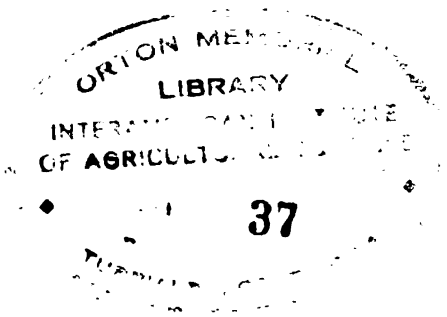
POLLINATION OF CACAO IN COSTA RICA

by

ANTON G. SMIT



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BIOGRAPHY

Anton G. Smit was born on January 4, 1926 in Paramaribo, Surinam and attended the primary and secondary schools in that country. His secondary education was interrupted when he obtained a Junior Teacher's certificate in 1942 and worked as a teacher in a primary school in Paramaribo for one year.

In 1945 he obtained a scholarship and went to the Imperial College of Tropical Agriculture in Trinidad from which he graduated in 1948.

After the completion of his studies in Trinidad, he was employed by the Agricultural Department of Surinam.

In January 1949 he enrolled in the Cacao Center of the Inter-American Institute of Agricultural Sciences in Costa Rica.

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INTRODUCTION

Only a small percentage of the flowers produced by a cacao tree develop into fruits, this percentage varying in the different countries, but seldom passing 5%. Van Hall (5) cited the following figures: 1.4% (Jones), 4.3% (v. Hall), 1.8% (Harland), 5.7% (Stahel).

Dropping of the flowers is not due to a defective constitution of the ovary or of the pollen, but probably to lack of pollination. It may be assumed that the majority of the cacao flowers never receive sufficient pollen to develop into a fruit.

An increase in production might thus be effected if this percentage could be increased. To achieve this a knowledge of the mechanism of pollination is necessary.

Early workers were content to suggest that self-pollination occurred either in the bud or with the aid of wind. These theories have since been largely discarded because the former assumption was not founded on facts, while the flower-structure and sticky nature of the cacao pollen make wind pollination unlikely.

The discovery of self-incompatibility, in 1931, brought out the importance of the ways in which the cacao flowers are pollinated and made investigation on the subject urgent.

Although evidence on the methods of pollination has accumulated, it is still not conclusive. That cross-pollination occurs, and that both crawling and flying insects are involved,

has been definitely indicated, but comparatively little is known regarding the actual agents of pollination.

Investigators in the different cacao producing countries have found different insects to be involved in pollination. The insects are, however, limited to a few groups, comprising but a few species of aphids, thrips, ants, and a Ceratopogonid midge.

The lack of agreement on any particular insect as the major agent of pollination of cacao necessitates investigation of the subject in each country separately. This work is a study of factors influencing pollination, and is intended to collect evidence regarding the agents of pollination in Costa Rica.

As no work has been done, to the knowledge of the author, on the time the cacao pollen remains viable, and he thought this important, especially with regard to cross-pollination, he carried out some experiments to determine this. Further he thought this important in breeding programs where pollen might have to be transferred from one country to another for crossing purposes.

REVIEW OF LITERATURE

Opinions are divided as to the method of pollination. De Haan (4) wrote that insects are of minor importance as pollinating agents in Java, and that pollination takes place with the aid of wind. Van Hall (5) believed wind to be one of the most important pollinating agents and stated in support, that the number of flying insects visiting the cacao flowers, both in Surinam and in Java, was exceedingly small. On the other hand, he mentioned that pollen falls from the hanging flower rather easily when the flowers are shaken. No experimental evidence was, however, accumulated by them to support their belief, so that these can be classified as just personal opinions.

Harland (7) and Stahel (12) have blown on and shaken flowers to simulate wind action, but have never transferred pollen to the stigmas in this way. They both concluded that insects are the pollinating agents. Stahel indicated that ants, aphids, and an unidentified flying insect were the likely pollinators.

Voelcker (13) reviewed the literature on pollination up to 1937. He indicated ants, thrips, and aphids as the insects responsible, these being the only insects found in large numbers in the flowers.

Cope (2), in Trinidad, followed the trends of fruit-setting and of insect populations in the flowers of self-sterile and self-fertile trees. He did not find any

significant difference in the insect population of the two types. The correlation between aphids (Toxoptera aurantii B. de Fons) and fruit-setting was negative; that between ants (Wasmannia auropunctata Reg.) and thrips (Frankliniella parvula Hood) positive. He pointed out that the correlation between ants and fruit-setting on self-sterile trees was probably an anomaly. This also casts doubt on the significance of the correlation between thrips and setting.

Voelcker (14) concluded that at least 25% of the fruit-setting on self-fertile trees at Ibadan, Nigeria, was the result of cross-pollination, provided that the trees were uniformly surrounded by others. He concluded further that cross-pollination probably takes place between neighbouring trees. This is at variance with Cope's (3) conclusion, that in Trinidad, self-sterile trees are not dependent on their immediate neighbours for fertile pollen.

In Trinidad, Voelcker (15) estimated up to 50% of cross-pollination on self-compatible trees, using Harland and Frecheville's (6) hypothesis regarding the genetics of axil spot. The variation between results in Nigeria and Trinidad indicates a difference in the effectiveness of the cross-pollinating agents.

Posnette (9) found two species of Crematogaster ants pollinating cacao in the Gold Coast, but also showed that flying insects were responsible for at least half the fruit-setting on the experimental trees and concluded that these flying insects were more effective pollinators.

Billes (1) conducted experiments which indicated that wind and water are not pollinating agents. He investigated the insects associated with cacao flowers in Trinidad and confirmed earlier findings that sticky bands have little effect on fruit-setting. He expressed doubt that aphids played an important role in the pollination and setting of fruits. He stated further, that although thrips action might result in some self-pollination, it was doubtful that they carried sufficient pollen to cause cross-pollination. The thrips population showed marked seasonal variation. Further he drew attention to the Ceratopogenid midge, Forcipomyia sp., which Posnette (11) called the most important contribution to the literature on the pollination of cacao flowers.

Posnette (11) concluded that Forcipomyia was responsible for most of the effective pollination of cacao flowers in Trinidad.

Muntzig Arne (8) stated that aphid-visited flowers were better pollinated in Ecuador than flowers not visited by aphids.

MATERIALS AND METHODS

Location of Investigations

The investigations were carried out with materials from two locations namely El Chino experimental plot and La Lola farm.

El Chino: This is an experimental and demonstration cacao plot consisting of eight clones of the United Fruit Co.

selections. The trees are about four years old. It is located at Turrialba on the Institute grounds near the Reventazon river at an elevation of approximately 600 meters. The total rainfall over the year 1949 was 3255.1 mm. and the average temperature 22.3° C.

La Lola: This farm is located near Madre de Dios some 55 kilometers from Turrialba, in the Atlantic coastal lowlands. Prior to 1948 this was a commercial cacao farm. The majority of the cacao trees are between 32 and 34 years of age. The total annual rainfall for 1949 was 4024.63 mm. Elevation of the farm is from 24 to 56 meters.

