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The Low Fruit Set that Follows Conventional Hand Pollination in Hevea brasiliensis: *Insufficiency of Pollen as a Cause*

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Hand-pollinated female flowers were collected and the pollen grains deposited on the stigmas were counted by incident ultraviolet-light fluorescence microscopy. The counts indicated that the fruit set that normally follows conventional hand-pollination is low partly because too few pollen grains are deposited on the stigma. In agreement with this proposition, a new method of hand-pollination, whereby a larger number of pollen grains was deposited on the stigma than in the conventional method, resulted in a higher percentage of fruit set.

The fruit set in *Hevea* after hand-pollination is low. For the crosses carried out in the Rubber Research Institute of Malaysia from 1969 to 1980, the average percentage fruit set is 3% for the first (or main) flowering season and 8% for the second season*.

Heusser[1] and Maas[2] studied the floral biology of *Hevea* and attempted artificial pollination. They noted that the majority of hand-pollinated flowers abscind by three weeks, and that pollination failure could be the cause. Morris[3], Wycherley[4-6] and especially Ross[7] investigated the problem of low fruit set in *Hevea* and made generally similar observations. Other workers, who studied the nature of the pollinating agent and other aspects of natural and artificial pollination[8-13] and pollen germination[14] in *Hevea*, have also made references to the low fruit set.

Conventionally, a female rubber flower is hand-pollinated in the following way[1,15]: A staminal column is dissected out from a closed male flower which is about to open. A closed female flower also due to open the same day, is opened manually by parting the perianth tips with a pair of forceps. The staminal column is inserted within the female flower. A small wad of cotton wool is then dipped in latex and inserted in the female flower near the tips of the perianth lobes which are closed again by bringing them together against the cotton wool. A pre-requisite for successful fruit set would be that during pollination, a sufficient number of pollen grains fall on the stigma of the female flower. Each anther in *Hevea brasiliensis* contains 150 to 200 pollen grains[1,16] and a staminal column has ten anthers attached. Therefore, by the conventional method of pollination described above, a theoretical maximum of 2000 pollen grains is made available for pollinating a female flower. Warmke[9,10] studied the natural pollination of *Hevea* and concluded that female *Hevea* flowers were

*Calculated from data presented in the Annual Reports of the Rubber Research Institute of Malaysia, 1969 to 1980.

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adequately pollinated and that the low fertility of cultivated *Hevea* was not due to failure of natural pollination. He based his conclusion on his data which showed that 77% of the stigmas in female flowers from Puerto Rico and 68% and 48% of stigmas examined at two locations in Brazil bore pollen grains**.

In the present investigation, pollen grain counts were made on stigmas from hand-pollinated flowers to determine whether adequate amounts of pollen were deposited on stigmas by the conventional hand-pollination technique.

EXPERIMENTAL PROCEDURES AND RESULTS

Pollen Count

Female flowers were hand-pollinated by the conventional method as described above. The flowers were collected the following day and in some cases fixed in Carnoy's fixative (3 : 1 ethanol : acetic acid) overnight and then transferred to 70% ethanol. A preliminary study indicated no significant difference in the number of grains observed between fresh and fixed samples of hand-pollinated female flowers. The perianth was removed from each flower and the stigma was cut with 1–2 mm of stylar/ovarian tissue attached. The stigma surface was then scanned for pollen grains by incident ultraviolet-light fluorescence microscopy. A Leitz Dialux microscope was used in which light supplied by a 50w mercury lamp was filtered through a 2 mm UG1 filter. Samples were observed through K 430 and K 400 suppression filters. The pollen grains could be easily distinguished from the other tissues by virtue of a characteristic blue auto-fluorescence displayed by the pollen grain wall. The microscope was re-focused constantly during the scan, to ensure that all pollen grains were counted.

Figure 1 shows the frequency distribution of female flowers in relation to the total number of pollen grains observed on the stigma, style and upper portion of the ovary. The results of the two experiments were generally similar despite the fact that different crosses were involved. About 15% to 20% of the female flowers had a total of 0 to 2 pollen grains. *Figure 2* shows the distribution of pollen grains on the stigma lobes in *Experiment 2*. In each female flower, the lobes were ranked according to the number of pollen grains found, *Lobe 1* having the fewest grains. More than 70% of the female flowers had no pollen grains at all on at least one stigma lobe.

