FIELD OBSERVATIONS AND EXPERIMENTS

ON THE

POLLINATION OF HEVEA BRASILIENSIS.

By L. E. MORRIS.

1. Introduction.

In the improvement of a crop by selection or breeding the first steps to take are to study the natural methods of reproduction of the plant concerned and to find out how far the individuals already in our fields display the character we desire to intensify and preserve, so that the most suitable stock for selecting or breeding from can be chosen.

With Hevea we wish to grow trees that will yield large quantitites of rubber and also possess the advantageous characters of disease resistance, suitable tree shape and so on. It is well known that the bulk of the crop from any particular field comes from only a small proportion of the trees and it is from such high yielders that we must generally start in any attempts to improve the general standard of plantation rubber, whether by budding, seed selection, or the breeding of new and better types.

It is with the last of these methods that the present note is concerned.

2. The Structure of the Flower and the Development of the Bud.

During the flowering season the rubber tree produces masses of flowers, which are borne in bunches (inflorescences) towards the end of the leafy twigs where the new season's growth is starting. Each inflorescence is much branched and bears two kinds of flowers. The female flowers are situated at the ends of the central axis and of the main side branches, while the male flowers occur in much greater numbers on all the lesser shoots as indicated in the much simplified diagram—Figure 1. The flowers of both sexes are surrounded by a bell-shaped corolla of usually five yellowish petals. The female flowers can be distinguished not only by their terminal position but also by their greater size and by the small green button-like expansion at the base of the petals. When the bud opens the round green ovary can be seen inside carrying on top three whitish lobes. These are known as the stigmas and form the receptive portion of the female sexual apparatus; Fig. 2 shows a diagrammatic sketch of a female flower cut through longitudinally. The male flower is yellow right down to its stalk, and on opening exposes a central white slender column surrounded by two rings of tiny yellowish rounded projections. These are the anthers, which soon split and set free the pollen as a slight yellow dust. (A section of a male flower is shown in Figure 3.) The pollen grains carry the male generative cells and if transferred to the stigmas under the proper conditions (pollination) they may germinate and fertilise the female. Subsequently the ovary will grow and become a fruit containing three or, rarely, more seeds. The whole of the woody fruit wall and the outside layers of the seed, including the hard outer coat with its characteristic colour and markings and shape, arise from purely female tissue, and, though development does not take place without fertilisation, no male substance enters into them. The embryo, which lies in the middle of the seed and later grows into the seedling, is, on the other hand, the direct product of fusion between the male and female germ cells, and inherits a mixture of the characters of both parents.