Germinating tests were carried out in the Institute laboratory.

Water and Wind Pollination

Examination of dew water in the flowers and of water accumulated in the flowers after rain was done by approaching the drop with a dry microscope slide and touching it with the slide to transfer the water from the bottom part of the drop to the slide. The slides were then taken to the laboratory for examination to determine the presence of pollen.

Stickiness of pollen in the anthers: To determine the degree of stickiness of the pollen in the anthers, open anthers, full of pollen, were placed under a dissecting microscope and clamped firmly with the four pollen sacs pointing upwards. Someone then blew on the anthers horizontally against the pollen, while the behaviour of the pollen grains

was watched constantly by the observer through the microscope. The blowing was done softly at first, then harder, and finally in soft and hard puffs.

Hand Pollination

For a proper understanding of hand pollination, the structure of the cacao flower must be considered. This is also of importance when considering wind pollination.

Structure of the cacao flower: The cacao flower is pentamerous with five, white or rosy coloured sepals and five petals. The petals have a very peculiar form, consisting of a basal, cup-shaped part, to which is attached a ribbon-shaped ligule spatulate at its end. The basal part is white or rosy with three dark carmine ridges running longitudinally on the inside. The center ridge starts higher up in the cup. The ribbon-shaped part is yellowish. The five stamens are arranged alternately with five staminodes. The stamens and staminodes are united at their base to form a tube. The awl-shaped, hairy staminodes are dark carmine coloured and protrude above the petals and the pistil like five little horns. The five stamens are whitish, much shorter than the staminodes and reflexed, so as to hide the anthers in the cup-shaped part of the petals in such a way, that the anthers face upwards in spite of the natural downward position of the flowers. Each stamen bears two 2-celled anthers. The ovary is superior, comprising one compound pistil with five indistinct stigmas more or less coalesced.

Techniques: Whenever artificial pollination had to be done the open flowers were removed the day before the pollination was carried out, with a twofold purpose: (i) to have fresh flowers for the tests and (ii) to assure that no flowers already pollinated the day before were included in the tests. This last precaution was of great importance as the flowers were in no way protected to avoid natural pollination.

To carry out artificial pollination, the petal was pinched loose from the receptacle at the junction with sharp-pointed forceps, then carefully lifted from the stamen so as not to lose more pollen than necessary. Once the stamen was free, this was pinched off and the anthers rubbed against the stigmas. When necessary, several anthers were used, until the pollen could be observed with the naked eye. This, though it increased the accuracy, could sometimes be misjudged when the stigmatic lobes separated, but was the most accurate way transferance of pollen could be effected and judged.

In selfing with pollen of the same flower, the anthers of the same flower were used. In cross-pollinations the anthers came from a different flower.

Collection of Insects

In collecting insects to determine the insect population in the cacao flower, the following method was used: A wide-mouthed bottle, containing 80% alcohol, was held under the flower without disturbing it in any way. The flower was then rapidly cut off with a pair of scissors at about the middle

of the pedicel, and on falling into the alcohol, the lid was replaced rapidly on the bottle. Each sample collected for insect counts consisted of 10 flowers.

The samples were taken in 2 different sections of La Lola; section #7 consisting of upruned trees, and section #1 with pruned trees. Fortnightly collections were made consisting of 5 samples in each section. The samples were taken fortnightly because the life cycle of Frankliniella parvula Hood is about 14 days. The collections for the general insect counts were always made at about 10 a. m.

Some collections were made at about 7 a. m., 12 noon, and 5 p. m. to find out if the density of insect population in the flowers differed at different times of the day. In this case 3 samples of 10 flowers each were taken at each time.

After the samples were obtained they were taken to the laboratory and the insects carefully separated from the flowers, grouped by classes, and counted. It was observed that very seldom an insect remained in the flower itself, all floating in the alcohol, so that a dissection of each flower was discontinued and only a superficial examination of each carried out. If any insect was present in the petal cup, this could easily be observed as it stood out as a dark spot against the cup.

Germination of Pollen

The pollen used in the germinating tests was collected

from clonal trees in El Chino.

Collection of samples: The samples were collected at about 9 a. m. On the preceding day, all open flowers were removed from the trees selected for sampling. Each sample contained 3 flowers, which were placed in a test tube and taken to the laboratory. The test tubes were left open all the time.

Agar preparation and arrangement of experiments: Several media were tried before the tests were set out. A saturated atmosphere gave a good but low percentage of germination. Liquid water failed to give any germination, either pure or as an extract of macerated stigmas and styles. Two percent agar with 5% sucrose gave excellent germination, but commercial white sugar was used and as this may contain contaminations, it was decided not to use it in the tests. It was finally decided to use 2% agar and 5% dextrose which gave a good germination.

After the agar-dextrose was prepared it was put in Erlenmeyer flasks before sterilization. For each experiment another flask was used since it had been noticed that continual boiling to liquify the agar for setting up the tests could have a detrimental effect on germination.

To prepare the agar for setting out the pollen samples for germination, it was boiled some hours before, then poured on a slide and covered with another so as to obtain an even, flat surface to deposit the pollen on.

For each test, a small square of agar was cut out and put on a slide. Then a stamen was removed from the flower and touched slightly on the agar so as to deposit pollen. If necessary, several anthers were used. The samples were then put into covered petri-dishes to avoid fungus contamination. After at least 3 hours a germination count was made of the samples.

In counting, a distinction was made between the grains with a germination tube longer than 1 pollen grain diameter and those grains with a germination tube shorter than 1 pollen grain diameter. In the analysis, however, the total of the two was used.

Three sets of experiments were laid out. In the first test, a random pollen grain sample was taken to determine the germination life of the pollen. Each flower sample consisted of 3 individual flowers from three different clones. The design of this experiment was that of a randomised plot-layout with six treatments and five replications. Taking 7 a. m. as the time the anthers are completely opened in El Chino, the actual time falling somewhere between 5 and 7 a. m., samples were put in at 7 a. m., 7 p. m., etc. giving a time interval of 12 hours between treatments. The treatments included pollen 12, 24, 36, 48, 60, and 72 hours old. This experiment was duplicated one day later.

The second test was similar in layout, but here the treatments used pollen 36, 48, 54, 60, 72, and 78 hours old.

The third set of experiments was a test of pollen viability differences between clones, laid out as a 3 x 6 factorial experiment with 3 clones: 613, 11, and 12 and six ages of pollen as in experiment 1. Four replications were used for each clone. The "clones" 11 and 12 are not pure clones, but in this experiment are referred to as clones. The implications of this are discussed later. This experiment was repeated.

RESULTS: AGENTS OF POLLINATION

In considering the agents of pollination, the assumption had to be made, that pollen of a flower can cause pollination in that same flower. To check this assumption, hand pollinations were carried out. of 32 flowers selfed in this way on several trees, 26 developed into fruits, giving a total of 81.25 %.

Cross-pollination between a self-incompatible tree and a self-compatible tree, using the self-sterile tree as male parent was shown to be possible. Of 25 flowers pollinated, 13 developed into fruits i.e. 52 %.

