UNITED FRUIT COMPANY

DEPARTMENT OF RESEARCH

ANNUAL REPORT

1958

VOLUME I

DIVISION OF TROPICAL RESEARCH

General Office
Boston, Massachusetts
April 1, 1959

Mr. K. H. Redmond
President, United Fruit Company

Dear Mr. Redmond:

The work of the Department of Research during 1958 is summarized in the following two volumes.

Volume I includes the work of the Division of Tropical Research at La Lima, Honduras; Changuinola, Panama; Sevilla, Colombia; Guayaquil, Ecuador; and Coto, Costa Rica.

Volume II reports on activities at the Central Research Laboratories, Norwood, Mass.; New York Laboratory; Transportation and General Research, New York; and on studies undertaken for the Company at several American universities and experiment stations.

During 1958 improvement and expansion of staff and facilities continued, notably at Central Research Laboratories. The Department's total professional staff increased from 47 scientists at the end of 1957 to 70 at the end of 1958. Staff at Central Research Laboratories numbered a dozen scientists at the end of its first year of operations.

Much information of immediate and potential value to the Company is contained in these reports of research findings in 1958. We can expect an increase in the flow of valuable information from research investigations as new staff members are integrated into the accelerated program and as new facilities and equipment become fully operational.

Careful review of the past year's research program impresses me with the number and scope of serious Company problems requiring factual information, and with the progress which is being made toward economic solutions to them. There is satisfying evidence of the increased support the Company is giving to its research activity.

Biological research often appears disappointingly slow, compared with the rapid advances in knowledge and skill which is achieved in other scientific fields. Many of the investigations reported on in this annual report must continue for months and even years before reliable facts can be determined. I believe, however, that the Company can take real satisfaction from the indications of progress reported here.

Sincerely yours,

Jesse E. Hobson

Vice President
and Director of Research

DIVISION OF TROPICAL RESEARCH

La Lima, Honduras
March 25, 1959

Dr. J. E. Hobson
Vice President and Director of Research
United Fruit Company
Boston, Massachusetts

Dear Dr. Hobson:

I am pleased to transmit herewith the Annual Report of the Division of Tropical Research for the year 1958.

A full year of research on the part of a larger staff than we have ever had before has resulted in a lengthy but informative report. Part 1 covers La Lima, Lancetilla, Changuinola, Sevilla and Guayaquil stations. Part 2, the Coto Station. Many procedural details have been omitted, in order to reduce the report to manageable size. Further information is, of course, immediately available.

During 1958, our staff made significant advances in understanding and in techniques of banana production. It was a year of problems; it was also a year of progress. I feel sure we have laid the groundwork of future advances that will contribute significantly to our Company's operations.

Yours very truly,

N. C. Thornton

N. C. Thornton
Director

ANNUAL REPORT

1958

UNITED FRUIT COMPANY

DIVISION OF TROPICAL RESEARCH

PART 1

VINING C. DUNLAP LABORATORIES
LANCETILLA EXPERIMENT STATION
CHANGUINOLA RESEARCH STATION
SEVILLA RESEARCH LABORATORY
RESEARCH DEPARTMENT - GUAYAQUIL, ECUADOR

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ANNUAL REPORT

1958

UNITED FRUIT COMPANY
DIVISION OF TROPICAL RESEARCH

PART 1
SUMMARY

Agronomy

Growing one extra sucker per mat and removing it when 7-8 feet tall (rhizome weight 8-10 pounds) can be used in good areas as a method for obtaining seed.

Average bunch weight increases slightly and maturation period decreases slightly when clean, perforated polyethylene bags are placed over fruit within 7 days after the first hand is exposed. At present, these differences are statistically but not economically significant.

Fertilizer performance of the Lacatan, Valery and 67 varieties of banana is quite similar to that of Gros Michel - nitrogen produces significant response in terms of growth rate, fruit production and fruit quality - phosphorus and potassium do not.

Panama Disease

Inter-flooding moldboard plowing greatly reduces incidence of Panama disease. One discing between floodings also reduces disease incidence, but not as much. Results vary from one area to another.

Sigatoka Disease

Leaf reaction to oil spray may be evident within 30 minutes after application. Elongated dark green streaks appear on treated leaves. Oil controls the Sigatoka pathogen within the confines of the sprayed area only. Application of oil to either the upper or lower leaf surface is effective, again, within the confines of the area of application.

There are statistically significant differences in fruit weights between oil-sprayed and Bordeaux-sprayed areas, in favor of Bordeaux. Differences in favor of Bordeaux also exist in terms of days to harvest, number of hands, and number of leaves at time of shooting but these differences are slight.

If enough oil is applied, Sigatoka can be controlled even when adjacent to areas of heavy, uncontrolled infection. This has not been true with Bordeaux.

Moko Disease

Moko research in 1958 was centered in the Coto Station. (See Part 2). At La Lima, Moko extension work contributed to reduction of Moko cases in one district from a high of 5,000 cases to a low of 30 cases during 1958.

Nematodes

The burrowing nematode, Radopholus similis, and other nematodes have been found in various location in all division surveyed.

The life history of the burrowing nematode has been described. Preliminary findings indicate that commercial species of bananas have no resistance to these nematodes.

Economic significance of nematode infection remains to be determined.

A method of rhizome paring and chemical treatment to eliminate nematodes from seed has been devised.

Physiology

Initial weight of seed markedly affects subsequent germinations and growth. For Honduras Gros Michel sucker seed, nothing less than 1.5 pounds should be planted; where rapid growth is essential, seed weighing more than 2.0 pounds, trimmed, should be used.

Adventitious buds arise from calluses on a rhizome. These buds could be the source for a range of banana sports. Treatment of the callus with radiation or with chemical mutagens could further increase variation in the genetic material of the Gros Michel plant.

A new technique of rhizome trimming has been devised in which the rhizome is trimmed away to the zone of emerged roots. Germination is high; growth is satisfactory. Further work is required to prove the field practicability of the method.

A file of microscope slides of banana plant tissues has been established and is available on loan.

Entomology

Dieldrin applied in orchard oil spray by helicopter continues to control Red Rust Thrips. Perthane 4E or Malathion 5E alone or together has proved to be ineffective.

Dusting planting holes with Dieldrin 2.5% dust before and after the seed is introduced is effective in controlling root borers.

Timing has proven important in controlling leaf-feeding caterpillars. The proper time to apply insecticides is at the beginning of an infestation.

Baiting with cut green bananas dusted with strychnine alkaloid has proven to provide effective taltuza control.

Formulations tested of Diazinon, Toxaphene, Sevin and Malathion have proven to be phytotoxic. Dieldrin is the least phytotoxic material tested to date.

Soils and Chemistry

Preliminary studies of the Motagua River, Guatemala, reveal a striking relationship between river stage and sediment content. No such correlation has been found for the Ulúa River, Honduras.

Clean Seed Program

Late in 1958, a program to acquire disease - and pest-free seed in each Division was launched by means of a conference attended by Agricultural personnel from each banana-producing Division.

The study on seed infections revealed that healthy-looking rhizomes can carry Panama disease organisms which may or may not cause the plant to succumb to the disease.

Scientific Publications

Staff members of the Division of Tropical Research contributed 15 papers to the scientific literature during 1958.

Uptake and losses of nitrogen from banana soils and the effect of rate of urea fertilization on fruit production.

Inability to account for all of the nitrogen applied to crops has bothered agriculturists for many years. Lysimeter experiments have shown that the differences between the nitrogen applied and that accounted for are not entirely due to leaching. Allison (1955) has reviewed a great deal of nitrogen loss experimentation and calculates that from 15 to 50% of the nitrogen applied to a crop may be lost from the soil. Efforts to reduce these losses are generally unsuccessful, due to the fact that conditions most suitable to crop growth are usually those most conducive to nitrogen loss. On annual crops in the temperate regions, nitrogen nutrition can be made more efficient by careful consideration of time and placement of fertilizer. Gaseous losses due to microbial action will vary with the season and, during the three or four months that a crop is being grown microbial populations are increasing from low winter levels. Thus, the crop has a chance of obtaining a fair share of the nitrogen applied. In the tropics, however, there is probably only a slight variation in microbial numbers throughout the year and for most of the year conditions are optimum for microbial activity. As a result, any nitrogen applied to the soil can be attacked immediately by large numbers of microorganisms and the crop must make use of what is left. Denitrification is, therefore, practically impossible to combat. However, other forms of loss such as ammonia volatilization and leaching can be controlled to some extent by method and time of application.

Type of nitrogen carrier can also be exploited in this regard. Reducing the cost of application by more efficient methods can also help to defray a part of the loss which will be experienced when the nitrogen reaches the soil.

An ideal fertilizer material would be one that would not be attacked by denitrifying organisms but which could be utilized by the plant. Urea-form fertilizers may have some such quality but their cost may be too high to warrant their use.

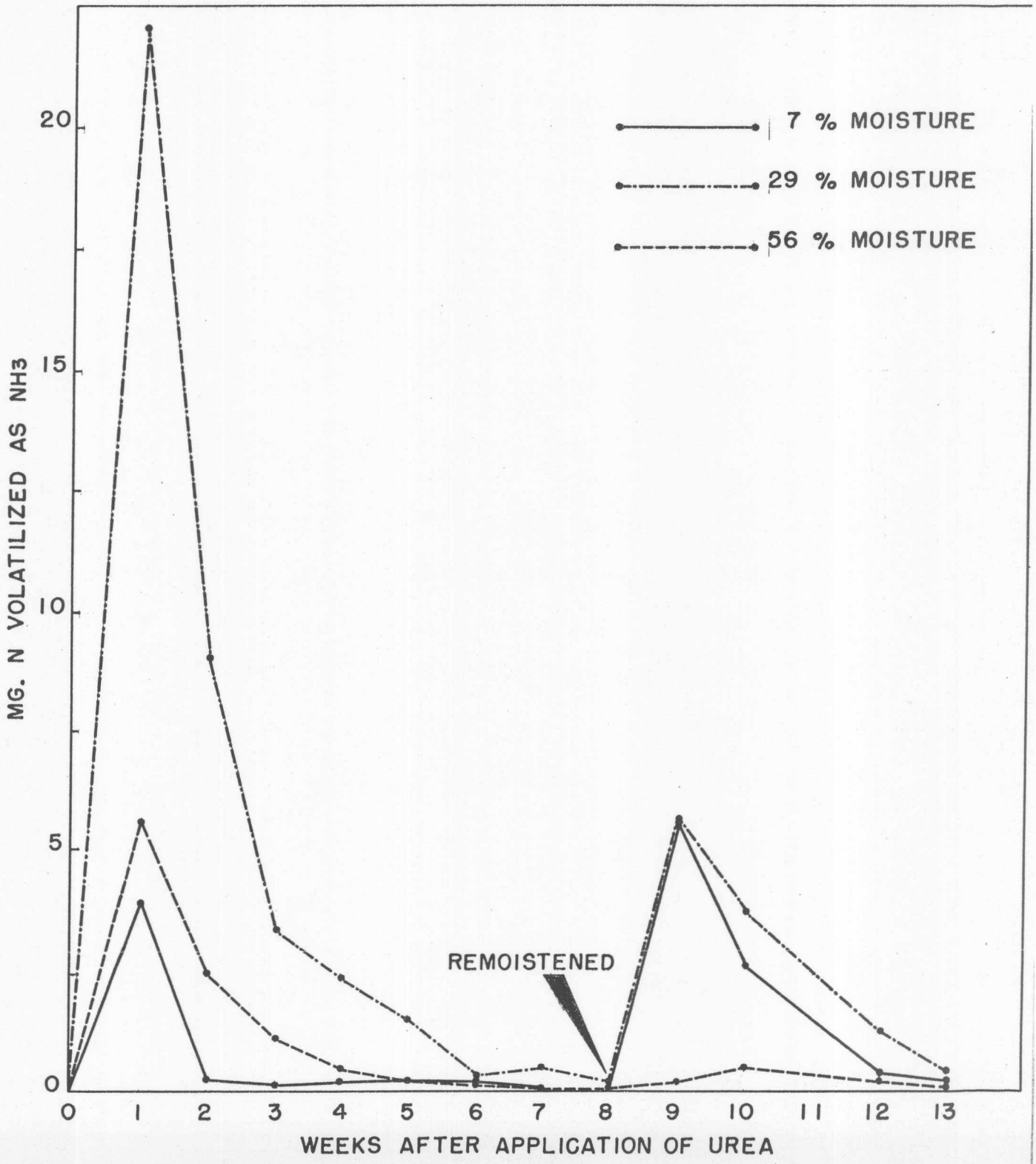
Factors affecting the rate of ammonia volatilization from urea-fertilized soils have been studied and the following observations made:

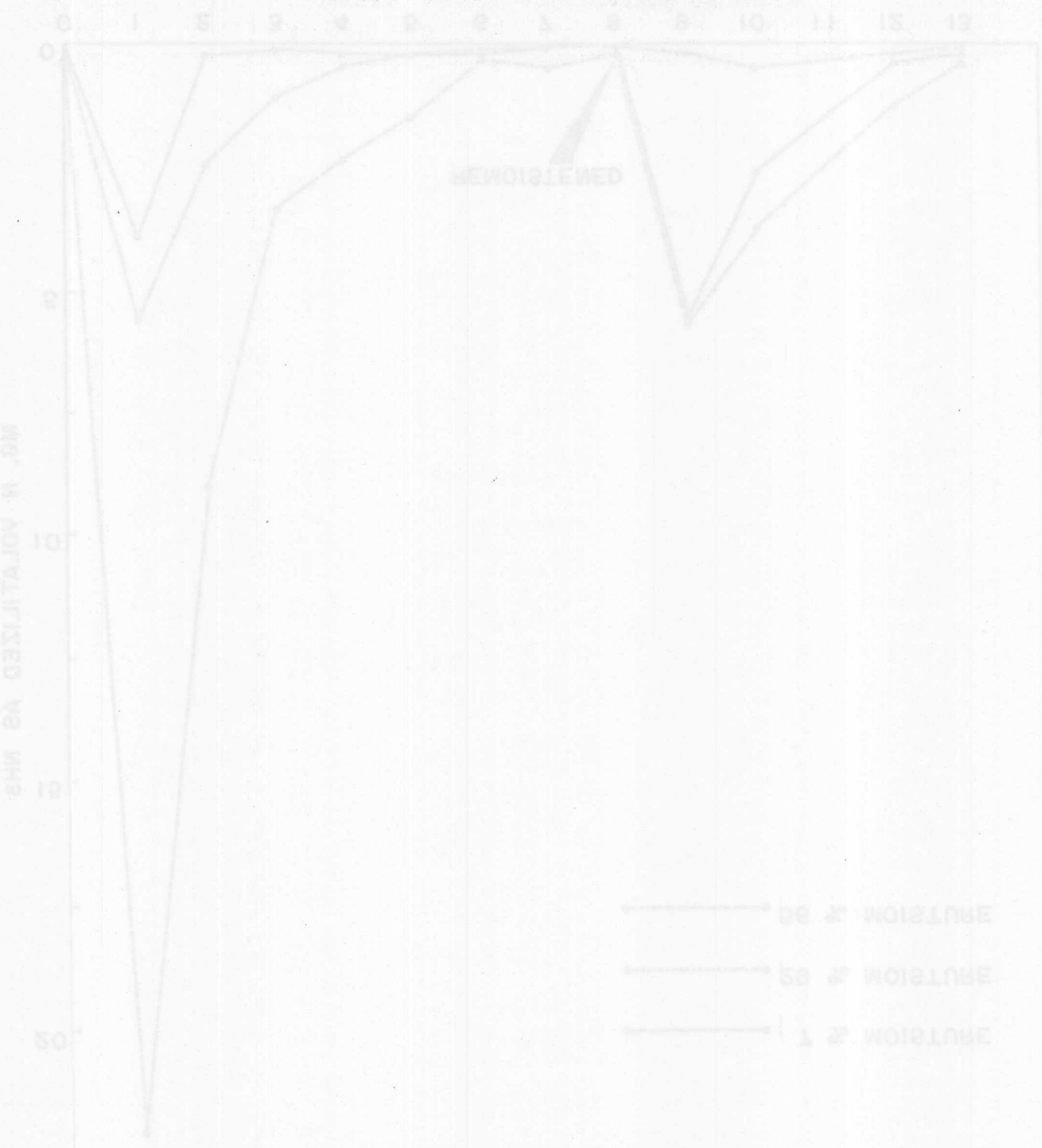
1. Increasing the temperature of the soil resulted in quicker volatilization of NH_3. However, after six days the differences in NH_3 volatilized for three temperatures were not very marked.
2. Applying urea as a solid resulted in greater loss of NH_3 than if the urea was applied in solution even though all soils were brought to the same moisture level following application. Soils that were allowed to dry out naturally lost more NH_3 than soils which were remoistened daily, regardless of the form in which the urea was applied.
3. Moisture level of the soil exerted an important influence on NH_3 loss, the maximum loss occurring at the optimum level of soil moisture for plants (Fig. 1).
4. The addition of a grass or banana trash mulch to the soil surface resulted in approximately twice as much NH_3 loss as from a bare soil. When sugar was applied instead of grass or banana trash, exactly the

FIGURE 1

EFFECT OF SOIL MOISTURE LEVEL

ON NH_3 VOLATILIZATION FROM UREA FERTILIZED SOIL





opposite effect was observed; that is, there was a sharp reduction in the amount of NH_3 volatilized.

5. Amoung three rates of urea compared, there seemed to be no effect of rate on per cent of added N lost as NH_3, the losses being approximately 20% for 167 ppm N, 333ppm N or 500 ppm N.

6. Texture of the soil appeared to have no effect on the rate of NH_3 volatilization when urea was applied. This was also true of Uramite. However, ammonium sulphate caused a far greater loss of NH_3 from a sandy loam than from either a soil loam or a silty clay loam. Calcium cyanamid showed somewhat greater loss of NH_3 from a silt loam or silty clay loam than from a sandy loam.

Preliminary tests have shown that from 20 to 35% of the N applied as aqua ammonia by spraying was lost during the spray application. Any losses from the soil following application would be in addition to this. Ammonia solutions varying in concentration from 42 to 294 ppm N were used and were not found to differ as regards per cent N lost.

The conversion of urea to ammonia, nitrite and nitrate was studied in conjunction with total nitrogen losses. Following an initial buildup of ammonia, there was an accumulation of nitrite which persisted for about one week before being converted to nitrate. However, at no time did the total ammonia, nitrite and nitrate exceed one half of the nitrogen added as urea. Maximum loss of total nitrogen appeared to occur at about the time that all of the nitrite disappeared and nitrate accumulated.

Comparison of urea with other nitrogen carriers as to total N loss on different textured soils showed that urea and ammonium sulphate lost over 40% of the applied N from a silt loam and a silty clay loam and from 20 to 30% from a sandy loam. Sodium nitrate lost about 25% from all soils, as did ammonium nitrate. Uramite resulted in a loss of only 8% from both the sandy loam and the silty clay loam. Calcium cyanamid lost about 12% from the sandy loam, about 27% from the silt loam and 38% from the silty clay loam. These losses include denitrification as well as ammonia volatilization.

Studies on biuret toxicity to banana plants indicated only slight marginal leaf burning when as much as 87 ppm biuret were applied to the soil. The effect was the same whether urea was present or absent. Biuret is apparently quite stable in the soil. At least it was not converted to inorganic forms of nitrogen after 21 days incubation. Where the enzyme urease was added to the soil along with biuret, there was a significant increase in NH_3 loss. However, this did not begin to occur until the sixth week following application and after twelve weeks only 7% of the added N had been volatilized as NH_3. This lag period may be due to the biuret having to be converted to some other organic form before it could be attacked by the urease. Where urease was not added with the biuret there was essentially no NH_3 volatilized, even after twelve weeks.

On two crops of sorghum in the greenhouse, it was observed that where no urea was applied, there was a 13% loss of soil nitrogen after crop removal had been accounted for; where 200 ppm N were applied, this loss was 15.5%, where 400 ppmN were applied it was 22.0% and where 600 ppm N

were applied it was 26.5%. After subtracting the loss from the checks, this means that the 200 ppm N application resulted in 35.1% of the applied N being lost, the 400 ppm N application resulted in 33.9% of the applied N being lost and the 600 ppm N application in 63.4% loss of applied N.

It must be stressed that all of the foregoing are laboratory and greenhouse observations and should in no way be applied to field conditions without first being thoroughly tested in the field.

The effect of pruning for seed production.

This experiment was established for the purpose of comparing the effect on fruiting performance of normal farm pruning with: (1) growing one extra sucker per mat for seed and removing it when 7-8 feet tall at which time the rhizome weighs 8-10 pounds and (2) removal of the old "bullhead" for seed the day following a fruit cut.

Results as of December 31, 1956 (Page 23, 1956 Annual Report) indicated that growing one extra sucker per mat for seed and removing it when the rhizome weighed 8-10 pounds had no effect on fruit production or quality whereas removal of the old "bullhead" for seed the day following a fruit cut caused a 3.6% decrease in mean fruit weight with respect to normal farm pruning. This difference was significant at P.05 and has continued to be significant at P.05 through 1957. In March 1957, the experiment was modified to the extent that two extra suckers per mat, instead of one, were permitted to develop for seed and removed when the rhizomes weigh 8-10

pounds. The other two treatments -- normal farm pruning and removal of the old "bullhead" for seed the day following a fruit cut -- remain the same.

As a result of Panama disease, there has been a variable reduction in plot population during the course of the experiment. In the 1957 Annual Report, we attempted to minimize the effect of this factor on the fruit production data by (1) computing an adjusted acreage factor for each plot (2) multiplying each plot-production value (number of stems shot, number of stems reaped, etc.,) by its corresponding factor and then (3) using the new production values also as a basis for the analysis for variance. At that time it was noticed (in a few cases) that plots having a certain number of cases of Panama disease in December, 1956, had fewer cases in December, 1957.

This is even more apparent at the present time. The primary reason for this variation lies in the fact that good agricultural practice permits healthy suckers to develop and a varying percentage of these produce marketable fruit even though the mother showed symptoms of Panama Disease. This has become a more important factor during 1958. In addition, some supplying may have been done. As a result, correction for reduction in acreage has little meaning at the present time and the analysis of variance has been carried out on the basis of actual production values.

The effect of the various treatments on fruiting performance is given in Table 1.

TABLE 1

FRUIT PRODUCTION - PRUNING EXPERIMENT

AUGUST, 1955 TO DECEMBER, 1958

	Stems Shot P/acre	Stems Shipped P/acre	Total Weight Shipped P/A	Mean Weight Per Stem
Normal farm pruning	1166	730	70809	97.00±.4345
Extra suckers per mat removed for seed*	1167	733	69931	95.40±.4432
Normal farm pruning - old "bullhead" removed for seed	1170	741	69347	93.59±.4162
F. Value - Treatments	.003	.025	.008	---
F. Value - P.1	2.81	2.81	2.81	---

* One extra sucker per mat grown for seed until March, 1957. Thereafter, two extra suckers per mat have been grown for seed.

The results continue to show no significant difference between treatments with respect to number of stems shot per acre, number of stems reaped per acre and total weight shipped per acre.

The adverse effect on mean bunch weight caused by the removal of the old "bullhead" for seed the day following a fruit cut is still evident. The decrease, which is significant at P.01, amounts to 3.64% with respect to normal farm pruning. This reduction can probably be attributed to either root disturbance during digging or removal of a large portion of the reserve food supply of the mat.

Previous results (1956 and 1957 Annual Reports) indicated that growing one extra sucker per mat for seed and removing it when the rhizome weighed 8-10 pounds had no significant effect on fruit production or quality. The data in Table 1 show that although the growing of two extra suckers per mat for seed and removing them when the rhizomes weighed 8-10 pounds has had no adverse effect on fruit production,

the practice has brought about a slight reduction in average bunch weight. This decrease, which is significant at P.01, amounts to 1.68% with respect to normal farm pruning.

It should be noted that the difference between the extra-suckers-per-mat-removed-for-seed treatment and the normal farm-pruning-old-"bull-head"-removed-for-seed treatment is also significant at P.01.

The data accumulated thus far lead to the following conclusions:

a) Removal of the old "bullhead" for seed the day following a fruit cut has a detrimental effect on mean bunch weight.

b) The growing of one extra sucker per mat for seed and removing it when 7-8 feet tall at which time the rhizome weighs 8-10 pounds has no adverse effect on fruit production or quality.

c) The growing of two extra suckers per mat for seed and removing them when 7-8 feet tall at which time the rhizome weighs 8-10 pounds has no detrimental effect on fruit production but gives rise to a slight reduction in fruit quality.

d) The growing of one extra sucker per mat and removing it when 7-8 feet tall at which time the rhizome weighs 8-10 pounds can be used as a method for obtaining seed. However, such an operation should be confined to good areas.

The effect of bagging fruit with polyethylene on fruit production.

Observations made by Agriculture Department personnel indicated that placement of a clear-perforated-polyethylene bag (3/4" holes-96 per side) over approximately two-week-old stems of fruit and leaving them in place until the stems are reaped will result in cleaner, more symmetrical and

heavier bunches. Furthermore, the maturation period may be decreased. An experiment was laid out in February, 1958. The experimental design was a randomized complete block with forty-eight replications. Each plot was 25 mats or 0.08389 acres in size. Treatments consisted of no bagging and placement of a clear-perforated (3/4" holes-96 per side) polyethylene bag over each stem of fruit as soon as possible after the first hand was exposed. Tagging and bagging cycles were conducted on a weekly basis.

The results with respect to fruit production, as of November 30, 1958 (17 months after planting -- 10 months after shooting), are presented in Table 2.

