<td></td>
</tr>
</tbody>
</table>

intermediate between the parents, perhaps resembling rustica somewhat more closely. In figure 1 are shown actual size drawings of typical flowers.
for comparison. It will be seen that the flowers of $F_1$ *rustica* × *paniculata* are intermediate in size and shape between those of the parent species.

The triple hybrid plants were tall, about 36 inches in the greenhouse pots, slender, and branching at the top something like *paniculata*. As already stated plants 3 and 6 were larger and more vigorous than plants 4 and 5. The leaves were ovate like *paniculata* in general shape, but acute at the base like *rustica*. They were petiolate.

The flowers were borne in compact panicles with racemose branches, resembling *rustica* somewhat more closely in this respect than did *rustica* × *paniculata*. The individual flowers, also pictured in figure 1, were shorter than those of the $F_1$ parent, more like pure *rustica* but more slender.
The absence of any mention of *Langsdorffii* characters will be noted, and it might be thought that the chromosomes of this species failed to enter the combination at all, the "triple" hybrids being produced by the F₁ mother plant parthenogenetically. However, it must be borne in mind that *rustica* and *Langsdorffii* have many characters of leaf, stem, and flower in common, and that the effect of the *Langsdorffii* genes could thus be easily masked. More especially is this true since *paniculata* is even more closely related to *rustica* than is *Langsdorffii*, necessitating that any distinctive characters of the latter species might have to overcome the combined effect of inheritance from the other two before they can express themselves in the triple hybrid. Furthermore, the cytological evidence to be presented later will show conclusively that these plants could be neither pure *rustica* nor *rustica X paniculata*.

**Figure 2.**—*rustica*-like derivatives, 
([(rustica X paniculata) X Langsdorffii] X rustica), compared with *rustica*.
The progeny of plant 6, the result of crossing it with *rustica* pollen, as might be expected, resemble the male parent very closely in all respects, as shown in figure 2. The habit of growth and all detailed characteristics are identical except size. Plants 331 and 431 are considerably smaller and have smaller flowers. But even then there is no more variation than between plants of a pure species when grown under similar artificial (greenhouse) conditions. These *rustica*-like derivatives could not be told from pure *rustica*.

In keeping with this close resemblance to the pure species, these plants exhibit much more fertility than their triple hybrid parent. Plant 131 was pollinated 4 times by each of the three parental species, and its pollen was used reciprocally on each of them 4 times. An equal number of attempts were made to self it. Plants 231, 331, 431 and 531 have been similarly tested, usually 5 pollinations being made in each case, but sometimes a few more. The numbers of capsules harvested are shown in table 2.

**Table 2**

*Capsules produced by rustica-like derivatives, ([rustica×paniculata]×Langsdorffii)×rustica.*

<table>
<thead>
<tr>
<th>φ</th>
<th>131</th>
<th>231</th>
<th>331</th>
<th>431</th>
<th>531</th>
<th>SELF</th>
<th>rustica</th>
<th>paniculata</th>
<th>Langsdorffii</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>231</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>331</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>431</td>
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<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>531</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><em>rustica</em></td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td><em>paniculata</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td><em>Langsdorffii</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
</tbody>
</table>

In addition, a number of other capsules were produced by natural selfing of 331, 431 and 531.

The number of seeds contained in the capsules included in the table varied considerably. For example, 5 capsules of 331 selfed contained only 4, 5, 5, 5 and 9 seeds. Another group of 5 contained together 72 seeds. In 3 capsules on the same plant pollinated by *paniculata* were found 12, 15 and 71 seeds. Three other capsules pollinated by *rustica* produced 20, 25 and 31 seeds. Likewise several capsules on 531 contained seeds varying in number from 9, in the case of a selfed capsule, to 92, this last one being a capsule of 531×*rustica*.

The viability of these seeds has been tested in three cases. Fifty-one selfed seeds of 331, 80 of 431, and 29 of 531 were planted. Of these 34 or...
66.67 percent, 57 or 71.25 percent, and 16 or 55.17 percent, respectively, germinated, and the young seedlings grew normally as long as observed. Naturally it was to be expected that the 5 plants, resembling *rustica* as closely as they did, probably had a nearly complete set of *rustica* chromosomes and would behave in all these crosses very much as pure *rustica* would. That they were not altogether *rustica* in constitution, however, is indicated by the fact that they were somewhat less fertile, producing fewer seeds per capsule, and in some cases, especially in 131 and 431, considerably less than the usual amount of pollen, so that several anthers were necessary to make a good pollination.