Water Pollination

Examination of dew water or rain water for pollen showed that pollen seldom floats from the anthers to the bottom of the drop and never a sufficient amount to cause pollination. Of 50 slides examined for pollen (25 from dew and 25 from rain drops) 46 were without any pollen grains and in the remaining 4 only 8, 10, 11, and 13 grains were counted respectively.

Wind Pollination

Shaking 100 flowers without protecting them against natural pollination resulted in 3 sets i.e. 3 %.

Blowing against flowers from different directions resulted in 4 % setting (100 flowers with 4 sets).

Blowing horizontally from a flower held at about 1 foot at the same level of the flower to be tested, resulted in 4 % setting (100 flowers with 4 sets).

Blowing against anthers under the microscope never resulted in pollen leaving the anthers.

Insect Pollination

Tables 1 and 2 show the distribution of insects found in the flowers over the period May-November 1949 in La Lola. Thrips (Frankliniella sp.*) are by far the most common insects in the cacao flowers, followed by ants.

TABLE I. INSECT DENSITY IN CACAO FLOWERS IN LA LOLA OVER THE PERIOD MAY-NOV.1949 SECTION I PRUNED TREES.

Date	Average number per ten flowers			
	Thrips	Ants	Flies	Other insects
26- 5-49	44.6	0.0	0.0	0.2
9- 6-49	4.8	0.8	0.2	0.0
23- 6-49	2.0	[illegible]	0.0	0.2
7- 7-49	4.8	1.[illegible]	0.2	[illegible]
22- 7-49	1.6	[illegible]	0.0	0.0
18- 8-49	29.6	0.6	0.2	[illegible].0
8- 9-49	17.2	0.0	0.4	[illegible]
22- 9-49	3.2	0.8	0.2	0.2
7-10-49	28.7	0.0	0.0	0.0
21-10-49	116.0	0.3	0.0	0.3
4-11-49	25.7	0.0	0.0	0.0
17-11-49	19.3	0.3	0.0	0.3

*Identification done by USDA Bureau of Entomolo y and Plant Quarantine. One sp cies of thrips of both sexes.

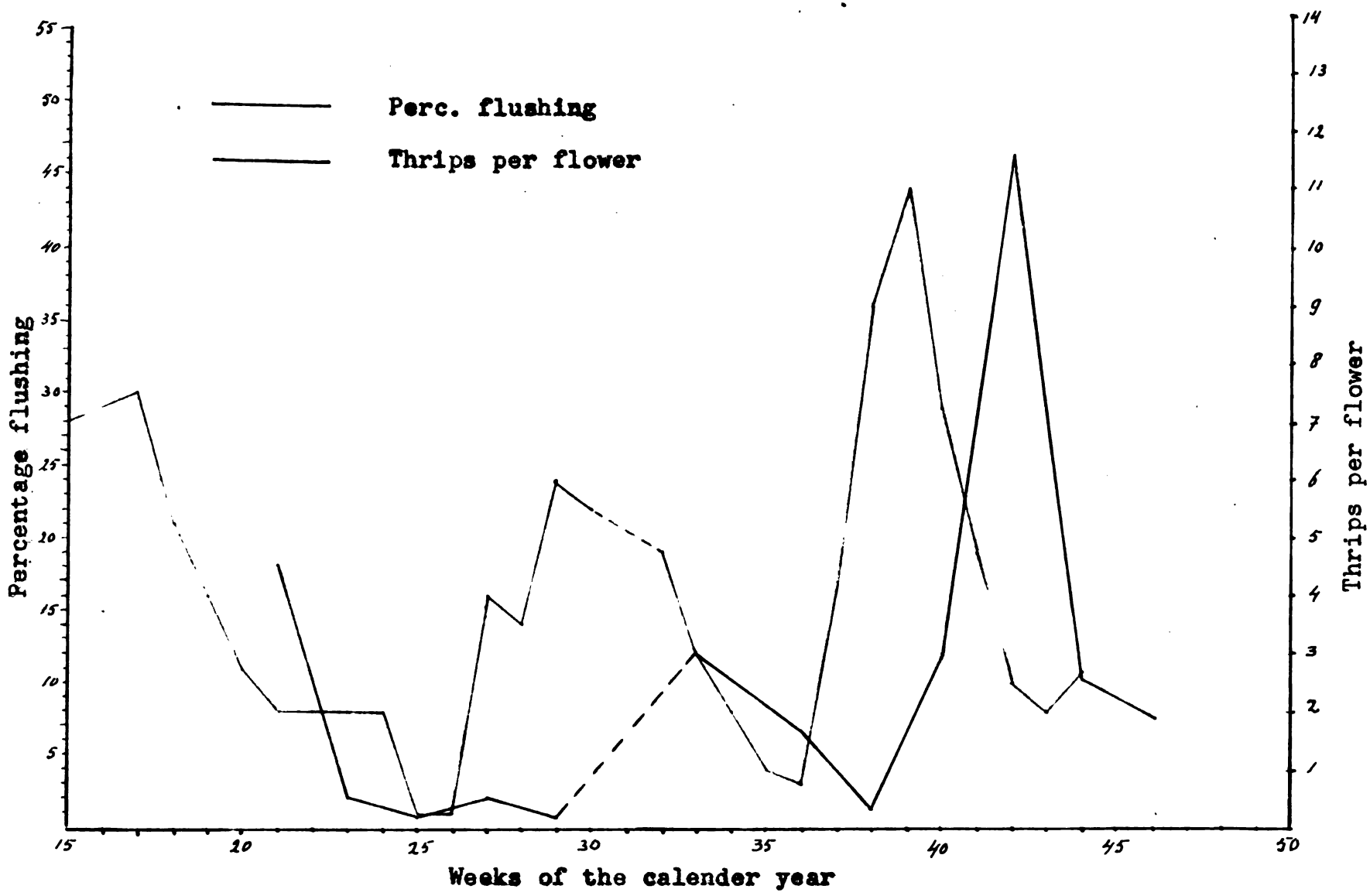
TABLE 2. INSECT DENSITY IN CACAO FLOWERS IN LA LOLA OVER THE PRIOD MAY-NOV.1949 SECTION VII UNPRUNED TREES.