TABLE 2

EFFECT OF BAGGING WITH POLYETHYLENE ON FRUITING PERFORMANCE

Treatment	Stems Shot*	Stems Reaped*	Weight Shipped Pounds*	Mean Weight Per Stem Pounds
Bagged with polyethylene	2108	1074	88344	82.26
Normal	2091	1086	86798	79.92
L. S. D. - P 0.05				1.05
L. S. D. - P 0.01				1.41
F. Value calculated	0.29	0.17	0.36	23.75
F. Value P 0.05	4.05	4.05	4.05	4.05
F. Value P 0.01				7.20

* Each value is the total for 48 plots or 4.02672 acres.

The summary shows an increase of 2.93% in average bunch weight for the bagged treatment. In addition, the bagged sample has also matured slightly more quickly than the non-bagged. This is shown in Table 3.

TABLE 3

EFFECT OF BAGGING WITH POLYETHYLENE ON MATURATION PERIOD

Treatment	Mean Maturity Period In Days
Normal	89.86 ± 0.1997
Bagged with polyethylene	89.12 ± 0.2087
Difference	0.74 ± 0.2888
"t" Value	2.560
"t" Value P 0.05	1.960
"t" Value P 0.01	2.576

The difference in maturation period amounts to only 0.83% but is almost statistically significant at P 0.01. This means that fruit growth has been accelerated by enclosing the bunch in a clear-perforated-polyethylene bag. The average daily weight increase of the bagged sample has been 0.923 pounds compared with 0.881 pounds for the normal farm fruit. This increase amounts to 4.77%. The differences observed have little significance at the present time from an economic point of view, but are of interest in demonstrating that some factor induced by covering the stem with a clear-perforated-polyethylene bag, has increased the speed of fruit development and average bunch weight.

Varieties of possible economic importance.

Variety plots were maintained to increase the acreage of each of our most promising varieties and use these plants for obtaining essential data necessary for the comparison of these varieties with each other and with Gros Michel.

In addition, during the period June through September 1958, the variety collection located in Section 17 of Guaruma 1, was dug up and replanted. Varieties and sports in the collection are as follows:

Morado Pula	Variety 67 (Kan Chiao)	Jamaica Unknown (probably Robusta)
Morado Puti	Inarnibal	Farm No. 8
Tadiao	Chuoi Cau Trang	Dwarf Cavendish
Guaruma 2 Unidentified	Kapas	Sauce Sport
Indianan Unidentified	Pisang Seroeanota	Giant Cavendish (probably Grand Nain)
Brazilian Banana	Pisang Mangsan	Sixaola Unidentified
Radja	Mamboef Diodi	Manang
Chek Tuk	Djantan	Guyuran
Guaruma 2 Sport	Morong Datu	Susu
Manchado Sport	Lidi	Kanara
Balsamo Sport	Mundan	Pomme Java
Honduras Plantain	Dorado	Masak Sahari
Iacatan	Radja Puri	Sonkel
Rotan	IC - 2	Balbisiana
Chuoi Tieu Hoong	Veinte Cohol	Siguatepeque
Bungulan	Galimba Pula	Red Plantain (Hembra)
Giant Fig	Rosacea	Red Plantain (Macho)
Vimama	Maia Oa (red type)	Horse Plantain
Valery	Pandok Beureum	Guineo Prieto
Kluithon Keo	Zebrina	Iaknau
Grand Nain	Bout Rond (probably Congo)	Maiden Plantain
Congo	Musa Ensete	DeCosta White Plantain
Tumoc	Dominican Dwarf Plantain	Cenizo Apple Plantain

Several months prior to digging up and replanting this collection, three seed of each variety and sport were planted in a small area of Section 21 in Guaruma 1. Thus far, the following varieties and sports have shown symptoms of Panama disease: Maia Oa (Red type), Manchado Sport, Guaruma 2 Unidentified, and Pomme Java.

The effect of various amounts and combinations of nitrogen, phosphorus and potassium on growth and fruiting performance of the Iacatan, Valery and 67 varieties of banana was studied. The results to date are very similar to those obtained for the various nitrogen-phosphorus-potassium experiments carried out during the period 1948 to 1952 in the same localities in which Gros Michel variety of banana was the indicator plant.

Regardless of variety or location, no significant response to either phosphorus or potassium was obtained for (1) growth rate, (2) fruit production - number of stems shot, number of stems reaped and total weight shipped, or (3) fruit quality as indicated by average weight per bunch.

Regardless of variety or location, a significant response to nitrogen was obtained for growth rate, fruit production and fruit quality. These components were found to increase progressively with an increase in the rate of nitrogen fertilization. Without exception, a highly significant result was obtained for the linear regression component of the nitrogen treatment source of variation, indicating a gradual increase in growth rate, fruit production and fruit quality as indicated by bunch weight with increasing rate of nitrogen fertilization. This suggests that under like circumstances the linear response curves should hold for even higher levels of nitrogen.

Because of new interest in the Iacatan variety, results for this plant are given here in some detail.

Fertilizer treatments were initiated in October 1957, using three levels of nitrogen (N_0, N_1 - 200 lbs. per acre per year and N_2 - 400 lbs. per acre per year), three levels of phosphorus (P_0, P_1 - 50 lbs. of P_2O_5 per acre per year and P_2 - 100 lbs. of P_2O_5 per acre per year) and three levels of potassium (K_0, K_1 - 200 lbs. of K_2O per acre per year and K_2 - 400 lbs. of K_2O per acre per year) singly and in all possible combinations. Carriers were urea (46% N), treble superphosphate (47-49% P_2O_5) and potassium sulfate (48% K_2O), respectively. The sidedress method of application was utilized. Nitrogen was applied on a quarterly basis. Phosphorus and potassium were applied on a semi-annual basis.

Growth rates.

Subjection of the basic data to an analysis of variance showed the nitrogen treatment to be the only highly significant source of variation. The nitrogen x phosphorus interaction was found to be significant at P.05. The relationship between rates of nitrogen fertilization and average monthly growth rate is shown in Table 4. The interaction of nitrogen levels x phosphorus levels with respect to average monthly growth rate is presented in Table 5.

The results in Table 4 show that the average monthly growth rate increased progressively with an increase in the rate of nitrogen fertilization. The improvements in growth rate for the N_1 over the N_0 and the N_2 over the N_0 levels of nitrogen amount to 6.0 and 9.0%, respectively. These differences are significant at P.01.

TABLE 4

EFFECT OF NITROGEN LEVELS ON AVERAGE MONTHLY GROWTH RATE (IN FEET) FOR THE PERIOD DECEMBER, 1957 TO NOVEMBER, 1958.

Nitrogen Levels	Average Monthly Growth Rate*
N_0	1.3058
N_1	1.3847
N_2	1.4233
Min. Sig. Diff. (P.05)	0.0462
Min. Sig. Diff. (P.01)	0.0632
F. Value (N)	20.98
F. Value (P.01)	5.61

* Each value is an average of 18 plots.

TABLE 5

EFFECT OF NITROGEN LEVELS X PHOSPHORUS LEVELS ON AVERAGE MONTHLY GROWTH RATE (IN FEET) FOR THE PERIOD DECEMBER, 1957 TO NOVEMBER, 1958.

	P LEVELS*			
N Levels	P_0	P_1	P_2	Total
N_0	1.2333	1.3631	1.3210	3.9174
N_1	1.4095	1.3534	1.3911	4.1540
N_2	1.4611	1.4100	1.3986	4.2697
Total	4.1039	4.1265	4.1107	12.3411
Min. Sig. Diff. (P.05)	Any two of nine means			0.0803
Min. Sig. Diff. (P.01)	Any two of nine means			0.1098
F. Value (N x P)				4.09
F. Value (P.05)				2.78
F. Value (P.01)				4.22

* Each value is an average of 6 plots.

The nitrogen treatment variance was separated into its linear and quadratic components. These variances were then compared with the variance for error. A highly significant result was obtained for the linear component, indicating a gradual increase in average monthly growth rate with increasing rate of nitrogen fertilization and suggesting that under like circumstances, this linear response may hold for even higher levels of nitrogen.

The data with respect to the nitrogen x phosphorus interaction show that for any phosphorus level, there is a tendency for growth rate to increase progressively with an increase in the rate of nitrogen fertilization. However, significant differences between rates of nitrogen fertilization were found only for the P_o level of phosphorus. The interesting feature of this interaction is the fact that we obtained a significant response to phosphorus at the N_o level of nitrogen, even though the analysis of variance showed the phosphorus treatment source of variation to be non-significant. The differences between the P_1 and P_o and the P_2 and P_o levels of phosphorus are significant at P.01 whereas, the difference between the P_2 and the P_1 levels was found to be non-significant. The data also show that the difference between any two phosphorus levels for either the N_1 or N_2 levels of nitrogen, is not significant. This indicates that applications of phosphate in conjunction with nitrogen fertilization, did not improve growth rate. We might speculate, therefore, that although a significant response to phosphorus was obtained for the N_o level of nitrogen, when phosphorus was applied at either the N_1 or N_2 levels of nitrogen, it may have partially

counteracted the effect of the latter nutrient. If this were not so, for any given level of applied phosphate and applied nitrogen, we would have expected the growth rate to be at least equivalent to, if not greater than, the growth rate for the corresponding level of applied nitrogen at the P_0 level of phosphorus.

Fruiting Performance

An analysis of variance showed the nitrogen treatment to be the only highly significant source of variation insofar as number of stems-shot per-acre, number of stems-reaped-per-acre, and total weight shipped per acre are concerned. The nitrogen treatment and the nitrogen x phosphorus interaction for the average-weight-per-stem unit of measurement were found to be significant at P.05. The relationship between the various units of measurement and nitrogen levels is shown in Table 6. The interaction of nitrogen levels x phosphorus levels with respect to average bunch weight is presented in Table 7.

The results in Table 6 show that regardless of the unit of measurement, fruit production and quality increased progressively with an increase in the rate of nitrogen fertilization. It will be observed that the increase in production with respect to the N_0 and N_2 levels of nitrogen amount to 7.75, 17.77 and 26.67% for the number-of-stems-shot, number-of-stems-reaped and total-weight-shipped units of measurement, respectively. These differences are significant at P.01. The improvement in fruit quality, expressed as average weight per stem, with respect to the same levels of nitrogen is equal to 5.67 lbs or 8.04%. This difference falls just short of being significant at the 1% probability level.

TABLE 6

EFFECT OF NITROGEN LEVELS ON FRUITING PERFORMANCE FOR THE PERIOD DECEMBER, 1957 TO NOVEMBER, 1958.

Nitrogen Levels	Stems Shot P/A*	Stems Reaped P/A	Total Wt. Shipped P/A	Average Weight P/Stem*
N_o	697	495	35342	70.50
N_1	728	546	40577	73.71
N_2	751	583	44768	76.17
Min. Sig.Diff. (P.05)	23	49	5080	4.21
Min. Sig.Diff. (P.01)	31	67	6945	5.76
F. Value (N)	29.36	6.75	7.24	3.78
F. Value (P.05)	3.40	3.40	3.40	3.40
F. Value (P.01)	5.61	5.61	5.61	5.61

* Each value is an average of 18 plots.

TABLE 7

EFFECT OF NITROGEN LEVELS X PHOSPHORUS LEVELS ON AVERAGE BUNCH WEIGHT FOR THE PERIOD DECEMBER, 1957 TO NOVEMBER, 1958.

	P LEVELS			
N Levels	P_o	P_1	P_2	Total
N_o	65.76	73.93	71.82	211.51
N_1	76.18	69.20	75.75	221.13
N_2	81.15	74.05	73.26	228.48
Total	223.09	217.18	220.83	661.12

Min. Sig. Diff. (P.05)	Any two of nine means	7.30
Min. Sig. Diff. (P.01)	Any two of nine means	9.98
F. Value (N x P)		3.87
F. Value (P.05)		2.78
F. Value (P.01)		4.22

Each value is an average of 6 plots.

The nitrogen treatment variance associated with each unit of measurement was separated into its linear and quadratic components. Each variance was then compared with its corresponding error variance. In each case, a highly significant result was obtained for the linear component, indicating a gradual increase in production with increasing rate of nitrogen fertilization, and suggesting that under like circumstances the linear response curves may hold for even higher levels of nitrogen.

Results for the nitrogen x phosphorus interaction parallel those obtained for the growth data discussed previously. Consequently, the interpretation in terms of average bunch weight is the same. It should be noted that the difference between the P_0 and P_2 levels of phosphorus for the N_2 level of nitrogen is just significant at P.05.

PATHOLOGY

Panama Disease

Effect of land working and plowing in conjunction with flood-fallowing on incidence of Panama disease.

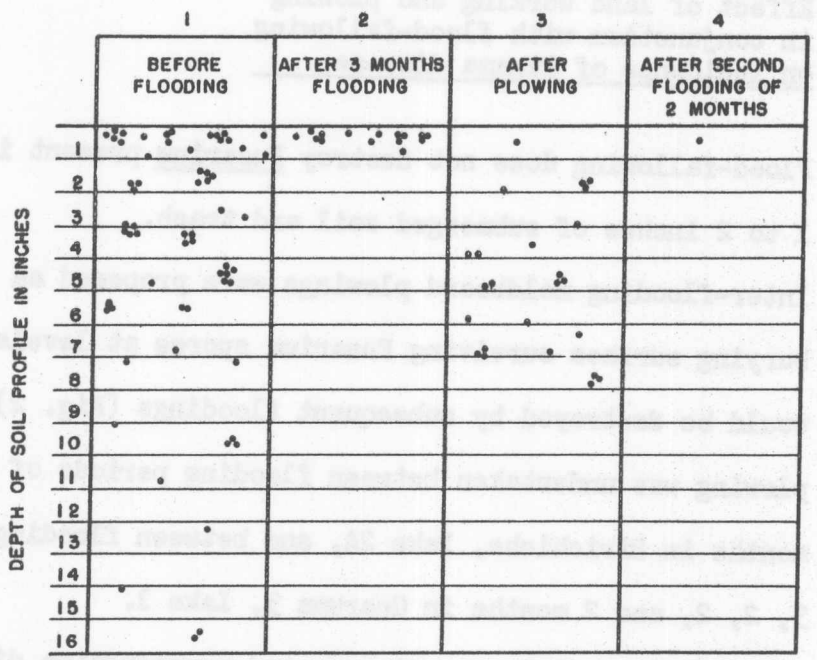
Flood-fallowing does not destroy Fusarium present in the surface 1 to 2 inches of submerged soil and trash.

Inter-flooding moldboard plowings were proposed as a method of burying surface surviving Fusarium spores at levels where they would be destroyed by subsequent floodings (Fig. 2). Moldboard plowing was undertaken between flooding periods of 3, 2, and 2 months in Birichiche, Lake 2A, and between flooding periods of 3, 2, 2, and 2 months in Guaruma 3, Lake 1.

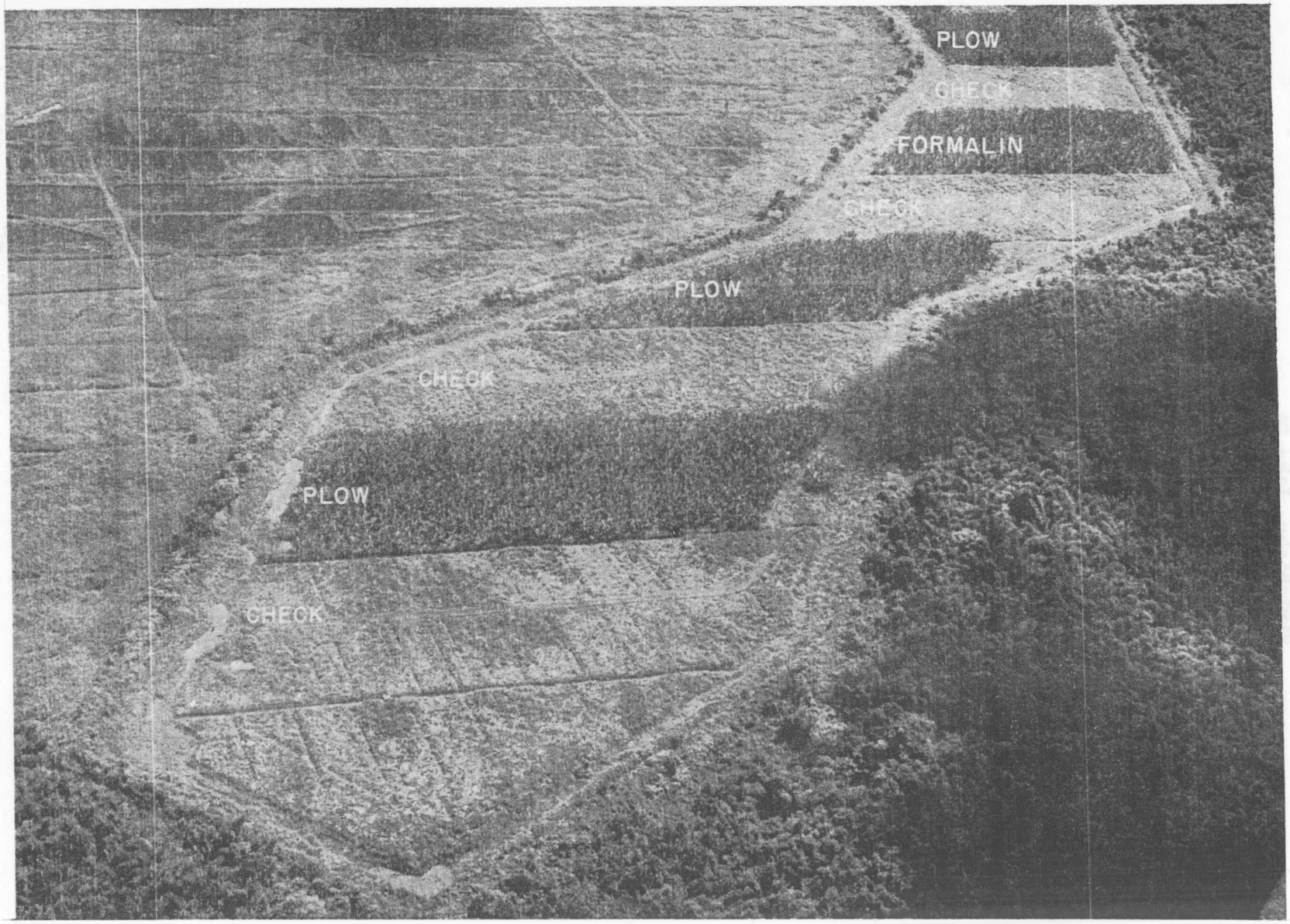
The incidence of Panama disease and unproductive diseased mats in Birichiche, Lake 2A are shown in Table 8 and Figs. 3 and 4. Disease is developing slowly in the plowed plots whereas the surrounding non-plowed plots have mostly been abandoned. Difference in disease incidence between plowed and non-plowed check plots is highly significant statistically.

The incidence of Panama disease and amount of fruit harvested to December 15 in the Guaruma 3, Lake 1 plowing-discing experiment are shown in Tables 9 and 10, and Figs. 5 and 6. Data in Table 9

Figure 2



Distribution of F. oxysporum f cubense, in soil profiles before and after flood-fallowing alone and in conjunction with plowing. Each dot represents a Fusarium spore.

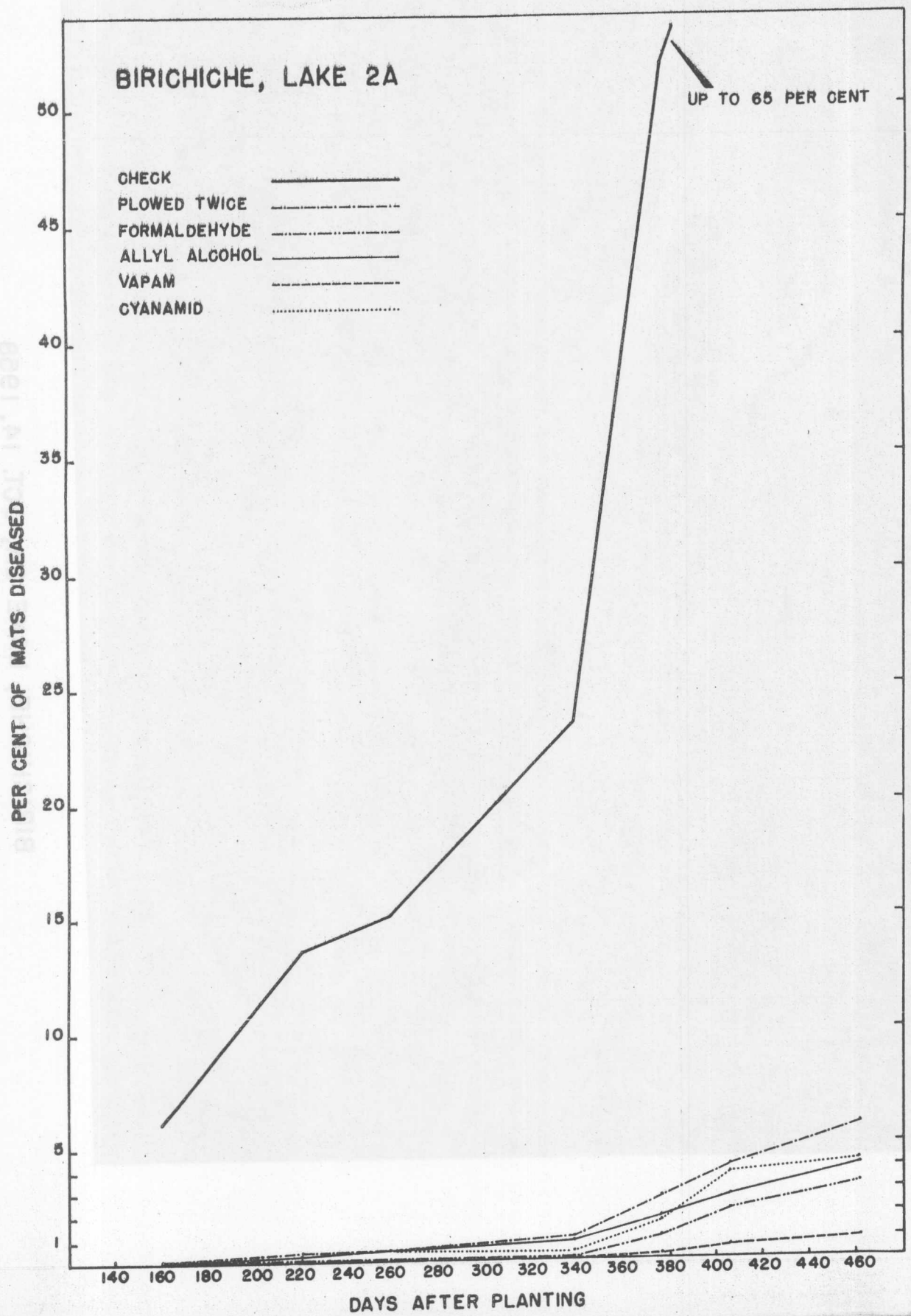


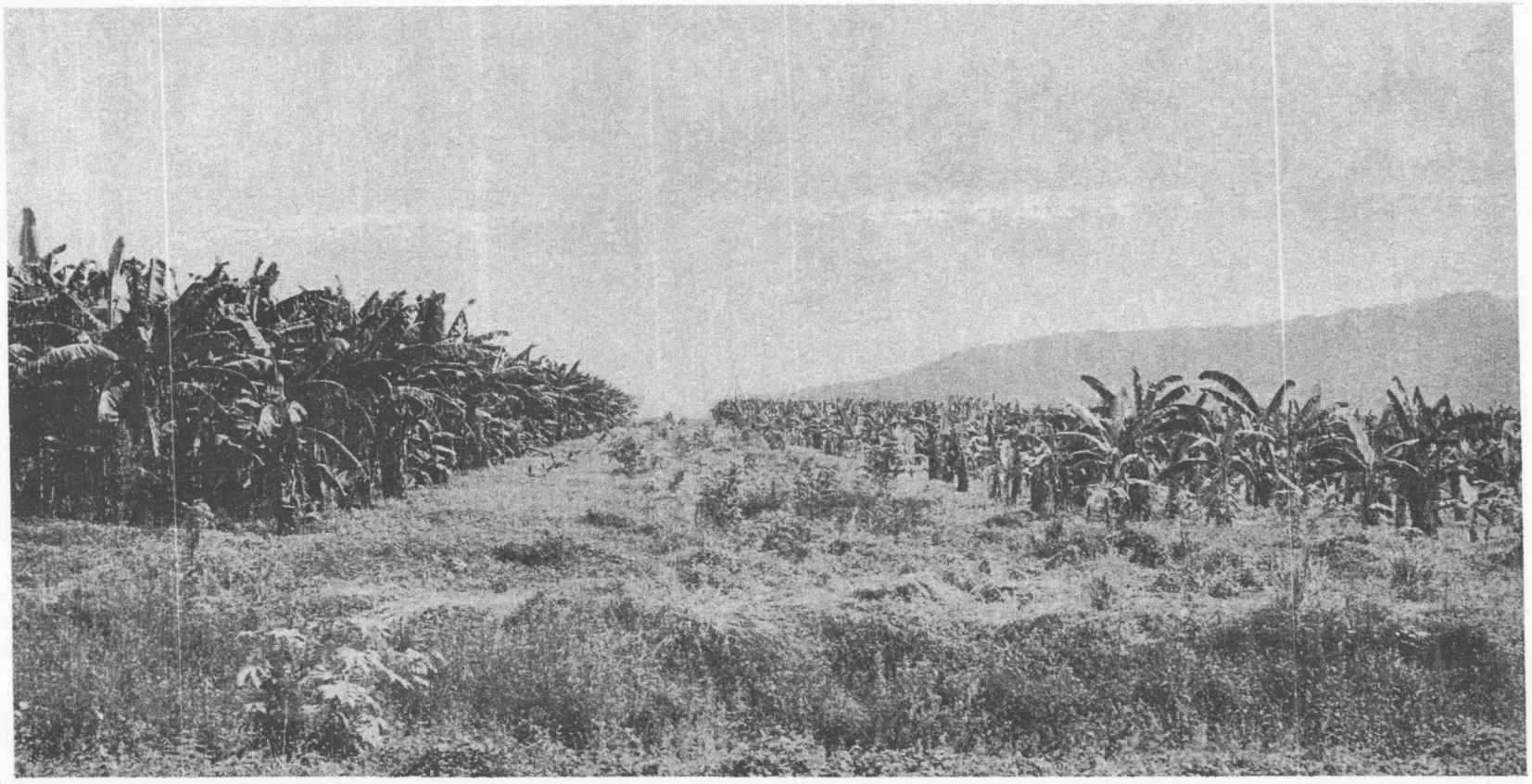
BIRICHICHE, LAKE 2A, OCT. 14, 1958

FIGURE 3. Aerial photograph of a portion of Birichiche, Lake 2A, 406 days after planting showing check areas abandoned and cut down because of high incidence of Panama disease, and plowed areas still in produc

Figure 4

Development of Panama disease incidence in check and treated plots
Birichiche, Lake 2A





PLOWED 3 TIMES NOT PLOWED

Guaruma 3, Lake 1, Oct. 14, 1958

FIGURE 5. Plots of bananas in Guaruma 3, Lake 1, 331 days after plowing. Check plot on right was 100% diseased, plot on left was plowed 3 times between 4 floodings and was about 15% diseased.