Buds and root tips of the 5 plants just discussed have been imbedded for cytological investigation, and may shed some further light on the compatibility of the species involved, or at least on the genetic make-up of the triple hybrid parent.

The cytology of the triple hybrid plants themselves shows pretty clearly, however, how they came into being, and possibly why they were partially fertile while Kostoff's were sterile. It should be recalled at this point that, as shown by Christoff, and by Goodspeed, Clausen and Chapman, in the F₁ *rustica* × *paniculata* at the metaphase of the reduction division there are usually 12 bivalent chromosomes and 12 univalents, the 12 *paniculata* chromosomes apparently having paired with 12 of the *rustica* chromosomes, leaving the other 12 *rustica* chromosomes as univalents, as is the case in a number of other hybrids in this and other genera. In the anaphase the univalents are distributed to the two poles at random, and appear in the gametes in frequencies from 0 to 12.

If these gametes, then, with 12 to 24 (12+0 to 12) chromosomes should unite with 9 *Langsdorffii* chromosomes, the resulting plants should have 21 to 33 somatic chromosomes. If only 21 are present there should be little or no pairing. Christoff (1928) states that there is no pairing between *paniculata* and *Langsdorffii* chromosomes in the F₁, and it may be considered doubtful whether the *Langsdorffii* chromosomes would pair with the *rustica* chromosomes, although there seems to be no real evidence on this point. If, however, the larger number (33) of somatic chromosomes (24 *rustica* or *rustica* and *paniculata* + 9 *Langsdorffii*) is present there would no doubt be some pairing, and there might be as many as 12 bivalents, reducing the number of chromosomes in I M to 21.

Before describing the actual cytological facts found in these plants let us suppose further that in the F₁ parent the gametic number of chromosomes (12 to 24) should be doubled by failure of a cell wall to form between the granddaughter nuclei. Then when one of these gametes unites with a
Langsdorfiī gamete carrying 9 chromosomes the resulting plant should have from 24 to 48+9, or from 33 to 57 somatic chromosomes. In this case most of the chromosomes at I M would doubtless be bivalent, and the total number visible at this phase, assuming complete pairing of all except Langsdorfiī chromosomes and that they refused to pair at all, would be from 21 to 33 (12 bivalents+9 univalents to 24 bivalents+9 univalents). And since most of the chromosomes are paired there would be nearly as many chromosomes in the II M plates as in the I M plates, especially if the number approaches the maximum, 24 bivalents 9 univalents. Of course there might not be complete pairing of the rustica and paniculata chromosomes, or a few of the Langsdorfiī chromosomes might conceivably unite with rustica, or with a bivalent, forming a trivalent group. At any rate these theoretical considerations show that the normal expectancy is about 21 to 33 chromosomes in somatic tissues and at I M, and smaller numbers at II M if the two divisions take place in the mother plant as usual; or from 33 to 57 somatic chromosomes with about 21 to 33 appearing at both metaphase stages if, in the megasporogenesis, doubling of the chromosome number has taken place during the second (mitotic) division.

With these possibilities in mind we may examine the available facts in regard to the cytological conditions in the triple hybrid plants. In plant 6, 30 was the most common number of chromosomes observed in the pollen mother cell at both I M and II M. This number was also observed at diakinesis. In different cells counts of from 29 to 33 were obtained at I M, and from 28 to 32 at II M. Two counts of 31 and 30 (?) were also made at I A. The exact number is often difficult to determine because not all the chromosomes lie in the same plane. Several may be a little higher or lower than the equatorial plate. Of the II M plates observed one of the best showed 29 (or possibly 30) chromosomes. Two cells at least showed both II M plates quite plainly. In one the counts were 24 and 24 (or possibly 25); in the other 28 and 28 (or possibly 29). These facts are illustrated in figures 3 and 4, which show I M and II M respectively in plant 6.

This variability in the number of chromosomes is probably due to variability in the amount of pairing, such as has been observed in gametogenesis in a number of F₁ hybrids (compare East 1928).

In the anaphase of the reduction division the chromosomes progress to the poles at a rather uneven rate as is typical of hybrids. Figure 5 shows 2 or 3 chromosomes lagging on the spindle at this stage. However, as a rule, all of the chromosomes are included in the nuclei at interkinesis. The same is true of the II A, and the telophase and tetrad stages present quite a normal appearance in most cases, although irregularities were observed.
occasionally. Figure 6, for example, shows dyads appearing in the place of tetrads in one of these triple hybrids.