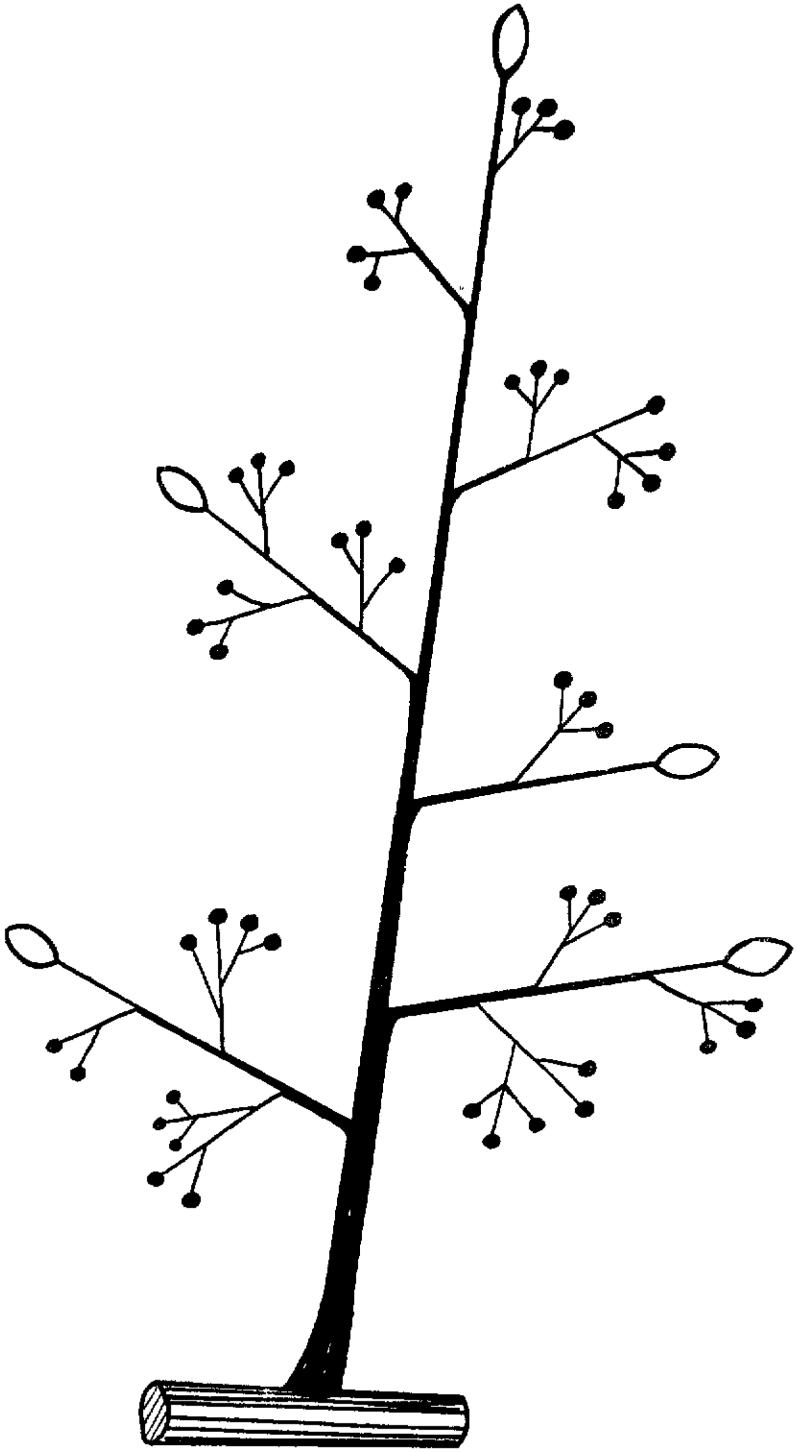
3. The Natural Pollination Process.

The method by which the natural pollination of the flower is brought about determines the parentage of the seeds, that is whether they are the offspring of but one tree or of two different parent trees.

Two general types of pollination occur in plants that have separate male and female flowers. In one of these the pollen is transferred from the stamens to the stigmas by wind, and in the other by insect visitors. The structure of the flower of Hevea, the stickiness of its pollen and stigmas and the presence of an attractive scent and colour indicate that insect pollination is the most probable method, although so far positive evidence from actual observation is lacking.

Many kinds of insects may be seen flying round the inflorescences and several workers in the Dutch East Indies have observed the flowers being visited by bees, flies, moths and beetles of several families, but they do not state that these insects were actually seen to enter female flowers.

During the flowering season of 1928 the same families of insects have been seen on Hevea inflorescences in Malaya. Three