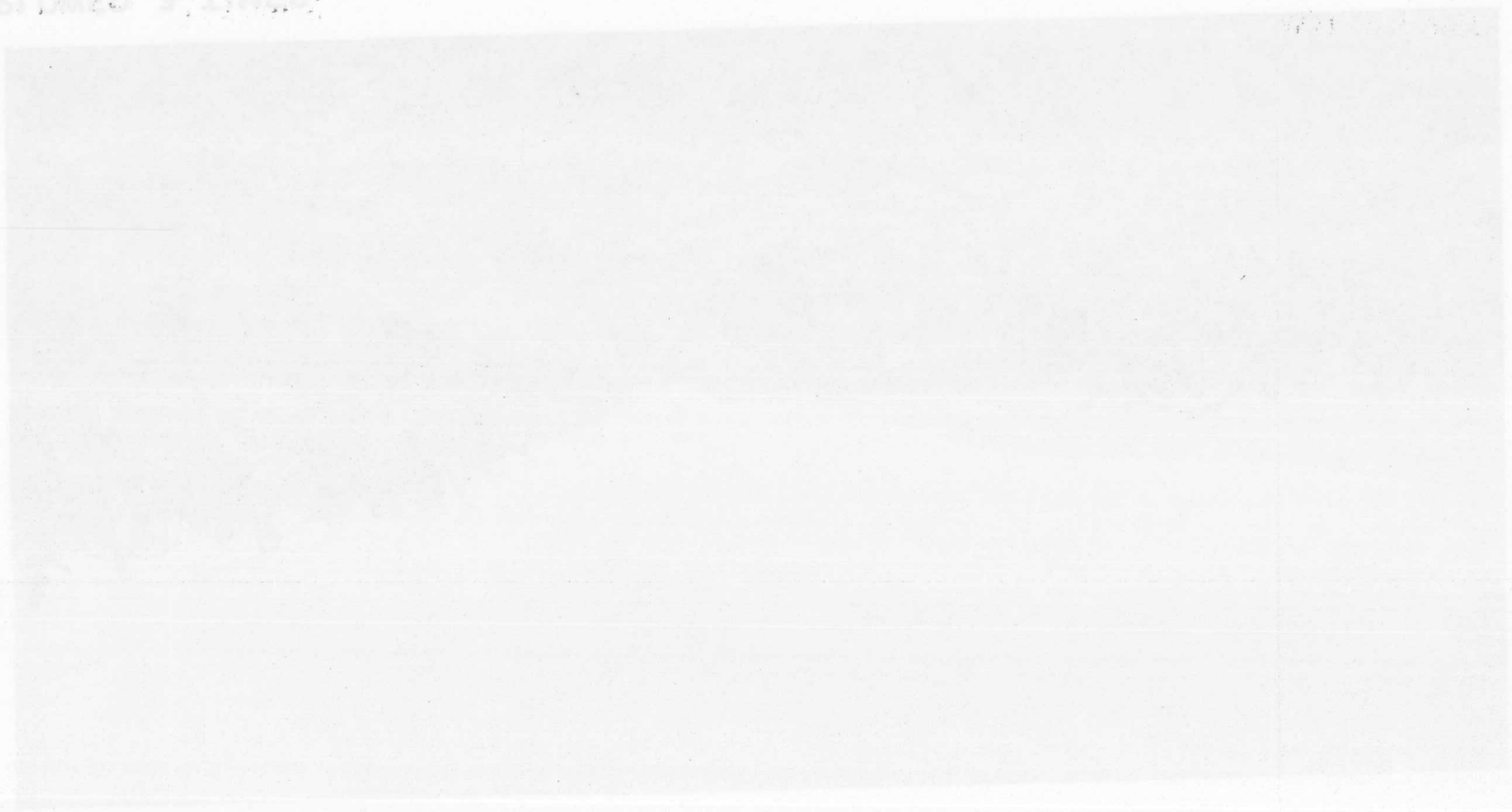


Figure 6

Development of Panama disease incidence in check and treated plots in Guaruma 3, Lake 1.

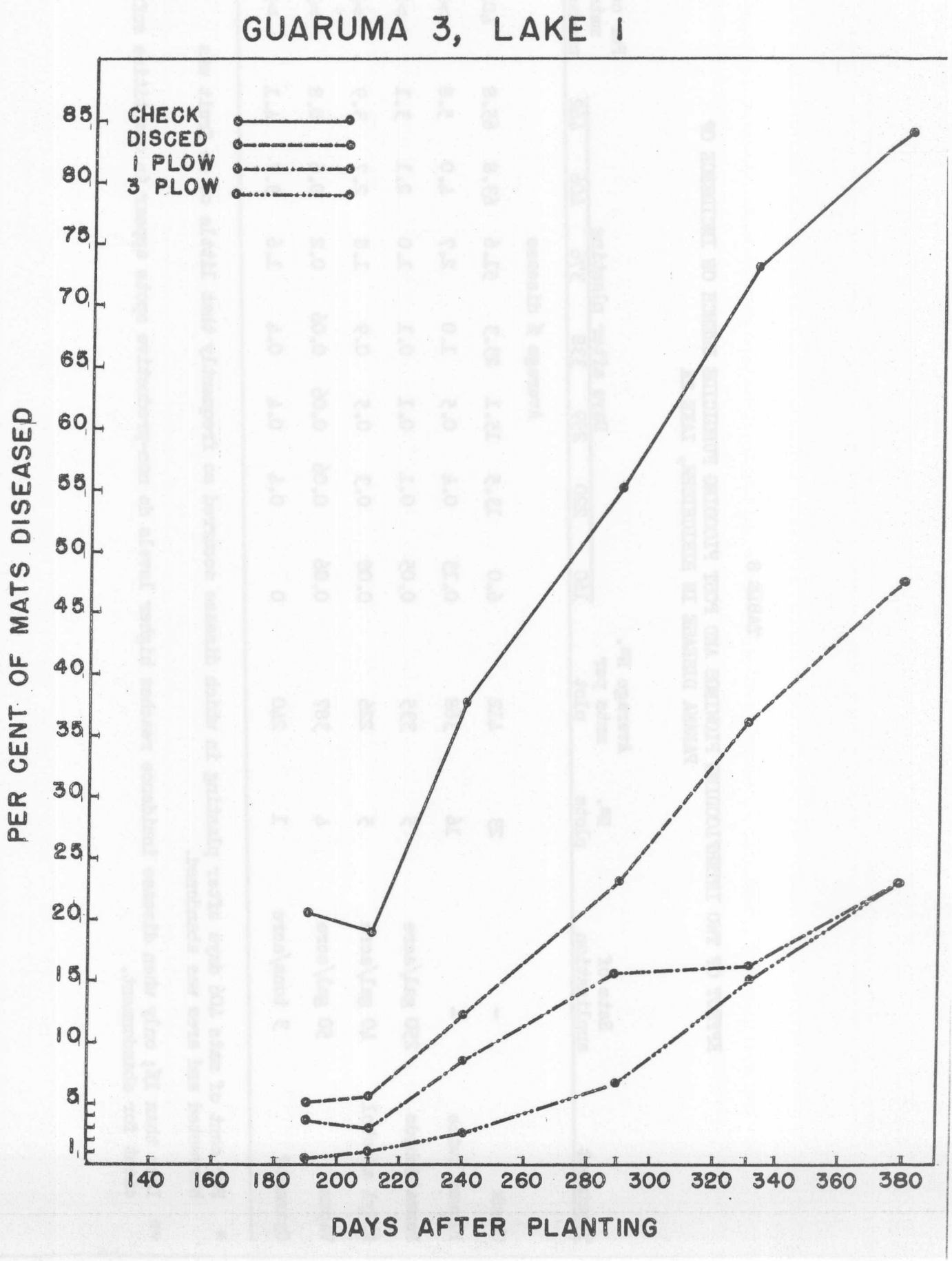


TABLE 8

EFFECT OF TWO INTERFLOODING PLOWINGS AND POST FLOODING FUNGICIDE DRENCH ON INCIDENCE OF PANAMA DISEASE IN BIRICHICHE, LAKE 2A

Treatment	Rate of application	Nº. plots	Average Nº. mats per plot	Days after planting							Per cent of mats not productive *
				160	220	259	338	376	406	462	
				Average % disease							
Check	-	23	412	6.0	13.5	15.1	23.3	51.6	63.8	65.8	40.0
Plowed twice	-	16	368	0.13	0.4	0.5	1.0	2.7	4.0	5.8	>1 **
Formaldehyde	250 gal/acre	5	335	0.05	0.1	0.1	0.1	1.0	2.1	3.1	>1
Allyl alcohol	40 gal/acre	5	226	0.08	0.3	0.5	0.9	1.8	2.7	3.9	>1
Vapam	50 gal/acre	4	387	0.06	0.06	0.06	0.06	0.2	0.6	0.8	>1
Cyanamid	3 tons/acre	1	240	0	0.4	0.4	0.4	1.6	3.7	4.1	>1

* Per cent of mats 406 days after planting in which disease occurred so frequently that little or no fruit was harvested and area was abandoned.

** Less than 1%; only when disease incidence reaches higher levels do non-productive spots appear in quantities sufficient for abandonment.

TABLE 9

EFFECT OF LAND-WORKING BETWEEN FLOODING ON INCIDENCE OF PANAMA DISEASE 378 DAYS AFTER PLANTING IN GUARUMA 3, LAKE 1

Treatment	Average No. cases of Panama disease*	
Check	391.0	
1 discing	319.5	
1 plowing **	121.5	
3 plowings	103.0	
* Per 462 mat plot replicated 6 times in a complete block design.		
LSD .05	55.5	(for comparing check,
LSD .01	118.6	1 discing - 3 plowings)
** One plot missing		
LSD .05	91.5	(for comparing check,
LSD .01	126.9	1 discing - 3 plowings and 1 plowing)

TABLE 10

STEMS OF BANANAS HARVESTED UP TO 385 DAYS AFTER PLANTING IN LAND-WORKED AND NON-LAND-WORKED PLOTS IN GUARUMA 3, LAKE 1

	Average No. stems harvested per 100 mats planted	Standard error*
Check	2.9	1.09
One discing	16.5	1.88
One plowing	17.8	2.09
Three plowings	24.0	4.28

* Based on 6 replicates in randomized complete block design; discing and plowing yields are significantly different from the check at P = .01.

show that there is a highly significant difference in disease incidence between plots plowed between floodings and check plots not land-worked. Disease incidence in plots disced once between floodings is significantly different from check plots at P = .05. Also, differences in disease incidence between 3 plowings and 1 discing between floodings is significant at P = .05, and almost significant at P = .01. There was no significant difference in disease incidence between 3 and 1 plowing. Disease is now developing rapidly in the disced and plowed plots although considerable fruit is being harvested. Little fruit has been harvested from the check plots. Although one discing between floodings reduced disease incidence initially, it soon built up to uneconomic proportions. However, one discing greatly increased the amount of fruit harvested (Table 9). At 387 days after planting there was 23% disease in Guaruma 3, Lake 1 plowed plots versus about 3% disease in the Birichiche, Lake 2A plowed plots, whereas the checks were 84 and 51% diseased, respectively. The greater disease incidence and rate of disease development in Guaruma 3, Lake 1 may be partly attributable to a more virulent clone of Fusarium in that area.

In Honduras about 1,200 acres of flood-fallowed projects have been moldboard plowed once, and in some areas twice, between two and three floodings on a production scale. Farm 12, the first project, was planted in July, 1958 and six months later only one case of Panama disease was found. Further studies are warranted, particularly with combined treatments such as fungicides and/or green manures with interflooding plowing.

Effect of fungicides and fumigants on incidence of Panama disease in flood fallow lakes.

Birichiche Lake 2A

Post-flooding fungicide drenches were proposed as a method of killing Fusarium spores known to survive flooding in the surface inch of submerged soil and trash. All fungicides except Cyanamid were applied through a portable sprinkler irrigation system immediately after the final flooding and after a shallow rotovating. Cyanamid was applied between the last two floodings with a fertilizer drill and disced into the top 4 inches of soil. Flooding periods were for 3 months, 2 months, and 2 months. Plots were from 1 to 1½ acre in size and replicated at least 4 times except Cyanamid.

Results of fungicide drench experiments in Birichiche Lake 2A are shown in Table 11 and Figs. 7 and 8.

All materials used were highly effective in reducing disease incidence. Although differences between treatments are not statistically significant, Vapam appears to have an advantage in effectiveness (fungicidal and nematocidal), ease of application, and in price. Vapam applications of 50 gallons per acre will cost between 170 and 190 dollars per acre depending on amount of land preparation required. The economics of fungicide applications will depend on the rate of which Panama disease re-invades the treated areas. Thus far, this has not been rapid in Birichiche Lake 2A when contrasted with the relatively rapid re-invasion of fungicide plots in Guaruma 2, Lake 1 (1957 Annual Report).

TABLE 11

EFFECT OF TWO INTERFLOODING PLOWINGS AND POST FLOODING FUNGICIDE DRENCH ON INCIDENCE OF PANAMA DISEASE IN BIRICHICHE, LAKE 2A.

Treatment	Rate of application	No. Plots	Average No. mats per plot	Days after planting							Per cent of mats not productive *
				160	220	259	338	376	406	462	
				Average % of mats diseased							
Check	-	23	412	6.0	13.5	15.1	23.3	51.6	63.8	65.8	40.0
Plowed twice	-	16	368	0.13	0.4	0.5	1.0	2.7	4.0	5.8	>1 **
Formaldehyde	250 gal/acre	5	335	0.05	0.1	0.1	0.1	1.0	2.1	3.1	>1
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Vapam	50 gal/acre	4	387	0.06	0.06	0.06	0.06	0.2	0.6	0.8	>1
Cyanamid	3 tons/acre	1	240	0	0.4	0.4	0.4	1.6	3.7	4.1	>1

* Per cent of mats 406 days after planting in which disease occurred so frequently that little or no fruit was harvested and area was abandoned.

** Less than 1%; only when disease incidence reaches higher levels do non-productive spots appear in quantities sufficient for abandonment.

Guaruma 3, Lake 1

a) At the end of the first experimental year, fungicides as used in conjunction with flood fallowing are more effective in the control of Panama disease than flood fallowing alone, if one accepts significance at the 5% level. Vapam is generally superior to other fungicides tested.

b) Four flooding cycles of approximately 255 days total together with three between-cycle discings, without fungicide treatment have resulted in a 25% incidence of Panama disease during a one-year experimental period. When this incidence is considered together with the rate of disease increase apparent at the end of one year, flood cycles in combination with three discings did not give satisfactory control of Panama disease.

c) A 30-foot unplanted border around each plot has effectively reduced encroachment of disease into plots. Relatively small plots have been used successfully in field evaluation of fungicides insofar as border effect did not become apparent during a one-year experimental period.

d) A sizeable coefficient of variation indicates that a considerable proportion of the mean treatments is not represented by this mean. A significant part of the variation apparent in analysis is due to non-conformity of disease incidence among replicates. Therefore, the experiment demonstrates the necessity of adequately replicated design, particularly if effect of fungicides rates is to be accurately evaluated.

It is recommended that fungicide and other field experiments designed to investigate Panama disease control, including the experiment reported herein, be used predictively in the design of future fungicide experiments adequate to furnish the demanded precision of results.

A fungicide experiment designed to investigate control of Panama disease involves a consideration of soil and drainage factors, as well as the influence of fungicides on growth and yield of the banana plant. It is recommended that a method of coordinated planning be adopted whereby these factors are considered by soil specialists, agronomists, nematologists, and pathologists. An experiment might thereby also yield information of value to several specialists.

It is recommended that methods of application of Vapam and/or allyl alcohol be investigated to determine the practicality of repeated fungicide application without the destruction of an existing planting, i.e without flooding.

During a one year period, 203 out of a total of 333 mats (61%) which became diseased were isolated, in the sense that they were not adjacent to a mat previously recorded as diseased. The odds favoring the appearance of isolated diseased mats decrease as the incidence of diseased mats in the plots increases. It is of interest that 364 days after planting 38% of mats recorded as newly diseased were isolated cases. These data indicate that mats became diseased as the result on ingress of roots into soil containing fungus residuum. This concept is of importance since it does not lend credence to the spread of Panama disease from mat to mat following flooding.

Effect of deep plowing on incidence of Panama disease in non-flood fallow areas.

Deep plowing has been used with some success in the control of Verticillium wilt of mint. Where it is not possible to flood-fallow some means of reducing Fusarium infestations in banana lands is desirable. Therefore, it was proposed that deep plowing be used on non-flood fallow Fusarium infested soils. Deep plowing would bury the upper 9-12 inches of soil (the area of heaviest infestation) to a depth of 24-36 inches where the Fusarium might be destroyed. Three areas previously abandoned because of Panama disease were selected for experimentation as being representative of different soils: Campin Farm, Honduras (clay loam soil), Guaruma Farm, Honduras (clay soil) and Aztec Farm, Bananera, Guatemala (loam soil). Following plowing, lands were fallowed from 7 to 12 months to allow sufficient time for Fusarium destruction to take place.

In Campin Farm Sections 24 and 26, Panama disease has been very slow to appear. After 18 months the plowed plots have an incidence of 9.9% and the non-plowed check plots have 17.4% Panama disease. This area is still in production.

In Aztec Farm (Bananera, Guatemala), Panama disease started appearing after the sixth month and thereafter monthly surveys of disease incidence were made. The experiment was terminated in August, 1958. The deep plowed plots had 7.5% Panama disease and the non-plowed check plots had 10.0% disease. The area is still in production and is expected to have a life of about five years.

In Guaruma 2 Farm Section 36, the deep plowed plots had 18.9% Panama disease while the non-plowed check plots had only 1.0% disease.

Apparently, some soil climatic, or fungus factor was responsible for the failure of deep plowing to reduce Panama incidence in this area. Deep plowing may suppress Panama disease incidence to a limited degree in some soils. However, this reduction in disease incidence is not sufficient to economically deep plow areas on a production basis. The Campin and Guatemala areas will be observed to determine the total life of the deep plowed versus non-plowed plots.

It is suggested that further experiments be designed using deep plowing in combination with fungicide and/or green manure applications. No efforts should be spared to find a Panama disease control measure which is cheaper and more successful than flood fallowing.

Effect of various organic soil amendments on survival of Fusarium in the laboratory.

It is known that there is competition and antagonism between organisms in soil which may inhibit or destroy certain species of fungi. Also, a reduction in incidence of several plant diseases has been obtained by adding green manure and other organic amendments to soil. Preliminary laboratory investigations in La Lima and Coto have shown that green plant material and sugar can reduce Fusarium survival. Therefore, it was proposed to add to soil various organic materials and attempt to establish a physical, chemical or biological alteration which would inhibit or destroy Fusarium.

In glass tumblers containing equal amounts of light clay soil from Camp 2 amended with quantities equal to 20 tons per acre of Crotolaria, Kudzu, Cow Peas and Sorghum, Fusarium survival was significantly reduced after two weeks as shown in Table 12.

TABLE 12

FUSARIUM SURVIVAL IN A LIGHT CLAY SOIL AMENDED WITH 20 TONS/ACRE OF CROTOLARIA, KUDZU, COW PEAS AND SORGHUM

Treatment	Total Fusarium survival (25 plates)
Check	991
Crotolaria	148*
Kudzu	493**
Cow Peas	459**
Sorghum	207*

* L.S.D. P.01 = 537
** L.S.D. P.05 = 394

When Mopala and San Alejo soils were amended with molasses, lime and cotton seed meal, Fusarium survival after two weeks was reduced significantly in both soils (Table 13). It is interesting to note that Fusarium survival was lessened in the San Alejo soil even without amendment.

TABLE 13

FUSARIUM SURVIVAL IN TWO SOILS AMENDED WITH MOLASSES, LIME AND COTTON SEED MEAL

Treatment	Total Fusarium survival (25 plates)	
	Mopala soil	San Alejo soil
Check	689	298**
Molasses (10 T/A)	101*	0 *
Lime (3 T/A)	370**	160*
Cotton Seed Meal (3 T/A)	80*	53*

* L.S.D. P.01 = 418
** L. S.D. P.05 = 312

When Mopala, San Alejo and Camp 2 soils were amended with 20 tons/acre of Velvet Beans and Jack Beans, Fusarium survival after two weeks was significantly reduced in some instances (Table 14).

TABLE 14

FUSARIUM SURVIVAL IN THREE SOILS AMENDED WITH 20 TONS/ACRE OF VELVET BEANS AND JACK BEANS

Treatment	Total Fusarium survival (25 plates)		
	Camp 2 soil	Mopala soil	San Alejo soil
Check	815	999	379*
Velvet Beans	1158	438*	66*
Jack Beans	431*	462*	164*

* L. S. D. P.01 = 477
** L. S. D. P.05 = 356

The incorporation of lime alone at 3 tons/acre and in combination with Cow Peas at 20 tons/acre gave no increased advantage over the use of Cow Peas alone. The Fusarium survival differences obtained were not significant for any of the treatments (Table 15). Heavy clay soil from Guaruma 2 Farm was used for this experiment. Both lime and Cow Peas reduced Fusarium survival in lighter soils as described in Tables 12 and 13.

TABLE 15

FUSARIUM SURVIVAL IN A HEAVY CLAY SOIL AMENDED WITH COW PEAS AND LIME ALONE AND IN COMBINATION

Treatment	Total Fusarium survival (25 plates)
Check	745
Cow Peas (20 T/A)	623
Lime (3 T/A)	1004
Cow Peas and Lime	642

L. S. D. P.01 = 539
L. S. D. P.05 = 747

Data from numerous experiments involving the use of concrete tanks have been reported previously (1957 Annual Report), in which chopped plant

material was incorporated into the soil but the plants themselves were not grown in the soil. To determine what effect growing the test plants in the tanks and then incorporating them into the soil when mature would have on Fusarium survival seemed to be the next logical step.

Plants of Sorghum, Cow Peas, Soybeans, Velvet Beans, Sesbania, Kudzu and Crotolaria were each grown in concrete tanks for two months. At that time, they were pulled up and a chopped portion equal to 20 tons per acre was re-introduced into the top 8" of the soil and Fusarium infested Rossi-Cholodny slides were also introduced. Slides were removed after one month and Fusarium survival was determined by dilution plate techniques. Each treatment contained five replicates and each replicate was analyzed by 25 dilution plates. The results are presented in Table 16.

TABLE 16

FUSARIUM SURVIVAL IN GUARUMA 2 SOIL PLANTED TO AND AMENDED WITH 20 TONS/ACRE OF SEVEN GREEN MANURE CROPS

Treatment	Total Fusarium survival (per treatment)
Check (non-treated)	7468
Sorghum	4640*
Cow Peas	5511*
Soybeans	4758*
Velvet Beans	2929*
Sesbania	4338*
Kudzu	3813*
Crotolaria	5400*

* L. S. D. P.01 = 194
L. S. D. P.05 = 144

In the laboratory, the incorporation into the soil of certain organic materials significantly reduces Fusarium survival. There appears to be a pronounced variation in survival results obtained, dependent of course, on the amount of organic material incorporated but also dependent to a very great extent on soil factors. Results of field trials will be necessary to substantiate the practicability of these laboratory experiments.

Pathogenicity tests with Fusarium isolates under controlled environment.

Tests are needed to determine quantitative and qualitative differences in pathogenicity between isolates of F. oxysporum f cubense. Methods tested thus far include: (1) the insertion of vials containing viable cultures of Fusarium on the cut ends of large fleshy banana roots in situ and measuring the growth of the fungus up the roots, as indicated by vascular discoloration, over a period of 4 to 12 weeks; (2) the application of 350 cc. of a 2-week old 10% corn-meal-in-sand culture of Fusarium to the sides and bottom of a trench about 6 to 8 inches deep and 2½ to 3 feet long around the base of a disease-free banana mat in the field. The trench is dug to expose the cut ends of banana roots to the inoculum. Above-ground disease symptoms begin to appear 6 to 12 weeks after inoculation.

Using the root vial insertion techniques some isolates of F. oxysporum from banana roots caused the same amount of root vascular discoloration as known virulent strains of F. oxysporum f cubense. The same root isolates did not induce disease by the trenching method. There appear to be isolates of F. oxysporum that can invade banana roots but do not become systemic with resulting Panama disease symptoms.

