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EXPERIMENTAL PLANTATIONS INC.
FINCA EL NARANJO
CHICACAO, SUCH.

GUATEMALA OFFICE
CIUDAD VIEJA
CALLE REAL Nº 91. TEL. 4042
CABLE ADDRESS:
ANAPU

Guatemala City,
December 4, 1940.

MEMORANDUM

Re : Research Program for Experimental Plantations

Although Dr. Popenoe has already enumerated the points worthy of inmediate investigations in a research program at El Naranjo, I nevertheless wish to review the matter from the point of view of giving greater detail with reference to the status of present knowledge, the method of attack, and tying up the major items to the seasons of the year.

The planting program in Guatemala has far outstripped the progress in developing horticultural techniques. The research program must be aimed, therefore, at overcoming our most pressing problems in the order of their importance and with the view of getting into commercial production as quickly as possible. As already pointed by Dr. Popenoe our most inmediate objectives include :

1st. Combating Red-Mottling : This apparent mineral defficiency threatens to destroy many of our most valuable trees and to exclude from production large areas of land which would otherwise be suitable for growing Cinchona.

2nd. Developing seed beds and nursery practice : There is room for improvement in our present methods. Routine with reference to the seasons of the year will have to be established.

3rd. Improving present grafting methods : Grafting threatens to become a bottleneck in the propagation of our best clones. Our best trees are grafted with great difficulty and cion material for subsequent grafting is thereby limited.

4th. Combating Fungus disease : We recognize tentatively two fungus diseases : A rosellinia-like disease which rings the bark of old trees near the surface of the ground, and a canker disease which apparently originates near the soil, splits the bark of the trees and travels upwards through the trunk sometimes as far as the leaves. The first disease is fatal but not widespread. The second, seems much more widely spread but the trees seem to be able to tolerate it to some extent. Both diseases are a threat to our best ledger trees and we may be spreading the canker disease by present methods of grafting and seed-bed practice.

5th. Establishing a more scientific standard for trees worthy of grafting as clones and developing methods of judging good trees from bad in the field : The tremendous heterogeneity of the plant material now available in Guatemala and the necessity of making successive yearly bark analyses of each individual tree in order to determine its desirability, will swamp us with work unless restrictions of some sort are exercised. These restrictions must be intelligent, or otherwise, our most valuable trees [illegible]

see page No. 2

Memorandum Re- Research Program for Experimental Plantations :

6th. Establishing comparable plantings at different altitudes : Although we now have three plantings at El Naranjo at different altitudes (roughly at 4, 5 and 6,000 feet), we do not know the altitude limits for growing Cinchona in Guatemala. It is desirable to extend our experimental limits to include from 3,000 to 7,000 feet and to make the plantings strictly comparable as to plant material used, time of planting, etc. Since this is a long term proposition an early start is in order.

PROGRAM FOR COMBATING RED MOTTLING

This problem is to be approached in two ways : (1) by trying to save valuable trees by more or less hit or miss methods, and (2) by a systematic study of the fundamental problem in an effort to determine the exact cause of the trouble. "Hit or miss" methods include such practices as applications of chemical fertilizers; much of which already has been done; applications of Peruvian guano, and mulching with Inga leaves. The systematic approach will consist primarily of painting the leaves with single mineral salts and similar nutrient deficiency diagnoses. In view of the prolongued dry season which is beginning at this time, additional applications of fertilizers can not be expected to do much good in overcoming red-mottling. Applications of chemical fertilizers, particularly those containing phosphorus, seem to some extent to have increased the vigor of the treated trees. Therefore, in spite of the dry season it would seem wise to apply phosphoric fertilizer to valuable trees which have not as yet received any, like those in the Zapote Finca. However, in the case of trees which have already received two doses of it, the addition of guano, which will increase the moisture retaining capacity of the soil, and mulching with Inga leaves would seem to be indicated. It is hoped the latter will help carry weakening trees through the dry season and save them until better treatments are devised.

PROGRAM FOR SEED-BED AND NURSERY PRACTICE

Small-scale experiments will be undertaken immediately. Since the season of year greatly affects results obtained in nurseries, experiments of this sort will have to be carried out with a view towards developing methods suitable to the different times of year and developing a calendar of seed beds and nursery practice. The tentative program in mind at this time includes the following : 1st., the construction of seed beds in strict accordance with the recommendations of Stoffels; 2nd., the use of sphagnum moss in seed beds; 3rd., the addition of dilute nutrient solutions to both seed beds and nurseries; 4th., the use of cloth covering as shade and protection from drip in nursery practice; 5th., the use of natural shade for nurseries during the dry season, and 6th., disinfecting both seed and seed-beds soil.

PROGRAM FOR GRAFTING INVESTIGATIONS

The tentative program is as follows : 1st., Approach grafting in an effort to save trees suffering from disease; 2nd., the use of shield-grafting or budding in an effort to make best use of small amounts of budding material; 3rd., control experiments in the grafting of scions suspected of being diseased; 4th., experiments designed to develop a calendar for grafting, and 5th., grafting under shade in dry season; 6th, grafting higher on rootstocks to avoid "rosellinia" infection.

PROGRAM FOR COMBATING FUNGUS DISEASES

see page No. 3

Memorandum Re : Research Program for Experimental Plantations

PROGRAM FOR COMBATING FUNGUS DISEASES

As already mentioned, experimental measures will include :

1. Disinfection of seed and seed-bed soil.
2. Approach grafting.
3. Experiments with grafting of disease-suspect cions.
4. Grafting higher on rootstocks to avoid "rosellinia" infection.

PROGRAM FOR TREE SELECTION

Relatively simple statistical analyses of the present individual tree records will be undertaken with the hope that they will yield further clues which will enable us to distinguish good trees from bad in the field and decide more exactly whether a tree is worthy of further examination or not.

PROGRAM FOR ESTABLISHING OBSERVATION GARDENS AT DIFFERENT ALTITUDES

The planting of observation gardens will be undertaken with Krukoff seedlings, as soon as they become available for planting out. Thesewill be supplemented with grafted trees when available in sufficient numbers.

William Pennock.

WP/prp

December 24, 1940

Mr. William Pennock
Finca El Naranjo
Chicacao, Such.
Guatemala, C. A.

Dear Mr. Pennock:

I have your letter of December 4, 1940 and will make every effort to obtain the books requested by you for the library at Naranjo.

With regard to the research program which was also attached to your letter of December 4th, I have little to add. I believe that this covers the field very well.

As you know, Mr.Perkins is planning to visit Guatemala shortly and I would suggest that you review the program with him.

With best regards, I am

Very truly yours,

R. P. Lukens

RPL:CS

cc Mr. Perkins
Dr. Popenoe
Mr. Rosengarten

Copy for Dr. Popenoe

February 6, 1941.

Mr. R. P. Lukens,
Merck and Co., Inc.
Rahway, New Jersey,
U. S. A.

Re: Report on Experiments for the Month of January, 1941.

Dear Mr. Lukens :

The following is a report on the progress of experimental work in Cinchona in Guatemala up to and including the month of January, 1941. This is the first of the monthly reports of the type requested by Mr. Perkins.

Experiments on Supplementary Inorganic Fertilization of Nursery Beds

Experiment No. 2 (x)

On September 11, 1940, at my suggestion, Mr. Benítez initiated an experiment on the use of Ammo-Phos as a supplementary fertilizer in nursery bed soil. Ammos-Phos containing 16.5% nitrogen and 20% of available P_2O_5 was applied to the soil of the beds in powder form at the rate of 1 1/3 oz. per 10 sq. ft. It was broadcast as evenly as possible over the surface of the soil and then worked into the soil to a depth of 1 to 2 inches. Small seedlings, 3/4 to 1 1/2 inches in height, were then brought from the seed beds and planted to the nursery beds at 2" by 2" spacing.

Two of the empty "lámina" (sheet iron) covered seed beds were used as nursery beds in this experiment. In one bed with the high side facing South, an area of 240 sq. ft. was fertilized and planted with 8,400 small seedlings of K # 200. In the other bed, with the high side facing West, a fertilized area of 180 sq. ft. was planted with 6,300 seedlings of K # 116 and another fertilized area of 90 sq. ft. was planted with 3,150 seedlings of K # 35. In all, 510 sq. ft. were fertilized and 17,850 Krukoff seedlings were used in the experiment. No unfertilized nursery to serve as check or control was planted at this time.

About 10 to 15 days after the planting and for about a month later, heavy losses occurred. The dead plants were not all scattered hapzardly throughout the bed but were particularly plentiful in certain small areas. After about two months the dying of plants stopped, and by the end of December, approximately 110 days after the planting, the seedlings were observed to be much larger and more vigorous than would generally be the case with similar but unfertilized plants.

On January 6, 1941, all of the K # 35 plants were transplanted to another bed at 6" X 6" spacing and on January 9th. all but 700 of the K # 116 were also similarly transplanted. The 700 K # 116 and all of the K # 200 plants remained in the experimental nursery. A rough idea of the increase in size and vigor brought about by the fertilizing, may be got from the fact that plants of K # 116 which were vigorously (continued on page 2)

(x/21 the experiments are numbered chronologically.

established at 2" x 2" spacing when this experiment was initiated, and which received no fertilizer, are now 1 to 2 inches shorter than the fertilized plants.

On February 6, 1941, survival counts of the fertilized plants were made : Of the K # 116 plants, 2,095 or 33 2/9% of the original number survived and were in excellent condition; of the K # 35 plants, 1,391 or 44 1/6% survived; and of the K # 200 an estimated 10% survived. A total of about 4,300 or about 24% of the 17,850 fertilized plants survived.

Undoubtedly, most of the losses were directly attributable to the ill effects of the Ammo-Phos, which we now know was applied too liberally. However, some of the losses must have occurred as a consequence of transplanting, since in this operation a small percentage of loss is as yet unavoidable.

Despite the lack of a check for comparisons, the experiment has contributed the following points to our store of information :

1. It is risky to use inorganic fertilizer, particularly Ammos-Phos, which may liberate Ammonia, on small plants 1 inch or so high. The element of risk is quite probably increased when the beds are protected from rain by "lámina" and when the high side of the bed faces South. As compared with other nursery beds these two features decrease the amount of water which leaches through the bed and increase the surface evaporation thereby tending to increase the concentration of the soil solution.

2. Appropriate supplementary inorganic fertilization may be expected to speed up growth and increase the vigor of plants in the nursery.

3. The tendency of plants to die out in specific portions of the treated area would seem to indicate that broadcasting the powdered fertilizer over the surface of the bed does not distribute the fertilizer evenly. When using an active fertilizer such as Ammo-Phos it may not be safe to apply it in this way since irrespective of the amount to be used per area, toxic concentrations may inadvertantly be placed in parts of the area. The application of a solution or fine suspension of such a fertilizer to the soil should give a much more satisfactory distribution.

Since some concern has been shown as to the fate of all of our K 116 seedlings, the following inventory as of February 6, 1941, is included:

Plants which survived fertilizer treatment and which are now planted at 6" by 6" and are the most vigorous of our Krukoff seedlings	1,395
Plants which survived fertilizer treatment, now planted at 3" by 3"	700
Unfertilized plants, now in nursery, at 3" by 3"	10,446
Plants remaining in original seed bed (estimate)	1,000
TOTAL k # 116 seedlings at "El Naranjo"	13,541

Experiment No. 3

On December 28, 1940 another experiment on the use of supplementary chemical fertilizing of nursery bed soil was initiated. A solution was made up as follows :

1 level teaspoonful	(about 5 gm.)	superphosphate (16% P_2O_5)
1 " "	" 5 "	sodium nitrate (agricultural grade)
1 " "	" 5 "	potassium sulfate (agricultural grade)
1 gallon tap water		

The solution was applied at the rate of 1 gallon per 10 sq. ft. of soil surface after the plants were already planted in the nursery. Following the application of the solution, the plants were sprinkled with about 1/2 gallon of water to wash off any of the salts adhering to the leaves and thus prevent burning.

One plot of 10 sq. ft. was treated in the following three beds :

Bed A 16 which had been planted on Nov. 5, 1940 with K # 45 seedlings received from Washington. The seedlings were 2 to 6 inches tall and planted at 6" by 6" spacing. Forty plants were included in the area treated.