FEMALE FLOWERS *O* MALE FLOWERS

FIG. 1

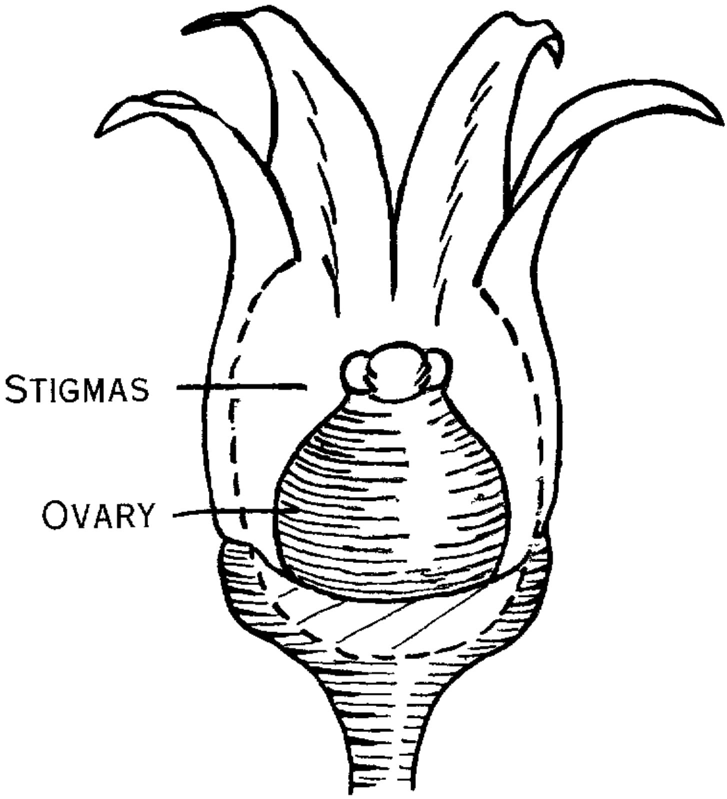


FIG. 2

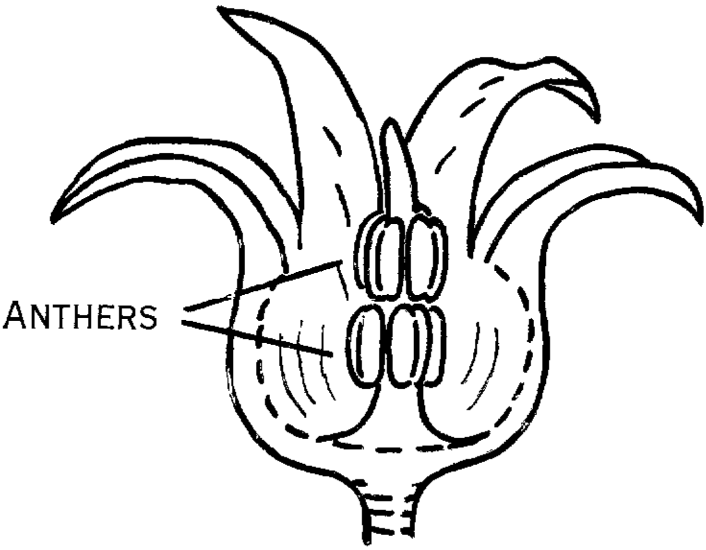


FIG. 3

or four sorts of bees have been frequently watched while visiting the male flowers for pollen and have then been captured and examined. The baskets on their legs have been observed to be filled with pollen, which under the microscope has been identified as that of Hevea. Nevertheless, so far, not one has been seen to enter a female flower, the nearest cases being two which paused on the edge and then turned away.

Smaller numbers of bugs, caterpillars, weevils, ladybirds and other beetles, flies and ants have also been observed. The bugs and weevils may puncture the flowers, and the caterpillars and some of the beetles eat them but the ants appear to take no interest whatever in the flowers. Occasionally flies have been seen entering male flowers, but the only insects seen with certainty in a female flower have been two minute beetles and one tiny fly, which walked all round the petals without touching the ovary and so could not have carried pollen to the stigmas. It appears probable, nevertheless, that the more numerous and active bees are responsible for pollination.

The part played by insects in general has been demonstrated, in the Dutch East Indies by Maas (7) and now in Malaya, by covering inflorescences with muslin bags to keep out insects and so preventing the flowers from setting fruit. In the course of pollination experiments that were started on Pilmoor Estate, Batu Tiga, Selangor, in the first flowering season of this year over thirty inflorescences with about two hundred female flowers and very many more males were enclosed in insect proof bags and not one fruit was formed.