The trenching technique, utilizing mature banana mats in disease-free areas in the field, continued to yield valuable data on the relative virulence of different clones. The technique is being improved by utilizing a randomized complete block design. Five plants in each of 4 plots (20-plant total) are each inoculated with 350 cc. of a 2-week old culture of Fusarium. The criteria utilized to determine virulence subsequent to inoculation are: (1) rapidity of appearance of disease symptoms, (2) rapidity of spread to adjacent non-inoculated mats, (3) number of inoculated and adjacent mats that do not recover sufficiently from initial disease to produce fruit.

Previous workers such as Wardlaw found that Fusarium would not enter wounded banana rhizomes directly but infection occurred via root bases. Attempts were made to infect rhizomes directly. Holes were punched in banana rhizomes in situ in the field and in the laboratory with a cork borer. The openings were ½ inch in diameter and about 1½ to 2 inches deep, and penetrated to the stele in some cases. These holes were packed with a fresh corn-meal-in-sand culture of Fusarium and sealed with paraffin or plasticine. Examinations 4 and 8 months after inoculation showed that Fusarium remained viable in the wound and penetrated the surrounding tissue as deeply as ¼ inch and occasionally followed a vessel for 1½ to 2 inches from the wound. Infection did not become systemic, and did not move into the pseudostem, and no external disease symptoms developed.

Studies of "Resistant" varieties.

Studies with banana seedlings showed that in addition to Musa schizocarpa, M Balbisiana seedlings could be infected in the greenhouse by artificial inoculation with F. oxysporum f cubense. This was most surprising as this species has never been observed to be infected under natural conditions even when growing in highly infested soil. These results indicate that artificial inoculation of seedlings must be used with caution in determining the resistance or susceptibility of a species, variety, or clone. Seedlings that are infected under artificial conditions may be highly resistant or immune under natural conditions, or when propagated vegetatively.

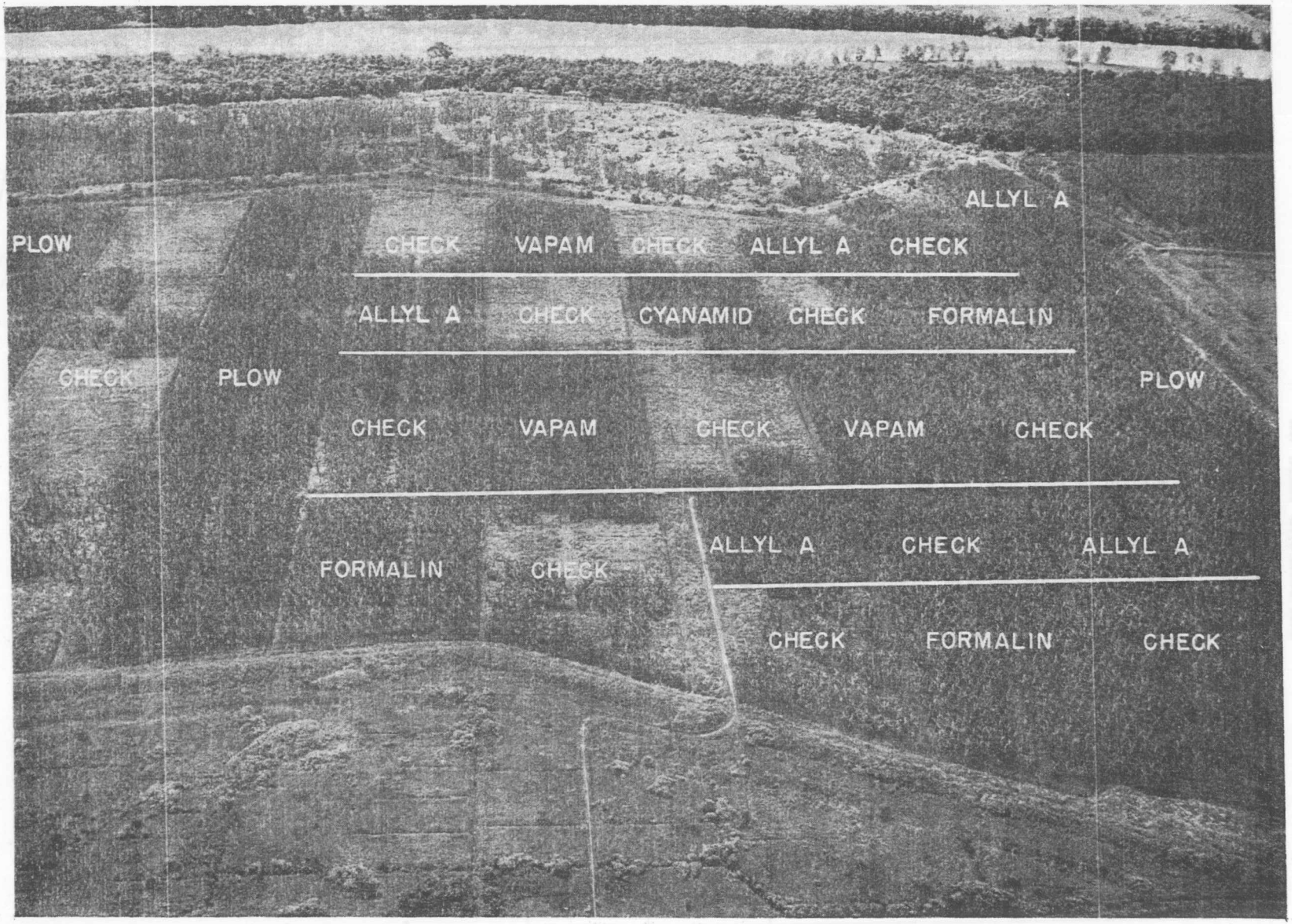
In Martinez Farm at Choloma numerous mats of Lacatan and Dwarf Cavendish contain shoots exhibiting symptoms of Panama disease. These plants all yielded F. oxysporum f cubense 'Inodoratum'. This area differs in the following ways from most banana growing areas:

(1) Only the 'Inodoratum' cultivar of F.oxysporum f cubense is present.

(2) Between 3 and 5 feet below the surface is an almost impervious layer of very compacted, plastic, and structureless soil. The water table was at about 3 feet in November and fluctuates from rainy to dry season.

(3) The area is heavily infested with Radopholus similis.

Whether the above factors contribute to the "breakdown" of resistance of these normally highly resistant varieties is not known. Preliminary studies indicate that there is a race of F. oxysporum f cubense that destroys bananas and Chatos but that the 'Odoratum' cultivar from bananas invades Chatos weakly or not at all.

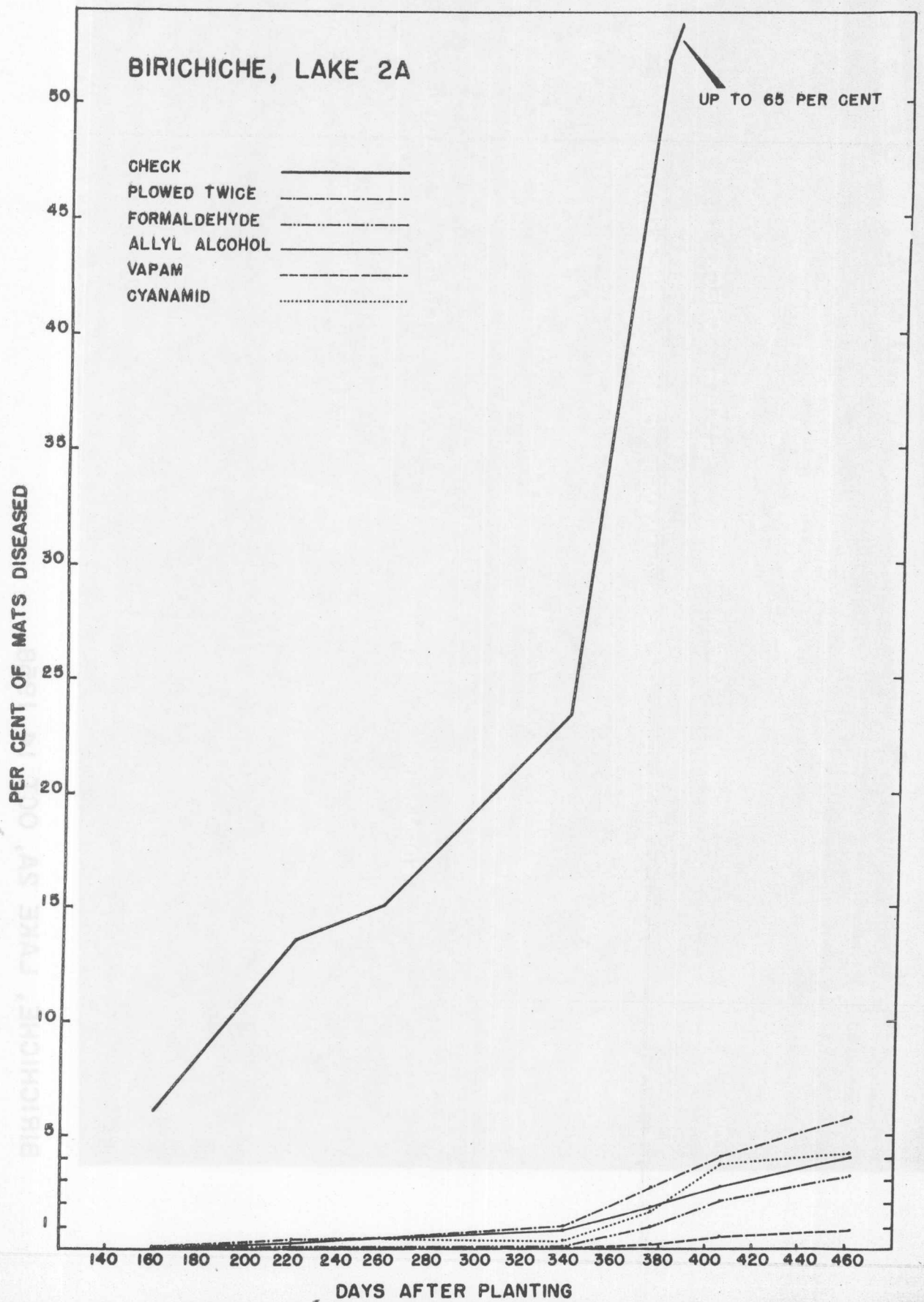


BIRICHICHE, LAKE 2A, OCT. 14 1958

FIGURE 7. Aerial photograph of a portion of Birichiche Lake 2A 406 days after planting showing check areas abandoned and cut down because of high incidence of Panama disease, and fungicide treated areas still in production.

Figure 8

Development of Panama disease incidence in check and treated plots
Birichiche Lake 2A



Miscellaneous studies with Fusarium isolates under controlled environment.

A project was originally outlined to study pathogenicity of Fusarium isolates under controlled environment. In the laboratory, surface sterilized banana seeds were germinated, plants produced in flask culture, and inoculated. It became apparent that any such approach did not permit development of the Panama disease syndrome which is the reflection of Fusarium pathogenicity.

Considerable information was available concerning the mechanism of action of F. oxysporum f lycopersici in the development of the tomato wilt syndrome. For example, in the tomato wilt studies two principal mechanisms were reported to account for symptom expression: a) pathogen-induced tracheary obstructions which caused a water shortage and loss of turgor, and b) systematic vivotoxins which inhibited certain plant functions such as respiration. It seemed desirable to investigate the Panama disease syndrome by paralleling these two general approaches.

It was known at the commencement of the study that variation existed in F. oxysporum f cubense. During the study of syndrome development, the importance of variation became increasingly evident. As a consequence, while variation was considered at the commencement of the study, an increasing emphasis was placed on variation during progress of this study.

It was found that when one-year-old healthy versus diseased Gros Michel plants were severed, then weighed, they lost weight at a different rate (Fig. 9). This weight loss was due to transpiration and to the flow of exudate from a severed pseudostem. Total volume

flask

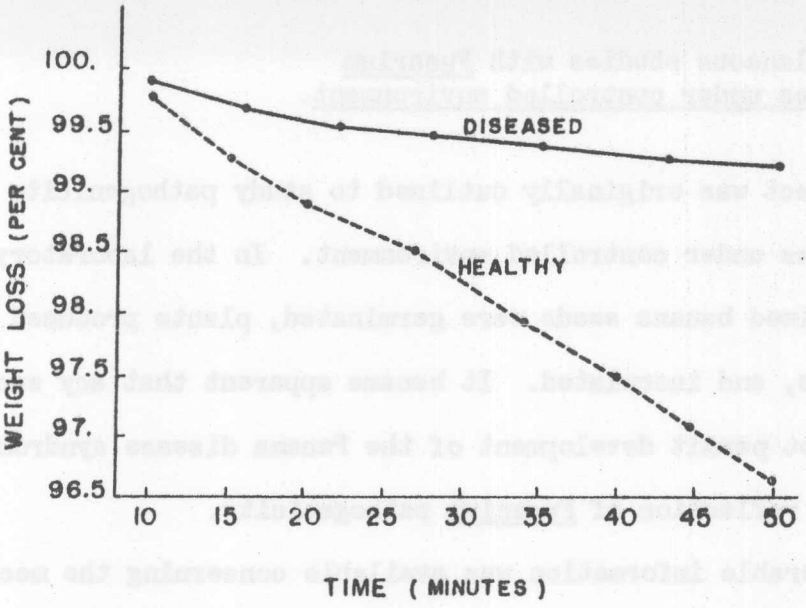


FIGURE 9.

Per cent weight loss with time in a healthy banana plant and a diseased plant of similar size.

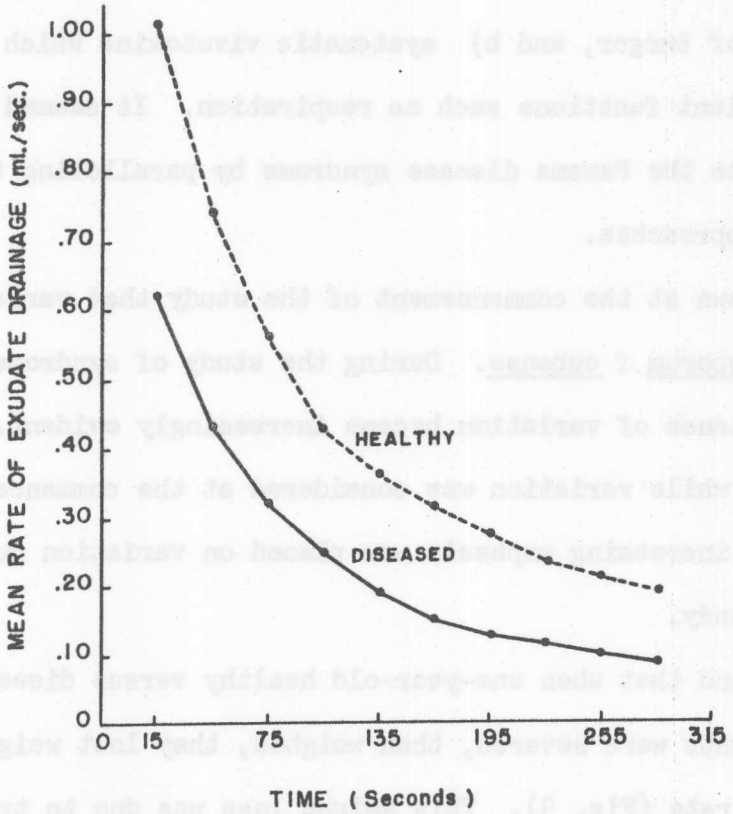
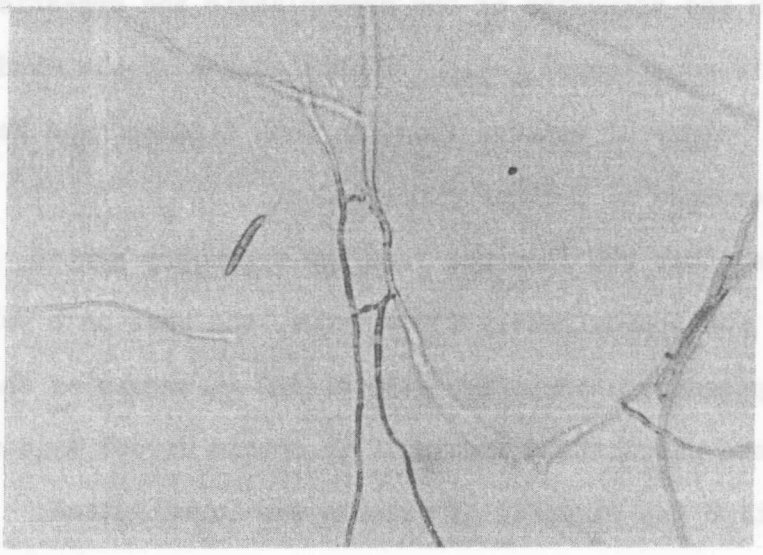


FIGURE 10.

Mean rate of exudate loss with time from the butts of healthy and diseased pseudostems of similar size.

FIGURE 11.

Anastomoses of hyphae of variant F.o.c. 1 growing on water agar.



and rate of exudate flow from diseased and from healthy plants were calculated. The mean rate of exudate drainage was higher from healthy pseudostem butts than from diseased butts of comparable size (Fig.10). Flow of exudate continued from stumps after cutting, and also from severed pseudostem butts tilted above the horizontal during drainage. These observations indicated that the exudate flowed under pressure. Furthermore, only the tracheary elements have sufficient capacity to permit continuing aqueous flow. Other ruptured tissues should contribute no more than the aggregate of the protoplasmic and ergastic contents within their constituent cells. Tissue source of the exudate and its relative volume of aqueous flow, in both diseased and healthy plants, were determined in further experiments.

It was established that the flow was from the tracheary system, and that the exudate was approximately 95% aqueous, and that on a volume basis a healthy pseudostem contained 2.35 ul/cm^3 in excess of that present in diseased pseudostems sampled. To obtain direct evidence of xylem dysfunction the plugging of vessels was investigated.

Flow rates were measured through cores of the axis removed from the center of a pseudostem. Such cores had an abundance of vascular bundles, separate from one another, and continuous throughout the length of the core. It was established that flow rate was slower through diseased vessels than through healthy vessels.

Since external symptoms of Panama disease usually develop first in the outer older leaves, and then only after vascular invasion of the pseudostem is well advanced, transpiration rate should subside before the disease is expressed in the inner younger leaves. This hypothesis was investigated experimentally. It was found that transpiration rates were

significantly higher in laminae of healthy plants than in laminae of diseased plants. When laminae from healthy plants were grouped with laminae from diseased plants they appeared identical; vascular discoloration or other disease symptoms were not apparent in selected laminae from diseased plants. A reduced rate of transpiration was demonstrated in laminae in advance of external disease expression. Although it cannot be concluded that a water shortage resulting from tracheal obstruction is the only cause of an imbalanced water economy, an important adjunctive role is ascribed to vessel dysfunction in the Panama disease syndrome.

Systemic vivotoxins have been proposed as a mechanism operative in certain fusariose syndromes. Since the phytotoxic complicity of fusar(in)ic acid (FA) has been demonstrated in several other wilt diseases, filtrates from cultures of F. oxysporum f cubense were assayed for the presence of FA.

Preliminary to chromatographic determination of whether FA was present in culture filtrates, a known sample of FA was chromatographed. Since FA forms stable inner metal complex salts, FA was complexed on the chromatogram with $CuSO_4$; the resulting complex was colorized with rubeanic acid, a reaction sensitive to ca 1.0 ug FA. Copper rubeanate was detected as an olive gray spot, R_f 0.93.

Two isolates of F.oxysporum f cubense were cultured on modified Richard's medium in darkness at 30° C. Neat filtrate was chromatographed alone and in combination with known FA. By visual com-

xylem - a complex vascular tissue found in higher plants that forms the woody supporting part of their stem and conveys water and nutrients.

parison with known levels of FA, both variants produced relatively low levels of FA during the first 16 days of incubation on Richard's medium. Thereafter variant F. o. c. 1 produced considerably more FA, until a level was reached on the 28th day following inoculation approximately 10 times the level produced by variant F. o. c.

To demonstrate the presence of FA in diseased Gros Michel plants, tissue samples were taken from infected rhizome stele. Juice was expressed from stelar tissue dissected from a rhizome. After centrifuging the juice it was acidified, extracted with chloroform, and the combined chloroform extracts dried in a flash evaporator. The extract residue soluble in ethanol was chromatographed as a copper-FA chelate.

When Richard's medium was further modified by the addition of zinc, there was marked reduction in the formation of certain organic acids, including some amino acids and FA. FA was not detected in filtrates from zinc-amended medium when zinc was present at levels of 10 mg zinc/liter of medium.

As investigation into the mechanics of syndrome development progressed it became evident that a better understanding of variation in the fungus was essential. It became necessary therefore to define experimentally the inherent variability in forma cubense as workable, though artificial, units. Such units or variants were distinguished from other variants by investigation of morphological and physiological traits. A variant was defined then on the basis of phenotypic expression; phenotypic stability of a variant was considered to reflect genotypic stability. An understanding of genotype is of considerable significance in any future investigations of resistance of banana plants to F. oxysporum f cubense.

Sub-cultures of single spored isolates, tentatively designated as "yellowing" and "non-yellowing" were obtained from Dr. Stover. Later 5 other sub-cultures designated "A","B", "C", "D", and "E" clones were obtained. Subsequent work with these seven sub-cultures has resulted in grouping them as follows: Variant F.o.c. ("yellowing isolate, and clone "B"); Variant F.o.c. 1 ("non-yellowing" isolate, and clones "D" and "E"); Variant F.o.c. 2 (clones "A" and "C"). As the study progressed isolations were made directly from diseased plants. Such isolates have also been artificially categorized as belonging to one of the three above variants. In the present report variants F.o.c. and F.o.c. 1 are considered since studies with these variants have been most extensive.

Insofar as can be determined, Brandes first recognized variation in F. cubense and proposed the variety "Inodoratum" based on the absence of an agreeable aromatic odor evolved by F. cubense on steamed rice. It would be desirable to relate present variants to the variants encountered 40 years ago; a marked stability in variants would thus be established. Furthermore, it is sound taxonomic principal to relate by appropriate criteria variants previously described in the literature. However, it seems unreliable in this instance to hold to this principal, the criterion of olfaction being unsatisfactory in that:

A) Brandes reported that the aromatic odor was similar to"orange peel", "ripe watermelon", "lilacs", etc., and yet this was the same odor, according to Brandes, that Lathrop previously attributed to propionic aldehyde. Although individual responses to odor differ markedly, it seems unlikely that the odor of propionic aldehyde (described as "suffocating" by Merck's Chemical Handbook) would be

considered aromatic. It is thus not possible to determine if Brandes was dealing with more than one variant, or whether the variant(s) was the same as that of Lathrop.

B) In the present study, two variants produced different odors on steamed rice, the odor evolved by F.o.c. being considered agreeably aromatic by some individuals, while F.o.c. 1 produced a pungent, disagreeable odor. Considering A) above, it does not seem possible to establish with certainty which of the present variants agrees by olfaction with F. cubense.

C) The two variants in question gave a positive Baeyer test, indicating the presence of unsaturated volatile compounds. By this test it was determined that quantitative differences existed between variants with respect to liberation of unsaturated volatiles. While available facilities permitted limited quantitative determinations to be made, more elaborate instrumentation would be required to establish the identity of the volatiles. It does not seem justified to further complicate identification by odor without associating an odor with a definite compound.

Concomitant with investigation of FA production two variants which were arbitrarily designated F.o.c. and F.o.c. 1, were evaluated with respect to the following:

a) Growth determined by dry weight of mycelium

b) Carbohydrate utilization-rate determined by iodometric titration of reducing sugar.

c) Nitrogen utilization-rate determined colorimetrically, nitrates by reaction with brucine in the presence of sulfuric acid, and ammonia by reaction with Nessler's reagent.

mycelium = the mass of interwoven hyphae (threadlike filaments that forms the body of a fungus.

d) Other cultural features - (i) pH changes by Beckman pH meter
(ii) color changes in medium by spectrophotometer.
(iii) pigment production by visual inspection and chromatography
(iv) inorganic salt precipitates-analyses by Chemistry Department

Variant F.o.c. 1 differed from F.o.c. in relative rate of growth, carbohydrate utilization, and nitrate utilization, as well as pH changes in culture medium during incubation.

During the first three weeks F.o.c. 1 grew more slowly than F.o.c.; autolysis became apparent in variant F.o.c. between the third and fourth week of incubation.

A colorless crystalline precipitate developed in filtrates from 21 and 28-day cultures of variant F.o.c. 1 upon storage at 10 C. This precipitate did not form in filtrates from isolate F.o.c. The precipitate, which produced an ammonia odor on heating, was identified as $NH_4MgPO_4.6H_2O$.

At the biochemical level the variants were found to differ quantitatively in at least 7 features. One of these features was the production of pigment endogenously by variant F.o.c., and the general absende of pigment in the mycelium of F.o.c. 1.

Brandes reported that F. cubense imparted a pink to blue color to a substratum of steamed rice. Since pigment production was constantly associated with cultures of F.o.c. throughout the experiments described above, it appeared to be a reliable marker whereby variant F.o.c. could be distinguished from variant F.o.c. 1. Pigment production was investigated further.