Unfortunately root tip material from this plant was not saved. However it is clear from the amount of pairing shown by the near equality of chromosome numbers in I M and II M that there must have been over 50 somatic chromosomes.

Plant 3 revealed much the same condition. In both I M and II M the chromosomes line up at the equatorial plate in quite the usual way. Still several chromosomes were often observed above or below. Numerous
plates showing the same range in chromosome numbers as in plant 6 were counted. The early I A was often very irregular (figure 7). Nevertheless in

later stages, as shown in figure 8, nearly if not quite all the chromosomes reach the poles and doubtless enter into the daughter nuclei. An excep-
tional case is shown in figure 9, which represents one pollen mother cell in this plant in which are formed two perfect spindles. Root tip counts also were made for this plant. Figure 10 shows a metaphase plate on which can be seen 53 chromosomes. This being assumed the correct number for this plant where 29 chromosomes appear in I M, they are evidently 24 bivalents + 5 univalents. Cells that contain 33 chromosomes are probably of the constitution, 20 bivalents + 13 univalents.

DISCUSSION

The chromosome constitution of these triple hybrid plants has already been explained, as a theoretical consideration. All that is necessary, therefore, at this time, is to call attention to the close agreement between the facts as observed and the theoretical possibilities. Plants 3 and 6 at least had over 50 somatic chromosomes, which could be accounted for only by a doubling of the gametic chromosome number in the second division of the megaspore mother cell, and adding to this enlarged number the haploid number of _Langsdorffii_ chromosomes. This duplication of many of the chromosomes is just what is necessary to account for the large number of bivalents, which, in turn, is proved by the approximate equality of chromosome numbers in I M and II M. To be sure, this phenomenon could be explained by postulating a double mitotic division of the univalent chromosomes, but there is no evidence to support the idea.

This is the first time, so far as I have found, that doubling during the second division has been reported, although it has often been observed as taking place during the first division.

Of course one can not be sure that all 9 _Langsdorffii_ chromosomes went into the make-up of these plants. Some of them may have been eliminated from the fusion nucleus. But from the fact that the maximum number of chromosomes that the F1 egg cell could have, even after doubling, is only 48, it seems clear that some of the chromosomes in these triple hybrids are indeed _Langsdorffii_ chromosomes.

In some of the metaphase figures a few of the chromosomes were larger than the others, and it is possible that these were trivalents. Kostoff (1930) concluded that in one of his similar triple hybrids there might be one trivalent and in the other probably four or five. The chromosome numbers he observed in somatic tissues and in pollen mother cells make these suppositions reasonable.

These conclusions regarding gametogenesis of the F1 _rustica × paniculata_ agree nicely with data of Doctor W. R. Singleton (1932), gathered in his study of the F2 from selfing the F1. For example he found one plant
of this second generation having 33 to 36 chromosomes, most often appearing at I M as 19 bivalents and 17 univalents, accounting for 55 somatic chromosomes. Others showed at I M, 18 bivalents and 6 univalents, 14 to 17 bivalents and 14 to 16 univalents, and 35 to 36 bivalents, respectively. In each of these cases, as in mine, it is most likely that one of the gametes, and in the case of the last plant both gametes, must have been diploid. The doubling in these cases however could have taken place during meiosis.

Kostoff’s triple hybrid plants already mentioned had 24 and 32 somatic chromosomes respectively, and showed 12 to 15 and 14 to 18 chromosomes in the heterotypic metaphase. If, as he supposed, these plants had 1 and 4 or 5 trivalents, then in order to harmonize these observations, there must have been in each case 6 or 7 bivalents, whereas in my plants most of the I M chromosomes were clearly bivalent.

It will also be recalled that Kostoff’s plants were sterile while mine, at least 3 and 6, were partially fertile. This fertility was probably due to the duplication of chromosomes in the F₁ mother plant which resulted in a preponderance of rustica or rustica and paniculata chromosomes in the triple hybrid plants. That their genetic constitution was of this type is also evidenced by the extremely close resemblance of all the progeny of plant 6, when pollinated by rustica pollen, to pure rustica, as already noted.

This study affords another instance, of which there are many, of practically pure plants being segregated out of even rather complex crosses, and explains the phenomenon on a cytological basis. It also adds another bit of evidence to that already available on why some plants are so much more fertile than others.