In *Experiment 2*, the number of pollen grains observed was 1515. Only 64.9% of all the pollen grains (984) fell directly on the upper surface of the stigma lobes where presumably, the pollen would have an opportunity to effect fertilisation. The mean number of grains deposited on the stigma, stylar and ovarian surface was 25.5, equivalent to only eight per ovule *(Table 1)*. The mean number of grains falling on the upper surface of the stigma lobes, 10.0 was even smaller. Of particular significance is the number deposited on the lobe with the fewest grains : the mean per lobe was 0.7. Of the pollen which fell on the lobes, 68.6% fell on the lobe with the highest number of grains (675), indicating that the distribution of pollen on the lobes was very uneven, one lobe receiving more than two-thirds of all pollen.

A New Method of Hand-Pollination

As insufficiency of pollen is being suggested as a contributory factor to

**Calculated from the data presented by Warmke[9].

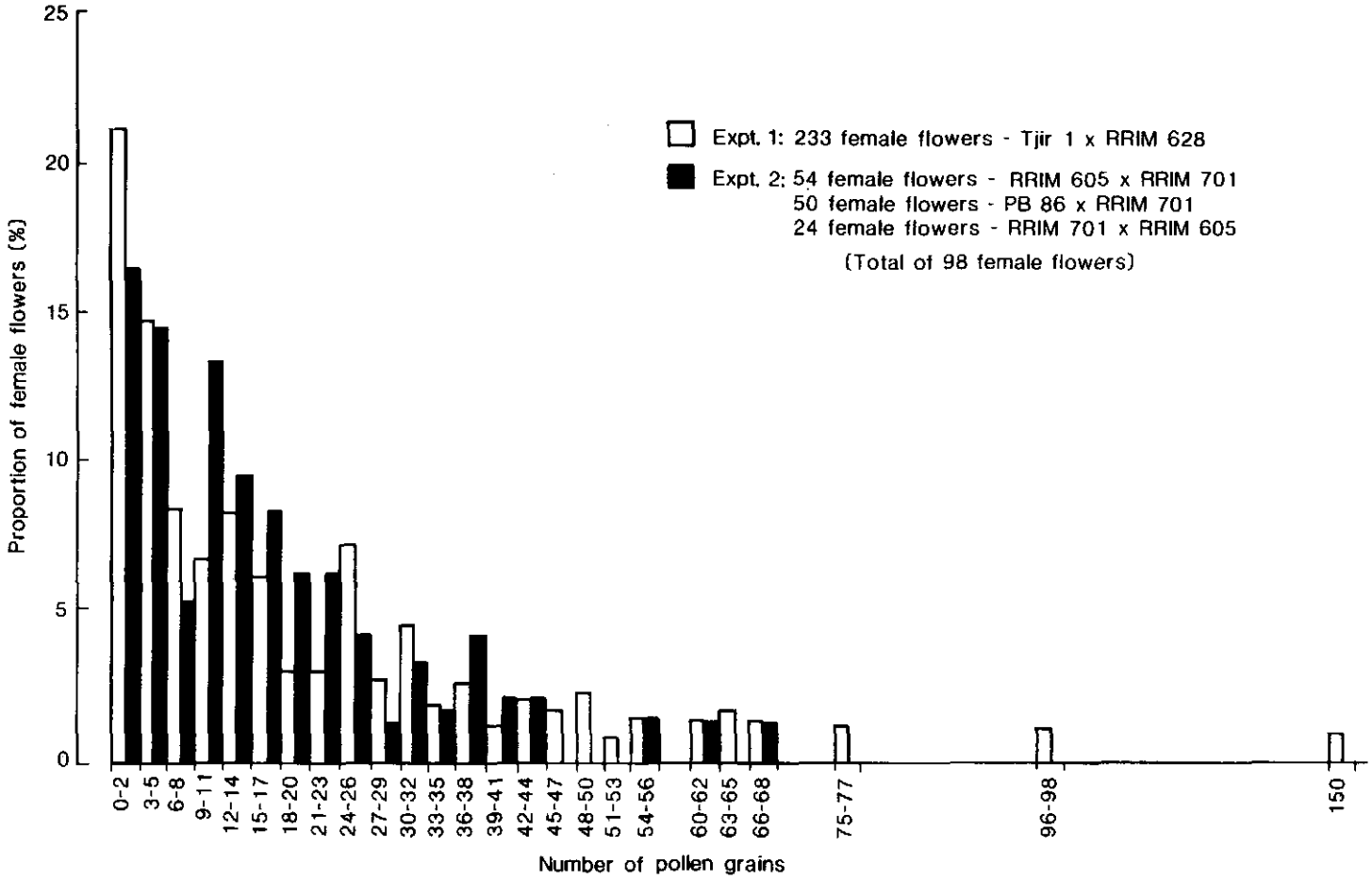

Figure 1. Number of pollen grains on the stigma, stylar and upper ovarian surfaces of hand-pollinated flowers.