Nine media ere investigated with respect to pigment production by variants. The most suitable medium consisted of finely ground

ere = before

polished rice (particle size less than 300 microns) 15 g.; Bacto agar 10 g.; and distilled water, 1 liter. On this medium variant F.o.c. imparted a red color to the medium, whereas variant F.o.c. 1 imparted no color, or a scarcely discernible pigmentation of scattered rice granules in old cultures. Further study has shown that the pigment produced in association with F.o.c., while initially red, will respond to changes in pH to become blue, the shift is reversible. The change in color of steamed rice inoculated with F.o.c. had been noted previously, the pigment imparted to the medium undergoing a color change with age of culture. The color imparted to the substrate eventually colored the walls of the mycelium, initially red and finally blue. It now seems possible to account for the variable color reported by Brandes as a color change due to pH shift.

One of the media investigated in the foregoing study was an amylodextrin-zinc-agar medium. In the absence of zinc, both variants, F.o.c. and F.o.c. 1, were able to grow at approximately the same rate. With increase in zinc content of the medium from 0 to 80 mg/liter the growth of F.o.c. remained essentially unchanged. Growth of F.o.c. 1 was scarcely apparent at a level of 80 mg zinc at the end of a 7-day incubation period.

The influence of zinc on fusaric acid production was noted previously.

A technique was devised whereby portions of a single tracheary element could be removed from an infected host. This technique permits determination of spatial distribution of variants within an infected host, as well as determination of fungus variation within a single tracheary element, the ultimate unit of cells invaded by the pathogen. It has been previously shown by Dr. Stover that variants of the fungus occur

within a single shoot. However, previous work had considered that a variant induced a definite syndrome, i.e., either "yellowing" or "non-yellowing" syndrome. That more than one variant of the pathogen may be present within a shoot might be reasonably expected since:

a) Infections are frequently multiple. Assuming variation of the fungus in the soil, the possibility exists of infection by different variants.

b) Within the host, and particularly within the rhizome, vascular anastomoses occur. A mechanism is thus available whereby variable fungus genotypes may be brought into intimate association.

c) Hyphal anastomoses occur (Fig. 11), and the potential of heterkaryon formation exists.

Portions of single tracheary elements were planted on medium inducing exogenous pigment formation. Usually portions of 3 elements were assigned to one Petri plate, or a single element was washed on a plate with a small volume of distilled water to disperse microconidia or mycelial fragments. Variation was determined by pigment deposition in the medium adjacent to an infected element or dispersed fungal colony.

Variants F.o.c. and F.o.c. 1 occurred in tracheary elements from shoots of one-year-old mats. These mats had in many instances shot fruit, and the isolations were made from the true stem in the center of the pseudostem. The development of disease in mats in the area (Guaruma 3, Lake 1) had been relatively rapid, that is, less than one year. Records were kept of the presence of bacteria in segments of true stem from 14 consecutive mats brought into the laboratory. 253 out of 348 tracheary elements yielded bacteria; all segments yielded bacteria associated with F. oxysporum f cubense. It seems unreliable, therefore, to ascribe a distinct syndrome-variant relationship, in view of the presence of at least two variants as well

as bacteria demonstrable within a single shoot. A range in Panama disease symptoms has been observed repeatedly in the field.

When portions of 3 tracheary elements are cultures in one Petri plate, a more dense zone of mycelium has been observed frequently to occur along the line(s) of contact between fungus colonies arising from one such portion, or between colonies arising from 2 or 3 portions of tracheary elements. Mycelial transfers from such colonies may or may not interact. Miller (Can. J. Res. C, 23: 16-43: 1943) observed that, "among mutants derived from the same parent some interact with the parent and some do not". If the diagnosis of Miller is accepted such diagnosis bespeaks a relatively high rate of mutation in colonies of F. oxysporum f cubense arising from host tissue; the use of cultural interaction in the separation of variants therefore seems unreliable.

Concomitant with investigations of variation in the host and on various agar media, a technique has gradually been evolved permitting a study of variation at a more fundamental level. This study developed from the observation that culturally defined variants under controlled environmental conditions produced different levels of FA. By consideration of a group of organic acids chromatographically, a variant can be delimited at one time by a group of convenient markers. Furthermore, variants can be cultured together in a common container and the influence of several variants on metabolite production determined. A tool is also available to study the results of heterokaryosis somewhat more effectively than by a consideration of only one or two morphological features.

The following method has therefore been evolved and to the present has permitted separation of variants by comparison of profiles of ninhydrin-reactive materials produced.

ácido fusárico

A) Modified Richard's medium, 1.5 ml per 10 ml screw-cap bottle inoculated by single spore or mycelial transfer. The cultures are then inoculated in a water bath at 30°C. Precision of results has been shown to depend upon minimizing evaporation losses normally encountered in a cotton plugged flask.

B) Following incubation for desired interval, culture medium is filtered and filtrate spotted on a chromatogram.

C) Circular chromatograms have been found to be very useful. Whatman #1 filter paper washed in dilute HCl and then in water is cut to suitable size. Three chromatograms are spotted identically with 5 filtrates and suitable control.

D) Chromatograms are then irrigated in 3 different solvents at 17°C. It is thus possible to compare 5 profiles under 3 different conditions relatively rapidly.

Sigatoka Disease

Observations on oil applications to leaves

Field observations of helicopter spraying and counting data indicate that leaf reaction to oil spray may be evident within 30 minutes after application. Elongate, dark green streaks appear on oil sprayed leaves; the top leaf is least affected. No such streaks appear on areas protected from oil droplets.

Texas 522 oil when applied to upper or lower banana leaf surface, controls the Sigatoka pathogen within the confines of the sprayed area only. Any movement of oil in leaf tissue is insufficient to

effect control of Sigatoka away from point of application.

Oil spraying for control of Sigatoka Disease

Texaco 522 Oil versus Bordeaux

An oil versus Bordeaux experiment was set up in Mopala Farm with six replications for each treatment - each treatment having a total of approximately 40 acres. Texaco 522 oil and 5-3.75-50 Bordeaux plus Triton X114 + Marasperse C are being used. The experiment was set up in November, 1957 with the oil being applied by knapsack type mist-blowers at the rate of 1.5 gallons per acre on three week cycles. It was necessary to change helicopter application on a two week cycle in August due to heavy outbreak of Sigatoka in the oil plots. This outbreak was evidently caused by lack of penetration of oil spray through the heavy canopy of banana leaves. Helicopter applications have ranged from about 1.25 to 1.45 gallons per acre.

Since the experiment had not been laid out for helicopter application of oil, difficulties were encountered with oil drift. Most difficulties have been ironed out by having the helicopter fly 80 feet inside the border of the oil plots. Two or three rows of the Bordeaux plots are now left as buffers and data is not taken from these. Only one set of permanent flag markers is used since alternate markers for alternate cycles caused too much confusion.

Leaf spots counts were begun in January on a monthly basis. Two hundred random plants per plot were counted and the per cent of leaves showing light, medium, and heavy infection recorded. After the serious outbreak

in August counts were made every 10 to 14 days. At the beginning of the experiment infection was approximately the same for all plots. An increase in medium and heavy Sigatoka infection was noted in mid-July and by August it had increased to serious proportions in oil plots. Helicopter applications on two-week cycles began in August and the medium and heavy infection has dropped considerably but rather slowly in comparison to the July-August increase. As of the last leaf spot survey on December 13th, amount of infection in Bordeaux and oil plots is similar, infection having risen in Bordeaux plots.

Fruit weights in all plots were similar in April when records first began but differences in weights between treatments subsequently became greater (Table 17). The four pounds difference between oil and Bordeaux treatments in May was significant at P = .05 using a t test. This difference was also significant for the period up to the end of May. Differences did not become significant again until September when oil treated bananas had an average weight per stem of 81.60 lbs. and Bordeaux treated bananas 87.59 lbs. (significant at P = .05). In October differences between treatments were approximately 8 pounds in favor of Bordeaux and significant at P = .01. These significant differences between fruit weights after July, 1958 can be attributed at least in part to leaf spot disease. For November, the differences in fruit weights between treatments were approximately 9 pounds in favor of Bordeaux.

Days to harvesting the fruit have always been slightly longer for oil sprayed fruit ranging from 1 to 2.7 days. For the period to

TABLE 17

AVERAGE FRUIT WEIGHTS PER STEM, FROM OIL VS. BORDEAUX PLOTS, MOPALA *

	Bordeaux Average weight (pounds)	Oil Average weight (pounds)	Decrease of oil over Bordeaux (%)	Significance t test
April	91.90	91.57	0.36	
May	89.92	85.69	4.70	0.05
Period	90.65	87.37	3.62	0.05
June	84.14	82.75	1.65	
Period	88.72	85.95	3.12	
July	85.53	81.96	4.17	
Period	88.15	85.21	3.34	
August	89.96	86.80	3.51	
Period	88.59	85.53	3.45	
September	87.59	81.60	6.84	0.05
Period	88.47	84.75	4.20	
October	97.91	89.53	8.56	0.01
Period	90.19	85.51	5.18	0.05
November	96.19	87.41	9.13	0.01
Period	91.18	85.84	5.86	0.05

* Stems examined each month ranged from 1286 to 2857; total stems for the period were 16,446.

to date the difference between oil and Bordeaux is 1.59 days.

The number of hands has ranged from 0 to 0.3 hands less for oil sprayed fruit. For the period to date the difference is 0.15.

The number of leaves per plant at shooting has ranged from 0 to 0.4 less for oil sprayed plants than for Bordeaux. For the period the difference is 0.27 less for oil.

Chalcid wasp damage for the period has varied from 4.33% of harvested fruit in oil plots to 13.3% in Bordeaux plots. Differences in damage could be attributed to the great variation in chalcid population between locations in the experimental area.

Of 8059 stems checked for oil blotching, 69.98% have been clean, 24.51% have less than 1/3 of hands blotched, and 5.51% have more than 1/3 of the hands blotched. Of these same stems checked for maturity stain, 88.86% have been clean, 9.65% have 1/3 or less of the hands bronzed and 1.49% have more than 1/3 of the hands bronzed.

Oil screening (field plots)

In screening plots of ½ acre size replicated 4 times, six oils are being sprayed using Texaco 522 oil as the control. These oils are: Shell 20, Shell E, Shell 21, Sunvis 7, and Texaco TL 3578.

The experiment was begun in March, 1958 using knapsack type mist-blowers to apply 1.5 gallons of oil per acre on a four-week cycle. Leaf spot infection was about 1.5% medium and 42% total in all plots at beginning of the experiment. Infection increased in August and cycles were changed to two weeks. Since infection was very severe in the 3/4 acre screening plots nearby, heavily diseased mats were chopped out of the ½ acre plots and the nearby 3/4 acre plots.

Also, the rate of application was changed from 1½ to 3 gallons oil per acre at the end of September. Because of lack of oil supply, it was necessary to spray two cycles with Texaco 522 oil in all plots. At the present time, the oils can be put in the following order for performance in leaf spot control, although differences are not outstanding:

1. (Sunvis 7
   (Shell 21
   (Shell 20
2. (522
3. (TL 3578
4. (Shell E

Shell E caused the least blotching and slightly less maturity stain than the other oils.

In 3/4 acre oil screening plots, two additional oils were compared with Texaco 522 and an unsprayed check; each treatment was replicated 4 times. This experiment was set up in March but was left unsprayed until July 11. The first leaf spot count was made shortly after the last cycle of Bordeaux, in March. At this time total infection showed about 42%, heavy infection 0.1 to 0.3%, and medium infection 1.4 to 2.4%. There was a gradual increase in infection through mid-July in all plots but in August it rose rapidly to epidemic proportions. Although infection increased greatly in the oil treated plots, there was a definite measure of control over the non-sprayed checks. The first cycle was 4 weeks, after which the cycle was changed to two weeks. At the end of September rates were raised from 1.5 to 3 gallons per acre, and Texaco Rabtex oil was sprayed on the check since it was decided that heavy infection in the non-sprayed checks was strongly influencing oil performance in adjacent plots. The oils could be put in the following order performance in control of leaf spot, but differences are very slight: (1) Sun Superior

Oil 11, (2) TY7, and (3) Texaco 522 Oil. The general disease pattern since the greatest increase in September has been a gradual decrease in medium and heavy infection in all plots to the end of November but the disease was still at a high level, and began to increase again in December.

In another type of screening experiment that began in July, four oils, Prorex 1 and 2 (Socony Oil Co.) with and without inhibitor, are being sprayed on 3 replications of 10 plants each of Lacatan bananas. Between replications there are 3 rows of bananas which are left unsprayed. A check is also included in the experiment and is randomized the same as the four oils. Cycles have been at two week intervals at the rate of 1.5 gallons per acre up until the end of September when the rate was doubled.

Sigatoka infection at the beginning of the experiment was about the same for all plots. By the end of August, medium and heavy categories rose to some extent in all oil plots but disease in the check was much greater than in oil plots. Medium and heavy infection in the oil plots has been held around 10%. Prorex 2 has shown better performance than the other oils. Control is readily visible in the field and suggests that if enough oil is applied leaf spot can be controlled even when adjacent to areas of heavy uncontrolled infection.

Oil Screening ($\frac{1}{4}$ leaf)

Oils were applied to two alternate and opposite quarters of individual leaves. Three applications on a 2-week cycle were applied with a glass atomizer or microsprayer until an oil film was clearly visible.

The first two opened leaves on each of four plants were sprayed with each oil making a total of 16 different leaf areas for observation. Leaf spot and phytotoxicity, as indicated by flecking, spotting, and pitting of leaves, were recorded 2 weeks after each spray cycle and 4 weeks after the third or final cycle.

Oils were screened during February to March dry season, and during the season of the year (July to September) more favorable to Sigatoka infection. All oils were effective in controlling Sigatoka, and most caused some phytotoxic response. Phytotoxic responses increased with each application of oil, suggesting a cumulative effect from repeated oil applications and possibly an influence of leaf age on phytotoxicity. However, the technique used was not capable of distinguishing between oils as to degree of Sigatoka control or phytotoxicity. The quarter leaf technique at its present stage of development is useful to indicate gross failures to control Sigatoka, but it is not sufficiently refined to distinguish among a wide range of oils as to degree of control or phytotoxicity. More refined and accurate techniques and modifications must be developed.

Moko Disease

Moko Disease research in 1958 was centered in the Coto Station. At La Lima, the extension aspect was stressed. In the Pantano District of Honduras, remarkable progress in Moko Disease control was made in the last few months of 1958. This success can be attributed to the following three reasons:

1. Improved detection, sanitation and eradication methods.

2. The assignment of one individual with complete responsibility for the division control program.

3. Great cooperation on the part of Agriculture personnel in carrying out recommended control practices.

The incidence of Moko Disease has been reduced to the level of a minor problem in the Pantano District and should soon be on the decrease in the Mezapa and Higuerito Districts as a result of improved methods (Table 18). Strict vigilance will be necessary to maintain this control.

TABLE 18

MOKO DISEASE INCIDENCE - 1958
CASES BY
DISTRICT

Month	Pantano	Mezapa	Higuerito
January	982	175	2400
February	1428	104	6638
March	1605	81	1107
April	1065	84	381
May	5043	145	250
June	309*	68	181
July)		- **	3468
August)	2984	195	6389
September)		285	2144
October	134	454	3870
November	30	149	5605
December	39	319	7830
	TOTAL	55,941	

* Blowdown records incomplete and large infested area abandoned without counting.

** Blowdown - no records taken.

Nematodes

Distribution of plant parasitic nematodes and their importance in growth and production of bananas

The results of initial surveys indicate that several plant parasitic nematode species are associated with the roots of bananas and are probably causing damage. The root knot nematodes, Meloidogyne spp., and the spiral nematodes, Rotylenchus and Heliocotylenchus spp., are widespread in all of the divisions. The burrowing nematode, Radopholus similis, is common in the Cocos variety in all divisions and on both Cocos and Gros Michel in the Tiquisate Division, and in at least one planting of Gros Michel in the Honduras and Armuelles, Panama divisions. Preliminary findings indicate that there is no resistance to R. similis in commercially grown species of Musa. Gros Michel, Cocos, Lacatan, Dwarf Cavendish bananas, Horse plantains, Chato plantains, and Abaca have been found infected with this nematode.

It is apparent that the burrowing nematode has been transported in the planting stock of the Cocos variety. Since this variety is being propagated as rapidly as possible, it is important to rid the seed of nematode infection at this relatively early age. In the absence of tabulated proof of damage to bananas by this nematode, the chance of damage being done is too great to allow further spread on planting stock.

The root and rhizome damage in the Almirante Division is more or less self evident and tabulated proof of losses in fruit weight is being developed. However, in the other divisions good fruit is being produced in spite of nematode infections. It now becomes extremely important to

obtain more details on the distribution of plant nematodes and the damage caused by them in order to develop economic control programs where required.

The burrowing nematode was found in all divisions surveyed. With the exception of the Tiquisate Division and Esquinas District, however, the occurrence was limited to the Cocos variety. The occurrence of R. similis in such a high percentage of the Cocos plantings in areas where it was absent on adjacent Gros Michel indicates that this pest was transported along with the seed. In Esquinas, the nematode was found in a planting of Gros Michel adjacent to the railroad. In the Tiquisate Division, the burrowing nematode was found in soil, roots, and rhizome tissue of all Gros Michel and Cocos mats sampled. The burrowing nematode was associated with shrunken lesions extending through the cortex to the stele of the roots, and with lesions extending several millimeters into the rhizome. A red discoloration was present in all lesions from which this nematode was extracted.

Spiral nematodes were found in practically all farms sampled. Usually a shallow reddish discoloration confined to the root cortex was correlated with the presence of these nematodes.

Lance nematodes were confined largely to samples taken in the Tiquisate Division. These nematodes were also present in reddish discolorations on root surface.

Coco Farm, the site where the original Cocos plant was found in Armuelles in 1948, was sampled and no R. similis infestation was found. The sites to which the variety was moved prior to shipment to Almirante were sampled. From Coco Farm the variety was moved to Palo Blanco and

from there to Zapatera (Section 16) and Jobo farms. From Zapatera and Jobo farms the variety was shipped to Almirante. Volunteer plants in Zapatera and Jobo farms were dug and planted together in Majagua Farm and from there planted into production plantings. The site in Zapatera to which the variety was moved was probably the origin of the infestation of the Cocos in Armuelles. The Gros Michel now growing there are infected with this nematode.

In Honduras, the major emphasis in survey work has been directed toward the Cocos variety. Most of the Cocos have been surveyed and found to be infected with R. similis. The introduction of the variety from Almirante in 1955 into Honduras was at Guaruma 2 Farm and all plantings with Guaruma 2 as a seed source are infested. Two seed pieces were brought from Armuelles in 1955, and planted in back yards in the residence areas of the division. One of the resulting mats has been dug, split and planted in an isolated section in Farm 16 of the Progreso District. This planting now consists of about an acre. A close check is being kept on this planting for possible infestation since it is hoped that this will be the nucleus of a clean seed bed for the Honduras Division. When attention was called to this planting there were 169 plants. Seed has been stripped from all these plants and observations were made of the seed at the time of stripping. These plants are lightly infested with root knot nematode but no Radopholus has been found as yet. (Table 19).

TABLE 19 DISTRIBUTION OF FOUR NEMATODE GENERA ASSOCIATED WITH BANANAS

Division or District and Farm	NEMATODES PRESENT			
	Radopholus sp.[1]	Meloidogyne sp.[2]	Rotylenchus sp.[3]	Hoplolaimus sp.[4]
ARMUELLES				
Jarillo	-	≠	≠	-
Nispero	-	≠	?	-
Zapote	-	≠	≠	-
Zapatero	-	≠	≠	-
Bongo	-	≠	≠	-
Manaca	≠	-	≠	-
Toreto	≠ *	≠	≠	-
PROGRESO				
Cuajada	-	≠	≠	-
Almendro	-	-	≠	-
COTO				
44	-	≠	≠	-
45	-	≠	≠	-
46	-	≠	≠	-
48	-	≠	≠	-
52	-	≠	≠	-
53	-	≠	≠	-
47	≠ *	≠	≠	-
55	-	≠	-	-
54	-	≠	≠	-
QUEPOS				
Bartola	≠ *	≠	-	-
Llorona	-	≠	≠	-
Maritima	-	≠	≠	-
Los Rios	-	-	-	-

1 - Burrowing nematode
2 - Root knot
3 and 4 - Ectoparasitic nematodes

*Cocos variety

Table 19(Continued)

Division or District and Farm	NEMATODES PRESENT			
	Radopholus sp.	Meloidogyne sp.	Rotylenchus sp.	Hoplolaimus sp.
ESQUINAS				
Seed Bed*	✓	✓	✓	-
Limon	-	✓	✓	-
Alajuela	-	✓	✓	-
Heredia	-	✓	✓	-
San Jose	-	✓	✓	-
Cartago	-	✓	✓	-
Guanacaste	-	✓	✓	-
Jalaca	-	✓	✓	-
*Radopholus also found in confirmation samples				
PALMAR				
12	-	✓	✓	-
9	-	✓	✓	-
10	-	✓	✓	-
4	-	✓	✓	-
3	-	✓	✓	✓
4	-	✓	✓	✓
5	✓*	✓	✓	-
6	-	✓	✓	-
18	-	✓	✓	-
*Cocos variety				
TIQUISATE				
Antigua	✓**	✓	-	✓
Peten	✓	✓	✓	✓
Solola	✓	✓	✓	✓
San Marcos	✓	✓	-	✓
Izabal	✓	✓	✓	-
Ipala	✓	✓	✓	✓
Moyuta	✓	✓	✓	-
Zunil	✓	✓	✓	✓
Ixtepeque	✓	✓	-	✓
Tacana	✓	✓	-	-
Ticanlu	✓	✓	-	✓
Primavera	✓	✓	-	✓
Ceiba	✓	✓	✓	✓
Jutiapa	✓	✓	-	✓
**Cocos and Gros Michel				

Control

Two flood fallow projects have been surveyed for nematode survival. Lake 1 of Guaruma 2 Farm was flooded for three months, drained and plowed. Seven samples were taken and examined for nematodes. Spiral nematodes, root knot nematode larvae and several specimens of non-parasitic nematodes were found. Twelve samples were examined from the Campin lakes which had been flooded for six months. The only nematodes found were non-parasitic forms. The San Juan lakes, to be used as an improved seed bed area, are being surveyed. These lakes have been flooded for 3 months, drained for 4 months followed by another flood of 6 months. One lake has been surveyed, numerous free-living nematodes, a few root knot nematode larvae and a few Helicotylenchus were found.

On the basis of the surveys of flood-fallow lakes, experiments were initiated in tanks to determine the length of time that Radopholus will survive under continuous flooding. Infested soil was brought from Choloma, placed in concrete tanks, and flooded to a depth of about one foot. A total of 16 tanks are included in the experiment. Alternate tanks received about 10 pounds of infested rhizome tissue chopped to about the size left in the field after discing. After two months soil was taken from each tank, placed in a smaller pot and planted to corn, an excellent host plant. Future samplings will utilize tomato instead of corn due to a greater susceptibility of tomatoes to root knot nematodes and an equal susceptibility to R. similis. Plantings will be made every month until no more Radopholus can be detected in the roots. At the end of the experiment, bananas will be planted in the tanks, left as long as possible and

checked for infection by R. similis.

Flood-fallowing, if effective, is only a partial solution to the nematode problem since there are areas that cannot be flooded. In such places, cultural practices or chemical treatment of the soil will be necessary to control these pests. Limited success has been obtained in controlling nematodes by the use of cultural practices such as crop rotation, trap crops, and dry fallowing. Usually such practices are satisfactory only where an annual crop follows. An annual crop can be grown and harvested before the nematode population again builds up to a damaging level. In bananas more satisfactory results are likely to be obtained by the use of chemical nematicides which can be applied to the soil and the growing plants. Even though a complete control cannot be accomplished, it is possible that the population can be reduced by chemicals in a much shorter time than by the use of cultural practices. When the loss of production incurred by the cultural practice is considered, the cost of chemical treatments is by no means large.

Methods of obtaining nematode free seed are being investigated at La Lima. Treatments that have been successful in cleaning planting stock of nematode infestations and infections are being tried. It is hoped that a chemical dip can be developed that will eliminate the nematodes without necessitating paring of the rhizome or will allow good germination of pared rhizome pieces. The paring of the rhizome creates additional portals of entry for organisms such as the rhizome rot bacterium, thereby greatly reducing germination and survival. Several chemicals have successfully eliminated nematode infestations from bulbous plants. Among these are Dowicide B, Dowicide 2, Aaventa and Aabulba. The first two are in trial at present and the latter two are on order. Other chemicals still in experimental stage are being obtained for trials.

Various treatments will be tested with pared and unpared seed including applications of the above mentioned chemicals in hopes improving the germination and growth of the planting stock.

Microbiology

Interactions of banana plants, Fusarium, and Scaptocoris talpa under field conditions.

The results presented in the 1957 Annual Report indicated that odoriferous matter produced by S. talpa was able to control the growth of Fusarium oxysporum f cubense in vitro as well as in the soil.

An experiment was started in 1957 to find out whether Scaptocoris talpa is able to protect banana plants from the attack of Panama disease under field conditions of general banana plantation practice. Three locations were selected for this experiment:

1. Omonita Lake, Section 19, flood-fallowed area
2. San Juan, Lake 7, Section 19, area abandoned just before planting time (August 1957) due to Panama disease
3. Mopala, Section 27, area abandoned during last two years due to Panama disease

The reason for selecting these areas was to evaluate the protective ability of S. talpa in soils with different density of initial inoculum of Panama disease organism. Thus, San Juan area was considered as an area with active virulent pathogen; Mopala with mild disease activity and Omonita, after flood-fallowing, an area "without disease".

Treatments were arranged as follows:

1. Insect infestation during planting time
2. Insect infestation at beginning of root formation
3. Insect infestation at the time of well-developed root system
4. Treatments 1 + 2 + 3
5. Control - no treatment

At the time the 1957 Annual Report was presented, the plants were four months old and did not show symptoms of Panama disease.