Bed A 13 which was planted with K # 1 seedlings moved from our seedbeds on Nov. 29, 1940. The plants were 1 1/2" tall and planted at 3" by 3" spacing. The 10 sq. ft. treated included 150 plants. Both this bed and the A 16 were shaded with a flat cover of "Bilil" stems and fern and palm fronds.

Tablon No. 21 which was planted with K # 1 seedlings moved from our seedbeds on Dec. 5, 1940. This bed was originally a seedbed and was covered with "lámina". The high side faced south-west. The plants were 1/2 to 3/4" tall and planted at 1 1/2" by 1 1/2" spacing. The 10 sq. ft. of area treated included 450 plants.

All three beds contained the rich topsoil thought to be best for nursery purposes.

The beds to date have been treated with three successive applications of the fertilizer solution at 15 day intervals as follows :

1st. application	- Dec. 28, 1940
2nd. "	- Jan. 16, 1941
3rd. "	- Jan. 31, 1941

On January 31, at the time of making the third application, the treated plants already showed a slight increase in growth and vigor when compared with similar plants in the untreated portions of each bed.

The experiment is intended to continue for an indefinite period. Following the third application, additional applications will be made at approximately 15 day intervals, to one half of each treated area. In this way a comparison may be made in each of the 3 plant size groups between the effects of 3 applications and that of an indefinite greater number which will continue to be applied until possible toxic effects first begin to appear. If no toxic effects

are forthcoming, the applications will continue until the plants are shifted to other nursery beds at wider spacing.

The experiment has shown that applications of a fertilizer solution of the above formula did not injure the plants in any way but increased the growth and vigor of recently planted seedlings in the nursery in three different size groups, namely : 2 to 6 inches tall at 6" by 6" spacing; 1 1/2"tall at 3" by 3" spacing, and 1/2 to 3/4 in. tall at 1 1/2" by 1 1/2" spacing. This improvement in growth and vigor occurred in the richest topsoil available for nursery purposes in the plantation.

The experiment is further expected to yield information on the following points :

1. Whether continued applications of the solution at 15 day intervals will have toxic effects during the period that the plants remain in their present nursery location.

2. Whether additional applications will increase the growth rate and vigor of plants beyond that obtained with only 3 applications.

3. What precautions and experimental techniques are desirable in experiments of this nature and approximately how many applications should succeeding experiments include in an effort to determine the optimum number of applications for commercial practice.

In addition to the two foregoing experiments, a few miscellanous trials of an exploratory nature in the use of supplementary nursery fertilizer were made as follows :

1. On Dec. 28, 1940, all of bed C11 was fertilized with superphosphate at the rate of 1 oz. per 10 sq. ft. The bed was subsequently planted with Krukoff seedlings, approximately 1 1/2" tall at 3" by 3" spacing. Bed C10 containing similar plants is to serve as the untreated check.

2. On Dec. 28, 1940 superphosphate at the rate of 1 1/3 oz. per 10 sq. ft. was applied to a nursery bed where Succirubra plants, approximately 1 1/2 ft. tall were subsequently planted at 10 " by 10" spacing. A similar unfertilized bed was kept as check.

3. On Dec. 28, 1940, superphosphate at the rate of 2/3 oz. per 10 sq. ft. was applied to two beds which were subsequently planted with Succirubra seedlings about 2" tall, at 6" by 6" spacing. A similar untreated bed was kept as check.

In all three trials the superphosphate, in powdered form, was broadcast on the surface of the soil and later worked in to a depth of 1 to 2 in.

These trials are intended to speed up the growth rate of the Succirubra plants involved so that stocks for grafting may be available as quickly as possible and also to furnish information on the use of superphosphate rather than Ammo Phos as a phosphorus carrier. The low solubility of phosphorus fertilizers and the tendency of this nutrient to quickly become fixed in the soil at the place of application, make it desirable that special

emphasis be given to the manner of applying it. Heavy applications made in advance of the planting, so that it may be worked in to sufficient depth, are indicated. These trials indicate that superphosphate is not toxic at the rate used to date and that it is better than Ammo-Phos for this purpose. However, we are now convinced that a more homogenous application of superphosphate may be made by sprinkling a fine suspension of it over the soil rather than broadcasting it in powdered form. A convenient method for applying such a suspension inexpensively on a large scale is now being worked out and a thorough investigation of the use of superphosphate is planned.

Seed-Bed Investigations

Experiment No. 7 :

On January 28, 1941, an experiment involving a new type of seed-bed and testing the advantages of several seed-bed media and the use of nutrient solutions was initiated.

A seed-bed 3 1/2 ft. wide by 83 ft. long was constructed and partitioned off into 27 sections. Each section or plat was 35" long and encompassed roughly 10 sq. ft. of effective seed-bed surface. The length of the bed extended from East to West and the protective shelter was constructed so that the high side of the roof was due North. The roof was made of "teja manil", placed as shingles, making the roof impervious to rain. The roof was pitched at a 45 degree angle, the high side being 7 1/2 ft. above the soil.

Nine different treatments involving changes in seed-bed media and the addition of nutrient salts were given as shown in the following table :

SYMBOL	TREATMENT
A	Media of subsoil covered with 1/2" layer of dead sphagnum moss and later sprinkled with 1 gallon of nutrient solution at 15 day intervals.
B	Media of subsoil covered with 1/2" layer of live native moss and later nutrients added as above.
C	Media of subsoil covered with 1/2" layer of live native moss; nutrients not added.
D	Media of subsoil covered with 1" layer of live native moss; nutrients added.
E	Media of subsoil covered with 2" layer of live native moss; nutrients added.
F	Media of topsoil covered with 1/2" layer of live native moss; nutrients not added.
G	Media of subsoil, without moss layer; nutrients not added.
H	Media of topsoil " " " " " "
I	" " " ; " added.

The moss in all cases was rubbed through a 1/4" mesh screen and tamped somewhat when placed on the plats. The nutrient solution contained, per gallon of water, :

5 gm. superphosphate
5 gm. potassium sulfate
5 gm. sodium nitrate

Three replications were made of each treatment and located at random in three sections of the bed, each section containing 1 replication of each treatment as shown below :

Section # 1									Section # 2									Section # 3								
A_1	B_1	C_1	D_1	E_1	F_1	G_1	H_1	I_1	C_2	G_2	I_2	D_2	A_2	E_2	F_2	B_2	H_2	G_3	D_3	B_3	F_3	C_3	I_3	A_3	H_3	E_3

On January 28, 1941, when all the plats were prepared, seeding was started. Ledger seeds received from Helvetia on Jan. 14, 1941, were used and were sown at the rate of 2 gms. per 10 sq. ft. Plats in Section # 1 were all sown with seed from tree No. H-1213; those of Section # 2 were sown with seed of tree No. H-866; and those of Section # 3, with seed from trees Nos. H-965 and H-1147, which had been mixed thoroughly together, so that all plats in a given section were sown with comparable seed. The seed was broadcast by hand over the wet surface of each plat and subsequently watere d to prevent its being blown away by wind. The entire seed-bed was then shaded on all sides and kept well watered. Water was applied twice daily with a watering can which had a very fine spray; each plat received about 3 gallons per day. The first application of nutrients was given to the specified plats on February 6, 1941.

Following germination, watering and shade will be reduced in accordance with present seed bed practice. Data will be taken on the percentage of germination and of subsequent growth till the plants are removed to the nursery. It is planned to thin out to comparable stands a small specified area of each plat by transplanting some of the very small plants when germination is complete. This will eliminate to some extent the factor of spacing so that plats having high germination may be compared (with reference to subsequent growth) with plats having low germination.

This experiment is expected to yield information about the following points :

1. The advantages, if any, of using a layer of chopped-up moss on the surface of seed beds.

2. The kind of moss and the thickness of the moss layer giving best results.

3. Whether differences occur in the incidence of damping-off and similar diseases in a topsoil medium, as compared with a subsoil medium.

4. Whether the addition of nutrient salts to topsoil and subsoil media is desirable.

5. The advantages, if any, of a "Stoffels" type of seed bed, having a narrow planting area, a steep pitched roof, and 7 feet of open light space facing North.

Experiments on Mineral Defficiency Diagnosis and Field Fertilization

Experiment No. 1

This experiment, initiated on Aug. 22, 1940, and already described in the memorandum of the same date, involved the fertilization, with AmmosPhos of eleven trees in various stages of "mineral defficiency".

On Nov. 27, 1940, the treated trees were compared with the similar untreated trees serving as checks and results noted as follows :

Pair No: 1 : No perceptible difference; both trees rather poor.

2 : Treated tree much better than check; both fairly healthy.

3 : No perceptible difference.

4 : Treated tree slightly better than check; both poor, nearly dead.

5 : Check better than fertilized tree; both rather poor.

6 : No difference; both poor.

7 : Treated tree alive, check dead; however, treated tree in poor condition and new growth gave no evidence of recovery.

8 : Slight improvement in treated tree, none in check, both trees poor.

9 : Treated tree much poorer than check.

10 : Treated tree much better than check, showed marked improvement in new growth, whereas check showed none.

11 : This tree, for which no comparable check could be found, showed a remarkable recovery. All leaves of old growth were markedly yellow mottled, whereas all new growth, was deep green and vigorous.

This experiment, showing marked positive results in three, slight positive results in four, negative results in two, and doubtful results in four instances, is certainly not conclusive. It does, however, give a fairly good indication that the application of Ammo-Phos increases the vigor of trees suffering from mineral defficiency, provided the tree is not too far gone when the application is made.

A common sense interpretation is that most of the trees entering in the experiment were too far gone to be able to take up the Ammo-Phos and that the negative and doubtful results were a consequence of the impossibility of selectin g exactly duplicate trees. The three trees that showed definite reaction were in better condition than the others and a more extensive root system probably enabled them to make better use of the Ammo-Phos.

Experiments Nos. 4 and 5 :

On Dec. 30, 1940 and Jan. 8, 1941, two experiments were carried out in an effort to diagnose the cause of "mineral defficiency by foliar treatments.

Squares of bandage gauze soaked with single mineral salt solutions were placed on part of both surfaces of young leaves showing mineral defficiency symptons. The gauze was then covered with celluloid sheets and clipped in place. The gauze and celluloid were removed after 12 hours, and several observations were made thereafter at weekly intervals.

In experiment No. 4 the solutions contained the equivalent of 1 mg. per liter of mineral being tested. In experiment No. 5, the equivalent of 1 gm. of each mineral per liter was used. the minerals tested included :

Potash	as	$K_2 S O_4$
Phosphorus	"	$Na H_2 P O_4$
Magnesium	"	$Mg S O_4$
Copper	"	$Cu S O_4$
Iron	"	$Fe_2 S O_4$
Zinc	"	$Zn S O_4$
Boron	"	$H_3 B O_3$

The results were negative in all cases. It seems probable that good penetration of the minerals was lacking since in no cases was injury apparent. Copper and Boron would, in all probability be toxic, in the last concentrations used.

Further experiments with a modified technique are planned, and calcium will be added to the list of minerals.

Experiment No. 6 :

On Jan. 15, 1941, an experiment was initiated in the Merck area of Los Andes, Panamá, on the use of Peruvian guano fertilizer and a mulch of Inga leaves. Both of these treatments are calculated to benefit the trees during the dry season when inorganic fertilization would not be expected to be advantageous and would at best be a risky procedure.

Six rows including a total of 176 trees were selected for the experiment. These were classified as to vigor and segregated into four equivalent groups, each of which received one of the following treatments :

Symbol	Treatment
G	Guano was applied at rate of about 8 oz. per tree.
M	A mulch or covering of dry Inga leaves, about 2" thick and 1 1/2 ft. radius, was placed around each tree.
GM	A combined treatment of guano and mulch, applied as above, was given.
Z	Untreated check.

The classification as to vigor was done by measuring the height of the tree and then assigning the following symbols or combinations thereof to each individual:

Symbol	Criterion of Vigor
A or a	Vigorous trees having approximately a normal number of leaves for a tree of its size.