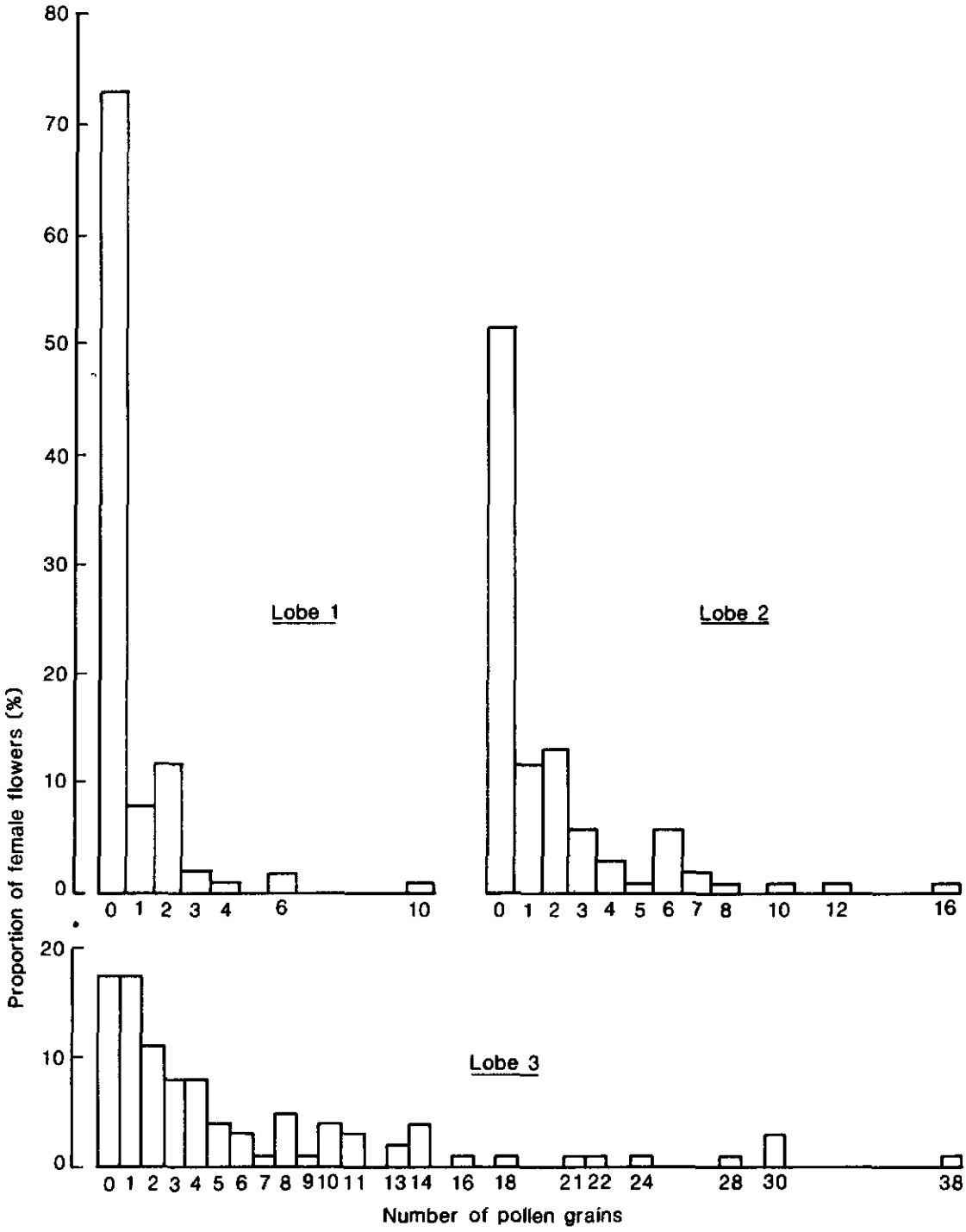

Figure 2. Distribution of pollen grains on individual stigma lobes of ninety-eight hand-pollinated flowers. Data are from Experiment 2 *(see* Figure 1 *for details). Data for the fourth stigma lobe (found in only sixteen out of the ninety-eight flowers) are not presented.*