During the first quarter of 1958 (March) a survey on the population of S. talpa of treated plots indicated that the insects did not establish themselves in San Juan, while in Mopala, insects were found on 95% of the plants, and in Omonita insects were found on all plants except those of Treatment 1, in which the rhizomes were treated with Dithane.

During the second quarter of 1958 (June) a more thorough survey on the insect population was made but approximately the same results were obtained. Therefore, San Juan plots received a second insect infestation. Approximately 50-75 insects were placed at each mat and certain mats (marked) received 200-300 insects each.

During the year the plots were surveyed 3 times for prevalence of Panama disease. The number of cases of Panama disease on Mopala and San Juan plots increased until June but toward the end of the year there was a considerable improvement in the stand; however, further data will be required before one can say whether or not the insects protected the plants against the disease.

On the other hand, in an insect survey of agronomy spacing and fertilizing experimental plots at Mopala Farm, Lake 2,Section 7, definite reverse correlation of density of insect population around the roots of healthy and diseased banana plants was established; that is, healthy banana plants harbored around the roots 15.3 to 69 insects per cubic foot of soil, whereas diseased plants harbored only 1.3 to 0 insects per cubic inch of soil.

Effect of different soils on survival of S. talpa

San Juan and Omonita soils were passed through a 2 mm sieve and 500 g of soil and 64 insects were placed in each of several quart glass jars and kept at 30° C. There was no difference in numbers of insects surviving in San Juan or Omonita soils.

Effect of odoriferous matter on nematodes

A weak flow of air was passed over 200 insects and then bubbled through water containing nematodes. After one hour of such treatment, the nematodes appeared to be very sluggish, and some mature animals were dead. However, small larvae were still alive (barely convulsing) after 24 hours of treatment.

Extraction of odoriferous matter with carbon tetrachloride

One pint glass jars were filled with insects and CCl_4 was added to cover. The portions of the solvent were then filtered out after 15, 30, 60, and 120 minutes in contact with the insects. The color of the solvent obtained was pale straw yellow. If these extracts were left in Erlenmyer flasks covered with glass beakers, to stand at room temperature, the solvent was evaporated, leaving an oily residue. This residue was of strong insect odor and contained antifungal activity.

Extraction of odoriferous matter from soil

To find out whether S. talpa excretes the odoriferous matter into soil, 500 insects mixed with 500 g of soil were placed in a quart glass jar and left to stand for 24 hours at room temperature. The insects were then separated from the soil and soil was extracted with CCl_4. The extract obtained was of pale straw yellow color. When this extract was left in Erlenmyer flasks covered with beakers, to stand at room

temperature, the CCl_4 evaporated and the remaining residue was of stong insect odor. This residue contained antifungal activity.

Preparation of 2,4-dinitrophenyl-hydrozones from CCl_4 insect extracts

To a freshly prepared 2,4-dinitrophenyldrazine reagent a 5 ml of 30 minutes CCl_4 insect extract and 15 ml of 95% ethanol were added and formation of crystals began about 15 minutes later. The mixture was allowed to stand overnight at room temperature. The precipitate was then filtered out, washed with cold 95% ethanol and was resuspended again in 30 mls of 95% ethanol. This suspension then was heated on the steam cone till precipitate was dissolved. The hot solution was filtered through Whatman's No. 42 filter and allowed to stand overnight. The precipitate then was filtered out, washed with cold 95% ethanol, and dried at 37° C. The powder prepared from this precipitate melted at 123.5 - 125.0° C, uncorrected. The melting point was determined according to Fieser's method.

According to Roth et al the melting point of 2,4-dinitrophenyl-hydrazone, prepared from 2-hexenal of cockroach origin, was 139.5 - 141.5° C, uncorrected.

If our determination of the melting point of 2,4-dinitrophenyl-hydrazone is correct, the odoriferous matter produced by S. talpa sould be different from 2-hexenal (trans) produced by Euricotes floridana.

The odoriferous matter of several non-fossorial insects was assayed against F. o. f cubense, and it was found that one other insect (family Coreidea) produced odoriferous matter with antifungal activity.

Effect of composts containing antagonistic organisms on the survival and the virulence of Fusarium oxysporum f cubense

The results presented in the 1957 Annual Report indicated that among the microbial population of the soil there are considerable numbers of antagonists. Among the actinomycetes isolated there were 9.5% antagonistic to F. o. f cubense, 33.3% antagonistic to Pseudomonas solanacearum (Moko) and 13.5% to Xanthomonas species. Furthermore, it was also found that F. o. f cubense isolates produced different pigments when assayed against the same antagonistic organism. This observation was utilized and a new method for differentiation of F. o. f cubense isolates was outlined. It was also shown that some of the isolated antagonists were able to produce antifungal antibiotics when grown on liquid media.

When the interaction of antagonistic organisms and F. o. f cubense was investigated in the soil, it was found that the dilution plate method aerobic conditions was inadequate to detect a low population of Fusarium in soil. The minimum dilution that could be counted after 3-6 days of incubation was 1:1000.

It was demonstrated, however, by Timonin and Bisby et al (1935) that certain soil-inhabiting fungi, including the genus Fusarium, are able to develop growth under anaerobic conditions on solid media. These findings were utilized for development of a method by which low density populations of Fusarium in the soil could be determined.

Determination of viable spore incidence in soils with potentially low Fusarium population

The numbers of fungi per gram of soil were estimated by dilutions. The Petri dishes received 1 ml of 1:10, 1:100 and 1:500 soil dilutions in sterilized distilled water and were poured with Rose bengal-strepto-

mycene-peptone-dextrose agar and incubated under anaerobic conditions at 29-30° C.

The anaerobic conditions were obtained by introducing CO_2 into a chamber, (Fig. 12-4) from a cylinder (Fig. 12-1) until the air was displaced. The soil dilution plates were incubated in the chamber, closed except for two tubes, through one of which CO_2 constantly entered slowly, (Fig. 12-3) the other (Fig. 12-5) serving as an exit through a water seal (Fig. 12-6). When chambers were loaded with dilution plates and leads secured by means of nuts, the clamp on the bottom tube (Fig. 12-7) was opened and air in the chamber was rapidly replaced with CO_2. Then the flow of CO_2 was adjusted by the same clamps to the desired speed.

To check that conditions in each chamber were anaerobic, one test tube containing reduced methylene blue was placed in each chamber at the time of loading. This apparatus was assembled in the incubator. The temperature during incubation was maintained at 29-30° C. It was found that under anaerobic conditions the fungi grew more slowly than under aerobic conditions and required 7 to 9 days of incubation to produce a colony which can be unmistakably seen by the naked eye. When chambers were opened, the tubes with methylene blue indicated that the conditions in the chamber had remained anaerobic. The plates were then immediately counted and each colony was checked under microscope for rough identification. Colonies proved to be Fusarium sp. were subcultured for further studies. If the plates were left overnight under aerobic conditions they were overgrown with aerobic fungi and count was impossible. To verify this method, several sterilized, and unsterilized soil samples in one-quart glass jars were inoculated with F.o. f cubense conidial suspension in sterile distilled water. Each 200 gm of soil received 30 ml of

conidial suspension. The soil originally contained 5% of moisture (at 105° C); with addition of 30 ml of inoculum, the soil contained 20% (w/w) of moisture.

The inoculum was prepared from 7-day-old cultures and contained 1,872/ml of viable spores. Therefore, each 200 gm of soil received 56,160 spores or 351 spores per gram of moisture-free soil.

After addition of inoculum the soil was thoroughly mixed by means of rotating and shaking the jars so that homogenous distribution of inoculum was obtained. The jars were incubated overnight at 29-30° C and analyzed next morning.

The results summarized in Table 20 indicate that using the outlined method it was possible to make a count of F. o. f cubense in soil with low potential inoculum. This method was also used satisfactorily last summer for the determination of Fusarium population in flooded soils.

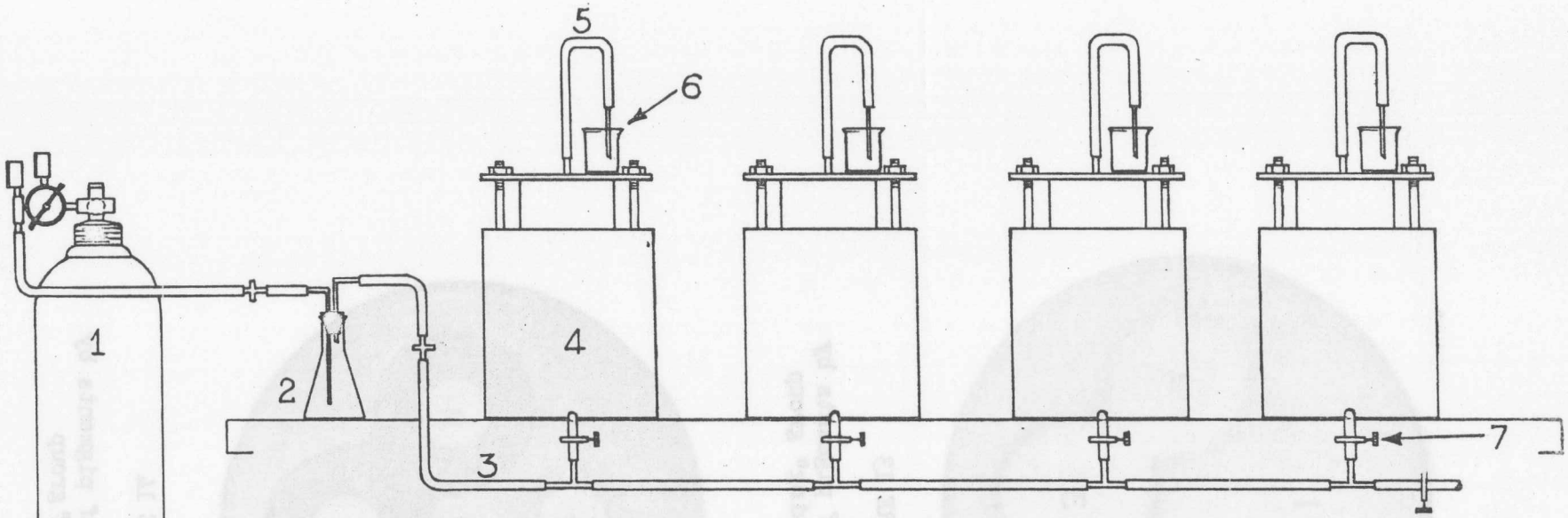
TABLE 20

NUMBER OF COLONIES OF FUSARIUM OXYSPORUM F CUBENSE PER GRAM OF MOISTURE-FREE SOIL

Soil Sample	Replicate	1:10 Dilution Total Count	Fusarium Count
Sterilized	1	27.57	27.57
	2	29.16	29.16
	3	27.48	27.48
	4	31.95	31.95
Unsterilized	1	13.10	9.36
	2	14.98	9.67
	3	15.82	9.31
	4	14.00	9.02

FIGURE 12

ANAEROBIC CHAMBERS



1 CO_2 CYLINDER

2 TRAP

3 DISTRIBUTION LINE

4 ANAEROBIC CHAMBERS

5 EXAUST TUBE

6 WATER SEAL

7 CLAMP ADJUSTING THE CO_2 FLOW

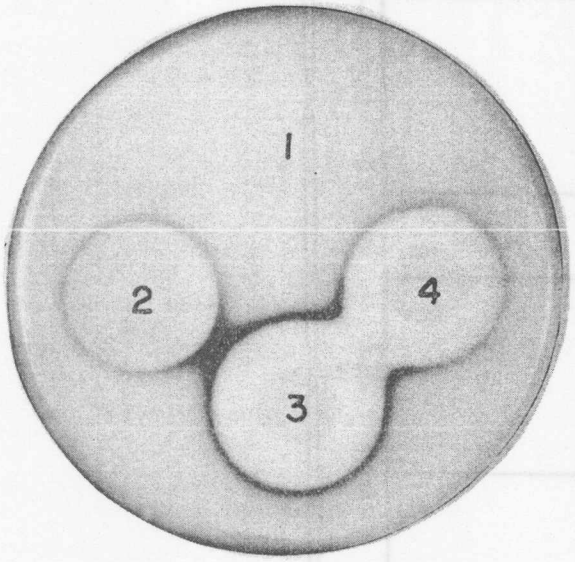


FIGURE 13

Production of pigments by
"non-yellowing" group

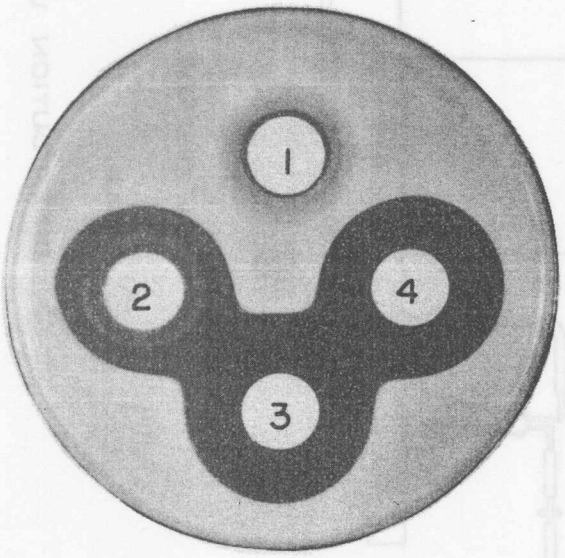


FIGURE 14

No production of pigments by
"yellowing" group

It was reported in the 1957 Annual Report that 12 isolates of F. o. f cubense received from Dr. R. H. Stover were differentiated into four distinct groups. At present, Dr. Stover classifies similar isolates into 7 clones which possess a varying degree of virulence to banana plant variety Gros Michel.

These 7 clones were run through the test with antagonistic organisms and the results are summarized in Table 21. From the data presented it is quite evident that all isolates were differentiated, according to the pigment production, into 3 groups:

Group 1. Clone A, C
Group 2. Clone B
Group 3. Clone D, E, F, G

Therefore, the method failed to agree with Stover's new classification but rather agreed with his old one, namely: yellow, non-yellow and interactants 1 and 2. The yellow and non-yellow isolates of F. o. f cubense could be very easily differentiated by the antifungal antibiotics produced by organism 1025. In this case when the antibiotic was assayed against isolates of non-yellow group, on the perifery of the zone of inhibition, they produced dark greenish pigment (Fig. 13), whereas isolates of the yellow group produced no pigment (Fig. 14) or very weak hyssop violet pigment.

TABLE 21

DIFFERENTIATION OF ISOLATES OF FUSARIUM OXYSPORUM F CUBENSE ACCORDING TO PIGMENTS INDUCED BY ANTAGONIST 1025

Clone	Pathogenicity	Syndrome	Czapek's	Soil Extract	Peptone Dextrose
A	Moderately virulent	Yellowing	DGBS	DVB	DHV
C	Very highly virulent	"	DGBS	DVB	DHV
B	Very slightly virulent	"	N	HV	DHV
D	Moderately virulent	Non-yellowing	DGBS	DVB	N
E	Highly virulent	" "	DGBS	DVB	N
F	Moderately virulent	" "	DGBS	DVB	N
G	Slightly virulent	" "	DGBS	DVB	N

RIGWAY COLOR STANDARD

Plate No.	Color	Abbreviations
XXXVI	Hyssop Violet	HV
XXXVI	Deep Hyssop Violet	DHV
L	Dull Violet Black	DVB
XLVIII	Dark Green Blue Slate	DGBS
	No Color	N

Production and Concentration of Antibiotic 1025

The antibiotic 1025 was produced by growing the organism on peptone dextrose, soil extract (containing yeast extract), and medium containing NA glutamate in shake culture or stationary. In the case of shake culture it required 5 to 7 days of concentration at 26-27° C to obtain a 18-20 mm of zone of inhibition, whereas in stationary cultures it required 20 to 30 days to attain the same concentration. The broth was periodically tested by assay disc method and when the broth reached the 18-20 mm inhibition zone potency it was extracted (mycelium included) with wet n-butanol.

Butanol was then concentrated under vacuum to dryness and obtained solids extracted with 95% methanol.

The concentrates with 700 F. oxysporum f cubense and 500 Candida albicans units were obtained.

Phytotoxicity and Translocation

The preparations with 700 F. o. f cubense units m/l proved to be not phytotoxic to Gros Michel banana leaves. However, the results indicated that the antibiotic was not translocated in leaf tissues.

Effect of Antibiotic 1025 on Nematodes

Antibiotic 1025 containing F. o. f cubense units m/l in buffer solution (pH-7.1) kills nematodes in two minutes to ten hours. It was observed that small larvae were more resistant than mature nematodes and required ten hours of contact with antibiotic to be killed.

The results also indicated that 700 F. o. f cubense units m/l concentrations of 1025 antibiotic in buffer solution is active in Gros

Michel rhizome tissues infested with burrowing nematodes (Radopholus similis). The infested areas of rhizome were sliced into ½ x 5 x 5 cm blocks and immersed into antibiotic solution. It was observed that adult nematodes were killed first and small larvae were more resistant.

Identification of Antibiotic 1025

The antibiotic 1025 retained its activity after immersion for 30 minutes in boiling water. It is soluble in M/5 phosphate NaOH buffer (pH 7.1), ether, methanol, ethanol, ethyl acetate and chloroform. It decolorizes $KMnO_4$ and in concentrated H_2SO_4 it changes color from violet to dark coffee brown. It does not reduce Fehling's solution and does not change color with $FeCl_2$.

In n-butanol or 95% ethanol it gave 291-304-318.5 mμ ultra violet absorption maxima (Fig. 14). Therefore, it should be placed with the polyene antibiotics of the tetraene group. As far as the author is aware, only five antibiotics belonging to this group have been reported, namely: Amphoterecin A, Antimycoin (complex), Chromin, Nystatin and Rimocidin.

The antibiotic 1025 differs from:

1. Amphoterecin A - is not soluble in ether and it is not stable in boiling H_2O.
2. Antimycoin - is not soluble in ether and chloroform.
3. Chromin - labile in acid and alkaline solutions.
4. Nystatin - not soluble in ether and ethyl acetate, and not stable in boiling H_2O.
5. Rimocidin - no information

When the activity of antibiotic 1025 was compared with the activity of mycostatin (Nystatin) against isolates of F. o. f cubense, mycostatin did not induce the non-yellowing strain to produce characteristic pigment such as produced with antibiotic 1025. Secondly, it is not as active against F. o. f cubense as antibiotic 1025 (Table 22).

TABLE 22

ACTIVITY OF NYSTATIN AND ANTIBIOTIC 1025 IN DIFFERENT SOLVENTS AGAINST FUSARIUM OXYSPORUM F CUBENSE

			F. o. f cubense strains	
			Non-yellowing	Yellowing
Antibiotic	Solvent	Units/ml	Zones of inhibition in mm	
Mycostatin*	Ethanol[1]	500,000/15	17-16**	23-22**
1025	Ethanol	700	27-27	32-31
1025	Methanol	700	27-27	32-31
1025	Chloroform	?	27-27	30-28
1025	Ether	?	27-26	28-28
1025	Ethyl acetate	?	24-23	25-25
1025	Buffer (pH-7.1)	?	25-25	30-29

* Mycostatin is the trade name for Nystatin.

** Assay was performed with assay paper discs 12.7 mm in diameter

1 Unitage determined by Squiib & Sons, Div.

Physiology

Banana growth and propagation

The subject of growth in the banana plant has been investigated from the agronomic standpoint to good effect for some time. However, our knowledge of some of the fundamental but nonetheless significant and important growth characteristics is far less complete. It was to fill this gap that the growth studies have been undertaken.

Although banana propagating materials have been used for many years within the Company, the potentialities of this "seed" have only been examined in the most cursory manner. A considerable saving in both labor and transportation could result from a more detailed consideration of this material. Germination failures could be reduced.

Foliar growth

Growth is initiated from an apical meristem that is central in the pseudostem atop the true stem. Each plant organ must grow up from this basal position through to the top of the pseudostem. To elucidate the manner of this growth 15 significant and many observational dissections were made. These have led to the concept of growth presented below.

When a banana leaf has completely unfurled it has attained, at least in a slow growing plant, very nearly its maximum laminar size. This is being re-assessed on more rapidly growing plants. In a 9-foot plant at the time of this completed unfurling (Fig. 15), the tip of the next youngest leaf, No.2, is about 18 inches exposed, the blade is about 75% expanded and the leaf has attained 57% of its final height although petiolar elongation stands only at 26%. Central to No. 2, leaf No.3 is

but 25% of its final height with the blade but 34% of its ultimate length and 21% of its matured width. Leaf No. 4 is developed only to 11% in total height, with its blade only 16% in length and 3% in width. Leaf No. 5, the last to show significant development, is 5% of its final height, has the blade 8% of the ultimate length, but less than 1% of a final width. While numerous other leaves have been cut off by the meristem, the flower bud is not present. In essence this means that in some 32 days leaf No. 5 will grow 241 inches in length, meanwhile expanding its blade by 32 inches in width and 111 inches in length. This growth will be made at a rate precisely coordinated with the rapidity of development of leaf No.4, governed by No. 3, governed by No.2. (The factors that govern the growth of leaf No. 2, light, rainfall, humidity, temperature, will be studied in 1959). The various portions of leaf No.5 will be enlarging at independent but closely correlated rates, for example, while the petiole elongates by 64% in the last 8 days, and while the leaf is in the "candle" stage, only 25% of the blade expansion occurs during the same period. The bulk of the laminar development is completed having started most extensively some 16 days prior to the unrolling.

Foliar growth can be visualized as a coordinated expansion of several leaves, each growing independently but functioning together as a solid cone that forces up through the heart of the pseudostem. That the growth is directly upward is demonstrated by the insertion of a fine capillary diametrically through the pseudostem followed, after 3 days, by a dissection; there is no spiralling. The sheared glass traces the direction and extent of the growth by scoring the leaf bases. Similar techniques have indicated the

FIG. 15

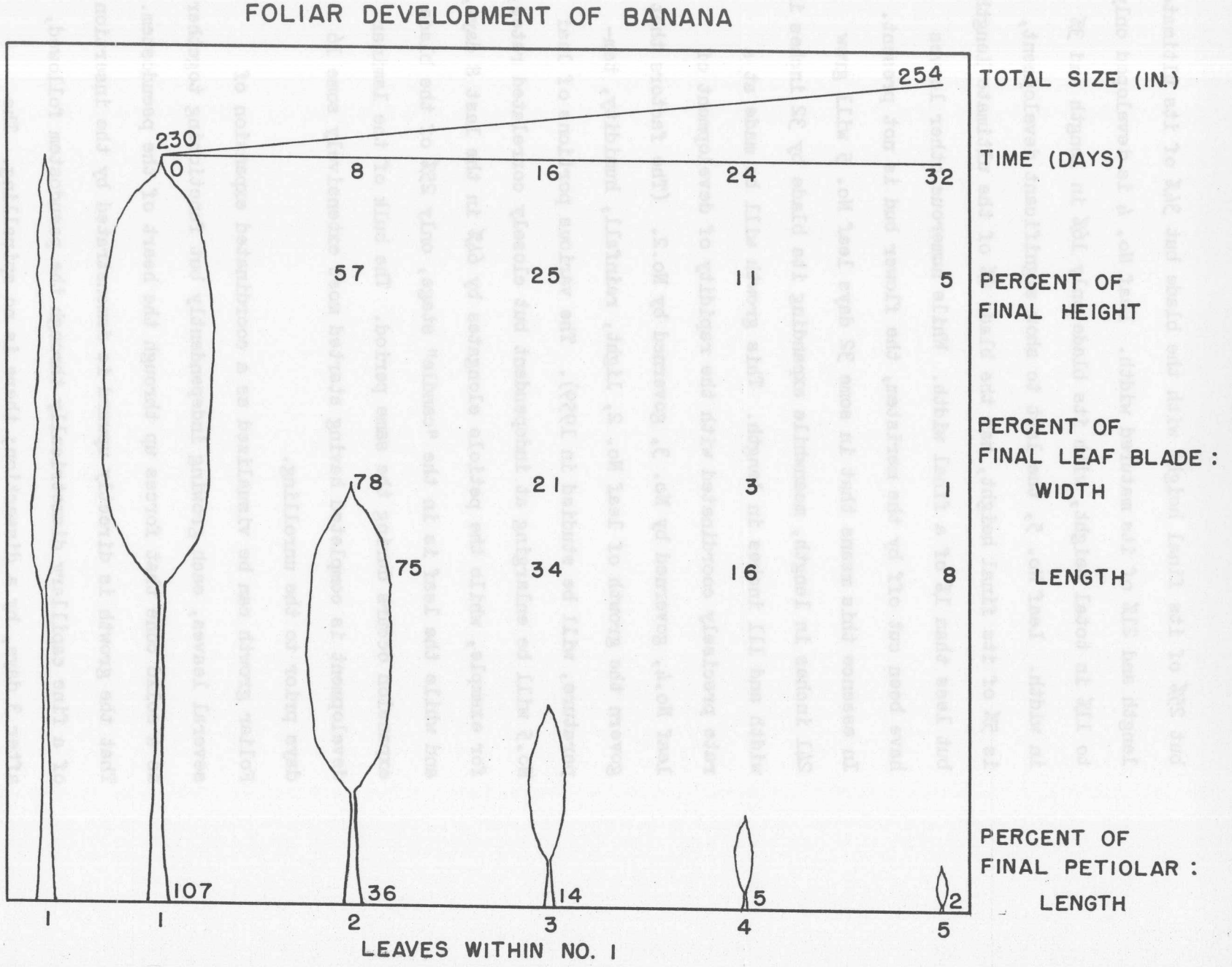
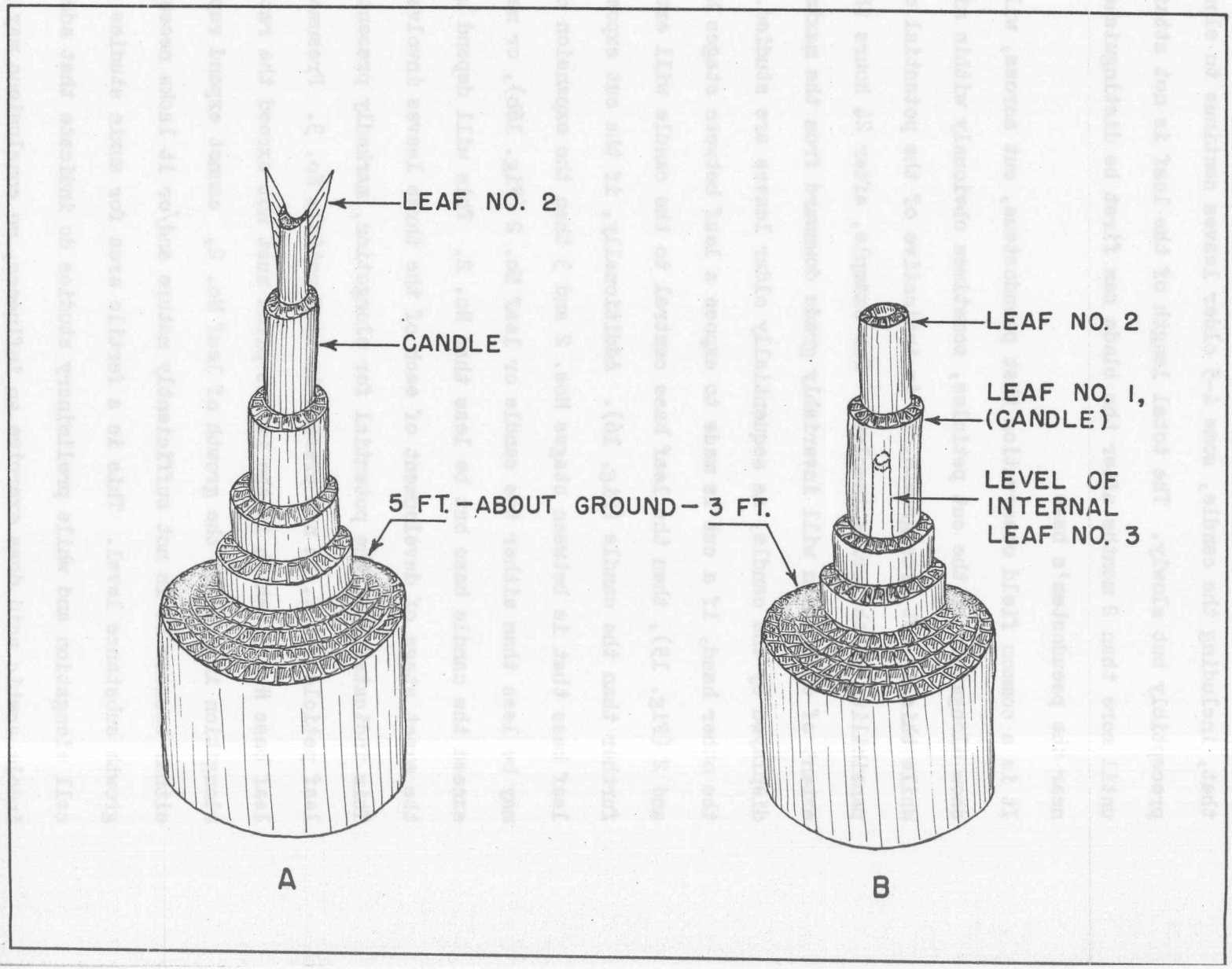