Date	Average number per ten flowers			
	Thrips	Ants	Flies	Other Insects
26- 5-49	40.8	0.0	0.0	0.8
9- 6-49	10.2	1.0	0.4	0.2
23- 6-49	3.0	0.6	0.2	0.2
7- 7-49	8.2	0.6	1.2	0.2
22- 7-49	5.8	0.0	0.2	0.2
18- 8-49	31.2	0.4	0.4	0.0
8- 9-49	6.2	0.2	0.4	0.0
22- 9-49	5.6	0.0	1.6	0.0
7-10-49	74.7	0.0	1.7	0.3
21-10-49	121.0	0.0	1.3	0.0
4-11-49	15.3	1.3	1.0	0.0
17-14-49	14.7	1.0	1.0	0.0

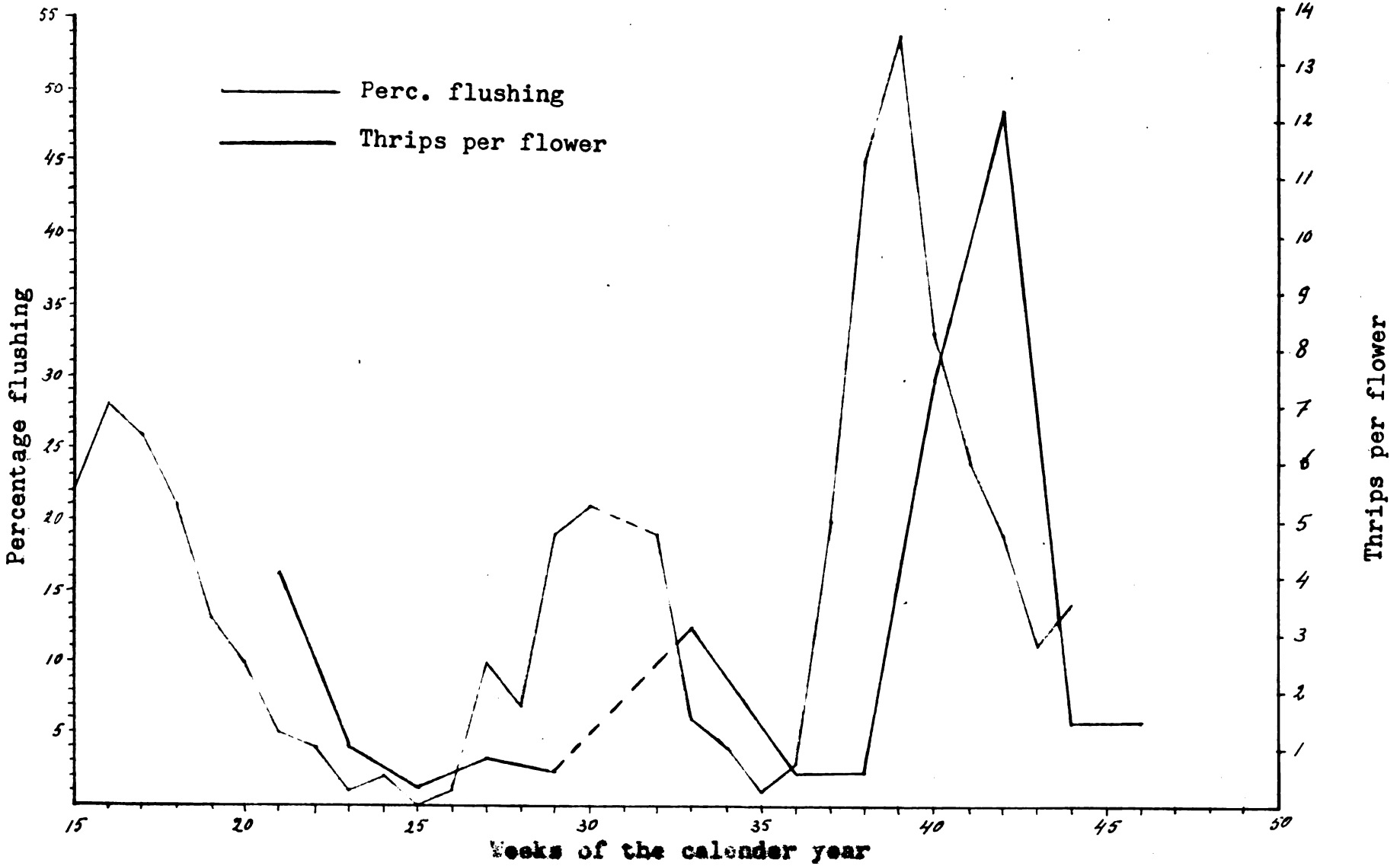
Unfortunately, the ants were not divided up into different species so that the larger black ants as well as the small red ones are included. Likewise, the flies were not divided up into species or genera so that their number gives only an idea of the flying insects visiting the cacao flowers. Some answered the description of Forcipomyia given by Billes (1).

In the column "other insects" are included those commonly encountered such as nymphs and adults of Jassids and Membracids plus small spiders, but these were usually present only on the flower stalk.

The density of the thrips showed a close correlation with flushing as shown by the graphs 1 and 2. The flushing percentages represent averages from 50 trees in each section, the averages



GRAPH. 1. PERCENTAGE OF CACAO TREES FLUSHING AND NUMBER OF THRIPS PER FLOWER IN LA LOLA FROM APRIL 1949 UNTILL NOV. 1949. SECTION I, PRUNED TREES.



GRAPH. 2. PERCENTAGE OF CACAO TREES FLUSHING AND NUMBER OF THRIPS PER FLOWER IN LA LOLA FROM APRIL 1949 UNTILL NOV. 1949. SECTION VII UNPRUNED TREES.

being determined from visual judgements classified as 5, 25, 50, 75, and 100 % flushing for each tree.

The insect population showed a variation at different times of the day only in the case of thrips, see Table 3.

TABLE 3. DENSITY OF INSECTS PER 10 FLOWERS AT 7 a.m. 12 NOON AND 5 p.m. AT LA LOLA.

Date	Thrips			Ants			Flies			Other insects		
	7	12	5	7	12	5	7	12	5	7	12	5
*3-6-49	17.3	52.5	26.5	2.3	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16-6-49	5.3	3.7	10.7	0.0	0.0	0.0	0.0	0.0	0.3	1.3	0.0	0.0
30-6-49	1.3	6.3	8.7	1.0	0.3	0.7	0.0	0.0	0.3	0.0	2.7	0.0
14-7-49	2.7	6.3	6.7	1.7	0.3	0.7	0.0	0.0	0.0	0.3	2.3	0.0

This variation, however does not show a statistically significant difference.

TABLE 4. ANALYSIS OF VARIANCE OF DENSITY OF THRIPS IN CACAO FLOWERS AT 7 a.m. 12 NOON AND 5 p.m. AT LA LOLA.

Factor	S.S	D.F.	Variance	F
Treatment	498.7	2	249.35	1.56
Error	4793.8	30	159.79	
Total	5292.5	32		

* Because of insufficient flowering only 2 samples of 10 flowers each were taken.

DISCUSSION: AGENTS OF POLLINATION

Water Pollination

In the early morning, especially following clear nights, the flower is often filled with dew. Pollen could float from the petal hood to the bottom of the dew drop which because of the hanging position of the flower is around the stigma and staminodes. On drying, the pollen could be deposited on the stigma and cause pollination. Estimates of pollination percentages made by Posnette (9) showed that pollination does not occur before 9 a.m., though Stahel as cited by v. Hall (5) put the time pollination takes place between 6:30 a.m. and 9 a.m.

Water accumulated in the flowers after rain could effect pollination in the same way as dew water. Billes (1) estimated pollination before and after rain, did not find any differences and concluded that rain did not have any effect either favourable or detrimental.