TABLE 1. MEAN NUMBER OF POLLEN GRAINS DEPOSITED ON THE LOBES OF THE STIGMA

Distribution	Mean number of pollen grains
Per flower	
On stigma, style and upper portion of overy	25.5
On stigma lobes	10.0
Per stigma lobe[a]	
All lobes	3.2
Lobe 1	0.7
Lobe 2	1.7
Lobe 3	6.1
Lobe 4	9.3

[a]Of a total of ninety-eight female flowers, sixteen had four-lobed stigmas. The lobes were ranked so that *Lobe 1* had the smallest and *Lobe 4* the largest number of pollen grains.

low fruit set, trials were carried out on new methods of hand-pollination aimed at increasing the number of pollen grains deposited on the stigma. Initial attempts involved repeated pollination of the same flowers but these methods gave only a slight improvement in pollen grain count. Insertion of several staminal columns into each female flower during pollination posed difficulties because of the limited space in the perianth tube. It was decided, therefore, to convey the stigma to pollen rather than *vice versa.*

Staminal columns were detached from male flowers and placed in 6 X 18 mm gelatine capsules. The open half-capsules were placed under a lamp (≏ 30°C) for approximately 2 h to enhance dehiscence of the anthers. The pollen-staminal column mixture was used on the same morning for pollination. During hand-pollination, the petals of the female flower were completely removed and any exuding latex was blotted with a filter paper strip. The de-petalled flower was dipped into the mixture of pollen and staminal columns in the gelatine capsule *(Figure 3),* and this enabled large numbers of pollen grains to adhere on to the stigma. A 6 X 40 mm tube of plastic or waxed paper was slipped over the pollinated flower and then sealed at both ends with non-absorbent cotton wool to prevent extraneous pollen from reaching the stigma *(Figure 3).* The tubes were removed two days after pollination as they tended to collect rain-water during heavy showers.

Two experiments were carried out : the first during the main flowering season (March/April) and the second during the secondary flowering season (August/September) that followed. In the first experiment, each gelatine capsule contained sixty staminal columns and was used to pollinate nine flowers. In the second experiment, the number of staminal columns per capsule was reduced to forty, and each capsule was used for four pollinations. In the second experiment, a slight variation of the new hand-pollination method was also tested. Pollen grains were isolated from the staminal columns by shaking the gelatine capsule vigorously back and forth along its axis so that the pollen grains shaken loose were collected at either end of the capsule. The stigma of the female flower was thus brought into contact with the isolated pollen rather than a mixture of pollen and staminal columns.

In comparisons between the methods of pollination, the new and conventional (control) methods of hand-pollination were carried out on the same parental clones on the same days and by the same hand-pollinators.