FOLIAR DEVELOPMENT OF BANANA
254
230
0
8
16
24
32
TOTAL SIZE (IN.)
TIME (DAYS)
57
25
11
5
PERCENT OF FINAL HEIGHT
78
21
3
1
75
34
16
8
PERCENT OF FINAL LEAF BLADE : WIDTH
LENGTH
107
36
14
5
2
PERCENT OF FINAL PETIOLAR : LENGTH
1
1
2
3
4
5
LEAVES WITHIN NO. 1

FIG. 16

POTENTIAL GROWTH OF LEAF BASES

AT DIFFERENT LEVELS OF CUTTING (AFTER 24 HR.)



duration of the growth of the leaves already exposed and it is clear that, including the candle, some 4-5 older leaves continue to elongate preceptibly but slowly. The total length of the leaf is not attained until more than 2 months after the blade can first be distinguished near the pseudostem's base.

It is a common field observation that pseudostems, cut across, will show elongation of the cut petioles, sometimes obviously within minutes. While this is not true growth, it is indicative of the potential expansibility of the cells involved. For example, after 24 hours the extent of elongation will invariably grade downward from the maximum displayed by the candle, as sequentially older leaves are studied. On the other hand, if a cut is made to expose a leaf between stages No. 1 and 2 (Fig. 15), then the leaf base central to the candle will extend further than the candle (Fig. 16). Additionally, if the cut exposes a leaf base that is between stages Nos. 2 and 3 then the expansion of #3 may be less than either the candle or leaf No. 2 (Fig. 16b), or may exceed the candle base but be less than No. 2. This will depend upon the exact stage of development of each of the three leaves involved. This indicates that the potential for elongation, markedly present in leaf petiole No. 2, is not present in leaf petiole No. 3. Presumably leaf base No. 3, which in the intact plant must not exceed the rate of elongation imposed by the growth of leaf No. 2, cannot expand rapidly either because it is not sufficiently mature and/or it lacks necessary growth substance level. This is a fertile area for auxin studies on cell elongation and while preliminary studies do indicate that added indole acetic acid does exercise an influence, no conclusions may yet

be drawn. The coordinated growth concept, proposed because of the dissections, is beautifully supported by this rapid expansion following the cutting of pseudostems.

Growth of five plants

The foliar growth studies have been further elucidated by the leaf elongation and growth work carried on beside the greenhouse of the Research Building in La Lima. Here by means of a vertical pole, scored every 20 cm, and a bubble-levelled horizontal marker, leaf elongation can be measured. Unfortunately the plants in the Research grounds were excessively slow growing and so the study is of less interest than it should be.

TABLE 23

GROWTH AND FOLIAR DEVELOPMENT OF TWO GROS MICHEL PLANTS
MAIDEN HEAD SEED PLANTED OCTOBER 21, 1957.

	Plant No.1 (14.6 kg.*)					Plant No.2 (6.0 kg*)				
		BLADE					BLADE			
Date 1958	Leaf	Length (cm)	Width (cm)	Period** Days	Plant Height cm	Leaf	Length (cm)	Width (cm)	Period Days	Plant Height cm
Jan.1	12	117	49	6	84	12	104	43	6	82
June 1	27	175	55	8	196	28	158	57	10	153
Nov.11	43	290	78	11	365	44	275	73	11	337
Fruit	Shot Nov. 25, 1958 - 9 hands					Shot Dec. 1, 1958 - 6 hands				

* Planting weight

** Time from first appearance of a leaf to its completed expansion

Table 23 records the essentials of growth of two of the five plants. As a trend, the leaves continue to increase in size, both in length and width of the balde, and in petiolar length. This is not strictly true in the petiole since the last seven (or so) leaves attach to the elongating stem of the reproductive plant. Rate of leaf outturn

varies from plant to plant and, at the moment, no close correlation with weather is possible. Once exposed, the leaves fail to expand their blades appreciably, although total length continues to increase through petiolar elongation. It is interesting that the date of fruiting and the number of hands both appear to reflect initial seed weight. This possibility is being studied in the field at present. Some 43-47 leaves appeared prior to fruiting and no morphological change was observed that would serve to distinguish the vegetative from the reproductive plant.

Short term growth measurements

The short term measurements of growth studies were extended to the field where more rapidly growing plants were selected. The following information regarding bananas grown in Honduras was obtained in July.

A. There is a marked diurnal-nocturnal cycle with the growth falling off during the night hours and rising sharply at first light.

B. There is a marked depression in growth at midday when the temperature is highest, the relative humidity lowest and the light intensity high. The noon temperature approximated 95+ degrees. It is of interest that in March 1955, similar studies using auxinometers did not show a noon time depression at Coto, Costa Rica. Presumably all three of the variables mentioned above differed and it is believed particularly that the temperatures were lower in Costa Rica.

C. Growth of an individual leaf, on a 6-7 day outturn cycle, increases in daily rate to a maximum on the third day, after the unrolling of the previous leaf permits the first measurement; and thereafter decreases gradually.

D. Maximum per hour growth was slightly more than 1.0 cm (compared to a maximum of 2.8 cm in Coto in March, 1955).

With the coming of predictable weather these studies will be intensified and the effects of shading and temperature examined.

Relationship of growth and environmental factors.

In 1956, Freiberg reported a relationship between sunlight intensity and both fruit weight and height growth. He reported that a change in sunlight intensity was followed one month later by a corresponding change in fruit weight. In 1957, this relationship could not be corroborated - at least prior to June (Fig. 17) when the picture was complexed by a blowdown. Again in 1958, this relationship is not to be observed, although another blowdown, again in June, further confused the situation.

It appears that we would not be able to predict fruit weight on the basis of sunlight intensity with any degree of certainty.

It is also noted that growth in height for 1957 and 1958 does not follow sunlight intensity - indeed it is difficult to relate the two curves in any way. Again much of the difficulty could relate to the two June blowdowns spaced one year apart. However, the growth in 1957 was not measurably slowed in 1957 by the blowdown as appeared to be the case in 1958.

The past two years present a confused situation for analysis. However, they do indicate that sunlight intensity and growth relationships are not so simple as was earlier indicated and that other environmental factors must be considered with regard to banana growth phenomenon.

It is unlikely that further data will be gathered on this experiment since Panama disease will have so decimated the experimental area as to make it unusable by next December.

Propagating Materials

Sucker seed nursery

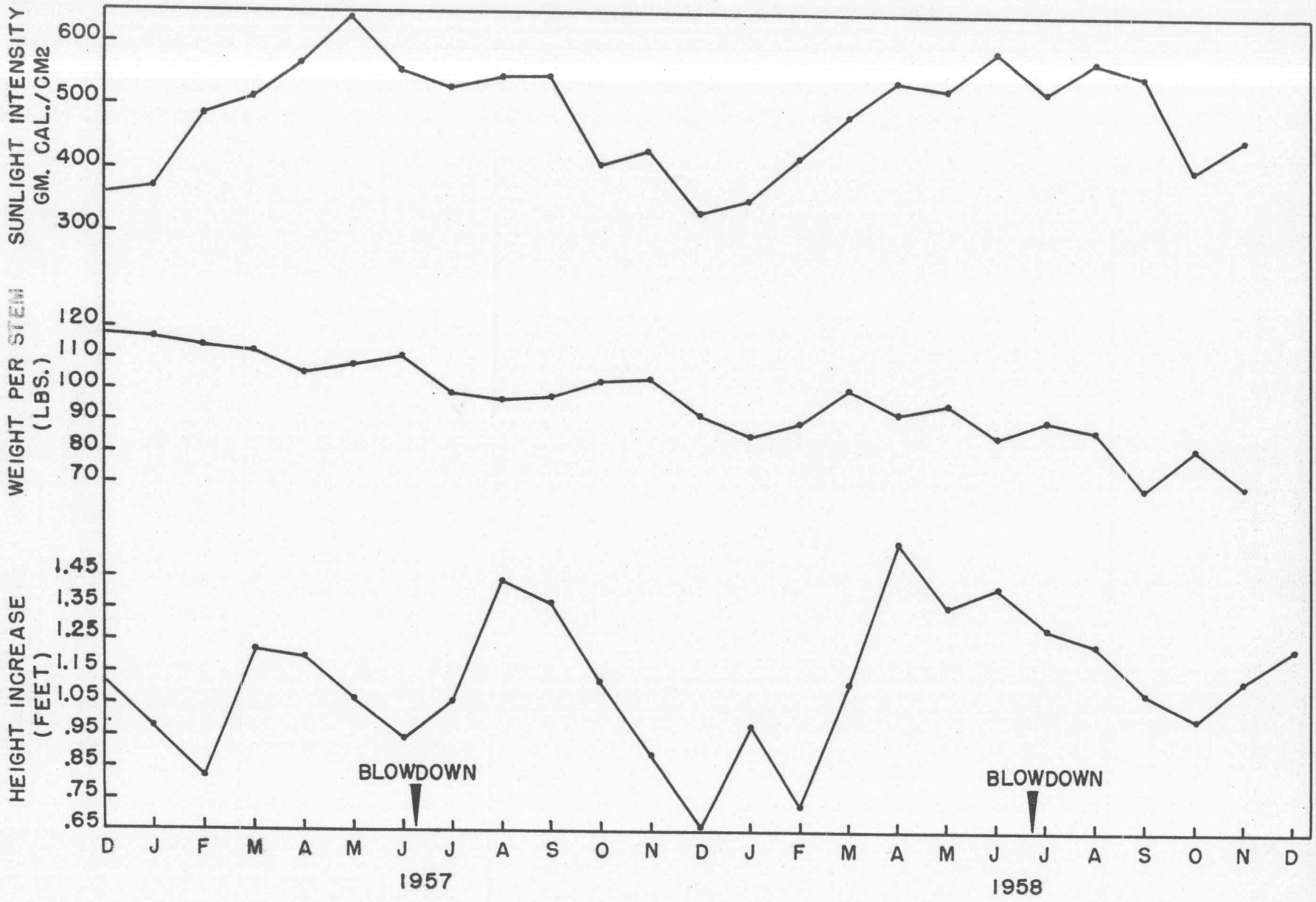
On November 9-15, 1957, an experiment was established in Guaruma 2 to study the potential of closely planted seed as a source for commercially acceptable seed. The weight of seed planted ranged from 0.5 lb. to 5.5 lbs. with a mean weight of 2.6 lbs. After five months the plants spaced at 1 meter hexagonal and 1 meter square, had grown to a mean height of 8.39 and 8.14 ft. and to a mean diameter of 4.0 and 4.12 inches (at 3 ft.). After six months the rhizomes had attained mean weights of 9.69 and 10.50 lbs. Interestingly enough, the maximum weight recorded was a fabulous 31 lbs.
The hexagonally planted (more closely spaced) plants were taller, thinner, and produced a smaller rhizome. This is expected when light is withheld from a plant.

Sucker seed weight experiment

This successful use of sucker seed and the increasing use of the stripping technique indicated that information relating initial weight of seed to subsequent growth and germination would be valuable. Accordingly an experiment was laid out to assess these facts. After three months the results in Table 24 were obtained.

FIG. 17

COMPARISON OF SUNLIGHT INTENSITY WITH MONTHLY GROWTH INCREMENTS AND FRUIT WEIGHTS OF BANANA PLANTS



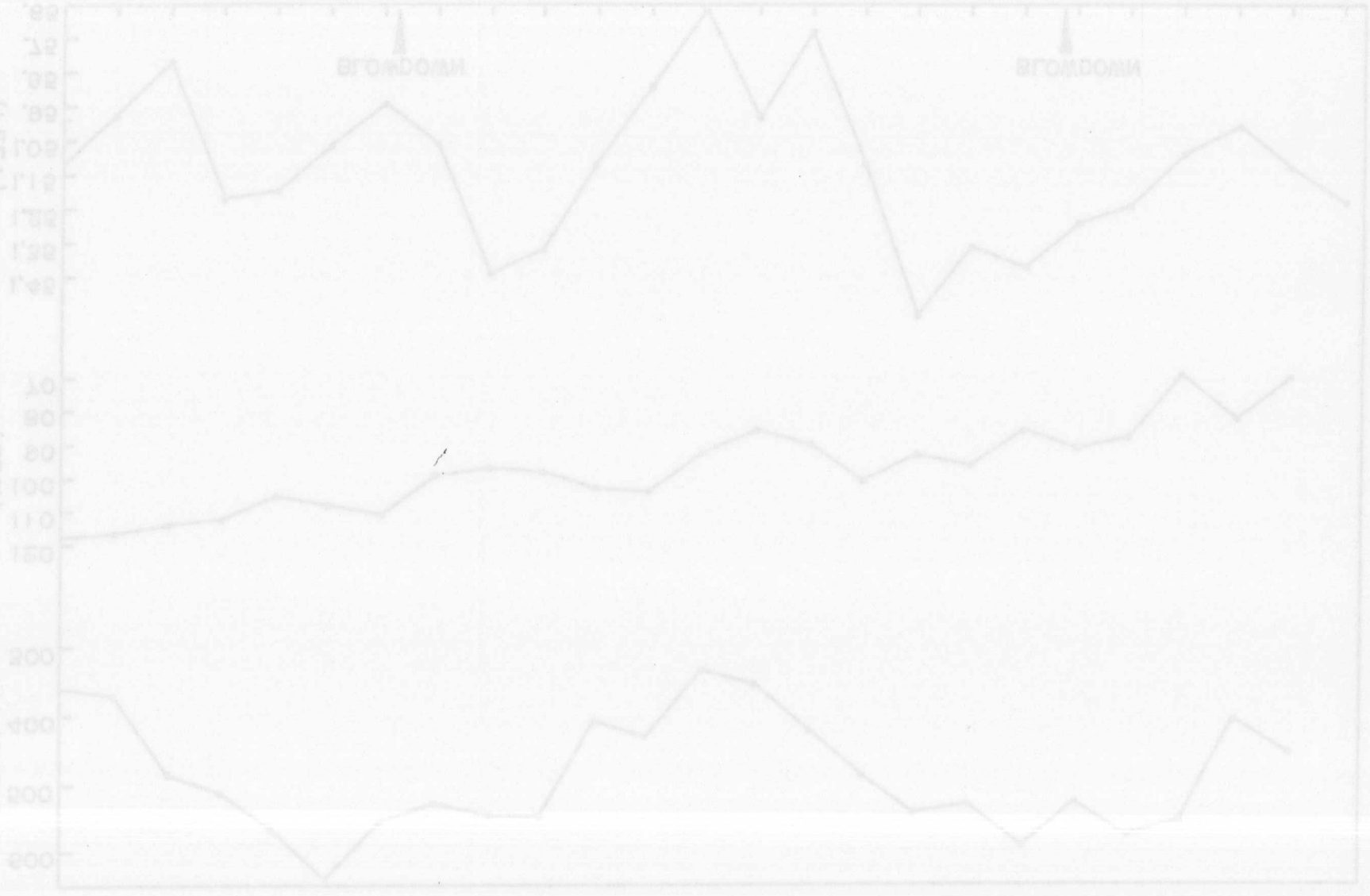


TABLE 24

GERMINATION, SURVIVAL, AND GROWTH IN HEIGHT OF BANANA SHOOTS FROM SUCKER SEED OF VARIOUS INITIAL WEIGHTS

Initial weight (lbs.)	Germ. (%) (30 day)	Survival (%) (90 days)	Growth in height (cm) 30 Days	60 Days	90 Days	Increase (cm) 30/60 Days	60/90 Days
0.5	31	25	5.7	20.8	53.9	15.1	33.2
1.0	67	75	7.8	27.4	58.0	19.6	30.5
1.5	89	91	12.2	36.3	66.8	24.1	30.5
2.0	93	86	17.4	46.8	82.3	29.4	35.5
2.5	97	98	24.3	55.6	97.2	31.3	41.6
3.0	99	97	24.4	58.6	95.2	34.2	36.6
L.S.D's (height)			0.05	2.69	5.20	8.35	
			0.01	3.54	6.99	11.02	

It is quite apparent that for Honduras Gros Michel sucker seed, nothing less than 1.5 lb. should ever be planted and, where rapid growth is essential, seed of more than 2.0 lbs. trimmed weight should be used. There is no difference in rate of growth or germination between seed of 2.5 and 3.0 lbs. However, per half pound increment of seed less than 2.5 lbs, there is significance that still persists (but only at the .05 level) even after 90 days.

The merit of sucker seed of 2.5 and 3.0 lbs is well illustrated when the 30 day increments are studied. Shoots from 1.5 and 2.0 lb seed are increasing in height between 30-60 days at the rate that shoots from 2.0 - 3.0 lb seed increased up to 30 days, while plants up to the 2.0 lb bracket are still increasing, between 60-90 days, at the rate displayed by the 2.5 - 3.0 lb seed from 30-60 days. The lesser

weighted seeds have had a very positive influence upon the subsequent growth rates. The weight of planted material obviously is important in sucker seed.

Large-seed weight experiment

On the basis of this experiment and particularly because of the level - ling off of significance at 2.5 - 3.0 lb a larger experiment has been planted in Guaruma 2. This is intended to elucidate relationships between seed of initial weights from 1.0 lb and by 2.0 lb increments to 13 lbs in germination and subsequent behavior including fruiting. This experiment has been carried for bullhead seed and for maidenhead seed. It is felt that the weight of seed planted commercially might be reduced - with a cash saving in labor and transportation - and it is hoped to establish this by these experiments for Honduras.

Bullhead bud selection

Continuing the seed studies, and in view of the fact that bullhead bits are increasingly being used as a seed source, a preliminary experiment has been completed to determine the potentialities of this material. Twenty seven bullhead bits were carefully examined and all existing buds re-examined for activity. It was obvious that only buds within 3 inches of the existing leaf sheath line were forcefully viable as a general rule. Buds lower down frequently failed to develop - generally because of injuries. Buds at the leaf line - either of the button type or less matured - grew well. Buds more than 2 leaf sheaths inward did not develop vigorously enough and would often be rotted out prior to growth.

It was also observed that where the vascular connection, bud-rhizome, had been cut within the cortex, or had entered the stele in a rotted area, shoot growth would be retarded and the young plant would probably perish.

In connection with this work the observation was made that where a bud had been wounded, including the excision of the apical meristem, a roll of what appeared to be typical callus would develop. Microscopically the proliferation appeared beneath a browned layer of dead cells through elaboration of adjacent cortical parenchyma. In the stelar region the amount of callus formed was less but involved parenchyma and possibly phloem elements of the vascular bundles. A typical cork formation was not observed and the callus activity appeared to derive wholly through proliferation of the indicated parenchyma.

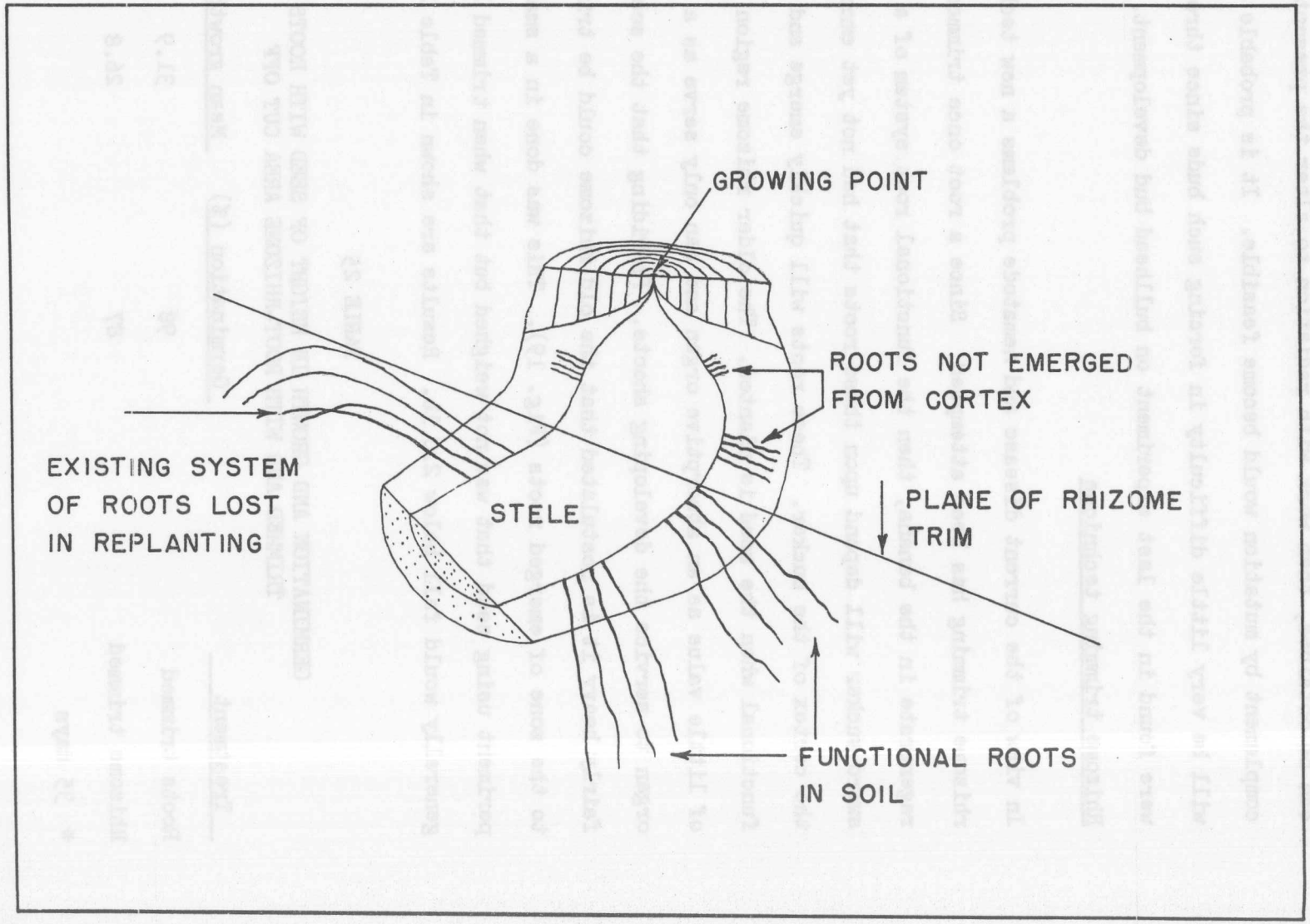
In conjunction with this callus a series of buds had appeared, (Fig. 18) apparently adventitiously. It is considered that these buds were those reported earlier by Dr. Knudson although his photographs left ample room for doubt. Similar shoots have apparently been observed at Coto. The true nature of the buds here found will be determined in the near future when the anatomy is worked out. It is considered that these buds, originating as they do from callus - known to vary genetically in other plants - could be the source for a range of banana sports. It is also considered that such callus could be treated with colchicine with a resultant increase in ploidy. Such a technique could result in plants with variations on the genetic complement already extant in the Gros Michel. It is also

FIGURE 18

Adventitious buds growing from a callus.