It might appear that the right insects for pollinating Hevea are not present in the East, for though the rubber tree produces such a mass of blossom very few female flowers develop into fruit. For example in Java Maas (7) found that only 2 to 3% of female flowers set seed—in one series of observations 20 out of 927—and Heusser (2) says that on the average only one fruit forms for seventy-two female flowers.

4. Artificial Pollination.

If insects do actually pollinate Hevea it is possible that when collecting pollen they will visit several trees in one excursion, and so the female flowers may receive pollen from males on the same or other trees. In that case seed picked from one tree will have a known mother but its male parentage will be unknown.

For accurate breeding experiments, however, it is essential to know both parents and recourse must be had to artificial pollination.

In the method used this year on Pilmoor Estate suitable inflorescences were selected and all open flowers removed. If the females were to be used all the male buds were cut off also, lest they should open later and their pollen reach the female flowers during subsequent handling. The prepared inflorescence was enclosed in a muslin bag supported on a light wire frame and left until the flowers began to open. Male flowers from another tree were taken to the mother tree and the bag opened temporarily, so that the anthers could be picked out with sterile forceps and transferred to the stigmas of the female flowers, which were enclosed again for a few days until the petals had withered and the stigmas had turned brown, showing that they were no longer suitable for the reception of pollen.

After carrying out these operations everything may be left to proceed naturally. If pollination has not been successful the female flower withers and falls within two or three weeks but if fertilisation has ensued the ovary begins to grow, slowly at first but more rapidly during the second month, until, unless previously damaged or diseased, it reaches full size after 2½ or 3 months. (Fig. 4) Internal growth and development of the seeds then goes on and the fruit ripens about 2 months later.

5. Breeding Methods.

With both plants and animals breeding methods generally fall into the two classes of "inbreeding" and "crossbreeding". In the first method individuals that possess the desirable characters in the highest degree are alone chosen as parents. The type is then purified by breeding only from close relations, as with animals, or in most plants between different flowers on the same plant or even from one flower alone. From each generation only those offspring that come nearest to the desired type are selected and used as parents for the next. Thus at each step the characters sought for are intensified, until after a few generations a strain is obtained that will breed reasonably true to type. It must be remembered, however, that no new hereditary factors can be brought in by this method, and if the full expression of a certain charactor is determined by the combination of several inherited factors (and many such characters are known in genetics) but all are not present in the original parents, then no amount of continued inbreeding will produce a race with that particular character developed to the fullest possible extent.

If, however, cross-breeding is employed between parents possessing different hereditary constitutions one of them may carry one or more of the determining factors not present in the other, and when these sets are re-combined in the next generation some of the seedlings may inherit the complete set and so exhibit the greatest possible development of this character. Subsequently the new strain may be preserved by inbreeding and continued selection.

It is very probable that the latex yielding capacity of Hevea is a complex character determined by all the hereditary factors that influence latex vessels, girth, quality of latex and so on, and thus one high yielding tree may be genetically different from another, neither having a complete set of factors and neither being the best ultimately possible.

6. Experiments in Self-Pollination.

With the limitation just mentioned selfing gives the quickest route to the production of a uniform race; but in the case of Hevea this could not be accomplished quickly as each generation takes several years from seed through tapping tests to seed again. The greatest drawback, however, is that not all Hevea trees are suitable for this method of breeding for most appear to be self sterile, i.e. pollen from the male flowers will not fertilise female flowers on the same plant.

Maas (7) and Heusser (3) in the Dutch East Indies have published the results of experiments in self pollination and their successful pollination in 1919 and 1920 respectively averaged 1.7 and 0.8 per cent. of their attempts, and though one tree gave as much as 6.9% of success, nine out of the fifteen studied gave none at all.