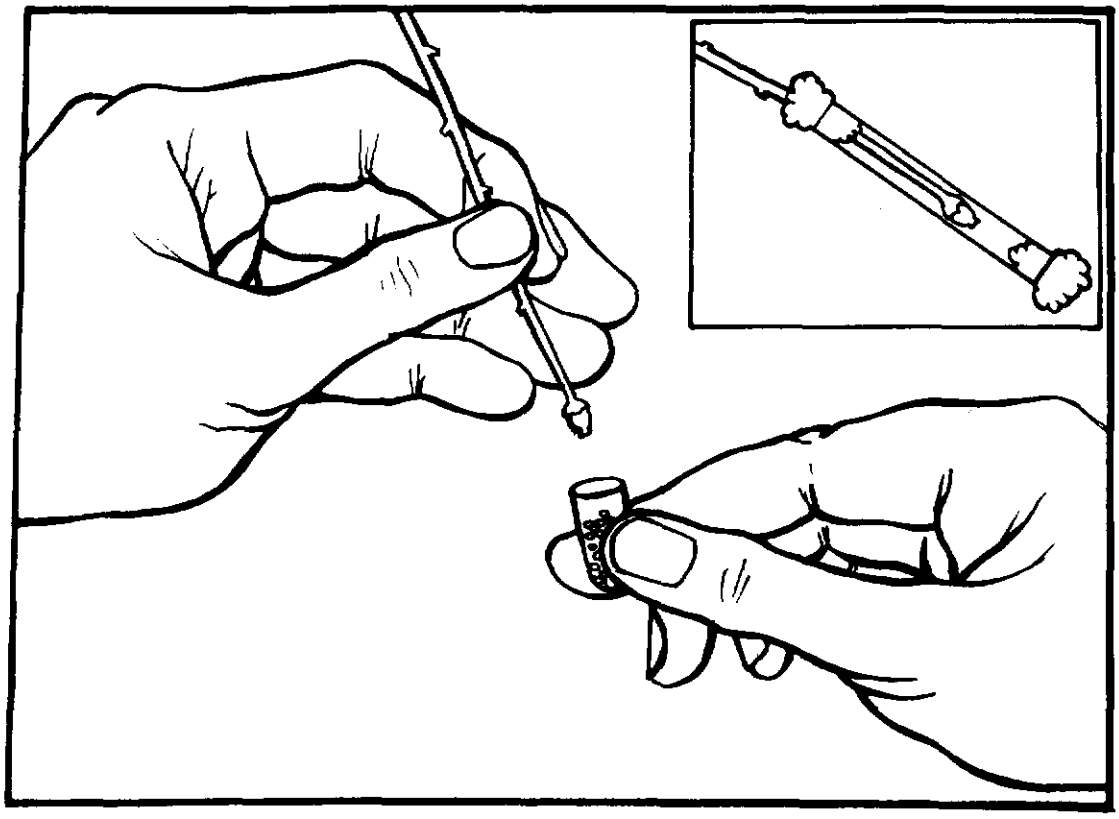

Figure 3. A new method of hand-pollination of Hevea.
Inset: A plastic tube sealed at both ends with non-absorbent cotton wool prevents extraneous pollination.

Female flowers pollinated by the new and conventional methods were regularly sampled for pollen grain count. In both pollen grain count and fruit set, no difference was observed between the two variations of the new method of pollination, where either isolated pollen or pollen-staminal column mixtures were used. As such, the results of the two variations have been combined in *Tables 2* and *3*.

Table 2 shows the number of pollen grains on the stigma of flowers pollinated by the conventional and new methods. In the first experiment, the total number of pollen grains on the stigma was increased 3.7 times when the new method of pollination was employed as compared to the control. The number of pollen grains on the stigma lobe with the fewest pollen grains was increased almost twleve times. In the second experiment, the results showed increases of five times and eight times respectively. Compared to conventional hand-pollination, the new methods increased the percentage fruit set 1.4-fold and 2.1-fold in the main and secondary flowering seasons respectively *(Table 3)*.

DISCUSSION

Pollen Count

Mature fruits of *Hevea* containing fewer than three seeds (*i.e.* fruits with one or

TABLE 2. NUMBER OF POLLEN GRAINS ON THE STIGMA OF FEMALE FLOWERS POLLINATED USING THE CONVENTIONAL AND NEW METHODS

Method of hand pollination	*Experiment 1* (Main flowering season)			*Experiment 2* (Secondary flowering season)		
	n	Mean number on stigma lobe with fewest grains	Mean total on stigma	n	Mean number on stigma lobe with fewest grains	Mean total on stigma
Conventional method	25	0.7	9.1	14	1.6	10.0
New method	25	8.4	33.6	23	13.3	53.2

n = Number of female flowers examined

The following crosses were carried out: (Figures in brackets are the approximate proportions expressed as % of the total)

Experiment 1: RRIM 605 × PB 86 (6), RRIM 628 × RRIM 623 (58), RRIM 628 × RRIM 605 (17), RRIM 600 × RRIM 501 (12), RRIM 628 × RRIM 501 (7)

Experiment 2: RRIM 605 × PB 86 (31), RRIM 623 × RRIM 605 (23), RRIM 605 × RRIM 501 (21), RRIM 501 × RRIM 605 (19), RRIM 623 × RRIM 701 (3), HPS × PB 86 (3)

more empty loculi) are rarely encountered. Muzik[8] noted that apogamy was not apparently involved in *Hevea* fruit set as no fruits were formed when young inflorescences were bagged. It seems improbable that unfertilised ovules commonly develop to mature-sized but non-viable seeds, since the viability of freshly matured seeds is high. For example, Sakhibun *et al.*[17] reported up to 100% germination in seeds of RRIM 600 harvested from fruit pods just before dehiscence. Hence, it may be surmised that all the three ovules of the female flower must form mature seeds for the fruit to develop to maturity successfully.