Direct examination of dew water and rain water in the flower showed that pollen grains are encountered very seldom in this water, and never in sufficient amounts to cause pollination, taking ten pollen grains as a minimum needed. Also, the few grains encountered have a great chance of being deposited on the staminodes, instead of on the stigma.

Wind Pollination

The structure of the cacao flower, especially the form of the petals and the enclosed position of the anthers, the hairy staminodes encircling the pistil, further, the sticky nature of

the pollen are strong indications that wind does not play an important, if any role at all, in the pollination of cacao.

The majority of the investigators exclude wind as a pollinating agent. However, some still consider wind pollination a possibility, e.g. de Haan (4) and v. Hall (5). The experiments carried out proved conclusively that wind does not effect pollination. The small percentage sets of 3 % and 4 % in the tests are without doubt due to natural pollination by insects, as the flowers were in no way protected to avoid this and the percentages coincide with what is to be expected, without any interference with the flowers.

Though by tapping the flowers some pollen can, in some cases, be seen falling from the flowers, this pollen is usually united into clusters and falls more rapidly than the single grain diameter suggests. The mean diameter of the pollen grain (see appendix F) is 21.4µ and falls thus in the group of good floaters 17µ-58µ as given by odehouse (17). Though the single grains can float thus for a considerable time, the amount of pollen produced per flower is comparatively small, and from this amount only a small part ever leaves the flower unaided by other means. The chances are further extremely small to negligible that a floating grain would reach a flower and even if it should reach the flower that it would reach the stigma, as the staminodes would probably intercept the grains.

The examination of open anthers with pollen which were blown against shows conclusively that wind by itself is not

able to remove pollen grains from the anthers.

Insect Pollination

All the major insects quoted in the different countries as agents of pollination are present in Costa Rica, but their frequency here is different from that in other countries. Forcipomyia sp. was not directly proved to be present, but indirect evidence showed that it probably plays an important role here also.

Thrips: The Thrips were very abundant in the cacao flowers in Costa Rica over the period of the investigation. This does not prove that it always is like this, as the climatic conditions in 1949 in Costa Rica appear to have been different from an average year. This was evident in the small flower production of the cacao trees throughout the year. However, comparing the thrips population here with that of Trinidad over 1938-39 as given by Cope (2), one observes that the peaks coincide closely as far as time of year is concerned, but the density in Costa Rica was about 48 times higher. On October 21, 1949 the highest number collected here was 12.1 thrips per flower as against 25.3 thrips per 100 flowers in Trinidad on October 18, 1938. Billes (1) put the highest number of thrips per 100 flowers in Trinidad as being over 200. These figures show clearly that thrips play here a more important role than in Trinidad, at least in self-pollination.

As to how far they can effect cross-pollination is difficult to determine. According to Billes (1) they

vibrate their wings prior to flying and by so doing shed the pollen they carry scattered over their body. The truth of this is not established. However, they do not carry pollen only on their dorsal side, as pollen was observed quite often on the abdomen and legs of the thrips. If the thrips do not shed all their pollen in flying, they can play a much bigger role in cross-pollination than up till now attributed to them. About 50% of the flowers fall off before 24 hours after opening. Of 152 flowers observed, 70 dropped before 24 hours after opening (46.3%). In Trinidad (16) 80% of unpollinated flowers fall off the day after opening. The pollen at this time is still viable (see section pollen viability) and the thrips, falling with the flowers, have at least a 50% chance to reenter a flower on another tree, and so effect cross-pollination.

The thrips population shows a correlation with the flushing of the cacao trees (graphs 1 and 2), as the young leaves provide also a major source of food material for the thrips. When flushing is general there is an abundance of food. This builds up a large population of thrips which migrate to the flowers after the flushing stops and the leaves grow older, so we get large numbers of thrips in the flowers about three weeks after a peak flush, as at this age the leaves are hardening up and are not used again as food by the thrips.

Ants: Ants do not seem to play an important role in Costa Rica in the pollination of cacao. The highest number encountered per 10 flowers was 1.6 and a great part

of these were the big, active, black ants, which because of their size only can be excluded as pollinators of cacao. The small red ant can effect self-pollination, but its frequency in the flowers does not suggests it as an important pollinator.

Aphids: It is doubtful if aphids play any role as pollinators here. They occur mainly on the flower stalk and do not move about to any extent. Only on rare occasions were aphids actually encountered in the flowers. An individual flower could be pollinated by them, but as a general agent they are of no importance.

Flying insects: Various flying insects were trapped in the collections, but their role could not be established. The method used in collecting the insects was not a very good one for trapping flying insects. On several occasions, a fly answering the description of Forcipomyia was noticed, but direct proof could not be otherwise obtained of its importance. It has, however, a characteristic way of depositing the pollen in clusters or streaks on the stigma or style respectively, which was noticed before the midge was discovered. Indirect proof of its presence and importance was obtained by using this fact, on examination of styles and stigmas of flowers, about 20% showed the pollen in the characteristic cluster or streak. The rest had the pollen scattered over the pistill in a manner suggesting thrips action. The scattered pollen consisted in most cases of a limited number of grains, whereas the pollen deposited in clusters and streaks was

always plentiful. This confirms also the available reports that Forcipomyia is a very effective pollinator.

RESULTS: POLLEN VIABILITY

The data pertaining to the following experiments on pollen viability are added as appendices.

Experiment I: The results of this experiment are presented in Tables 5 and 6. Table 6 showing the mean percentage germination at the different ages. The pollen showed a highly significant difference in germinating power at the different ages. There is no significant difference in viability of pollen of 12, 24, and 36 hours old. The decline in viability starts at 48 hours, dropping rapidly at higher ages.

TABLE 5. ANALYSIS OF VARIANCE OF GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Factor	S.S.	D.F.	Variance	F.
Treatments	9006.64	5	1801.33	14.19 **
Error	3045.56	24	126.90	
Total	12052.20	29		

TABLE 6. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Age in hours	Mean in %
12	48.8
24	45.5
36	52.4
48	27.3
60	17.3
72	5.8

L.S.D. at 1% is 19.91
L.S.D. at 5% is 14.68

Experiment I-A: This experiment is a duplicate of experiment I and was carried out one day later. The results as shown in Tables 7 and 8 confirm the findings obtained in experiment I, showing no significant differences in germinating power in pollen of 12, 24, and 36 hours old and a decline starting at 48 hours.

TABLE 7. ANALYSIS OF VARIANCE OF GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Factor	S.S.	D.F.	Variance	F
Treatments	4922.67	5	984.53	10.24 **
Error	2072.68	24	86.36	
Total	6995.35	29		

TABLE 8. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Age in hours	Mean in %
12	31.5
24	37.8
36	30.3
48	9.6
60	11.6
72	4.1

L.S.D. at 1% is 16.44
L.S.D. at 5% is 12.13

Experiment I+I-A combined: The results of the combination of experiments I and I-A into one analysis (Table 9) show more clearly that the decline in germinating power starts at the age of about 48 hours. Further it shows that there is a difference in germinating power at different times. As these experiments were carried out with only one day interval, it shows that the pollen viability

fluctuates even from day to day.