The other triple hybrids which Kostoff (1931) has studied cytologically are Tabacum × (Langsdorffii × Sanderae) and Tabacum × (sylvestris × Rusbyi). The former was sterile, but the latter both vigorous and fertile. In this case there was complete pairing of the chromosomes, apparently the 12 from sylvestris and the 12 from Rusbyi each pairing with 12 of the Tabacum chromosomes. This result is not surprising since in both F₁ crosses, Tabacum × sylvestris and Tabacum × Rusbyi, the Drosera type of chromosome pairing is observed, there being in each case 12 bivalents and 12 univalents at I M. It is perhaps doubtful however, as Doctor E. M. East points out, whether these facts should be taken to mean that half of the Tabacum chromosomes are homologous to sylvestris and the other half to Rusbyi chromosomes. Goodspeed and Clausen (1928) conclude that the Tabacum chromosomes are half sylvestris and half tomentosa from similar reasoning in regard to their hybrids, sylvestris × Tabacum, tomentosa × Tabacum,
and \textit{sylvestris} \times \textit{tomentosa}. Obviously both these conclusions can not be sound unless, indeed, the \textit{Rusbyi} and \textit{tomentosa} chromosomes are practically identical. This is doubtful even though the species are quite similar. In my tests \textit{Rusbyi} failed to produce any hybrids with \textit{tomentosa} pollen after 5 trials. This is admittedly not positive evidence. But it must also be borne in mind that \textit{Tabacum} also shows a similar type of chromosome behavior with \textit{glutinosa}, as well as with \textit{sylvestris}, \textit{Rusbyi} and \textit{tomentosa} and probably with \textit{glaucua} and \textit{Sanderae} (East 1928). At least if both these conclusions as to the homology of chromosomes in the different species are correct, the identity of several chromosomes in all these 12 chromosome species should be established. Evidently pairing does not necessarily mean homology of chromosomes. In fact Yarnell (1931) was able to demonstrate pairing of non-homologous chromosomes in \textit{Fragaria}.

**SUMMARY**

1. A triple species hybrid was produced by using \textit{Langsdorffii} pollen on \textit{F}_1 \textit{rustica} \times \textit{paniculata}. Four plants, numbered 3, 4, 5 and 6, were grown, which resembled the \textit{F}_1 parent in being intermediate between \textit{rustica} and \textit{paniculata}, but which, in certain respects, were more like \textit{rustica}. No distinctive \textit{Langsdorffii} characters were found, but it is pointed out that \textit{Langsdorffii} and \textit{rustica} have many resemblances, as do also \textit{rustica} and \textit{paniculata}, so the absence of definite \textit{Langsdorffii} characters does not mean that the chromosomes of this species were left out, fertilization being replaced by parthenogenesis.

2. A cytological study of these triple hybrid plants, especially of one of them, bears out this conclusion, and affords an additional explanation of the lack of \textit{Langsdorffii} characters. The somatic chromosome number is so large, over 50, it can be accounted for only by assuming doubling of the chromosome number in the second division of megasporogenesis in the \textit{F}_1 female parent. This doubled number, 24 to 48, plus 9, the haploid number in \textit{Langsdorffii}, gives 33 to 57 as the expected number of chromosomes in the triple hybrid. If the actual chromosome number is near the upper limit of this range, the few \textit{Langsdorffii} chromosomes present would have many \textit{rustica} and \textit{paniculata} factors to overcome before the characters of this species could be expressed.

3. These chromosome numbers were found in plants 3 and 6. The best root tip plate in plant 3 showed 53 chromosomes. A variable amount of pairing in pollen mother cells caused these chromosomes to appear at \textit{I M} as 27 to 39, or more often 29 to 33. Since nearly all of these chromosomes were bivalent, \textit{II M} plates also showed 28 to 32 chromosomes.
4. These two plants, 3 and 6, were more vigorous and fertile than the others, which were not studied cytologically. Possibly plants 4 and 5, like Kostoff's similar sterile plants, were less successful because of having resulted from the union of haploid gametes so there was a less complete set of rustica chromosomes.

5. Capsules were produced when these triple species hybrid plants were pollinated by each of the three pure species, and also when selfed. Seeds of number 6 × rustica germinated well and vigorous plants closely resembling rustica, and at least partially fertile, were grown. These produced numerous capsules when pollinated by all the parent species and when selfed. Selfed seed of three of them germinated, and the seedlings grew normally as long as observed.

ACKNOWLEDGMENT

The writer wishes to express his indebtedness to Doctor E. M. East for plants and seeds which he kindly furnished in his laboratory, and especially for his helpful criticisms and suggestions given in connection with the experiments described.

He is also indebted to Doctor D. Kostoff for the F₁ plants which served as the immediate parents of the triple hybrid.

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