Heusser (4) states, however, that in some cases the sterility of the mother tree was lost to some extent between its budded offspring and pollination became successful. Unfortunately this does not occur always. During May and June of this year two of the highest yielding budded clones—A 44 and B 58 on Pilmoor Estate were tested for self sterility. Flowers on a number of trees were pollinated from other trees in the same clone and from male flowers on other parts of the same tree or on the same inflorescence. Altogether 154 flowers of A 44 and 255 of B 58 were pollinated but without a single success.

7. Crossing Experiments.

Experiments on cross pollination have been carried out for several years in Sumatra, and they show that, so far as raising seed is concerned, crossing gives better results than selfing.

In 1919 Maas (7) obtained 42 fruits from 751 cross pollinated flowers, that is 5.6% of successes. The results varied greatly from tree to tree; for example trees No. 4, 5, 6 and 7, gave about 2, 4, 13 and 12% respectively. In 1920 Heusser (3) and his assistants achieved 10.9% of successes from 6716 cross pollinations. With 35 different combinations of 20 parent trees the results again varied greatly. For example in the cross between tree 29 used as the mother and 141 as the father out of 239 pollinations the whole failed. At the other extreme when trees 138 and 146 were employed as the female and male parents respectively 83 successes out of 211, or 39.3% were obtained.

The fruit setting of one mother tree also depended on the father: tree 164 gave no successes with pollen from tree 144, but gave 8.9% with tree 142 while when tree 151 was the male parent 28 fruits were harvested as the result of 132 pollinations, i.e. 21.2% success.

In 1925 Schweizer (8) in West Java made 18% of successful crossings—187 out of 936, and van der Hoop (6) in Java gives his total results as 5% in 1924, 35% in 1925, 8% in 1926 and 6.9% in 1927.

In May and June of this year cross pollination was carried out on Pilmoor Estate between the clones A 44 and B 58. From 143 female flowers of A 44 two fruits are now ripe, and from 260 crosses with B 58 as the mother one fruit only was obtained, that is less than 1% of the pollinations were successful. To illustrate the mortality amongst young fruit, and the uncertainty of results in work of this kind, it may be of interest to follow the hopes of success from these two crosses. Growth of the ovary into the young fruit is first noticeable between two and three weeks after pollination. From 143 flowers of A 44 only 6 ovaries had started to grow in three weeks, and were then about 3mms. in diameter. Inflorescence No. 49 formed 2 young fruits of which the lower dropped off in five weeks and the other in seven weeks. The one fruit on inflorescence No. 50 grew well to 14mms. in seven weeks, but then its stalk was broken by the wind and it was dead a fortnight later. On inflorescence 90 two young fruits were growing three weeks after pollination and both have

