Some Experiments on the Storage of Cacao Seed in a Viable Condition.

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Introduction.

CACAO is usually propagated by means of seed, and under the right conditions this is a simple procedure. It is most important that the seed selected for planting be relatively fresh for although the viability of freshly-picked seed is good, the ability to germinate begins to fall rapidly after about ten days from the time of harvesting, under normal conditions in the tropics. This short period of viability of stored cacao seed at times is a severe handicap to the industry, for it means that some places cannot receive cacao seed from the recognized sources of seed, and that distant new areas are difficult to open up to cacao.

Pyke (1) and Pyke *et al* (2) in 1934 attempted to store cacao seed in the pod by subjecting the pods to various antiseptic treatments followed by a coating of vaseline or latex. A variety of storage temperatures was tried. They found that the antiseptic treatments were of no practical value; that vaseline treatment reduced water loss but only increased storage life where there was no fungal attack; that the optimum storage temperature was about 60 degrees F. (16 degrees C.); and that the most important fungus associated with the fungal attack of stored cacao pods was *Botryodiplodia theobromae.*

Evans (3) in 1949 attempted to store cacao seed both inside and outside the pod. He found that fully ripe pods kept better than under-ripe pods though initial germination of the seed within was excellent in all cases. The coating of pods with vaseline and the injecting of the pod cavity with sterile water were both found unsatisfactory. Pods were succesfully stored for up to two months by packing them in moist charcoal. Cacao seed was layered in powdered charcoal with a moisture content of 30 per cent. and after thirteen weeks' storage, seedlings were established successfully.

Experiments on the storage of cacao seed were carried out at the Lowlands Agricultural Experiment Station, Keravat, in 1950 (unpublished). These included the layering of seeds in damp sawdust and various attempts to reduce the loss of water from the seeds. No results of importance were obtained.

STORAGE OF CACAO SEEDS OUTSIDE THE POD.

Under normal conditions, once a cacao seed is taken out of the pod it will commence to grow if conditions are suitable or it will die if conditions are not suitable. Excessive cold, heat, desiccation and fungal attack will all kill the seed. The general aim of the following experiments was to induce in the seeds by various growth inhibitors a period of dormancy that was not harmful to their later growth.

Experiment A 1 (using "Tuberite").

The seeds from twelve ripe pods were divided into five similar lots which were treated as follows:—

Lot 1. Lightly dusted with "Tuberite" (a fine powder containing 2.4 per cent. isopropylphenylcarbamate), just sufficient to form a continuous layer on the upper surface of the seeds.

Lot 2. Heavily dusted on the upper surface of the seeds.

Lot 3. Completely covered on all sides by a heavy application of "Tuberite".

Lot 4. The mucilaginous layer was removed from the seeds by washing in water and shaking with glass beads. Then the seeds were completely covered by a heavy application of "Tuberite".

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Lot 5. Untreated.

The seeds were stored in damp charcoal. At regular intervals the condition of the stored seeds, including the amount of root growth and their viability was ascertained.

This experiment gave no evidence that "Tuberite" was able to induce a period of dormancy in cacao seeds. One fact of interest was the very obvious stimulation of the seeds when the mucilage had been removed, but the "Tuberite" was not responsible for this. A similar stimulation was obtained by removing the testae of seeds or the mucilage alone with no addition of "Tuberite". The radicles of such seeds grew up to three-eights of an inch in sixteen hours in a humid atmosphere at 27 degrees C.

Experiment A 2 (using isopropylphenylcarbamate solution).

A 10 per cent. solution of isopropylphenylcarbamate in acetone was diluted with water to concentrations of 0.25 per cent. 0.10 per cent. and 0.01 per cent. Eight similar lots of cacao seeds were prepared. The tips of the cotyledons were removed from the seeds in lots 1 to 6. In lots 7 and 8 they were left entire. The lots were then soaked as follows:—

Lot 1. 0.01 per cent. solution for one hour.
Lot 2. 0.10 per cent. solution for one hour.
Lot 3. 0.01 per cent. solution for 16 hours.
Lot 4. 0.10 per cent. solution for 16 hours.
Lot 5. 0.25 per cent. solution for 16 hours.
Lot 6. unsoaked.
Lot 7. 0.25 per cent. solution for 16 hours.
Lot 8. unsoaked.