TABLE 9. ANALYSIS OF VARIANCE OF GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Factor	S.S.	D.F.	Variance	F
Treatments	13111.68	5	2622.34	24.60**
Time	2172.20	1	2172.20	20.37 **
Interaction: Time x Treatm.	817.63	5	163.53	1.53
Error	5118.24	48	106.63	
Total	21219.75	59		

TABLE 10. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN AT DIFFERENT AGES. COMBINED FROM EXP. I AND I-A.

Age in hours	Mean in %
12	40.15
24	41.65
36	41.31
48	18.43
60	14.41
72	4.93

L.S.D. at 1% is 12.42
L.S.D. at 5% is 9.30

Experiment II: This experiment was carried out with a closer age interval of the pollen to check the degree of loss in viability of pollen older than 48 hours, but 48 hours still shows to be the critical age of cacao pollen with regard to its germination power.

TABLE 11. ANALYSIS OF VARIANCE OF GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Factor	S.S.	D.F.	Variance	F
Treatment	1965.89	5	393.18	8.3**
Error	1166.30	24	48.60	
Total	3132.19	29		

TABLE 12. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Age in hours	Mean in %
36	21.2
48	22.3
54	10.7
60	4.3
72	4.9
78	2.6

L.S.D. at 1% is 12.33
L.S.D. at 5% is 9.10

Experiment III: This experiment was carried out to find out if there existed any difference between clones with regard to pollen viability. The results as expressed in Tables 13, 14, and 15 show a highly significant difference, the pollen of Clone 12 showing a greater germination power than either Clone 11 or 613.

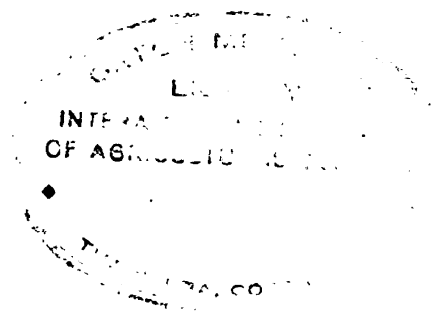


TABLE 13. ANALYSIS OF VARIANCE OF GERMINATION OF CACAO POLLEN OF DIFFERENT CLONES AT DIFFERENT AGES.

Factor	S.S.	D.F.	Variance	F
Clones	2693.29	2	1346.65	10.93**
Age	6584.33	5	1316.86	10.69**
Interaction:				
Age x Clones	2774.38	10	277.44	2.25*
Error	6651.85	54	123.18	
Total	18703.85	71		

TABLE 14. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN OF DIFFERENT CLONES.

Clones	Mean in %
613	12.16
11	7.39
12	22.08

L.S.D. at 1% is 8.58
L.S.D. at 5% is 6.44

TABLE 15. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Age in hours	Mean in %
12	26.01
24	20.15
36	22.11
48	10.98
60	3.40
72	0.60

L.S.D. at 1% is 12.16
L.S.D. at 5% is 9.12

Experiment III-A: To check the results obtained in experiment III this experiment was duplicated one day later. The results obtained as presented in Tables 16, 17, and 18 confirm the earlier findings with regard to differences

between clones and also to differences between ages.

TABLE 16. ANALYSIS OF VARIANCE OF GERMINATION OF CACAO POLLEN OF DIFFERENT CLONES AT DIFFERENT AGES.

Factor	S.S.	D.F.	Variance	F.
Clones	2913.95	2	1456.98	40.42**
Age	18215.70	5	3643.14	101.06**
Interaction:				
Clones x Age	2087.34	10	208.73	5.79**
Error	1946.47	54	36.05	
Total	25163.46	71		

TABLE 17. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN OF DIFFERENT CLONES.

Clones	Mean in %
613	9.23
11	12.70
12	24.08

L.S.D. at 1% is 4.63
L.S.D. at 5% is 3.47

TABLE 18. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN AT DIFFERENT AGES.

Age in hours	Mean in %
12	49.00
24	15.43
36	13.54
48	9.62
60	4.09
72	0.43

L.S.D. at 1% is 6.56
L.S.D. at 5% is 4.92

Experiment III+III-A combined: The combination of these two experiments into one analysis of the data as presented in tables 19,20, and 21 confirms the earlier

findings. Comparing these results, however, with Table 9 the striking difference is the lack of significance in the time factor.

TABLE 19. ANALYSIS OF VARIANCE OF GERMINATION OF CACAO POLLEN OF DIFFERENT CLONES AT DIFFERENT AGES AND DIFFERENT TIMES.

Factor	S.S.	D.F.	Variance	F.
Clones	5194.24	2	2597.12	32.62..
Age	21118.11	5	4223.62	53.05..
Time	78.33	1	78.33	--
Interactions:				
Clones x Age	2803.83	10	280.38	3.52..
Clones x Time	413.00	2	206.50	2.59
Age x Time	3681.92	5	736.38	9.25..
Cl. x Age x Time	2057.88	10	205.79	2.58 ..
Error	8598.33	108	79.61	
Total	43945.64	143		

TABLE 20. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN OF DIFFERENT CLONES OF EXP. III III-A.

Clones	Mean in %
613	10.70
11	10.05
12	23.10

L.S.D. at 1% is 4.78
L.S.D. at 5% is 3.61

TABLE 21. MEAN PERCENTAGE GERMINATION OF CACAO POLLEN OF DIFFERENT AGES OF EXPERIMENTS III+III-A.

Age in hours	Mean in %
12	37.50
24	17.79
36	17.83
48	10.30
60	3.75
72	0.51

L.S.D. at 1% is 6.76
L.S.D. at 5% is 5.11

DISCUSSION: POLLEN VIABILITY

The percentage values by themselves are of no great importance in these experiments, as another medium may give other values, and the artificial germination probably does not duplicate the process of natural germination on the stigmas.

The intention was not, however, to obtain germination values as such, but to arrive at comparative values. As such they are valid and general conclusions can be drawn from them.

As shown in Table 22 and 23 the pollen grains show a viability up to 48 hours, after which their germinating power declines rapidly. In general as shown by Tables 22 and 23 for the significances pollen of the age of 12, 24, and 36 hours have the same germinating power. Table 23 shows pollen 12 hours old to have a highly significant difference over pollen 24 and 36 hours old, but this might

be due to a time variability in germinating power of the pollen at the different ages as expressed by the significant age x time interaction (see Table 19) though this interaction failed to be significant in experiments I and I-A (see Table 9). However, even in this case where the 12 hours pollen was significant over 24 and 36 hours, the latter two ages kept their position relative to the higher ages as in the other experiments.

TABLE 22. SIGNIFICANT DIFFERENCES IN GERMINATION POWER OF CACAO POLLEN AT DIFFERENT AGES OF EXP. I, II, AND I + I-A.