B or b	Trees having about 1/2 the normal number of leaves.
C or c	Trees having about 1/4 or less of the normal number of leaves.
E or e	Trees showing yellow mottling.
F or f	Trees showing red mottling.

Small letter symbols denote lower limit of leaf number or slight mottling, as case may be.

When the trees were all classified, those having the same symbols and approximately the same height, were selected in groups of four to constitute a replication. Each individual in any group of four was designated to receive one of the different treatments. Trees receiving the same treatment were therefore, scattered haphazardly throughout the experiment. This is desirable in accordance with theory for experimental plot technique. An undesirable feature of the experiment is that the individuals in each replication were sometimes widely separated. Differences in soil and immediate environment might thereby be increased, spoiling the fairness of a comparison between the effects of the different treatments. This unavoidable fault may, however, be considered of minor importance since the vigor of the trees is largely a manifestation of soil and environmental conditions and trees showing similar symptons would be expected to be located in similar soil and local environment.

The experiment is intended to show the effect of guano fertilizing and mulching with Inga leaves on the rate of survival and changes in vigor occuring during the dry season.

The table which follows, on separate page, shows the location by row and number of each tree in the experiment, its height and vigor classification, and the treatment to which each individual was subjected.

Respectfully submitted,

WILLIAM PENNOCK.

WP/prp

Copy for Dr. Pogenee

March 15, 1941.

Mr. R. P. Lukens,
Merck and Co., Inc.,
Rahway, New Jersey.

Re: Experiments for the month of February, 1941.

Dear Mr. Lukens :

The following is the report for the month of February on the progress of the experimental work on Cinchona :

New Experimentation

Experiment No. 8 :

On February 6, 1941, an experiment on the fertilization of Cinchona Succirubra nurseries with mineral salt solutions was initiated. Three areas of 20 sq. ft. each were selected for treatment in tree beds where different conditions existed. The remaining parts of each bed served on this case as untreated checks. The mineral solution and the rate of application in this experiment were the same as those used in Experiment No. 3 and described in our report for January, 1941. The beds selected were : tablon No. 20, which had plants 3/4 in. high in the original seedbed; an unmarked bed containing 2 in. plants at 6" by 6" spacing, which, as described in the Jan. report, had been fertilized with superphosphate in powdered form; and an unmarked bed containing one inch plants at 2" by 2" spacing, which had not received any fertilizer.

The purpose of this experiment is to test the efficacy of present methods of fertilizing with solutions when applied to C. Succirubra.

Experiment No. 9 :

On February 6, 1941, an experiment on the use of "Sunraytex" cloth in nursery practice was initiated. The original seedbeds at Naranjo had been orianted in different directions in accordance with the slope of the land. This feature made it necessary to shade those beds facing South and West with "bilil" stems so as to protect the plants in them from direct afternoon sun. This shade proved excellent for the early growth period, when small amounts of light were desireble. But later when it became necessary to harden-off the plants previous to transplanting, this type of shade proved impossible to adjust when trying to avoid burning of the plants during the afternoons of extreme sunlight intensity and yet allow enough light to enter at all times so that the plants would get tough and wiry. Lath frames were tried as a substitute with some success. However, the extreme variations in sunlight intensity occuring during the winter months created the necessity of improving this type of shade. It was, therefore, thought that Sunraytex cloth, which is similar to cheese-cloth or tobacco cloth, might furnish a more suitable shade for hardening-off the plants.

A 25 ft. section of two of the original seedbeds containing 2 inch tall Krukoff seedlings was selected. These beds were : Tablon # 19, with opened sides facing due West and tablon # 4 facing South, South-West. Both beds con-

2 to 4 inches tall.

tained seedlings of K 100. The remaining portions of each bed, shaded with bilil stems or lath frames, were allowed to remain as they were to serve as checks. The cloth was attached to the high edge of the lámina roof and stretched tightly at approximately a 45 degree angle to the ground, so as to shade the opened side of the bed.

On February 27, the plants in the cloth shaded section of the two beds were observed to be harder and tougher that the plants in the rest of the beds. Although the sun had shone with great intensity on several afternoons during the intervening period, no burning was observed. Burning was, however, observed in a few plants that received full sunlight in a narrow unshaded strip between the cloth and lath shade.

The experiment shows that Sunraytex cloth can be used to advantage with present conditions and seedbed set-up, and also promises to be useful at other stages during the seedbed-to-planting-out period of Cinchona cultivation. The economies involved will have to be determined after continued usage and further experiments. The cloth, although expensive, can be easily and quickly placed in position and may be used repeatedly for short periods in different beds when hardening-off the plants is desired. Its durability under our conditions is still to be determined.

Two photographs, attached hereto, show how the cloth was used.

Experiment No. 10 :

On February 7th., a useful and simple laboratory method for testing seed germination was devised. The method consists of filling a 75 mm/ glass funnel with sand or soil, the stem being plugged with cotton. The funnel is then placed on a tumbler filled with water so that the rim of the tumbler supports it and the sand is wet by capillary action through the stem of the funnel. A 75 mm. filter paper of low ash content is then placed on the levelled surface of the sand or soil and wet down so that it makes good contact with it. The seeds are then scattered on the filter paper and may be prevented from blowing away by covering with another filter paper or by sheltering it with glass. When this method was used, very good, even germination was obtained in 30 days time with ledger seed received from Helvetia. Although neither the soil or filter paper was sterilized, fungus contamination did not interfere with the test. Some slight amounts of mycelium grew on four apparently dead seed in a lot of 100.

The method has the advantage of simplicity and adaptability for many different purposes, including germination counts, seed and soil disinfection studies, and possible root disease observation and work with nutrient solutions.

Experiment No. 11 :

On February 14, a grafting experiment was initiated by Mr. Benítez. Twenty-four grafts of ledger cions were made on Succirubra stocks using standard methods as described in the memorandum of February 8, 1941, dealing with grafting. When the grafting operation was completed, each graft was enclosed in wax paper. The paper was rolled into a loose cylinder enclosing the graft and then tied securely both above and below it to the stem of the stock. The purpose of the experiment was to try and develop a method for grafting in the dry season.

Experiment No. 12 :

In conjunction with our clearing and preparing nursery areas for the Krukoff

seedlings the desirability of using some of the methods of the Soil Conservation Service of the U. S. D. A. suggested itself.

In the construction of bench terraces with shoulders supported by split logs the method of maintaining a nearly uniform vertical interval and varying the width of each terrace in accordance with the slope of the land, was put into practice. Because of the saving in logs, labor, and more efficient utilization of the land, it was promptly adopted as standard practice in all terraces except those intended for grafting stock where uniform width is particularly desirable. Our present practice in terraces of this type is to vary the vertical interval within a limit of four inches and thus obtain fair uniformity in terrace width, and avoid sharp curves and angles which would later make the provision of shade more difficult. This method avoids extreme heights in the retaining log supports which would be necessary in terraces of uniform width.

In the preparation of the nursery area on "loma de la tubería" where a large area is being prepared for the 6" by 6" planting of Krukoff seedlings, the method of preparing bench terraces with shoulders supported by barrier plantings rather than with log barricades, was tried on a small scale. The risks involved and the desirability of knowledge concerning the behavior of local soil types in local rainfall conditions led to the adoption of a "compromise" method for standard practice and the initiation of a terracing experiment. The compromise method consists of bench terraces without "pitch" (level from edge of shoulder to back of terrace) and with a lengthwise grade of from 0.83% to 1.7% to be determined for each terrace as proves most convenient. The slope of the shoulder is to be arrived at by what seems safe at the time of construction and some log supports will be used in difficult places. "Isote", Yucca elephantipes, will be planted at 1 foot intervals to serve as barrier planting. The ideas of both Mr. Benítez and Mr. Hoehn were incorporated into this method.

On February 27, six experimental terraces were started in a section of the cleared field which had a slope of roughly 30%. Remnants of old terraces facilitated construction and the specifications of the new terraces were varied so as to make best use of these remnants.

The specifications of these terraces are given below. The width of the terraces was measured from the edge of the shoulder to the edge of the cut above the terrace. The "pitch" of the terraces, as listed below, refers to the slope of the terrace's surface across its width. Accepted practice is to grade the terrace slightly from the shoulder to the cut, in opposite direction to the original slope of the ground. It will be noticed that terraces Nos. 4 and 3 are sloped outwards with "unorthodox" pitch.

Terrace No.	Length	Lengthwise Grade	Width in Feet limits	Width in Feet Average	Pitch
1.	140 ft.	1.25% (1 1/2" in 10')	8 to 10'	9 ft.	Outward : 5%
2.	130 "	1.67% (2" in 10')	6 1/2 - 10'	8 1/2'	N o N e
3.	140 "	1.67% (2" in 10')	10 to 15'	10 1/2'	Outward : 10%
4.	150 "	1.25% (1 1/2" in 10')	8 1/2 - 10'	9'	Inward : 2%
5.	140 "	0.83% (1" in 10')	7 to 11'	8 1/2'	" 1%
6.	120 "	0.83% (1" in 10')	8 to 13 1/2'	9'	" 1%

The barriers between terraces, made up of approximately half fill and half cut, had an average slope of about 45 degrees. The cut was given about 60 degrees slope and the fill about 30 degrees. Both vertical and horizontal intervals averaged close to 3 feet. Because of differences in grade and pitch in the differe nt terraces and the unevenness of the terrain, both varied considerably as follows :

Barrier between 1st. and 2nd. Terraces

Length		130 ft.
Difference in grade between terraces	1/2" in	10 ft.
Vertical interval	3 ft. to	3 ft. 6 1/2"
Horizontal interval	2' 8" to	3 ft. 6"
" " average, about		3 ft. 2"

Because of the outward pitch of terrace No. 1, the proportion of fill to cut was slightly less than 1' to 1' in this barrier, the average horizontal interval was, therefore, shorter than the vertical interval.

Barrier between 2nd. and 3rd. Terraces

Length		130 ft.
Difference in grade between terraces		None
Vertical interval		3' 5"
Horizontal interval	2' 6" to	3' 9"
" " average, about		3' 5"

In this barrier variations in horizontal interval were caused solely by differences in the terrain; large fills increased the interval and vice-versa.

Barrier between 3rd. and 4th. Terraces

Length		140'
Difference in grade between terraces	1/2" in	10'
Vertical interval	2' 11" to	3' 6"
Horizontal interval	2' 8" to	4'
" " average, about		3'

In this barrier because of the outward pitch of terrace No. 3, the fill again was less than the cut and the horizontal interval, therefore, shorter than the vertical. The 10% pitch was expected to reduce the fill more than it actually did.

Barrier between 4th. and 5th. Terraces

Length		140'
Difference in grade between terraces	1/2" in	10'
Vertical interval	3' 7" to	4' 2"
Horizontal interval	3' to	3' 8"
" " average, about		3' 4"

Some shoring with logs and a hump where barrier was located were instrumental in keeping a low ratio of horizontal to vertical interval.

Barrier between 5th and 6 th. Terraces

Length		120'
Difference in grade between terraces		None
Vertical interval	4' 2" to	5'
Horizontal interval	4' 2" to	4' 3 1/2"
" " average, about		

The ratio between the two intervals is quite close to what might be expected in view of the inward pitch of terrace No. 5.

A barrier planting of yucca elephantipes will support the shoulders. Shallow ditches will be constructed along the inside of each terrace.

As mentioned above, the purpose of this experiment is to apply methods of the Soil Conservation Service in our preparation of nursery areas for 6" by 6" planting and to learn about the behavior of local soil. An innovation of providing the terrace with outward pitch was tried because it was thought that a considerable saving in soil hauling and horizontal interval might be attained thereby. These savings actually proved to be comparatively small when the construction was carried out. The two terraces with outward pitch are regarded as "horrible" examples in which washing of the soil may be observed and the efficiency of the yucca barrier planting may have a severe test.

A small scale trial of the adaptability of Mangum terraces and simple contour ditches in the flatter sections of land intended for these nurseries is also planned.