After treatment the seeds were layered in moist powdered charcoal. Samples of seeds were taken at weekly intervals from each lot and planted out to test their viability. The condition of the stored seeds and the amount that the radicle had grown were recorded. The results showed that under the conditions prevailing, isopropylphenylcarbamate was unable to induce a period of dormancy in cacao seeds. The removal of the end of the cotyledons had a small stimulating effect on the growth of the seeds. The presence of isopropylphenylcarbamate acetone also had a stimulating effect on growth.

Experiment B (using citric acid).

When citric acid was applied to cacao seeds as lemon juice it was too dilute to produce any significant effect, but when applied as pure crystals it produced a considerable effect.

Four similar lots each of 100 cacao seeds, were used in the experiment. Three lots had citric acid crystals sprinkled over the seeds in the quantities of 25, 50 and 100 grams respectively. The fourth lot of seeds was untreated. The seeds were then layered in damp powdered charcoal. At weekly intervals samples of seeds from each treatment were taken and washed to remove any citric acid, the amount of growth of the radicle was recorded, then they were planted out to test for viability.

At the two higher levels of citric acid the cacao seeds were rapidly killed. At the lowest levels however, growth of the seeds during storage was distinctly suppressed but the percentage of seedlings finally established was similar to that of the control. These results indicate that it may be worthwhile to experiment further with citric acid.

Experiment C (using coumarin).

Coumarin has been used successfully on various plants in order to induce temporary biological dormancy. Three similar lots of cacao seeds were treated as follows:—

Lot 1. A little petroleum jelly containing 1 per cent. by weight of coumarin crystals was applied to the radicle end of the seeds.

Lot 2. Coumarin crystals were sprinkled evenly over the seeds at the rate of 10 grams per 100 seeds.

Lot 3. Untreated.

The seeds were then layered in moist powdered charcoal. Samples of seeds from each treatment were taken at weekly intervals and their condition, including the amount of growth of the root, was recorded, and they were then planted out to test their viability.

Coumarin was found to be unsatisfactory. The results were similar to, though distinctly poorer, than, those obtained with citric acid.

In all the experiments described above the storage medium of damp powdered charcoal was kept in cardboard boxes. The cardboard boxes seemed to allow too much drying of the charcoal. Probably the use of perforated tins as used by Evans (3) would be more satisfactory.

Conclusions.

1. Both "Tuberite" and its active principle, isopropylphenylcarbamate, were ineffective in inducing a period of dormancy in cacao seeds.

2. Citric acid applied at the rate of 0.25 grams per seed seemed capable of retarding growth without being harmful to the seeds. Further investigation is necessary.

3. Coumarin seemed capable of retarding growth of the seeds but it appeared to be less satisfactory than citric acid.

STORAGE OF CACAO SEEDS INSIDE THE POD.

Under normal conditions in the tropics cacao pods have rather a short life, beginning to break down under the attack of fungi in from one to three weeks after harvesting. The viability of the seeds within the pod declines very rapidly soon after the breakdown of the pod commences. While the pod is in a healthy state almost all the seeds remain viable. Pyke *et al.* (2) suggested that age is an important factor in the decline in viability of stored cacao seed. However, it appears that for at least the first few months of storage, age is only of minor importance. The major factors seem to be (i) fungal attack and (ii) perhaps the presence of toxic bi-products of fungal growth. Loss of water from the pod does not seem to affect the seed directly but it is of great importance because it lowers the pods' resistance to fungal attack. The seed is killed by fungal attack before desiccation causes death.

Preliminary experiments.

Experiment D 1 (to determine the variation in viability of seeds due to time).

Nine unblemished pods were harvested from each of four trees and the seeds from one pod from each of the trees were planted out every two days. Only pods showing little or no fungal attack were used so as to minimize the effects due to the growth of fungi.