Age	Exp. 1				Exp. I-A				Exp. I+I-A			
	Sign.	Inferior age			Sign.	Inferior age			Sign.	Inferior age		
12	**	48	60	72	**	48	60	72	**	48	60	72
24	**		60	72	**	48	60	72	**	48	60	72
	*	48										
36	**	48	60	72	**	48	60	72	**	48	60	72
48	**			72					**			72
60									*			72

TABLE 23. SIGNIFICANT DIFFERENCES IN GERMINATION POWER AT DIFFERENT AGES OF EXP. III, III-A, AND III+III-A.

Age	Exp. III				Exp. III-A						Exp. III+III-A					
	Sign.	Inferior age			Sign.	Inferior age					Sign.	Inferior age				
12	**	48	60	72	**	24	36	48	60	72	**	24	36	48	60	72
24	**		60	72	**				60	72	**			48	60	72
	*	48			*			48								
36	**		60	72	**				60	72	**			48	60	72
	*	48														
48					**					72	**					72
	*			72	*				60		*				60	

Germination of forty eight hours old pollen shows significance over that of 72 hours old pollen. At 72 hours we can consider that the pollen definitely has lost its viability. Pollen at this age shows a small percentage germination which may be due to latent viability or germination in the anthers previous to culture, the latter being probably the major factor.

Regularly, in the early morning, condensation was noticed within the test tubes containing the flower samples. This suggests a saturated atmosphere around the flowers and, as previously mentioned, some germination can take place in a saturated atmosphere. No attempt was made to estimate this germination, as the influence of concentrated or artificial light on the germination of the pollen grains was not known. Exposure to this light would have been unavoidable if the microscope was used to estimate the germination in the anthers.

TABLE 24. SIGNIFICANT DIFFERENCES IN GERMINATION POWER OF CACAO POLLEN AT DIFFERENT AGES OF EXP. II.

Age	Sign.	Inferior Age		
36	**	60	72	78
	* 54			
48	**	60	72	78
	* 54			

Table 24 indicates that pollen older than 48 hours shows such a decline in germination power that, though small percentage germinations are obtained at higher ages,

these can be disregarded for all practical purposes.

These results open a possibly minor, but definite by new aspect in cross-pollination. Crawling insects in this light are not entirely excluded as cross-pollinating agents. When the unpollinated flower drops before it is 36 hours old, as happens with over 50% of unpollinated flowers, and crawling insects are present in the flowers, they could reach another tree and cause cross-pollination.

Their potentiality as cross-pollinators will depend on the role the crawling insects play generally as pollinators in the country concerned. This may explain why Voelcker (14) in Nigeria, did not consider the distance over which pollen is carried to be far, and considered one guard row sufficient in clonal blocks to avoid cross-pollination, whereas Cope (3) concluded in Trinidad, that self-sterile trees are not dependant on their immediate neighbours for fertile pollen.

Table 9 shows that there exists a difference in viability of the cacao pollen at different times. As this significant difference was arrived at with only a one day interval in time, its importance acquires added value. Though the time factor failed to be significant in Table 19 this does not unde the value of the significance earlier mentioned, as the time interval here also was one day, a very small time interval, and these experiments were carried out about one week later than experiments I and I-A. It rather stresses the time variability in germination of pollen, in that at some periods the germinating power does not change much, whereas in other periods it fluctuates considerably.

In Trinidad it was noticed that there exists a period during which no pollination takes place called "crazy flowering." Posnette (9) noticed a similar phenomenon in the Gold Coast in March 1940, and observed that hand-pollinations also gave poor results during this period. He concluded that this was possibly due to climatic factors effecting abscission of flowers. It is also possible, however, that the pollen at this time is not viable or poorly viable, through climatic or other factors.

Different clones show a difference in viability of their pollen. This, however, does not extend to differences between self-compatible and self-incompatible clones (see Tables 14, 17, and 20). Clone 613, which was used in the experiments is a self-incompatible clone and does not show any difference in germination from the self-compatible 11.

The clones 11 and 12 are not pure clones. In their original selection, 4 mother trees were used for the establishment of each clone. However, it was not considered of much importance in our work, as the chance is great that at least 50% of the trees in El Chino came from one mother tree. As only a few of the trees were used to collect the samples, it can be considered that most pollen came from one original mother tree. The results, showing the constant highly significant difference of 12 over both 613 and 11, adds value to this assumption.

Even if the samples were a mixture of pollen of 4 mother trees, it still shows that this group of mother trees produce

a more viable pollen, under the specific conditions, than the other clones and that there exist viability differences in pollen between clones. This might prove to be a factor worth while considering in future selections.

The results obtained with these germination tests suggest that further work should be done on viability of pollen. An attempt should be made to determine which factors are causing the variability in time and also the variability between clones should be studied in more detail.

CONCLUSIONS

1. Neither wind nor water play any role in the pollination of cacao.
2. Insects are the only natural agents of pollination of cacao.
3. Both thrips and a flying insect which probably is a Forcipomyia sp. play a major role in the pollination of cacao in Costa Rica.
4. Ants probably effect some pollination but are not of much importance in Costa Rica.
5. It is doubtful that aphids effect any pollination at all in Costa Rica.
6. Cacao pollen is viable up to the age of 48 hours and declines rapidly after that in viability.
7. Cacao pollen varies in viability at different times.
8. There exist clonal differences in viability of cacao pollen, which do not extend to differences between self-sterile and self-fertile clones.

SUMMARY

1. The literature on the pollination of cacao is discussed.
2. The structure of the cacao flower and the method of hand pollination used are described.
3. The possibilities of wind and water pollination were investigated and excluded.
4. It was concluded that insects are the only natural agents of pollination of cacao in Costa Rica.
5. Evidence was accumulated to show that thrips (Frankliniella sp.) are one of the major pollinating agents in Costa Rica.
6. The percentage flushing of the cacao trees and the average number of thrips per flower were put into one graph which showed that there is a correlation between the two factors.
7. No significant difference was found between the thrips populations in the flowers at different hours of the day.
8. A great fluctuation was shown in the thrips populations in the flowers from week to week.
9. It was shown that ants and aphids do not play an important role as pollinators of cacao in Costa Rica.
10. Indirect evidence was collected to show that probably Forcipomyia is a major cross-pollinating agent in Costa Rica.
11. The cacao pollen remained viable for about 48 hours. The implication of this in relation to flower abscission and cross-pollination is discussed.
12. It was shown that pollen viability varies at different times.

13. There exists a significant difference in pollen viability between clones, but not between self-incompatible and self-compatible clones as such.

SUMARIO

1. Se discute la literatura sobre la polinización de cacao.
2. Se describe la estructura de la flor de cacao y los métodos de polinización artificial usados.
3. Se investigó y excluyó la posibilidad de polinización por medio del viento y del agua.
4. Se concluyó que los insectos son los únicos agentes polinizadores naturales del cacao en Costa Rica.
5. Se acumuló evidencia para mostrar que thrips (Frankliniella sp.) son unos de los agentes polinizadores más importantes en Costa Rica.
6. El porcentage de brotación de los árboles de cacao y el promedio del número de thrips por flor fueron puestos en un gráfico el cual mostró que existe una correlación entre los dos factores.
7. Ninguna diferencia significativa fué encontrada entre las poblaciones de thrips en las flores en diferentes horas del día.
8. Se mostró una fluctuación grande en las poblaciones de thrips en las flores, de una semana a otra.
9. Fué demostrado que las hormigas y los aphidos no juegan un papel importante como polinizadores del cacao en Costa Rica.
10. Se colectó evidencia indirecta para mostrar que en Costa

Rica Forcipomyia es probablemente un agente importante en la polinización ajena.