A theoretical minimum of three pollen grains – one deposited on each of the stigma lobes – would then appear to be an absolute pre-requisite for successful fruit set. *Figure 1* indicates that 15%–20% of the hand-pollinated female flowers had fewer than three pollen grains deposited on the stigma, style and upper portion of the ovary. These flowers, therefore, could not possibly set fruit. Further, in those flowers bearing three or more pollen grains, it seems that for successful fruit set, the grains must be well distributed, that is, each stigma lobe must have a sufficient number of grains so that all the three ovules of the flower can be fertilised. *Figure 2* shows that about 70% of the female flowers had no pollen grains at all on at least one of the stigma lobes. Thus, in the present sample of flowers, 70% of the hand-pollinations appeared certain to fail from the point of view of pollen number and distribution alone.

The percentage viability of mature *Hevea* pollen grains is on average about 50%, although on occasion it could reach 85% to 90%, and the percentage germination is lower than the percentage viability[18]. Hence, about 50% of the pollen grains that do fall on the stigma lobes, would be non-viable and a propor-

TABLE 3. FRUIT RETENTION AND FRUIT SET WITH CONVENTIONAL AND NEW METHODS OF HAND-POLLINATION

Method of hand pollination	*Experiment 1* (Main flowering season)			*Experiment 2* (Secondary flowering season)		
	No. of *pollinations*[a]	Fruit retention 4–8 weeks *after* pollination	Fruit *set*	No. of *pollinations*	Fruit retention 4–8 weeks *after* pollination	Fruit *set*
Conventional method	740	13 (1.8)	9 (1.2)	589	38 (6.4)	13 (2.2)
New method	594	17 (2.9)	10 (1.7)	550	65 (11.8)	26 (4.7)

Figures within brackets are percentages of the pollinations carried out.

Parental clones are as given in *Table 2*.

[a]In *Experiment 1*, filter paper strips were not used to blot latex exuded when the petals of the female flowers were detached. A number of pollinations were spoilt when the exuded latex covered the stigma. The proportion of pollinations damaged this way could be estimated from the flowers sampled for pollen count. The figures given for the number of pollinations is based on an estimate of the flowers not damaged by latex.

tion of this only would germinate to produce pollen tubes. Further, it is to be expected that not all germ-tubes would be capable of elongating to penetrate the style to fertilise the ovule. Hence, although theoretically, a single pollen grain should be able to fertilise an ovule successfully, in practice, more pollen grains would be required. The pollen count data presented in this paper strongly suggests that the present method of hand-pollination results in poor fruit set at least partly because too few pollen grains fall on the lobe surface of the stigma for effecting fertilisation of all three ovules.

Previously, Warmke[9,10] has published some data on *natural* pollination of *Hevea* which are of particular interest. The reported percentage fruit set in Puerto Rico, 5% or less[9], is somewhat higher than the 0.3%–1.6% reported in Malaysia[11]. Warmke's figure for *natural* pollination is, however, comparable to the average percentage fruit set for *hand*-pollination of the first flowering season in Malaysia. It seems significant that he observed a mean of nine pollen grains per stigma*** which is similar to the mean number (ten) observed on stigma lobes in the pollen counts on hand-pollinated flowers in the present study. The percentage of stigmas not bearing pollen grains in Warmke's study[9] was 23%, compared to 18% in the present study. Hence, Warmke's observations appear generally similar to and compatible with those we have presented here. Significantly, however, while Warmke from his data inferred that *Hevea* flowers in his study were adequately pollinated, we have concluded just the opposite, based on the reasons mentioned above.

Improvement of Hand-Pollination

In both experiments, the modified pollination method resulted in an increased pollen count as well as fruit set, over that of the control. This confirms that insufficiency of pollen is a contributory cause of the poor fruit set that follows hand-pollination. However, the increased fruit set was still relatively low. This indicates that other factors are also important in the impediment of fruit set.

Further trials were required to determine if the new method(s) of hand-pollination can be put into routine practice. In this connection, the increased fruit set has to be balanced against the additional costs involved in removing staminal columns from male flowers and in removing the protective tubes from the pollinated female flowers.

CONCLUSION

While the influence of climatic and other factors should not be discounted, the pollen counts of the present study show that an insufficiency of pollen grains deposited on the stigma, and further, an uneven distribution of the deposited pollen grains among the stigma lobes, are probably contributory causes of poor fruit set following hand-pollination in *Hevea*. In agreement with this proposition, a new method of hand-pollination, whereby a larger number of pollen grains was deposited on the stigma than in the conventional method, resulted in a higher percentage fruit set.

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***Calculated from data presented by Warmke[9].

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