This experiment indicated that so long as the pods remained in a healthy state, the viability of the seeds within them does not change significantly. Germinations of 100 per cent. were recorded from the first to the eighteenth day after harvesting. Where there was much fungal rotting, the viability of the seeds fell rapidly. In later experiments in which pods were kept free from fungal attack for up to 96 days, germination was also 100 per cent. This showed more conclusively that age is not a primary factor in the death of cacao seeds stored in the pod.

Experiment D 2 (to compare the germination of seeds from unripe and ripe pods).

One full-sized but unripe pod, just-ripe pod and well-ripe pod were harvested from the same cacao tree and twenty seeds from each pod were planted out. The total germination of the seeds in each category was very similar. The seeds from the unripe pods showed the least uniform germination. This result is very similar to that obtained by Evans(3).

Experiment D 3 (to compare the keeping quality of unripe and ripe pods).

Three full-sized but unripe pods and three ripe pods were harvested from each of four trees. They were all stored at room temperature in the laboratory on a bench and were inspected regularly for signs of rotting.

After taking the average of the results for the pods in each category for each tree, it was found that the under-ripe pods commenced rotting and were completely rotted before the ripe pods on the same tree. There was a considerable variation in keeping quality

between pods from the same tree, and a greater variation between pods from different trees but in the same category.

Experiment D 4 (to compare the keeping quality of pods from different trees).

Six pods were harvested from each of six trees and were stored on the laboratory bench. At regular intervals they were inspected for signs of rotting. The average period for which the pods remained healthy varied from 10.5 days to 17.0 days for the six trees, so there was a distinct difference between the keeping quality of pods from different trees. As in the previous experiment there was also a distinct difference between pods from the same trees. For example, the pods from one tree remained healthy for from 11 to 26 days.

EXPERIMENTS TO INCREASE THE STORAGE LIFE OF CACAO PODS.

The Use of Fumicides.

As the breakdown of pods during storage always has a fungal component, an attempt was made to inhibit the growth of such fungi by the external application of fungicides.

Experiment E 1 (using various fungicides).

Ten pods from each of three trees were harvested and treated by immersing them in a solution of the fungicide for about half a minute, except in the case of Phemox which was applied as a dust. The five treatments used, each on two pods from each tree, were 0.1 per cent. Karathene WD, 0.2 per cent. Dithan Z - 78, 0.1 per cent. Mercuric Chloride, 10 per cent. Phemox powder, and a water control. The pods were stored at room temperature in open boxes and inspected regularly for rotting.

None of the treatments increased the storage life of the pods. In all cases fungal breakdown was as rapid as with the control. Apparently the fungi are present in the healthy pod in crevices or as a latent infection in the pod tissues and so they escape contact with the fungicides.

Experiment E 2.

It was observed that with most pods rotting begins either at abrasions or at the stalk end of the pod. Special effort was made to protect these parts.

Pods were harvested from six trees and the pods from each tree were divided into three lots. In the first lot the stalk end and any abrasions were sealed with melted paraffin wax at 150 degrees C. containing 0.1 per cent. by weight of Dithane Z - 78. In the second lot the pods were surface sterilized by immersing in 95 per cent. ethyl alcohol, then the stalk end was liberally covered with 6 per cent. copper oxychloride powder. The third lot was the untreated control. The pods were stored at room temperature and were inspected regularly.

The results gave no significant difference between treatments. Alcohol burnt the skin of the pod and thereby reduced the storage life. The fungicides served no useful purpose. Those parts of pods protected by paraffin wax usually took longer to rot but as the remainder of the pod rotted as quickly as the control, there was no significant overall improvement. A number of fungi were isolated from the shells of some of these pods. The fungus which was isolated most commonly was *Botryodiplodia theobromae.*

The use of Paraffin Wax.

Loss of water from cacao pods stored under normal conditions appears to be an important factor in predisposing the pods to fungal attack. In the following experiments paraffin wax was used as a means to reduce this loss of moisture.

Experiment F 1 (immersing the pods in paraffin wax).