Experiment No. 13 :

On February 28, an experiment on the use of glycerine and ammonium nitrate solutions in conjunction with the transplanting of small seedlings was initiated. Since considerable losses, particularly at places other than Naranjo, have been experienced when transplanting in the dry season, the trial of a slightly modified technique originally developed elsewhere for handling small tomato plants, was thought advisable. Glycerine and ammonium nitrate penetrate quickly into plant cells and when supplied in suitable concentrations increase their osmotic pressure and makes them less susceptible to drying out. Moreover, some nutritional benefit is probable. Five treatments each replicated four times, were given to lots of 25 plants, as follows :

1. Roots immersed in 0.1% (by volume) glycerine solution for 1 hour, rinsed by immersion in fresh water and planted immediately.

2. Same as above, using 0.7% concentration of glycerine.

3. Same as above, using NH_4NO_3, at 0.8% by weight.

4. Same as above, using tap water.

5. No treatment, plants were planted immediately after they were taken from seedbed and carrying in their roots as much or slightly more soil than is customary in usual transplanting.

To simulate conditions of extreme drought the treatments were then repeated as above but the plants were allowed to wilt in half shade for a period of one hour after treatment and were then planted. One hundred plants received each treatment, 50 were planted immediately thereafter, and 50 after one hour period of wilting. The plants were handled in lots of 25, each lot being planted separately, in random order, in the seedbed.

Seedlings of K100 about 4" tall were used. These seedlings had been hardened-off under cloth shade as described in experiment No. 9.

The plants, subjected to one hour of drying, were all badly wilted but those receiving the glycerine treatment were somewhat less wilted than others.

The experiment is an effort to improve our transplanting methods in the dry season. If marked success is obtained the method may be extended to transplanting at other stages and will be particularly useful when seedlings have to be transported over some distance.

Progress in Experiments previously Reported

Experiment No. 7 :

On February 26, germination estimates were made in experiment No. 7. Only partial germination had occurred and many of the seed were in intermediate stages thereby making actual counts impracticable. In previous experience early germination was a good index of ultimate percentage of germination. A rough comparative estimate of germination in the different plats may therefore, be of value, particularly, for comparison with more exact data which may be obtained later. The plats were ranked with arbitrary numbers from zero to 50 in accordance with the extent of germination. The data are tabulated below :

Symbol	Treatment	Replications			
		No.1	No. 2	No. 3	Total
		(germination index numbers)			
A.	Subsoil plus 1/2" sphagnum plus nutrients	25	8	8	41
B.	Subsoil plus 1/2" live moss plus nutrients	1	2	0	3
C.	Subsoil plus 1/2" live moss no nutrients	0	15	1	16
D.	Subsoil plus 1" live moss plus nutrients	50	15	10	75
E.	Subsoil plus 2" live moss plus nutrients	50	15	10	75
F.	Topsoil plus 1/2" live moss no nutrients	35	2	5	42
G.	Subsoil, no moss, no nutrients	25	10	15	50
H.	Topsoil, no moss, no nutrients	25	10	5	40
I.	Subsoil plus nutrients, no moss	20	10	10	40
	GRAND TOTALS	231	87	64	382

Because of the nature of the data few generalizations may be made at this time. However, it seems safe to point out the following :

1. The addition of nutrient solutions did not affect adversely early germination. The possibility of burning the plants in the tender stage during germination made it advisable to postpone the second application until after more accurate counts are made. The first application had been made before germination had started.
2. It is outstanding that those plats showing least early germination were plats which dried out more quickly than the others. On Jan. 31, when response to watering was observed, the following note had been taken : Plats B_1, C_1, B_2, and B_3 dried out before others, after all plats had received two gallons of water the night before. Again on

February 2nd., a note was taken to the effect that B3 dried out before other plats. It is notable in this respect that treatments D and E , in which thicker layers of moss were used than in other treatments, highest early germination occurred. These plats retained moisture better than plats having thinner moss layers. When the estimates were made it was noted that in all plats covered with moss a parallelism occurred between the green color of the moss (caused by young moss sprouts) and the germination of seed. Both of these things are regarded as further corroboration of increased early germination obtained with conditions of high moisture. The bare soil surface maintained moisture as well or slightly better than the moss surface, but proved much more difficult to water without washing the seed about. In part, the lower germination obtained in bare soil plats was caused by injury from watering in spite of all precautions.

3. It is of interest that considerable difference in early germination occurred between the different lots of seed. Seed in replication No. 1, which had higher germination, was larger seed. An indication of the size of the seed is given by the volume measurements below :

H # 1213 (Repl. 1) 3,000 seed (1 gm.) equals -------8.5 cc.

H # 866 (Repl. 2) 3,000 seed (1 gm.) equals -------6.0 cc.

H # 965 plus H # 1147 (Repl 3) 3,000 seed (1 gm.) equals -------7.5 cc.

The seed of replication No. 3 was somewhat larger than that used in replication No. 2 but a larger amount of chaff reduced the number of seed entering in 2 gm., hence all plats in replication No. 3 actually had fewer seed than plats in the other two replications.

A photograph is attached hereto which shows the general set up of the experiment.

Respectfully submitted,

WILLIAM PENNOCK.

WP/prp
Encls.
cc Dr. Popenoe Mr. Rosengarten.

March 31, 1941

Mr. William Pennock
Finca El Naranjo
Chicacao, Such.
Guatemala, C. A.

Dear Mr. Pennock:

As you know I have been away for a while and, therefore, the delay in acknowledging the receipt of your reports. Please be assured that these have been read with a great deal of interest.

In your letter of February 24, 1941 you requested that one-half of your salary be sent for deposit with the Bank of Manhattan Company, in Jackson Heights, and that the other half be sent to you in Guatemala. Mr. Gage has been instructed to carry out your wishes.

The aluminum labels and labeling machine requested will be ordered as soon as I can find a supplier.

We have requested information concerning the analyses of the fertilizers from the supplier and as soon as available will be passed along to you.

One other request contained in your letter was for felts for pressed botanical specimens. Have you any idea who might be able to supply these?

The other equipment will be ordered and sent to you.

The investigational work that you have outlined in your report of February 8th sounds to the writer to be extremely worth while.

Your letter of March 15th and report for the month of February dated March 15th. In your letter you speak about the books to be shipped to Naranjo and the fact that the tropics are extremely hard on them. I have checked with some of our people who have had considerable experience with books in the tropics and they

know of no chemical treatment to preserve books. There are several things, however, that can be done to keep them in good condition. The books should be kept in a tight, dry closet. They should be wiped off frequently to remove any mildew. During the rainy season you might install one electric light bulb in the bottom of the closet leaving this lit in order to furnish enough heat to keep the books dry. I might also suggest that a little Dichloricide be sprinkled in the bottom of the closet, which tends to act as a repellent to any bugs and worms. I am ordering the Dichloricide for you.

In one of your earlier reports you speak of the status of the Krukoff series 116. Some of this lot was started by Mr. Armstrong in the city and brought out to the plantation when he left the country. These plants had an excellent start when I saw them last September. How are these making out?

In regard to seed beds I would suggest that if you have not read the report that you try to find in the files the method used by Washington. This was given to Messrs. Armstrong and Rosengarten some time ago. Washington uses moss and claims that they obtain about 95% germination.

Your letter of March 8th. This letters concerns your plans for living at Naranjo, and Mr. Rosengarten has recently been given approval to go ahead with your house. The addition of a fireplace was also approved. We are quite delighted that you are going to live at the plantation, as we believe it vital to the project that you be as close as possible during its critical development stage.

Your letter of March 27th is acknowledged. The equipment that you wish for the laboratory will be ordered.

Very truly yours,

R. P. Lukens

RPL:CS

cc Mr. Perkins
Mr. Rosengarten
Dr. Popenoe

Copy for Dr. Popenoe

May 10, 1941.

Mr. R. P. Lukens,
Merck and Co., Inc.,
Rahway, N. J.

Re: Experiments for the months of
March and April, 1941.

Dear Mr. Lukens :

The following is the report for March and April on the progress of experimental work on Cinchona.

NEW WORK

On March 5 and 6, 1941, carefully graded ditches were constructed on an observation area with the view of observing roughly the effectiveness of this type of ditch in preventing soil erosion in cleared land. The lower half of the completely cleared area, at 5000 ft. where it is intended to make an observation field planting with Krukoff seedlings during the latter part of 1941, was ditched, and the upper half is regarded as a check area. Each of these two areas includes roughly about one acre of land with an approximate average grade of 30%.

The ditches were constructed at vertical intervals of approximately 15 ft., some adjustments being made to avoid the largest stumps and felled tree trunks. The distance between the ditches measured on the soil surface varied considerably, but averaged about 50 ft. Four ditches in all were constructed, the uppermost was given a grade of 3 1/3% and the others a grade of 1 2/3%. The grades were marked off with stakes at 10 ft. intervals using a Gurly level very kindly loaned to us by Dr. Popenoe. The shallow ditches, with a profile in the shape of a skewed half-moon, were dug with hoes, the dirt being piled up as a barrier ridge on the lower border of the ditch. The upper border was bevelled off at approximately a 50% slope. The deepest part of the ditch was 8 inches below the top of the barrier ridge, and the ridge and ditch together occupied roughly four feet of soil surface.

This type of ditch has proven very effective in preventing soil erosion in Puerto Rico and costs very little. With our conditions, however, its construction proved more difficult. The land was badly cluttered up with litter and felled trunks and the workmen were unfamiliar with this type of work, so that constant supervision of minute details, and even the construction of a templet, were necessary to insure proper construction.

If these ditches prove to be of decided value in preventing the wash of topsoil on cleared areas, undoubtedly cheaper construction may be worked out.

These ditches have an additional value besides that of preventing soil erosion. They make good paths for walking through a planting and are also useful as base lines for systematized contour planting. When it is intended to plant different varieties in a field, they may also serve as markers to separate one variety from the other.

Experiment No. 14 : On April 15, a small experiment was initiated on the use of glymocol as a soil disinfectant for seed-bed soil. Eight flats 14" x 24" x 4" were filled with seedbed soil taken from an old seedbed where damping off had occurred. Two flats were treated with a solution of glymocol of 1 part to 100 parts of water; 2,with a concentration of 1 to 500; two, with a concentration of 1 to 1,000, and two were treated with plain water. To insure thorough saturation of the soil, 4 liters of solution were applied to each flat. The flats were then allowed to drain and were placed under a shelter for a time to allow the glymocol to dissipate from the soil.

On April 23, the flats were examined. Flats treated with water and with 1 to 1,000 concentration of glymocol were free of odor. All four flats had a few weeds growing in the soil. The flats treated with 1 to 500 and 1 to 100 concentration of glymocol, still smelled of glymocol. None of the four had weeds growing in the soil. On April 28, the flats were again examined and observed to be in the same condition.

Ledger seed from tree H-973 was weighed out into eight lots of 1/2 gram each. In addition, an old lot of Succirubra seed was rendered sterile and non-viable by heatingthe seed under pressure in a mason jar. When the flats are free of glymocol odor the ledger seed will be freed of possible fungus contamination by immersion in a 1 to 5 solution of Zonite. Each lot of 1/2 gram will then be dried, mixed with about a gram of the sterile unviable succirubra seed, and sown by broadcasting on the surface of the soil in the corresponding flat. The purpose of the mixing with the succirubra seed is to achieve a more homogenous scattering of the seed than would otherwise be possible. It is intended to keep all eight flats very moist for a long period so as to promote possible damping off. Unless a very marked control of damping off is achieved by these means, the use of glymozol for soil treatment will be discarded because it is so troublesome to apply.

Experiment No. 15 : On April 18, an experiment was initiated dealing with the size of hole and fertilization practice for field plantings.

In conjunction with our proposed field plantings of Krukoff seedlings for 1941, in which it is intended to plant 1,000 seedlings in full sunlight and 1,000 seedlings in half shade, at four different altitudes, it was thought advisable to put in an experiment on the size of holes and fertilizing which would embark all of these conditions of shade and altitude.

Two experimental plots, one in shade and one in full sunlight, were selected at each altitude, each plot to include 64 trees.Thus, 128 trees at each of 4 altitudes or a total of 512 trees will be included in the experiment. At the time of initiating the experiment, the clearings at only three of the altitudes, namely 7,000, 6000 and 5000 ft., had been completed. So the work today is limited to those areas. Soon the clearing at 4,000 ft. will be completed and the same procedure will be carried out there.