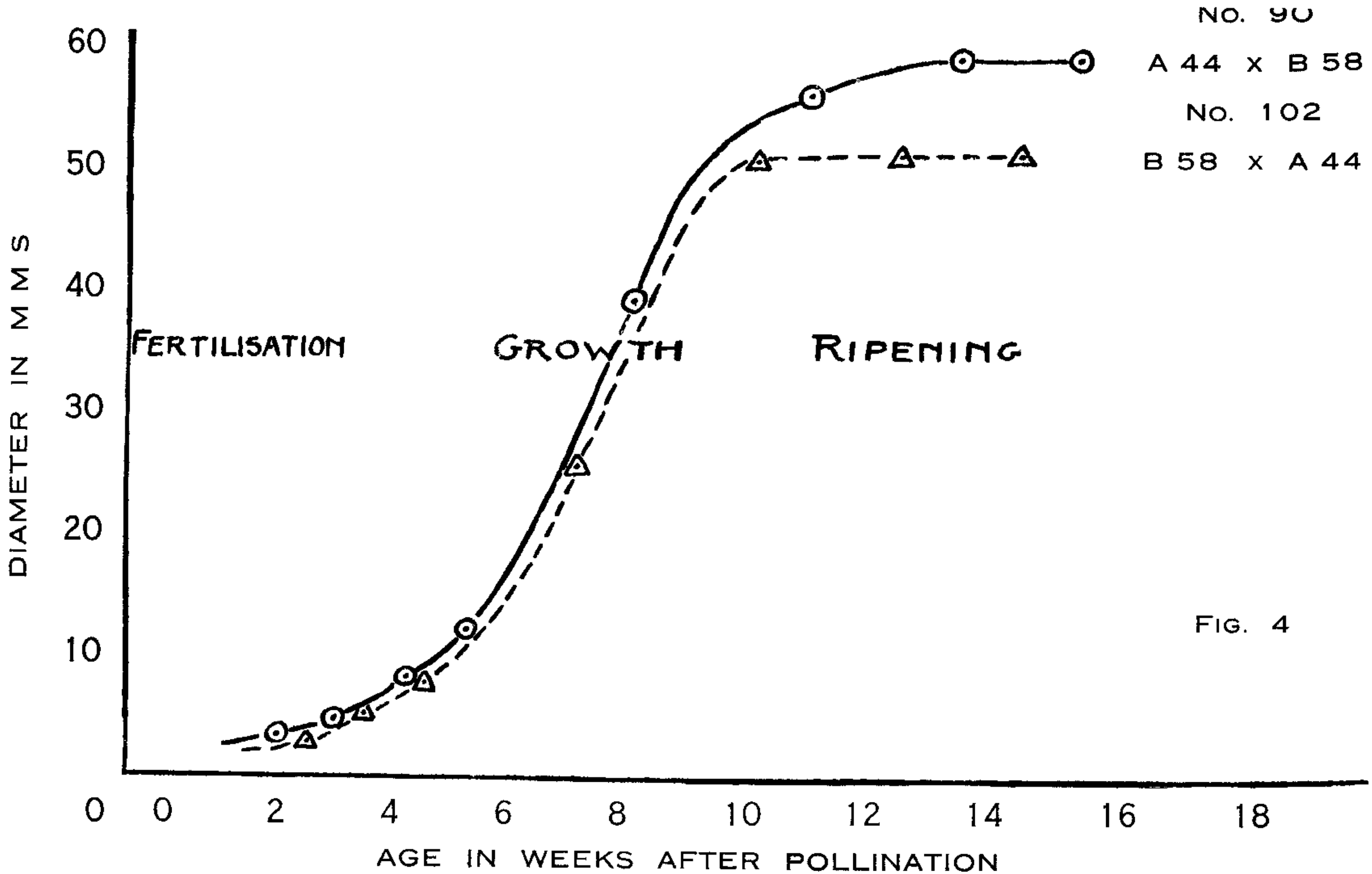

No. 90
A 44 x B 58
No. 102
B 58 x A 44
DIAMETER IN MMS
60
50
40
30
20
10
0
FERTILISATION
GROWTH
RIPENING
0
2
4
6
8
10
12
14
16
18
AGE IN WEEKS AFTER POLLINATION

FIG. 4

survived. The continuous curve of Fig. 4 shows the growth of one of them from the time it was first measured at $2\frac{1}{2}$ weeks after pollination, through the slow growing period and then through the time of rapid growth (from about 6 to 10 weeks) to the final stage of constant size when ripening is proceeding.

Of the reciprocal cross, in which the female parent was B 58, only 3 out of 260 flowers pollinated were alive 3 weeks later. The one on inflorescence 97 died during the next week, that on No. 96 grew to 5mms. and withered between 6 and 9 weeks and only the fruit on inflorescence 102 grew till it reached its full size of 53mms. in 11 weeks. The growth of this fruit for fifteen weeks is indicated by the dotted curve in Fig. 4 and it is of very great interest to note how this also shows the influence of the mother tree on the size of the fruit, for after natural pollination also B 58 fruits are smaller than those of A 44.

Thus of the seven young fruits that were growing one month after pollination only three survived and reached full size. In addition it still remains to be seen whether their seed will be fertile and germinate.