Some pods were harvested and the same day immersed in melted paraffin wax at a range of temperatures. The results were unsatisfactory because within three weeks all the pods had started to rot and most were severely rotted. The viability of the seeds within the pods after this period was negligible. Pods dipped in wax at 150 degrees C. had their shells browned from the heat and so should

have been effectively surface sterilized. However rotting still occurred. The only fungus isolated from these pods was *Botryodiplodia theobromae* and it was isolated at every attempt. It seems that the pods must have carried a deep-seated infection of this fungus before they were waxed. Pods dipped in wax at 120 degrees C. and at 80 degrees C. had a variety of fungi in them, the fungi isolated most commonly being a few species of *Aspergillus, Colletotrichum* and an unidentified white fungus as well as *B. theobromae.*

It was obvious that upsets in respiration had been a major factor in the death of the seeds, and that sterilization of the pods was of little practical importance. Keeping the pods in a healthy condition would appear to be the best way to combat fungal breakdown during storage and thus to increase the storage life of the pods.

Experiment F 2 (delaying the wax treatment of harvested pods).

Some pods were harvested and divided into five lots then stored in open boxes in the laboratory. After storing for one day, lot 1 was treated with paraffin wax by dipping both ends of each pod in melted wax at 100 degrees C. in such a way as to leave a central unwaxed band about half an inch wide. Lot 2 was treated similarly after storing for two days, lot 3 after storing for four days, and lot 4 after storing for nine days. Lot 5 was the untreated control.

The results indicated that the keeping quality of the pods declined very rapidly if the pods were kept for any considerable length of time before treating with paraffin wax. Pods stored for one day before treatment remained without any sign of fungal rotting for up to 40 days. The other treatments declined in their effectiveness as the length of the pre-treatment period of storage was increased. The pods stored for nine days before treatment had a keeping quality no better than the control. In both these cases all the pods had commenced rotting in about two weeks after harvesting. For best results the pods must be treated with paraffin wax as soon as possible after harvesting.

Experiment F 3 (using wax at different temperatures leaving an unwaxed band).

One hundred and twenty-five pods were harvested, divided into five similar lots then treated the same day with paraffin wax. Lot 1 was immersed in melted wax at 150 degrees C. for ten seconds. Lot 2 had the ends of the pods dipped at 120 degrees C. leaving an unwaxed central strip of about three-quarters of an inch in width. Lot 3 had the ends dipped at 100 degrees C. and lot 4 at 175 degrees C. Lot 5 was the untreated control. The pods were stored at room temperature on the laboratory bench and were inspected regularly. Two pods were taken each week from each treatment and seeds chosen at random planted out to test for viability.

The results of the treatment in which the pods were immersed in wax differed very little from those of the control. With both treatments, the viability of the seeds was very poor by the eighteenth day of storage. The percentage germinations recorded were 30 per cent. and 5 per cent. respectively and no seeds germinated after the eighteenth day. The results of the three treatments in which the pods had a central unwaxed band were very similar. This showed that the temperature of the wax is not critical, at least between 75 degrees C. and 120 degrees C. The average keeping quality of the pods in these three treatments was superior to that of the control.

Some of the pods in these three treatments deteriorated as rapidly as the pods of the control, but many lasted for much longer periods; one pod gave 100 per cent. germination after 56 days of storage. It was estimated that the pods of the control and those immersed in wax kept satisfactorily for about 14 days, whereas the pods having an unwaxed band kept satisfactorily for an average of about 27 days.

The beneficial effect gained by treating pods with wax appears to result from the reduction in transpiration of the pods. Precautions need to be taken to prevent too much of the surface of the pods being covered with wax thus seriously inhibiting respiration.

The chief fungus causing pod rot was found to be *B. theobromae* in every treatment, especially during the first few weeks of storage. After the first month of storage other fungi were dominant in pod breakdown. These were weaker parasites, the most common being *Colletotrichum* sp. and *Fusarium* sp.

It was noted that about half the pods which had a central unwaxed band had commenced to rot at this unwaxed portion, whereas the pods of the control commenced rotting usually at places other than the centre, particularly at the stalk end.

Experiment F 4 (varying the size of the unwaxed portion of the pod).