When the staking out for the entire planting was complete in each area, a nearly square plot including 8 stakes in 8 rows was marked off. Four alternate rows were marked for fertilization and large holes were dug at alternate stakes in every row, as shown in the diagram below :

O equals large hole

X " small "

(see next page for diagram)

DIAGRAM

Fertilizer	0	X	0	X	0	X	0	X
NONE	X	0	X	0	X	0	X	0
FERTILIZER	0	X	0	X	0	X	0	X
NONE	X	0	X	0	X	0	X	0
FERTILIZER	0	X	0	X	0	X	0	X
NONE	X	0	X	0	X	0	X	0
FERTILIZER	0	X	0	X	0	X	0	X
NONE	X	0	X	0	X	0	X	0

Each of the eight rows includes 4 large holes and 4 small holes. These groups of 4 holes are regarded as simple replications for each of the 4 treatments. The treatment and replications for each experimental plot are as follows ;

TREATMENT	NO. OF REPLICATIONS	NO. OF TREES
Large holes, fertilized	4	16
Small " fertilized	4	16
Large holes, not fertilized	4	16
Small " " "	4	16

The dimensions of large holes are 1 1/2' x 1 1/2' x 1 1/2' in every case. These holes will be left open for about a month or longer and then refilled 2/3 full about 2 weeks before planting.

The dimensions of small holes depend on what is considered practical at the different altitudes. At 7,000 and 6,000 feet where heavy timber was felled making hole digging difficult and expensive, and where the light texture of the soil suggested that large holes might not be of great advantage, no holes at all will be dug in advance of planting. At these two altitudes the small hole treatment will consist of small holes to be dug at the time of planting just sufficiently large to accommodate the root system of the plants. At 5,000 and 4,000 ft., where heavy felled timber is not so abundant, and where the soil is of a slightly heavier texture, the small hole treatment consists of holes 8" by 8" by 8". These holes will also be 2/3 filled following the same schedule outlined for the large holes. At all altitudes, the small hole treatment is what we have decided on as tentative, standard practice to be used for all plantings until possible improvement is indicated by experimentation.

The fertilizer treatment will also be modified in accordance with what is feasible at the different altitudes. At 7,000 and 6,000 feet, where nearby water for making up solutions is not available and where some of the plants to be fertilized will not have holes dug in advance of planting, a mixed, solid fertilizer consisting of 3oz. superphosphate, 1 oz. ammonium sulfate and 1 oz. potassium sulfate will be added to the soil in each hole at the time of planting. At 5,000 and 4,000 ft., 4 oz. of superphosphate alone will be added to the soil in each hole two weeks before planting, and in addition, a nutrient or "starter" solution will be applied at the time of planting. The nutrient solution will consist of 14 grams of ammonium nitrate (chemical grade) and 50 grams of monoammonium phosphate (chemical grade) per

gallon of water to be applied at the rate of 1/2 pint per plant.

At the time of planting, careful measurements of the height of all plants entering in the experiment will be recorded. Increase in height and survival rates at 3 and 6 months intervals, will be the data to be recorded in this experiment. Similar data taken after a period of several years, may also be of value.

This multiple experiment is primarily intended to make comparisons possible at each altitude and condition of shade, between our tentative standard practice and the most extreme feasible treatment in both size of hole and fertilizing, which theoretically might be expected to increase plant growth and survival. It will also provide a means of determining the effects of these two treatments separately. If marked improvement is obtained with either large holes or fertilization, intermediate, less expensive treatments may be worked out later.

A rough comparison involving the effect of altitude and shade conditions is also possible, but the validity of these comparisons will be largely a matter of estimate and they should be subject to careful, conservative interpretation. The data of the experiment will be analysed both as eight different experiments and as a simple complex experiment. In the latter case, when the effect of altitude and shade condition will be studied, the following three major considerations should be borne in mind :

1. The treatments vary with altitude.

2. The date of planting and, therefore, quite possibly the weather conditions at this critical time will differ with altitude.

3. The altitude and shade factors could not be randomized in the experimental set-up and there is, therefore, no way of seggregating the variance attributable to these two factors as such, from that occasioned by factors which vary with a plant's location in the field. These factors include such things as soil fertility, possible insect and disease injury and many others.

In a practical interpretation of results we can simply lump these factors together and consider for the time being that such variations as may occur in them are inseparable attributes of altitude or shade condition, as the case may be. However, we still have no measure of the relative importance of these factors, the extent to which some of them may vary in any given altitude or shade condition, and the fairness with which they were sampled at any given altitude or shade condition. For example, since we can not bring the soil from a higher altitude to a lower one, we would simply consider variations in soil as an attribute of altitude. The same would hold true for variations in the small hole and the fertilizer treatments, as well as differences in time of planting. Time of planting might conceivably influence growth and survival tremendously, but, it would simply register in this experiment as an undetermined portion of the difference in growth and survival rates observed at the different altitudes. Again, if for example, the plants in the experimental plot located in full sunlight at 5,000 ft. should become infected with root disease through some unfortunate circumstance, which could just as well have occurred in the other plots but did not, this would register as a part of the effect of this altitude and of full sunlight. There would be no measure of the likelihood of such a thing happening at any altitude or shade condition, as compared with others.

We can simply bear these objections in mind at the time of analysing the results of this experiment and design other experiments in the future which

may in part overcome these difficulties.

Progress in Experiments Previously Reported

Experiment No. 6 :

On April 22nd., when the rainy season could be safely said to have begun, data was taken for Experiment No. 6 in Finca Panama. As reported in the Report for January, 1941, this experiment deals with mulching and the addition of guano as field practices of possible benefit during the dry season. The procedure consisted of examining the trees for vigor and classifying them in accordance with the system already described. When all the trees had been classified, the trees were again examined individually so as to check and determine whether a difference between the first and final vigor ratings actually occurred or whether it was due to possible inconsistency in assigning the ratings. I was frankly surprised at how consistently the two readings were the same or differed in a way that was readily explained by obvious new growth, loss of leaves, or some other unmistakable change. In the entire experiment I amended the final reading in only two or three instances. These were border cases and the change in rating was not justified by any apparent recent change in the tree.

Since few changes occurred in vigor ratings, the replications as listed in the report for January, were lumped together into larger groups which will be used in tabulating and interpreting the data. Thus, the first ten replications in the former arrangement are now lumped together as a group of 40 trees, each ten receiving one of the four different treatments. Similarly, a second group includes former replications 12 to 19 included; a third group includes 20 to 23; a fourth includes 25 to 37, and a fifth group includes 39 to 52. In each case one fourth of the trees received one of the four different treatments. The trees formerly listed in replications 11, 24 and 38 were eliminated because they did not include trees subjected to all four treatments. The new groups are as follows :

GROUP NO. 1 Includes 40 trees all originally rated as "A" trees, 10 trees being subjected to one of the four treatments.

GROUP NO. 2 Includes 32 trees all originally rated as "A" trees but with additional symbols denoting some degree of either red or yellow mottling or both, Eight trees were subjected to one of the four treatments.

GROUP NO. 3 Includes 16 trees all originally rated as "B" trees; 4 trees to each treatment.

GROUP NO. 4 Includes 52 trees rated as "B" trees showing red or yellow mottling or both; 13 trees to each treatment.

GROUP NO. 5 Includes 56 trees rated as "C" and showing red or yellow mottling or both; 14 trees to each treatment.

To summarize the data and facilitate its interpretation the number of plants that died, and the net changes in leaf number, in yellow mottling and in red mottling were obtained separately for each group and treatment. Recourse was had to the following "point" system so as to be able to indicate the net effect when more than one tree in a given group and treatment changed in the symbol for one of these characters:

Changes in Leaf Number :

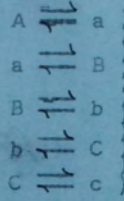
Where A or a trees had approximately a normal number of leaves :

See next page for diagram

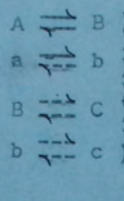
B or b trees had approximately 1/2 normal number of leaves.

C or c " " " 1/4 " " " "

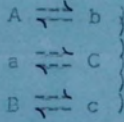
And where the small letter symbols denote the lower limit of leaf number for each group :



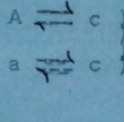
equals - plus or minus 1 point



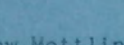
equals Plus or minus 2 points.



equals - plus or minus 3 points



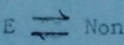
equals - plus or minus 4 points.



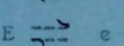
A c - equals - plus or minus 5 points.

Changes in Yellow Mottling :

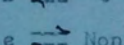
Where E signified severe symptons and e slight symptons:



equals plus or minus 2 points.



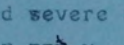
" " " " 1 point.



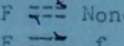
" " " " 1 " .

Changes in Red Mottling :

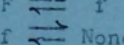
Where F signified severe symptons and f slight symptons:



equals plus or minus 2 points.



" " " " 1 point.



" " " " 1 " .

In all cases when the plants improved during the course of the experiment, the corresponding points were added; when the plant became poorer the points were subetracted.

The changes which occurred in the entire experiment are tabulated in Table I, which follows :

(see next page)

TABLE NO. I

A summary of changes occurring between January 15th. and April 22nd., 1941, in the vigor rating of the trees in Experiment No. 6. The number of plants that died, changes occurring in leaf number, in yellow mottling, and in red mottling of the leaves, are all listed separately in accordance with the point system outlined above.

PLANTS THAT DIED

TREE GROUP	ORIGINAL Vigor Symbol (2)	No. of Individuals in Group	TREATMENTS (1) G Plants Number	GM Plants Number	M Plants Died Number	Z Plants Number	TOTAL Unaccounted Number
I.	A	40	0	0	0	0	0
II.	A or a plus Q	32	0	1	0	0	1
III.	B	16	0	0	1	0	1
IV.	B or b plus Q	52	0	0	1	0	1
V.	C or c plus Q	56	3	2	1	6	12
	Totals	196	3	3	3	6	15

CHANGES OCCURRING IN LEAF NUMBER

			Points	Points	Points	Points	Points
I.	A	40	-1	-2	-4	-4	-11
II.	A or a plus Q	32	0	-1	+2	-3	- 2
III.	B	16	-2	0	+1	-2	- 3
IV.	B or b plus Q	52	+4	-2	0-	-2	+ 8
V.	C or c plus Q	56	+0	0	0	+0	+ 0
	Totals	196	-5	-5	-1	-9	-20

CHANGES OCCURRING IN YELLOW MOTTLING

			Points	Points	Points	Points	Points
I.	A	40	0	-2	-2	0	- 4
II.	A or a plus Q	32	-2	+1	+3	+2	+ 4
III.	B	16	0	0	0	-1	- 1
IV.	B or b plus Q	52	+1	0	+2	+1	+4
V.	C or c plus Q	56	0	0	0	0	0
	Totals	196	- 1	-1	+3	+2	+3

TABLE NO. 1 Continued

CHANGES		OCCURRING	IN	RED	MOTTLING	
			Points	Points	Points	Points	Points
I.	A	40	-1	-2	-2	-2	- 7
II.	A or a plus Q	32	0	+1	+3	+1	+ 5
III.	B	16	-2	0	-2	-2	- 6
IV.	B or b plus Q	52	+3	+7	+6	-1	+15
V.	C or c plus Q	56	+6	+4	+3	+2	+15
	Totals	196	+6	+10	+8	-2	+22

Explanations Notes of Table No.1 - Titles as Above:

(1) G equals guano GM equals guano and mulch M equals mulch
Z " check

(2) Symbols A and a denote trees having approximately a normal number of leaves; B and b , trees having about 1/2 the normal number of leaves; C and c, trees having about 1/4 the normal number. In all cases small letter symbols denote the lower limit of leaf number in that class.

Symbol Q refers to some qualifying symbol denoting a degree of red or yellow mottling or both.