Having emphasised the casualties that have to be reckoned with it may be permissible to quote the apparently greater success of some of the later crosses made in the second flowering season in August in further illustration of the tree to tree differences previously obtained by Heusser. From 261 female flowers of Clone A 8 crossed with A 44 16 young fruits have survived for five weeks. The four oldest have now lived for seven weeks and are about 18mms. in diameter. Of the 39 flowers of A 44 crossed in the reverse way all have died though one was still growing at 3 weeks. Other combinations of clones are still more hopeful. For example four weeks after pollinating 57 flowers of A 44 with D 61 pollen ten young fruits are alive, while the reciprocal cross has given about twenty potential successes from a hundred pollinations. Thus even allowing for severe casualties these crosses promise to be considerably more productive of fruit than those between A 44 and B 58, though on the other hand one or two others probably will be even less successful.

It is obvious that much more remains to be learned about the conditions necessary for success before a sufficiently satisfactory procedure for estate work can be recommended, but, on the other hand, it is distinctly encouraging that such a degree of success has attended the first efforts in this direction.

Although better results are obtained from artificial crossing than in nature or by selfing, still many female flowers have to be pollinated in order to obtain a few fruits. Various attempts have been made to find a method of increasing the chance of success or explaining the failures. Schweizer considered that his experiment was affected by the weather, and van der Hoop's variations from year to year taken with known results for other crops suggest that weather may exert a large influence. For instance, the time of day at which the flower buds open and at which the pollen is set free is affected, and is later on a dull and moist day than on a bright and dry one.

Maas (7) studied the effect of pollinating at various stages of opening and, on the whole, obtained the highest success by using male and female flowers on the morning after opening, less from just opened flowers, and least from females at the midday after opening with the use of fresh pollen. With one tree, however, these last conditions gave better results. The differences were, nevertheless, not great enough to balance the practical difficulty of using flowers at one stage only, for this reduces the number of pollinations that can be made, and any such programme is very liable to be upset by rain. It is hoped to obtain additional evidence on the effect of the conditions under which pollination is carried out from the results of this year's work on Pilmoor Estate, in which now over 2,200 flowers of 8 clones have been pollinated in all possible stages.

The hopeful possibilities of crossing as a means of producing high yielders are illustrated by Heusser's (5) results in Sumatra. Twenty seedlings from a cross made in 1919 between trees 36 and 35 averaged 11.2 grams per tapping in their first tapping period from May 1924 to October 1925, as compared with 8.7 grams for Clone 35, that is for buddings from the male parent of the cross. The best seedling gave 21 grams and the best budded tree only 14, while the seedlings have maintained their superiority in subsequent years. The best trees have been multiplied by buds, marcots and further crossings so that now tapping results are being obtained from a second generation cross made in 1923.

REFERENCES.

(1) A.V.R.O.S. (1927-28). Mededeelingen Algemeene Serie No. 34. *Director's Report.*

(2) HEUSSER, C. (1919). Over de voortplantingsorganen van *Hevea brasiliensis* Mull. Arg. *Archief voor de Rubber cultuur* **3**, p. 455.

(3) HEUSSER, C. (1921). Kunstmatige Bestuivingen bij *Hevea brasiliensis* in 1920. *Ibid*: **5**, p. 11.

(4) HEUSSER, C. (1924). Hevea Selectie. *Ibid*: **8**, p. 371.

(5) HEUSSER, C. (1926). Experimental Tapping on Hevea Seedlings and Buddings on Tjinta Radja Estate. *Ibid*: **10**, p. 637.

(6) VAN DER HOOP, D. (1928). Voordracht over de Excursie DD. 22 Mei J. L. Naar de P. en T-Landen. *De Bergcultures* **2**, p. 1623.

(7) MAAS, J.G.J.A. (1919). De Bloembiologie van *Hevea brasiliensis*. *Archief voor de Rubbercultuur* **3**, p. 288.

(8) SCHWEIZER, J. (1927). Superior Planting Material from *Hevea brasiliensis* in Besoeki. *Archief voor de Rubber cultuur* **11**, p. 99.