Eighty-four pods were harvested, divided into three lots and treated the same day. (Immediately before treating with wax the stalk of each pod was cut off with a sterile scalpel and any abrasions were cut down to a fresh surface.) Lot 1 was dipped in melted wax at 100 degrees C. leaving an unwaxed central portion which was crescent-shaped running about half way round the pods and was from a quarter to half an inch in width at the widest part. Lot 2 was treated similarly but the wax contained 5 per cent. by weight of the fungicide "Shirlan". Lot 3 was the untreated control. The pods were stored in the laboratory at room temperature and inspected regularly.

The results showed an overall improvement in the keeping quality of pods treated with paraffin wax, though for the first few weeks of storage the control was superior. A few of the waxed pods remained healthy for quite a long period, one pod still being unblemished after 90 days of storage. It seems that the small unwaxed portion was a limiting factor with many pods. This statement is further supported by the fact that in 45 out of the 56 pods treated with wax, rotting of the pod started at the central unwaxed portion. Once again about half of the pods of the control commenced rotting at the stalk end. The pods treated with wax containing "Shirlan" seemed to have a slightly better keeping quality than the pods treated with pure wax.

Experiment F 5 (varying the size of the unwaxed portion of the pod).

Seventy-five pods were harvested, divided into five lots and treated the same day. Before treating with wax the pods were brushed free of dirt and the stalk and any abrasions were cut to a fresh surface. Lot 1 had the ends of the pods dipped in melted wax leaving a small crescent-shaped unwaxed area at the centre running half way around each pod. Lot 2 was treated similarly except that the unwaxed portion was a central band about a quarter of an inch wide and running right around the pod. Lot 3 was treated similarly but with a half-inch unwaxed band. Lot 4 was treated similarly but with an inch wide unwaxed band. Lot 5 was the untreated control. The pods were all stored in the laboratory at room temperature and inspected regularly.

The results showed that all the treatments using wax were superior to the control but there was a noticeable difference between some of them.

The pods having the one-inch unwaxed band kept best for the first three weeks but then deteriorated rapidly so that the pods having the quarter-inch and half-inch unwaxed bands kept best over the last six weeks. The pods having the small crescent-shaped unwaxed area were the least satisfactory of all the waxen pods. It appears obvious that both respiration and loss of water are important factors in the keeping quality of pods treated with paraffin wax, and that, for best results, that size of unwaxed area must be found which gives a minimum loss due to the combined effects of these factors. It would appear from this experiment that a central unwaxed band about half an inch in width gives the best results. The size of the pod, the period of storage, and the conditions of storage would all be expected to modify the optimum size of this unwaxed area.

It was noticed that as the unwaxed area was made smaller than the half-inch band, the percentage of pods in which rotting was initiated at this site became greater.

Conclusion.

1. While the cacao pods remained free from fungal attack, the viability of the seeds within them did not change with time.
2. The germination of seeds from freshly-picked pods that were full-sized but un-ripe and pods that were ripe was very similar.
3. The average keeping quality of pods harvested from any one tree could vary considerably from that of pods harvested from a different tree.
4. The keeping quality of individual pods harvested from any one tree could vary considerably.
5. The keeping quality of pods harvested when unripe was considerably poorer than that of pods harvested when ripe.
6. The external application of fungicides was ineffective in lengthening the storage-life of pods.
7. Pods immersed in melted paraffin wax were rapidly decomposed by fungi with the consequent death of the seeds within.
8. Dipping the ends of pods in melted paraffin wax so as to leave an unwaxed central band significantly increased the average storage life of the pod if it was treated within a day of harvesting. The optimum size of the unwaxed central band was about half an inch in width.
9. When the fungicide "Shirlan" was added to the paraffin wax, the results seemed to be superior to those achieved when the wax was used alone.

Summary.

The storage of cacao seed outside the pod was attempted using the growth inhibitors "Tuberite" containing isopropylphenylcarbamate, citric acid, and coumarin, in order to induce a period of dormancy in the seed. None of these substances was found to prolong the life of the seed significantly.

The storage of cacao seed inside the pod was attempted using various fungicides and various paraffin wax treatments to increase the storage-life of the pod. The methods using fungicides did not prolong the life of the seed significantly. Using paraffin wax the average period for which the seeds in a pod could be kept in a viable condition was doubled, being increased from about fourteen days to twenty-eight days, with one pod lasting for ninety days.

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