Although twice as many trees died in the check treatment as in any of the other treatments, the number of dead trees is too small to warrant definite conclusions. The fact that all of the trees that died in the check treatment were in the group of poorest trees also detracts from the significance of these results. Trees in the "C" class were quite sickly and although it is possible that mulching may have reduced their mortality, it is more probable that those individuals that died were pretty badly off and would have met the same fate regardless of treatment. The fact that two vigorous trees in groups I and III died when subjected to a treatment that included mulching, and none of the vigorous trees died in either the check or none-mulch treatment, suggests that mulching might have created conditions favorable to wilt disease since in both cases wilt symptons were marked. Again, however, this is quite probably a consequence of chance infection rather than the effect of treatments. The data on dead trees quite obviously is lacking in usually accepted standards of significance and was not analysed statistically.

It also seems quite evident from inspection, that what differences occurred in leaf number of the trees were a consequence of chance rather than a result of the different treatments. Even though greater reduction of leaf number occurred in the check treatment than in any of the others, the difference is very small, particularly, when it is born in mind that a total of 196 trees were included in the experiment. Moreover, the differences are not consistent in [illegible] leaf reduction occurred in the mulch treatment

and less leaf reduction occurred in the guano treatment than in the check. If both types of treatment had actually been responsible for these differences then the combined treatment would logically be expected to reduce leaf loss even more. Again, the comparative leaf loss occurring with the different treatments varied in the different tree groups. If the treatments had exerted a significant influence, a marked degree of consistency would exist in this respect. A statistical analysis of this set of data was not considered worth the trouble.

The data for changes with respect to yellow mottling are very obviously lacking in significance.

The data with reference to changes occurring in red mottling show some indication that both the mulch and the guano treatments improved the trees and that the combined treatment was best in this respect. This set of data was submitted to analysis of variance. Table II which follows shows a summary of this analysis :

T A B L E
I I

VARIANCE DUE TO	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE
Total	21	171.8	
Between tree groups	4	115.8	
Between treatments	3	16.6	5.5 1/3
Error	15	39.4	2.62 2/3

$\frac{5.5\ 1/3}{2.62\ 2/3}$ equals 2.11

The required value for 20 to 1 level of significance equals 3.06

z

$\underline{z}$ value for data is .3733
and standard error for
$\underline{z}$ is .3818
probable error for $\underline{z}$ is .2575

As shown in Table II, the ratio of variance for treatment to that for error is less than an "F" value of 3.06, which is required for a 5% level of significance; the usually accepted level in work of this sort. In other words, the chances are less than 20 to 1 that the differences occurring between treatments were due to the effect of the treatments rather than to chance.

The $\underline{z}$ value is slightly less than its standard error and slightly above its probable error. This means that the chances are slightly better than 1 to 1 that the differences were due to the treatments rather than to chance. This is a very low level of significance.

To summarize the experiment as a whole, it may be stated that it showed some slight indication that mulching and the application of guano reduced the death rate of sickly trees, decreased the loss of leaves in trees of all

classes, and increased partial recovery, and kept down the incidence of red mottling. However, the significance level of the data is so low that little if any importance may be attached to these conclusions. Since material benefit is small, if any, the use of mulch and guano fertilizing just previous to the dry season, will be discontinued.

Respectfully submitted,

WILLIAM PENNOCK

WP/prp

Dr. Popenoe

Suggestions to W. Pennock, May 22, 1941.

1. Benitez will continue to be in charge of seedbed and nursery areas; Hoehn, as Administrator of finca will be responsible for this work.

2. Please make sure 2 bottles of Succirubra seed are sown each month.

3. a. When complete results come in from this year's Helvetia bark samples, please take Benitez to Helvetia to take our share (1/2) of the cuttings from all trees that will have analyzed 8% Q.S. or better.

b. When results come in from Zapote samples of this year, a trip should be made to Zapote to take our share (1/2) of available cuttings from trees 6% or over of the following numbers: Z-687 to Z-708 incl. (Monterey Succirubra in Plantation #3, Monterey.); Z-655 to Z-685 incl. (Various types from Washington in Plantation #2, Monterey.) Also trees Z-628, Z-629, Z-630 and Z-631 (Monterey Succirubra in Plantation #3) which already have analyzed high.

The above should be grafted at Naranjo if possible: if not at Panamá, or else at Naranjo in September, depending on the condition and growth of the Succirubra at both locations and how Benitez feels about these Succirubra stock.

4. Please follow the field planting plans as per memo.

5. Once a month a trip should be made to Panamá, Helvetia, Patzulín, Mocá and Zapote to maintain contact with the co-operators and give them helpful horticultural hints. Also to explain what we are doing at Naranjo.

6. In June, 25 of the Original Ledgers sampled last June in Helvetia will be re-sampled. Hartleben will help with these records (Individual Tree Records) . 4 copies of each; 1 for Naranjo, 1 for Rahway, 1 for Finca Helvetia, and 1 for Owen Smith, i. e. for the "General Smith files".

7. In case any ticklish questions of policy come up, please see Sanchez Also inform Rahway, and preferibly stall.

8. Cobán seed will be given to Owen Smith, Finca Helvetia, and L. L. Pettersen. (1 bottle "mixed", and one paper container of S-39 (13.3%) to each.

9. Owen Smith should be kept moving about shipment of Pito seed.

10. Please see to it high-yielding ledger seed from Samac are sown at Naranjo this June.

11. In the end of June, if we have on hand extra "mixed seed" from Cobán, it might be well to pay a call on Don Mariano Pacheco and give him a couple of bottles.

F. R Jr

F. Rosengarten Jr.

EXPERIMENTAL PLANTATIONS INC.

FINCA EL NARANJO

CHICACAO, SUCH.

June 26, 1941

Dr. Wilson Popenoe
Apartado Aereo 3710
Bogota, Colombia

Dear Dr. Popenoe:

Thank you for your letter of June 21.

I am enclosing herewith 10 gm. of C. succirubra seed, a photostat copy of Sand's article in English, its Spanish translation published by Don Mariano, and your two articles on Cinchona as requested. The Succirubra seed has proven of fairly low germination so you might sow it a little thick.

With the exception of a fungus disease that just recently broke out in the Krukoff nurseries everything has gone nicely here. At naranjo we lost about 15% of the plants but the big majority of these were of the 100 and 200 series of which we really have more than we can use to advantage. At Panamá the loss was very slight but at Patzulin and Zapote they suffered almost a complete fracaso where the K seedlings are concerned. We are convinced that it was the excessive rains that did it, having had 30 in. in May and 15 in. in the first week of June. We promptly dug ditches, removed infected material and did everything we could to increase evaporation and avoid the pounding impact of the rains even to the extent of covering many beds with Vita-pane, teja manil, tarpaper, etc. This seems to have stopped the disease, although the comming of a short "Canicula" probably did more good than all our efforts combined.

Your friend Mr. Kantor fom Burpee & Co. came here just in time to see the fun. Even at that I was able to take him around a little. He seemed well impressed with the possibilities in Guatemala.

George has certainly had his hands full lately what with everything coming at once. His wife presented him with a little girl just about a month ago. Both are doing fine and Mrs. Benitez is now back at the farm.

Don Pancho is behaving himself very nicely. He is now bringing the water from El Mono (near the Esterlina boundary) to add to the water supply and we plan to improve the electric facilities to the extent of getting a water turbine.

Cardona is working on the new house intended for myself and Pat on a contract basis. Pat is working on a temporary basis in Guate. for a wholesale drug outfit that just came here. She seems to like it fine, but we plan to move to the farm when the new house is finished.

Best regards to yourself and Mrs. Popenoe.

Sincerely, Bird

July 8, 1941

Mr. William B. Pennock
Finca El Naranjo
Chicacao, Such.
Guatemala, C. A.

Dear Mr. Pennock:

I wish to acknowledge your letter of June 27, 1941 with attached photographs.

We are all very much pleased to hear that the disease which has been endangering the Cinchona plants seems to be definitely under control.

I suppose by this time you have received the additional rolls of Vitapane which I sent to you.

I have passed the information concerning water pressure along to our engineers. They do not seem to be any too optomistic about getting additional power with the water that we have available.

Referring again to the Erythrina tree at Naranjo, Dr. Krukoff believes that he may be able to identify the tree on the basis of leaf material alone. Will you, therefore, send up a sample of these.

I have not seen Fritz lately but I understand that he is planning to leave the States on July 12th, driving down thru Mexico. Just when that will bring him to Guatemala is a little uncertain.

Attached you will find three copies of additional Cinchona assays. All of them are Helvetia samples, and on the average are extremely high. We wish to particularly call your attention to H-1196 which is 16.1% Quinine Sulfate and H-1239 containing 17.8% Quinine Sulfate. These two trees are the highest that we have had. Special consideration should be given to their care and propagation from these trees. As usual the Coban Ledgers covered by the last five samples listed are of little value.

When I was last in Guatemala you had not received the laboratory equipment consisting of water vacuum pumps, suction flask, Buchner funnel, glass dessicator, Erlenmeyer flasks, water faucets, laboratory cocks and bushings, which supposedly was sent to you in April. Did you ever receive this material?

With best regards,

Yours very truly,

R. P. Lozens

RPL:CS

cc Mr. Perkins
Dr. Popenoe
Mr. Rosengarten

July 8, 1941

CINCHONA BARK ASSAYS

Sample No.	Gms. per Cm^2	% Quinine Sulfate	% Cde. Sulfate
H-1075	0.12	4.1	0.8
H-1086	0.09	8.5	0.4
H-1124	0.14	8.9	0.7
H-1129	0.17	9.9	0.7
H-1132	0.12	12.5	1.4
H-1138	0.12	12.1	0.8
H-1149	0.12	7.4	0.8
H-1161	0.16	11.4	0.5
H-1170	0.13	7.1	0.5
H-1173	0.14	11.4	1.3
H-1175	0.12	11.7	0.5
H-1191	0.12	5.2	0.5
H-1196	0.09	16.1	0.7
H-1197	0.18	7.8	0.3
H-1205	0.13	11.4	0.4
H-1214	0.13	6.6	0.2
H-1217	0.14	11.4	0.6
H-1220	0.13	8.0	0.3
H-1224	0.16	6.4	0.4
H-1225	0.16	(3.3% Qui. & Cde. Sulfate)	
H-1230	0.16	10.2	0.8
H-1231	0.12	12.1	1.2
H-1232	0.14	6.2	3.5

July 8, 1941

CINCHONA BARK ASSAYS

Sample No.	Gms. per Cm^2	% Quinine Sulfate	% Cde. Sulfate
H-1239	0.14	17.8	0.8
H-1240	0.12	13.2	0.4
H-1242	0.10	9.4	0.5
H-1243	0.14	10.6	0.8
H-1245	0.12	8.2	0.6
H-1250	0.15	7.8	0.5
H-1251	0.13	10.1	0.7
H-1252	0.14	9.5	0.9
H-1255	0.14	8.9	0.3
H-1259	0.17	9.8	1.2
H-1260	0.15	12.9	0.5
H-1261	0.23	12.6	0.5
H-1262	0.12	8.2	0.2
H-1265	0.16	6.1	0.5
H-1266	0.12	8.1	0.7
H-1268	0.12	6.8	1.5
H-1269	0.14	8.7	0.4
H-1276	0.12	9.3	1.8
H-1279	0.12	5.9	0.3
H-1286	0.12	8.9	0.8
H-2025	0.15	(Slight traces)	
H-2229	0.12	(1.3% Qui. & Cde. Sulfate)	
H-2373	0.14	(Slight traces)	
H-2465	0.12	(1.9% Qui. & Cde. Sulfate)	
H-2483	0.16	(1.7% Qui. & Cde. Sulfate)	

July 9, 1941.

Mr. R. P. Lukens,
Merck and Co., Inc.,
Rahway, New Jersey.

Re: Experiments for the month of May, 1941.

Dear Mr. Lukens :

The following is the report for the month of May, 1941, on the progress of experimental work on Cinchona.