11. El polen de cacao permaneció viable por aproximadamente 48 horas. Se discute la implicación de este hecho en relación a la cortada de las flores y polinización ajena.

12. Se mostró que la viabilidad del polen varía en diferentes tiempos.

13. Existe una diferencia significativa en la viabilidad del polen entre clones pero ni la auto-incompatibilidad ni la auto-compatibilidad ejercen ninguna influencia.

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EXPERIMENT I

APPENDIX A.

GERMINATION PERCENTAGE OF CACAO POLLEN

Treatments	Replications					
Hours	I	II	III	IV	V	
12	46.2	39.0	75.2	37.4	46.4	244.2
24	50.3	34.8	64.5	36.8	40.9	227.3
36	45.3	45.5	43.4	60.9	66.7	261.8
48	41.0	34.0	18.6	33.9	8.8	136.3
60	23.1	8.6	15.9	9.0	29.7	86.3
72	7.6	4.9	7.3	7.9	1.3	29.0

EXPERIMENT I-A

APPENDIX B.

GERMINATION PERCENTAGES OF CACAO POLLEN AT DIFFERENT AGES

Treatments	Replications					Total	Mean
Hours	I	II	III	IV	V		
12	26.0	29.6	21.4	38.1	42.2	157.3	31.5
24	38.9	40.9	22.6	52.8	34.0	189.2	37.8
36	32.4	49.6	33.9	25.9	9.5	151.3	30.3
48	10.6	10.4	11.9	10.5	4.6	48.0	9.6
60	6.9	5.1	6.8	9.7	29.3	57.8	11.6
72	6.0	3.3	4.6	5.7	0.7	20.3	4.1

EXPERIMENT II

APPENDIX C.

GERMINATION PERCENTAGES OF CACAO POLLEN AT DIFFERENT AGES

Treatments	Replications					Total	Mean
Hours	I	II	III	IV	V		
36	9.2	24.4	24.4	23.9	24.1	106.0	21.1
48	14.3	41.5	15.7	19.8	20.0	113.3	22.3
54	11.9	14.3	16.0	1.0	10.2	53.4	10.7
60	4.1	8.4	4.9	1.2	2.4	21.0	4.3
72	0.5	12.5	3.2	3.6	4.8	24.6	4.9
78	2.2	0.0	0.0	3.4	7.6	13.2	2.6

EXPERIMENT III

APPENDIX D.

GERMINATION PERCENTAGES OF CACAO POLLEN OF DIFFERENT CLONES AND AT DIFFERENT AGES

Clones	Age	Replications					
	Hours	I	II	III	IV	Total	Mean
613	12	5.2	16.4	5.8	33.6	61.0	15.3
	24	24.2	27.3	24.4	5.3	81.2	20.3
	36	27.6	24.8	27.0	41.3	120.7	30.2
	48	1.8	7.6	4.7	3.8	17.9	4.5
	60	0.5	2.0	0.7	3.2	6.4	1.6
	72	0.4	0.8	3.4	0.0	4.6	1.2
11	12	39.7	12.0	1.2	59.8	112.7	28.2
	24	15.8	1.5	9.4	11.9	38.6	9.7
	36	2.0	1.0	2.3	2.2	7.5	1.9
	48	0.0	9.6	1.6	2.2	13.4	3.4
	60	1.9	0.0	0.7	0.0	2.6	0.7
	72	1.0	0.0	1.6	0.0	2.6	0.7
12	12	53.5	64.2	11.5	9.2	138.4	34.6
	24	22.8	52.2	28.2	18.8	122.0	30.5
	36	29.5	39.1	34.3	34.2	137.1	34.3
	48	29.7	14.1	25.4	31.3	100.5	25.1
	60	7.6	11.3	6.7	6.2	31.8	8.0
	72	0.0	0.0	0.0	0.0	0.0	0.0

Age totals	
12	312.1
24	241.8
36	265.3
48	131.8
60	40.8
72	7.2

Clone totals	
613	291.8
11	177.4
12	529.8

EXPERIMENT III-A

APPENDIX E.

GERMINATION PERCENTAGES OF CACAO POLLEN OF ..

CLONES AND AT DIFFERENT AGES

Clones	Age	Replications					
	Hours	I	II	III	IV	Total	Mean
613	12	23.6	30.8	35.8	54.2	144.4	36.1
	24	5.5	7.7	5.9	3.0	22.1	5.5
	36	6.4	5.5	1.4	5.9	19.2	4.8
	48	1.0	7.8	11.3	7.7	27.8	7.0
	60	0.0	0.6	2.0	4.1	6.7	1.7
	72	0.6	0.0	0.0	0.7	1.3	0.3
11	12	34.8	36.6	49.0	41.6	162.0	40.5
	24	18.1	11.8	9.8	7.0	46.7	11.7
	36	24.6	7.5	8.9	1.3	42.3	10.6
	48	7.0	17.4	5.1	9.5	39.0	9.8
	60	4.8	3.6	1.8	1.8	12.0	3.0
	72	0.0	2.2	0.0	0.6	2.8	0.7
12	12	72.6	67.8	81.5	59.7	281.6	70.4
	24	34.9	21.2	29.9	30.3	116.3	29.1
	36	23.3	16.8	37.1	23.8	101.0	25.3
	48	18.8	13.9	15.4	0.5	48.6	12.2
	60	8.4	8.0	6.8	7.2	30.4	7.6
	72	0.0	0.0	0.0	1.0	1.0	0.3

Age Totals	
12	588.0
24	185.1
36	162.5
48	115.4
60	49.1
72	5.1

Clones Totals	
613	221.5
11	304.8
12	578.9

APPENDIX F.

FREQUENCY DISTRIBUTION OF 100 POLLEN GRAINS DIAMETERS IN μ

Class	Frequency	
	Larger diam.	Smaller diam.
12.96 - 14.99		1
15.00 - 17.49	6	14
17.50 - 19.99	37	29
20.00 - 22.49	3	3
22.50 - 24.99	35	40
25.00 - 27.49	18	12
27.50 - 29.16	1	1

Total larger diameter: 2178.81 μ

Mean larger diameter : 21.79 μ

Total smaller diam. : 2111.63 μ

Mean smaller diam. : 21.12 μ

The measurements of the grains were taken at night angle. Of the 100 grains measured, 54 were round having the same diameter in both directions. The remaining 46 grains showed a difference in diameters as shown by the following frequency distribution.

FREQUENCY DISTRIBUTION OF DIFFERENCES IN DIAMETER OF SINGLE POLLEN GRAINS (expressed in μ)

Class	Frequency
0.40 - 1.19	21
1.20 - 1.99	20
2.00 - 2.79	2
2.80 - 3.59	3