New Work

Experiment No. 16:

On May 8, 1941, an experiment on grafting was initiated in the Loja nursery. This was purely an exploratory experiment in which but few grafts were made using material that was suspected of being diseased, material from a flowering tree, and material from a non-flowering tree. However, notes were taken about each individual graft both as to age of wood, diameter, and more particularly faulty technique in the grafting operation. Twenty-five grafts were made in all; 10 with cions from Tree H 1244G, which were suspected of being diseased; five from tree of clone Z 219G, which was not flowering, and 10 from another tree of clone Z 219G which was flowering. Benítez' grafting method was used throughout.
On July 8, counts were made and the stocks of the grafts which had taken were pruned back. The results were as follows :

H 1244G (diseased)	Z 219-G (not flowering)	Z 219-G (flowering)
3 dead	1 dead	4 dead
2 alive, unsprouted	1 alive, unsprouted	1 alive, unsprouted.
5 sprouted	3 sprouted	5 "
10 Total	5 Total	10 Total

The experiment is not significant as regards the diseased or flowering condition of the cion material. Its value lies in that it indicated that fairly large numbers of grafts will have to be used in any given group in order to get significant results; in that it was found that mature cions are difficult to cut and fit properly, and that cions whose cut surface exceeds that of the stock, are apt to dry out and die.

Experiment No. 19 :

On May 27 and 28, a slightly more complex experiment, but still largely exploratory, was initiated in the Loja nursery. Cions were taken from young grafted trees in Huasibamba as follows:

30 cions from Z 50-G (A ledger type with suberect, loose branching habit having normal pith; it had many slim, fast growing branches with long internodes).

30 cions from Z 51-G (A broad leaved hybrid type with suberect, compact branching habit, pith discolored).

20 cions from Z 68-G (A ledger type with suberect, compact branching habit, had short internodes and fairly thick terminals; occasional discoloration was found in the pith).

25 cions from Z 219-G (A ledger type with erect, compact branching habit; normal pith).

30 cions from Z 222-G (A ledger type with suberect, compact branching habit; discolored pith).

All of the above trees were flowering.

The cions were classified in accordance with diameter, age of wood, color of bark, length of internodes, and position in the original branch, particularly as regards the location of the leaves. Benítez' grafting method was used throughout.

On July 8, the grafts were examined. Since the age and original position of the cions in the branches are the only factors which noticeably affected the percentage of successful grafts, the results are tabulated below in summarized form as follows :

Cions	Z 50 G			Z 51 G			Z 68 G			Z 219 G			Z 22 G			Total			%
	A	B	X	A	B	X	A	B	X	A	B	X	A	B	X	A	B	X	T	X
1 1/2 to 3 yr. old cions	2	3	-	13	2	-	6	4	-	4	3	3	3	5	2	28	17	5	50	10
6 mo's to 1 yr. old cions	16	4	-	10	-	-	-	-	-	5	-	-	15	-	-	46	4	-	50	0
Terminal Shoots	4	-	1	5	-	-	9	-	1	9	-	1	2	-	3	29	-	6	35	17½
TOTAL	22	7	1	28	2	-	15	4	1	18	3	4	20	5	5	103	21	11	135	8½

A = Grafts that took and sprouted

B = " " " but were not yet sprouted.

X = " " died.

Cions that were 1 1/2 to 3 years old were from that part of the side branches near to where they joined the main trunk of the tree. Their bark was brown to gray in color and the wood was quite hard. Undoubtedly, the difficulty of cutting the cions easily, contributed to the losses. The "eyes" of this type of wood were quite dormant and lacking in vigor.

Cions 6 months to one year old came from that part of the original branch that supported healthy leaves or from a section just below it. Their bark was chocolate colored, particularly at the base, merging to green on the upper parts. The eyes were quite plump and vigorous. The diameter of these cions varied from 1/2 to 1 inch in circumference and the length of the internodes from 1 to

2 1/2 inches, yet all grafted equally well under the conditions of this experiment.

Cions from terminal shoots for the most part had green bark along their entire length, though a few with more mature wood at their base were chocolate-reddish at the base. Thin, less mature cions were poorest.

It is quite apparent from this experiment that highly successful grafting of cinchona may be attained using Benitez' method, particularly at this time of year when the weather is ideal. It is also apparent that the section of the side branches bearing leaves, or that section just below it, gives the best type of grafting material. The experiment further indicates that some trees or clones such as Z 51, in spite of abnormally colored pith, furnish excellent cion material at least in so far as making successful grafting possible. It may still be that the young grafts are potentially infected, and that disease may manifest itself at a later date, under less favorable growing conditions. The good results would also indicate that a tree's flowering is not a major impediment to successful grafting.

This experiment gives us no new information but merely confirms the experience and ideas of Benitez. He has lately had excellent results, having attained 100% of "takes" with material from different ledger clones, even when working with much larger numbers.

The matter of possible diseased grafting material is highly perplexing. Many of the trees from Zapote and Helvetia show a brown discoloration of the pith which in some cases disintegrates forming clots and cavities in the pith cylinder. The pith, particularly in old stems, is dead tissue and this sympton, though abnormal, is not typical of a pathogenic disease, but, rather suggests a nutrient deficiency of some sort. On the other hand, it is passed along in grafting and if found in the mother tree, all the trees in that clone will show it. Streaks in the tissue adjacent to the pith have been observed in some cases, and this suggests the possibility of a latent infection of some sort that may later manifest itself in more typical disease symptons. A budding experiment is planned to test out the possibility of avoiding abnormal pith in these clones by this method of propagation which avoids the transfer of pith tissue.

Experiment No. 18 :

On May 20, an experiment was started on the grafting of ledger cions on succirubra roots. Roots from an old succirubra tree at El Zapote were brought to El Naranjo, cut into 8 to 10 inch lengths and divided into 4 groups in accordance with diameter; each group being assigned a numerical symbol. Cions were taken from 4 different trees and classified into four groups in accordance with maturity; each group being assigned a letter symbol. The grafting was then carried out so that 4 cions of each group were grafted to four (4) roots of each type as follows :

	Symbols	
A - Cions of oldest wood	1- roots of 3-5"	circumference
B - " " 2nd. oldes wood	2- " " 2-3"	"
C " " 2nd. youngest wood	3- " " 2 "	"
D " " youngest wood (tops)	4- " " 1 1/2"	"

Four grafts were made having the following symbols :

A_1, A_2, A_3, A_4; B1, B2, B3, B4; C1, C2, C3, C4;
D1, D2, D3 D4 - making 64 grafts in all.

A side graft ("Java method") was used in all but a few cases.

The grafted roots were then buried in sand to the height of the graft and then moss was placed around them to a height even with the roots tops. An attached photo shows the arrangement.

On July 8th., the grafts were inspected. Seven grafts were found to have taken and of these, two had sprouted. All seven had different symbols; the sprouted ones were D1 and C3, and the others were C1, C4, B3, A2, and A3 respectively. The experiment is not conclusive as regards the type of material best suited for this type of grafting. However, it is thought that combinations C2 and C3 offer best chances.

In spite of the poor results we believe that the method still offers possibilities. The dead grafts were inspected carefully, and it was thought that many of the losses could have been avoided by using a slightly different technique and waxing more carefully. The principal advantage of this type of graft lies in the fact that roots from a high yielding hybrid type may be employed as stocks and thus get ledger types grafted on resistant yet high content roots. The necessity of growing seedling succirubra is eliminated. The disadvantages are the necessity of digging up the roots which is somewhat tedious, and also the need of nursing these grafts along for a while until the roots proliferate. It may be that this type of plant will not have a very desirable type of root system.

Experiment No. 17:

On May 14th., an experiment was started on the bagging of flowers to prevent pollination, in an effort to determine if Cinchona floweres are actually self-sterile. The flowers stalks of 12 trees (6 long style, 6 short) were bagged with stout waxed paper bags. About 25 flowers in each of four of these flower clusters were hand pollinated: Two clusters having long style flowers and two having short style flowers were pollinated, so that each type of flower was crossed with pollen of the two different types. Closed buds about to open were selected for this, so as to insure against their not having been pollinated before. All the other bagged flower clusters were kept free of pollen, and in all cases, open flowers were eliminated.

The bagged flowers were inspected on July 8th., and the results were found to be discourangingly inconclusive. None of the bagged flowers had set seed pods except in two cases where the bag had developed a hole and was invaded by many ants. In no case did the hand pollinated flowers set pods. The question s till remains unsettled, whether the bagging when intact prevented fruit setting, or whether the flowers are actually self sterile and the hand pollination was done imperfectly or at the wrong stage in the flower development. Further investigation along these lines is intended.

Experiment on Bark Yield :

On April 29, tree S 86, a ledger type in growth habit, was eliminated from the Samac planting because of its low content (about 1% total ppt.). It was first carefully measured and then the bark was stripped off from the roots, the main trunk and the branches; each lot of bark was kept and dried separately. The main trunk was sawed off square at soil surface and all bark below this level was designated as root bark. The branches were cut off flush and the upper part of the trunk 3" circumference at 7 ft. height was thrown in with the branches. On May 29, the dried bark was weighed; the dried bark

yields were as follows :

Main trunk	424	grams
Roots	436	"
Branches	753	"
Total-	1,613	" (56.9 oz. or 3.56 lbs.)

This tree was sown from seed July, 1934 and set out in the field July, 1936, thus making it approximately 7 years old from seed, and 5 years old from the time it was set in the field. In contrast with other ledger trees having a more desirable branching habit, this tree had many large sid e branches and the yield of branch bark was proportionately high.

S. H. Cross, in the Commerce Report for 1924, U. S. Department of Commerce; Trade Information Bulletin No. 273, gives the following figures on individual tree bark yields in Java :

4 yrs.	old	0.25 kilos
8 "	"	4. "
15 "	"	10 "
25 "	"	20 "

He does not state whether the age of the tree is taken from the time it is set out or from the time it is grafted or sown as seed. It would seem, however, that he refers to the age from the time of setting out the trees, since this would be a fairer basis for comparing seedling trees with grafted trees and is also the commonest way of referring to the age of orchard trees. At any rate, the interpolated values for a 5 and a 7 years old tree are 1186 and 3063 grams respectively.

Circumference measurements were made of the main trunk at soil level and at foot intervals as follows :

At soil level	-	13 inches
1 ft. high	-	10.5 "
2 ft. "	-	10.0 "
3 " "	-	8.75 "
4 " "	-	5.25 "
5 " "	-	4.75 "
6 " "	-	4.25 "
7 " "	-	3.00 "
(Average	-	7.4375)

The bark area of the main trunk was calculated (average circumference times length) and when converted to sq. cm. was found to be 4031. (The circumference was so small when compared to the length, that there would be but a very small difference if the bark area were calculated as the frustum of a cone, rather than as a cylinder). The weight of the dry bark of the trunk was divided by the calculated area in centimeters, and the average weight per sq. cm. of bark was found to be 0.105 grams. This agrees fairly close with the calculated value if one assumes that the bark weight varies proportionately with the circumference of the trunk. The bark sample taken in August, 1940, at 1 to 2 feet height, weighed 0.15 gms. per sq. cm. (Assuming an 8 inch circumference at the time of sampling - 2 1/2 in. diameter was recorded)

8: 0.15 = 7.435 : X
X = 0.139 gms.

This agreement is considered fairly close in view of the fact that a little of the bark was lost in peeling, that the former tree girth was recorded as diameter which is not as exact as a measurement of the circumference, and that the weight of the bark per sq. cm. undoubtedly registers high. (It must be very difficult to measure the sq. cms. of bark when it is dry and crinkled, moreover, the calculated area of bark is in terms of wet bark and considerable shrinkage must take place).

The assumption that the weight of the bark varies approximately with the girth of the trunk seems fairly well founded, both from a rough inspection of our ledger tree records and from recent micrometer measurements of wet bark.

It is proposed that subject to extensive checking, a formula for calculating roughly the dry bark yield per tree from circumference measurements, may be worked out somewhat as follows:

Average circumference of trunk in cm. - times - length in cm. - times-shrinkage factor - times - proportionate average weight per sq. cm.
equals
weight of dry trunk bark.
The root and branch bark could be got from a rough proportion.
Total yield of quinine could also be calculated.

It seems that such a formula would be a most valuable tool as a basis for tree selection and later as a basis for calculating field yields. It would, however, place a heavy burden on the accuracy of the weight per sq. cm. measurement.

Further investigations along this line are intended when the opportunity presents itself for harvesting the bark from similar trees. Shrinkage of the bark, proportionate weight to given girth, and the desirability of using large bark areas in obtaining unit dry weight will be examined. In this last connection it may prove desirable to trace the entire bark sample when fresh, on cm. graph paper, then record the dry weight of the total sample, and base the weight per sq. cm. on the area of fresh bark. This would seem to have some advantages over the present data that we enter into our individual tree records.

Respectfully submitted,

WILLIAM PENNOCK.

WP/prp

Encls.

cc Rosengarten
Dr. Popenoe
Files.

July 15, 1941

Mr. William B. Pennock
Finca El Naranjo
Chicacao, Such.
Guatemala, C. A.

Dear Mr. Pennock:

Attached you will find three copies of a report on additional assays of bark samples from Samac. Altho there are no outstanding assays of high yielding trees, the average is very satisfactory.

With this group we obtained two samples of bark from Honduras, one marked "Quina" and the other "Copalchi". These you will note contain no Quinine. I assume that you will report to the sender of these samples.

Yours very truly,

R. P. Pilkens

RPL:CS

enc.

cc Mr. Perkins
Mr. Rosengarten
Dr. Popenoe

7/11/41

CINCHONA BARK ASSAYS

Sample No.	Gms. per Cm^2	% Quinine Sulfate	% Cde. Sulfate
S-5	0.11	6.1	0.4
S-6	0.14	8.2	0.7
S-23	0.12	8.0	0.4
S-26	0.14	11.4	0.9
S-29	0.14	11.0	0.5
S-38	0.15	11.4	1.0
S-49	0.12	8.9	0.8
S-52	0.11	11.2	0.5
S-57	0.13	9.7	0.5
S-59	0.11	7.8	0.7
S-65	0.15	7.3	0.4
S-71	0.11	9.1	0.7
S-75	0.14	7.7	0.5
S-78	0.11	9.2	1.1
S-87	0.12	5.3	0.6
S-88	0.12	9.5	1.1
S-99	0.13	6.4	0.7
S-100	0.14	10.8	1.5
S-101	0.14	10.1	0.3
S-102	0.13	10.7	0.8
Quina) Bark from Honduras		(None)	
Copalchi)		(None)	

Photo 1

Experiment #3, Bed A16 Cinchona Calisaya (Krukoff) seedlings.
Photo shows untreated check (✓) plants between the white markers in the front and plants fed with nutrient salts in the back. As shown in the blackboards in back, part of the treated plants (rear, left) were given 3 applications and the others (rear, right) were given 8 applications. In this bed the treated plants showed considerable gain over the check plants at first but later many of the check plants grew quickly and caught up to the treated plants. The plants receiving only 3 applications are somewhat more vigorous than those given 8 applications. Perspective favors check plants. Photo taken June 5, 1941.

Photo 2

Experiment #3 bed A13 Cinchona calisaya (Krukoff) seedlings.

Photo shows untreated ( ) plants in front between white markers and plants fed with nutrient salts in back. In spite of the effects of perspective the much greater vigor of the treated plants is evident. Part of the plants (rear 5 rows across bed)were given 8 applications of nutrient and others(in front of white card in back) only 3,yet all treated plants were about equal in vigor. Photo taken June 5, 1941.

Photo 3

Experiment #3 Tablon No. 21 Cinchona calisaya (Krukoff) seedlings.

Photo shows untreated (✓) plants in foreground, and plants given 3 and 8 nutrient applications in back. The effect of perspective masks the difference in size and vigor. The treated plants are actually larger and more vigorous than the untreated plants. Those receiving 3 applications (between rear card & blackboard) are on a par with those receiving 8 (infront of rearmost card).
Photo taken June 5, 1941.

Photo 4

Experiment #8 Succirubra plants originally at 2" by 2" spacing.

Photo shows check (✓) plants in foreground and treated plants in back. The treated plants which received 5 applications of nutrients are actually much taller and more vigorous than the checks. Other plants in back of the treated ones are por on a with the checks.

Photo taken June 5, 1941, after plants had been thinned out.

Photo 5

Experiment #8, Succirubra plants which were all fertilized with superphosphate and later part of them were given additional nutrients. The check plants in foreground which did not receive additional nutrients were as tall and vigorous as the treated plants but not more so. Their apparent greater size is a result of perspective. The plants have been thinned out since the experiment was started.

Photo June 5, 1941.

August 21, 1941

Mr. R. P. Lukens,
Merck and Co., Inc.,
Rahway, New Jersey

Dear Mr. Lukens:

This is the report for the month of June 1941. This report is limited to giving the final results and conclusions for a number of experiments which were previously reported in part and are now terminated.

Experiments #3 & #8:

Five attached photographs show respectively the portions of certain beds that were given supplementary chemical fertilizing in liquid form. The first three are of experiment #3 and show beds A 16, A 13, and Tablon #21 which had Cinchona calisaya plants (Krukoff); the last two are of experiment #8 and show beds containing C. succirubra plants. Experiments #3 and #8 are described in detail in the January and February reports. Since the reports were written, 5 additional applications of nutrients were made: In experiment #3 only half of the treated calisaya plants were given these applications so that plants receiving a total of 8 might be compared with those that had received only 3 applications; in experiment #8 all of the treated succirubra plants received 5 applications. In all of the photos the check plants were placed in the foreground. Unfortunately the effects of perspective were much greater than had been anticipated and it has the effect of greatly minimizing or completely masking the larger size and greater vigor of plants in the background. This is pointed out in the legend attached to each photo so that they may be properly interpreted.

In summarizing these experiments, it may be pointed out that a solution containing 1 teaspoonful each of superphosphate, sodium nitrate, and potassium sulfate to a gallon of water and applied at the rate of 1 gallon per 10 sq. feet of soil surface stimulated the growth and vigor of both C. calisaya and C. succirubra seedlings when 3 or more applications at approximately 15 day intervals were made. Several different sizes and spacing between plants were included and the beds in all cases contained rich topsoil, thought to be best for nursery purposes.

In the case of bed A 16 a strong early response was noted up to and shortly following the third application, and thereafter, the untreated plants gradually caught up to the treated ones. In all the beds included in experiment #3 it was very evident that 3 applications of nutrients were as effective in stimulating growth and vigor as were 8 applications. In fact, as may be seen in photo No. 1, the plants in bed A 16 receiving only 3 applications were slightly more vigorous than those receiving eight. In experiment #8 a strong response to the nutrient solution was

obtained in a bed of plants which had not been fertilized previously (photo No. 4). No response was noted in the case of plants which had previously been fertilized with superphosphate even though the initial fertilization with superphosphate (in solid form) had apparently had no effect on the plants. (Photo No. 5). A possible explanation of the apparently anomalous results may be that the phosphate interfered with the absorbtion of nitrogen by the plants. In experiment #3 it is thought that the phosphorus as a result of its well known tendency to become fixed upon contact with the soil, may have accumulated therein during the first few applications of the nutrient solution and thereafter interfered with nitrogen absorbtion.

A well replicated experiment is now under way to test out this possibility.

Miscellaneous Fertilizer experiments described in the January report:

In the fertilization with superphosphate in which both succirubra and calisaya seedlings were supplied with superphosphate in powder form and dosis of 1 to 1.1/3 oz. per 10 sq. ft. were used, no evidence of either good or harmful effects of the fertilizing were observed. It may well be that the nutrient level or the balance of nutrient salts within the plant tissue may have been affected thereby. If so, this is not indicated by their appearance or rate of growth. No evidence of greater or lesser resistance to disease over that ofunfertilized plants was noted, though it must be emphasized that the experiment was not designed to detect small differences in this respect.

Experiment #11

The grafts of experiment #11 described in the report for February were examined for the last two times on May 8 and July 8; 83 and 124 days respectively after the grafting operation. Three of the 24 grafts had taken and on July 8 the growth that these had made was comparable to that of grafts in the field that were made on the last week of April and the first week of May! The delayed growth of these grafts is most remarkable.

Within the limits of this experiment and with the grafting technique employed, there was no advantage to be gained by grafting at this time. The use of waxed paper quite probably helped to prevent the 3 grafts to survive. Its use in further grafting in the dry season would seem to warrant further investigation.

It would appear that irrigation and the maintenance of a vigorous growing condition of the stocks which might be obtained thereby would be most desirable in further grafting attempts during the dry season.

Experiment # 13

The plants entering in experiment #13 were affected by the fungus desease which threatened our Krukoff nurseries on the first 15 days of June 1941. When these were last inspected in March there was no appreciable greater survival of the plants treated with glycerine or Ammonium Nitrate over the control plants or those treated with plain water.

Survival counts were not taken at that time because many plants were sickly looking and it could not be determined whether they would survive or not. Survival counts were made as of June 27th. These data are tabulated below as follows:

No. of plants surviving out of 25 originally planted

	Replications			
	Planted immediately		1 hr. drying	
Treatment	1	2	3	4
0.1% Glycerine	22	23	9x	21
0.7% Glycerine	17x	23	23	23
0.8% NH_4NO_3	13x	21	17x	25
Tap water	21	25	20	23
Untreated check	14x	25	24	14

A check mark was made next to the number of surviving plants in those groups where invasion by the fungus disease was evident. The fungus infection occured in spots and seemed correlated to the position of the plants in the bed rather than to the treatment they had received. The fungus disease was present in the areas included in replications 1 and 3 and apparently did not occur in replication 2 and 4.

From these data it seems quite evident that immersing the rootlets of small cinchona seedlings for 1 hr. in weak solutions of glycerine and Amonium Nitrate, within the limits used in the experiment, did not have any appreciable effect on the rate of their survival. This conclusion is consistent with the observations made of the extent of wilting in the plants just after they were planted. At that time no appreciable difference was noted between plants immersed in the different liquids though very marked wilting had occured in all which were left to dry out for 1 hr. in the shade. No differences were noted in vigor or resistance to disease consistent with the treatments. This line of investigation would not seem to warrant further experimentation.

Experiment No. 14:

This experiment on the use of glymocol for soil disinfection, whichwas described in the report for March and April, 1941, has been discarded. The seeds were sown on May 15, 1941, one month after the soil had been treated with the glymocol solutions. The flats were examined at 15 day intervals thereafter and germination was so poor as to make the experiment worthless.

The poor germination is difficult to explain, since seed from the same lot was used on several previous occasions with excellent results and the disinfection of seed with Zonite had been found to work very well in a small scale laboratory tests.

The test of the method of seed disinfection which has not been reported previously was as follows : Two lots, each containing 50 seed of Cinchona ledgeriana from tree H -973 (same source as used in experiment No. 14) were treated with a 1 to 5 dilution of Zonite and sown on wet filter paper placed in petri dishes. The treatment with Zonite consisted of placing the seed in a small vial with 10 to 15 cc. of solution and agitating the vial until all the seed was thoroughly wet and sank to the bottom of the vial. Lot No. 1 was sown immediately after it was thouroughly wet. Lot No. 2 was allowed to remain immersed in the vial for 1/2 hour. Both lots were sown while the seed was still wet with the solution. Excellent germination occurred in both lots; all but six seeds of Lot No. 1 and all but 3 seeds of Lot No. 2 germinated.

Under the circumstances, it is difficult to explain why the seed in experiment No. 14 which was identical with the above and was treated similarly, failed to germinate satisfactorily. I find it very hard to believe that the glymocol treatment of the soil killed the seed, since it was not sown until one month after the soil had been treated and no trace of glymocol odor could be detected. At this time a few weed seeds which had not been killed by the glymocol were germinating in all the flats. A possible, though not very convincing explanation, may lie in the fact that the seed which had been weighed out one month before sowing into 1/2 gram lots and each lot kept separately in corked test-tubes, may have degenerated during this period.

We are convinced that the most promising approach toward controlling fungus disease and damping-off in our seed beds lies in drying out the soil of the beds for an extended period before sowing, and in the careful control of the watering after sowing.

Experimentation with seed and seed-bed disinfection will, however, be continued and the use of glymocol will be tried again until some conclusive results are obtained.

Respectfully submitted,

William Pennock.

WP/prp
Encls.