FIG. 19

LONGITUDINAL SECTION THROUGH SWORD SUCKER



considered that if a fairly certain method of production of such buds can be obtained, then work with radiation to alter the present genetic complement by mutation would become feasible. It is probable that there will be very little difficulty in forcing such buds since three examples were found in the last experiment on bullhead bud development.

Rhizome trimming technique

In view of the current disease and nematode problems a new technique of rhizome trimming has been attempted. Since a root once trimmed will not regenerate in the banana, then the functional root system of a planted sword sucker will depend upon those roots that had not yet emerged from the cortex of the sucker. These roots will quickly emerge and become functional when the seed is planted. The older rhizome region will be of little value as an absorptive organ and can only serve as a storage organ to service the developing shoots. Providing that the seed is fairly heavy it is postulated that the old rhizome could be trimmed away to the zone of emerged roots (Fig. 19). This was done in a small experiment using seed that was not weighed but that when trimmed high generally would fall below 2.0 lb. Results are shown in Table 25.

TABLE 25

GERMINATION AND GROWTH IN HEIGHT OF SEED WITH ROOTS ONLY TRIMMED AND WITH ROOT-RHIZOME AREA CUT OFF

Treatment	Germination (%)	Mean growth (cm)*
Roots trimmed	98	31.9
Rhizome trimmed	87	26.8

* 35 days

While it seems likely that this type of trim will be satisfactory from the standpoint of growth it is felt that the study should be extended on the

basis of the planting weight work described earlier and on the basis of weathering, protecting chemicals, etc., after cutting. Thorough experimentation is indicated.

Sucker seed storage potential

Finally, in view of the necessity of handling and shipping seed the possibilities of storing sucker seed were assessed. It was found that sucker seed of 4 varieties (Gros Michel, Cocos, Vimama, and Variety 67) could be stored for 3 weeks trimmed to either ½ inch or 4 inches of the growing point (with a retrim prior to planting) under Honduran shade conditions and in a variety of packing materials with losses of less than 5% due to rot in storage. The surviving seed when planted, germinated at 97.6% and grew vigorously for the most part. Best storage techniques (in order) were, totally waxed, cut surfaces waxed, no treatment or packing but stored loosely. Paper wrapping and fine sawdust were the worst storage methods. Prior to planting it was found beneficial to cut away all traces of soft rot. It was noted that meristematic regions invariably were last to break down when the seed was attacked by soft rot.

Button bud dormancy

Button buds have been planted at 200 per month, following a 3-day storage, after being directly cut from the plant. This was done in order to detect dormancy, if present, and to determine whether such dormancy was seasonal. The buds were all taken from plants with well developed growth and the buds were free of the soil Results to date are shown in Table 26.

TABLE 26

PERCENTAGE GERMINATION OF BUTTON BUDS. 200 PLANTED EVERY MONTH

Month	1957	1958	Month	1957	1958
January		13.1	July	31.5	22.5
February		51.0	August	31.5	16.4
March		13.9	September	11.0	45.2
April		23.5	October	51.8	30.7
May		31.3	November	51.7	41.1
June	55.5	32.3	December	-	

It is immediately apparent that no correlation with season is possible. It is also evident that button bud dormancy is not absolute. It is probable that those buds that do grow are the older ones, closer to the ground. There does appear to be a suggestion of an increase in germination with rainy weather, i.e., June, October, November, but this is not by any means demonstrated. This experiment is discontinued.

Water requirements of the banana

In the past, Freiberg, following the methods of Thornthwaite, established a controlled irrigation system that was easily understandable and could readily be applied in the field. A study of actual water losses as determined by evapotranspirometer tanks has indicated that there are dissimilarities between these losses and those predicted by the Thornthwaite formula. The explanation of these differences appears to relate to sunlight intensity and also to the quantity of water available to the plants in the soil.

Water losses for banana plants grown in evapotranspirometer tanks have been obtained experimentally following the methods listed in the 1954 Annual Report. For the first time we have had, in 1958, a complete stand of bananas (Lacatan) in the mature state both in the tanks and in the surrounding acreage.

In Figure 20, the average water losses for tanks containing bananas alone, bananas + grass, and grass alone are compared with the computed potential evapotranspiration (P. E.); the comparison including information for the year 1957. The radiant energy and rainfall, both locally recorded, are included in the graphic presentation.

It will be noted that again the calculated value frequently is less than the actual water loss for more than half of the year. The observation, first made by Freiberg, that the peak values for actual measured P. E. follow by one month the periods of most intensive sunlight is not borne out this year - especially in June, where a very prominent peak of water loss is matched by a peak of radiant energy - also by increased rainfall. The trend of the two curves, water loss and light intensity, is similar.

The June peak is most prominent in those tanks commonly containing grass. The grass in the tanks is very dense and probably functions as a very efficient organ for water elimination. It will be noted that the effect of banana foliage appears of less significance than that of grass (June) since the bananas + grass line exceeds only slightly the grass-alone line but considerably, the banana-alone - this despite the fact that the average measured banana foliage is very similar in June in both treatments. This June peak also indicates that the relationship water loss/leaf area might be

FIG. 20

COMPARISON OF EXPERIMENTAL AND COMPUTED "P.E." WITH SUNLIGHT INTENSITY AND RAINFALL

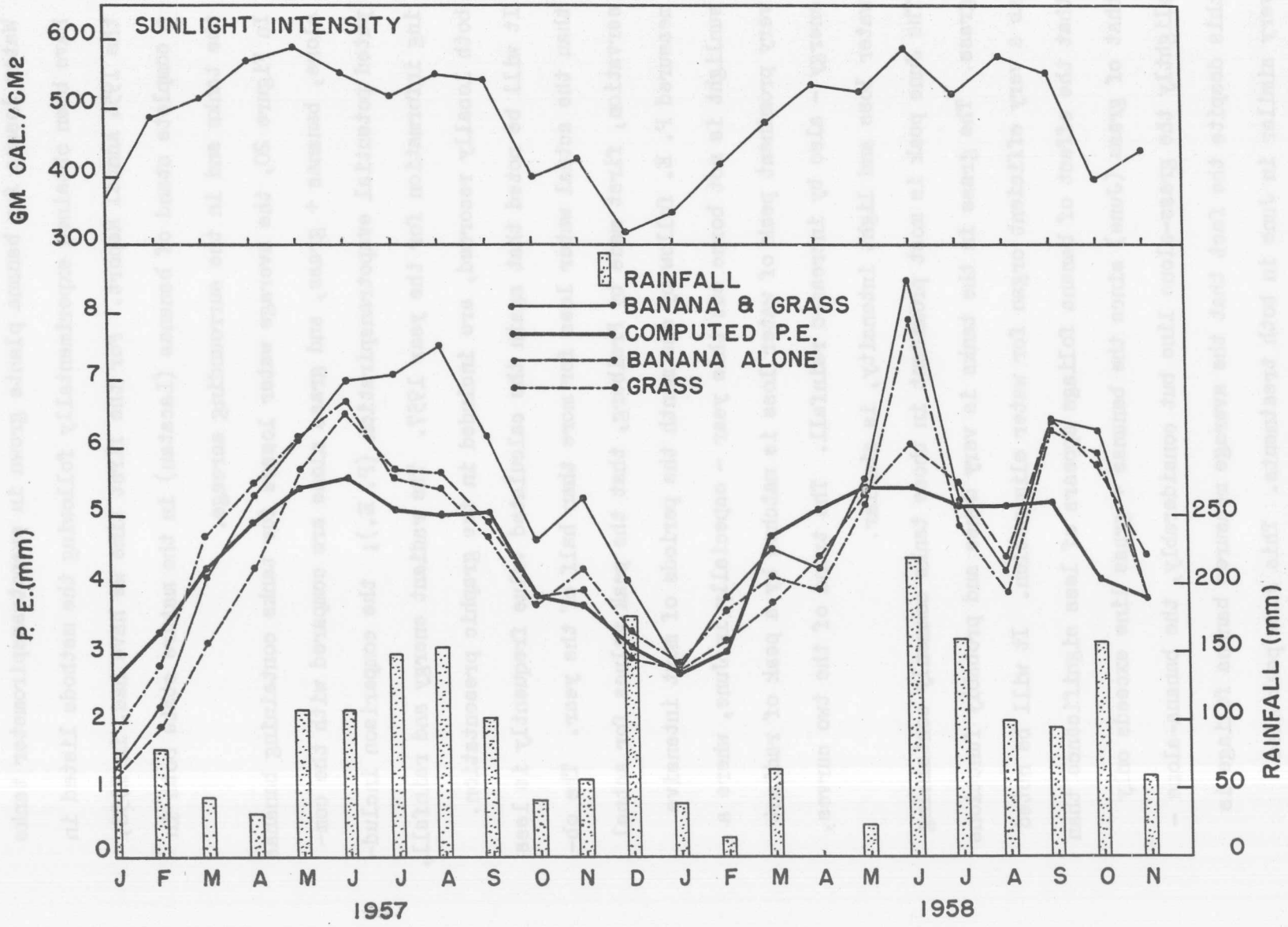
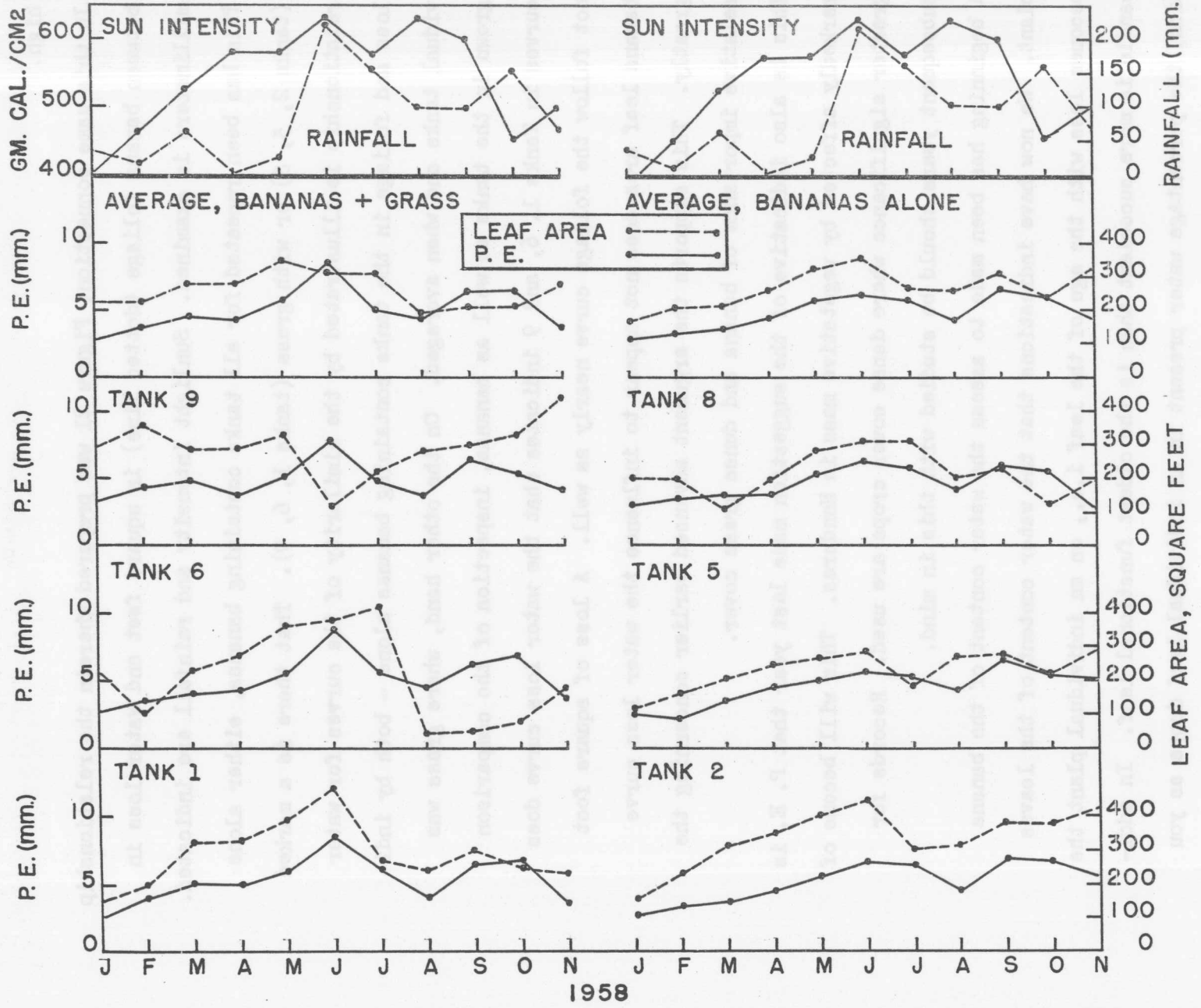


FIG. 21

COMPARISON OF EXPERIMENTAL "P.E." WITH LEAF AREA OF BANANAS, ALONE AND WITH GRASS, SUNLIGHT INTENSITY AND RAINFALL.



particularly important where sunlight intensity is maximal and rainfall high.

In this same connection, Figure 21 was prepared wherein the relationship between banana foliage (dotted line) in square feet and water loss in millimeters is examined. Sunlight intensity and rainfall are indicated. This has been repeated for all tanks containing bananas, either alone (tanks 2, 5, 8) or with grass (tanks 1, 6, 9). That there is a marked relationship is illustrated by the similarity of the curves for water loss and foliage in the tanks containing bananas alone - both by individual tanks and when averaged. On the other hand, where grass was grown in the tanks as well as bananas, inspection of the comparison curves in Tanks 1, 6, and 9 indicates that the water loss curve does not follow the foliage curve nearly as well. A loss of square foot banana leaf area does not appear to influence the water loss curve greatly. This supports the argument advanced earlier concerning the relative importance of banana and dense grass cover.

This is also indicative of the suggestion made last year that P. E. is markedly affected by vegetative mass in Honduras. This will become of greater significance where dense cover crops are used. Records for subsequent years should be studied with this in mind.

A beginning has been made to assess the water content of the banana plant. We now have indications that the water content of the leaves becomes less with the age of the leaf i.e., on an individual plant the candle is more succulent than is the oldest functional leaf. In addition, the percentage water present in an individual leaf drops as you move away from the midrib toward the margin and as you proceed away

from the base, and toward the apex.

With the development of improved sampling methods this study will continue.

An anatomical and morphological study
of important banana tissue and organs

There is no file of banana microscope slides available. This project is carried in an attempt to supply this need.

There follows, as Table 27, a list of slides on file at La Lima (stained in Safranine and Fast Green). In addition, there are a quantity of preparations in wax, ready for sectioning and staining, as well as a number of slides stained but as yet unclassified. Also, there are slides of a variety of banana tissues ready for staining.

The Physiology Department is now prepared to supply slides on loan from the list in Table 27, and to prepare additional slides upon request, within a reasonable period, of any banana tissue or organ stained appropriately.

TABLE 27

MICROSCOPE SLIDES AVAILABLE IN THE PHYSIOLOGICAL FILE.
STAINED IN SAFRANINE AND FAST GREEN.

No. of slides	Materials *
40	Long and transverse sections, G. M. and other banana roots
1	Stele or rhizome
7	Stele - root complex within rhizome
14	Leaf base - rhizome area, long sections
27	Healthy leaf sheaths - various maturities, transverse sections
34	Panama leaf sheaths - various maturities, transverse sections
1	Leaf prehensile appendage, transverse and longitudinal sections
18	Fruit - transverse, tangential, longitudinal; various maturities
7	Male flowers - G. M. and Lacatan
12	Growing point - vegetative and flower. Long section
10	Floral bracts, long and transverse sections
8	Peduncle, long and transverse sections
22	Other plants from Lancetilla

* Gros Michel (G. M.) unless specified otherwise.

Entomology

Insecticide tests for Red Rust Thrips control

The use of Perthane 4E or Malathion 5E at rates of 1 quart/100 gallons water have again proven ineffective in control of Red Rust Thrips when applied at the rate of 50 gallons per acre to hanging fruit. A combination of the two insecticides, with the reported synergistic interaction increasing its effectiveness, failed to give significant control compared to the chemicals used alone or controls. None of the treatments provided field control.

Hose applications of a mixture containing 1 quart Dieldrin 1.5 EC/100 gallons water to hanging fruit at the rate of 50 gallons per acre of 1 quart of Dieldrin 1.5 EC per acre in orchard spray oil by helicopter continue to give extremely good control of Red Rust Thrips.

Insecticide dips to control root borer

In an unreplicated experiment designed by the Agriculture Department, 51 acres of Coco Bolsa, Tibombo Farm were planted in June 1956, with material that received a Dieldrin dip prior to planting. The planting holes in 3 other 12-acre plots received a dusting with Dieldrin 2.5% dust. These applications were made in a manner that would place the dust beneath only, above only, or both beneath and above the seed at the time of planting.

Trapping results in the seed treatment areas of Coco Bolsa, planted in June 1956, indicate that the hole-dusting technique is providing better control than dipping or no treatment. The following bi-monthly trap averages also suggest that the application of a 2.5% Dieldrin dust above and beneath the seed piece furnishes the best control:

	Average Borers per Trap						
	Jan.	Mar.	May	July	Sept.	Nov.	Dec.
Seed dip	1.5	1.1	1.7	3.7	3.3	5.2	3.8
Dust above seed	0.8	0.2	1.0	2.1	2.8	5.6	4.2
Dust beneath seed	0.1	0.2	2.9	2.3	1.4	3.9	4.1
Dust above and beneath seed	0.2	0.8	1.6	1.9	0.8	1.7	1.8
Check	2.5	1.9	3.6	8.4	7.5	9.8	8.2

Systemic insecticides

Thimet at a 1:2000 concentration for an hour or 1:1000 for a 1/2 hour immersion can be used for pre-planting treatment of split bullheads averaging 25 pounds without detrimental effect to the developing plant. Germination was 100% in the experiment. Differences in number of leaves, growth, and insect damage between treatments were negligible.

Attempts to produce artificial infestations in one experimental area four months after planting failed but a natural infestation indicated that within 12 months from the planting date, Thimet 47.5% emulsifiable concentrate at concentrations of 1:1000 or 1:2000 in water as a seed dip no longer protected plants against root borer invasion.

Farm sanitation to control the root borer Cosmopolites sordidus Germar.

In theory, the rapid decay and dessication of fallen pseudostems realized by chopping and quartering should reduce the root borer population by eliminating a favorable site for larval development. This method of farm sanitation, suggested by Roberts and Roig (1956) for use in stalk borer control, may have surpressed root borer populations in Costa Rica. Similar measures have been suggested by Roberts (1955) for the control of the banana root borer. Mr. Roig reported that farm sanitation measures for stalk borer control similar to those used in Costa Rica did not materially reduce root borer populations in Bocas Division. In Bocas, however, chopping and quartering was done once every one and a half to two months.

In this experiment, an intensive sanitation program was used in 20 randomized 1/2 acre plots where reported favorable sites for larval development of the root borer were eliminated by chopping and quartering the pseudostems of freshly harvested plants each week. Twenty other plots served as controls subject to normal farm practices where harvested pseudostems were cut into 3 foot lengths every 30 to 45 days and left to decay.

Trapping results in both sanitation and check plots indicate that the adult borer population has continued a gradual reduction during the current year. The average number of adults taken in both treatments has decreased steadily to a level of 3 per trap and have continued to be so nearly equal each month during the course of the experiment that no significant difference has existed at any time.

With the termination of this experiment, the complete data for the full 16 months is presented:

Average Number of Root Borers per Trap

Sanitation

1957	No.	1958	No.
May	25*	Jan.	6
June	19	Feb.	7
July	11	Mar.	6
Aug.	6	Apr.	6
Sept.	7*	May	4
Oct.	5	June	4
Nov.	9*	July	3
Dec.	8	Aug.	4
		Sept.	3

No Sanitation

1957	No.	1958	No.
May	22*	Jan.	5
June	16	Feb.	6
July	11	Mar.	6
Aug.	6	Apr.	6
Sept.	5*	May	5
Oct.	5	June	4
Nov.	10*	July	3
Dec.	9	Aug.	3
		Sept.	3

* Blowdowns occurred during these months.

Leaf-feeding Caterpillars
in Honduras

In September, leaf-feeding caterpillars, Ceramidia butleri, were reported in Calan Farm. They were confined to a very small corner of the farm and were doing little damage. Later checks showed a reduction in the number of caterpillars and a 95% parasitization of the eggs.

The first week of November, leaf-feeding caterpillars were reported in Lupo Farm. There was an over-all average of 2.9 caterpillars per leaf, plus many viable eggs. Spraying with Dieldrin was recommended, and a small area of five sections was reserved for an experiment with Toxaphene. At the next Sigatoka spray cycle a larger experiment was initiated with a second application of Toxaphene and Dieldrin and an area of one application only of Dieldrin.

Niagara 60% Toxaphene, local purchase, at one quart plus one gallon of Texaco 522 oil was applied at 1-1/4 gallons per acre (1-1/2 lbs. Technical Toxaphene per acre) on November 14. Coahoma 1-1/2 lbs. per gallon Dieldrin was applied at the same dilution rate (0.375 lbs. technical Dieldrin per acre) on November 13 and 14. Results were as follows (averages of 5 Toxaphene sections 12 Dieldrin sections):

LUPO FARM CERAMIDIA BUTLERI PER LEAF

Large Caterpillars

	Nov. 10-12 Pre-spray	Nov. 17 3-4 Days	Nov. 24 10-11 Days	Dec. 1 17-18 Days	Dec. 8 24-25 Days
Toxaphene	2.70	0.10	0	0	0.83
Dieldrin	2.73	0.13	0	0	0.03

LUPO FARM CERAMIDIA BUTLERI PER LEAF

Small Caterpillars

	Nov. 10-12 Pre-spray	Nov. 17 3-4 Days	Nov. 24 10-11 Days	Dec. 1 17-18 Days	Dec. 8 24-25 Days
Toxaphene	0.40	0	1.10	14.62	4.00
Dieldrin	0.93	0.23	0.34	5.80	0.97

On December 12, the west side of the farm was treated with one gallon 90% Toxaphene plus nine gallons Texaco 522 oil at 1.1 gallons per acre (approximately 1 1/4 lbs. technical Toxaphene per acre). Dieldrin at the same rate as Cycle 1, was applied to the remainder of the farm except for six sections on the east side which received oil only. Results were as follows (average of 5 sections each treatment):

LUPO FARM CERAMIDIA BUTLERI PER LEAF

Total Caterpillars All Ages

	Dec. 17 5 Days	Jan. 8 27 Days	Feb. 9 59 Days
Toxaphene twice	0	0	0
Dieldrin twice	0	0.02	0
Dieldrin once	0	0.20	0

Several conclusions relative to field practice in caterpillar control are evident from this series of 8 counts of at least 5 replicates. Both Toxaphene and Dieldrin at the rates used are highly effective in quickly controlling Ceramidia butleri. Within 2 to 3 weeks, populations rose to levels well above pre-spray. However, these were entirely very small, newly-hatched caterpillars. Dieldrin was more effective than Toxaphene in killing these small caterpillars as they began to feed. In fact, one application of Dieldrin evidenced suf-

ficient residual activity in the leaf to kill all caterpillars soon after hatching, so that no re-infestation and no subsequent damage occurred. Two applications of Toxaphene, spaced 28 days apart, also gave complete control. No serious phytotoxic effects were noted, but by late January, Toxaphene injury (similar to that noted in Armuelles) could be found on some roadside exposed stems.

Shortly after Lupo was sprayed almost every farm in the Division had some degree of infestation. All farms near Lupo (Laurel, Los Limones, Las Mercedes, and Caimito) were damaged. However, surveys during late November and December showed these populations remaining static. Although considerable damage occurred, no spraying was done because of possible upset of biological balance and phytotoxicity. These farms served as checks to contrast to the excellent control obtained in Lupo. During January, these farms had a considerable rise in Ceramidia population, while Lupo remained at zero.

The infestation in Calan spread over the entire farm and was sprayed on November 21 with one quart of Dieldrin in one gallon of oil, by fixed wing plane. The caterpillars were very small, averaging 1.9 per leaf, and the timing was perfect. The population was reduced to 0.2 per leaf in 5 days, and one month later there were only 0.06 caterpillars per leaf, and the population never rose again.

Buena Vista had one of the heaviest infestations of any farm checked, with 10.8 caterpillars per leaf. On November 22, it sprayed with 1 lb of Malathion in 2 gallons of water, but the

insecticide was washed off by rain almost immediately. There was only a 20% reduction in caterpillar numbers, and a resurgence to 20.2 per leaf on December 2. It was sprayed again on December 3, with one gallon 90% Toxaphene to each 10 gallons of oil. One week after application the population was 1.43 per leaf, or 93% control. Subsequently, the population fell to 0.36 per leaf (2-weeks) and .08 per leaf (1 - month). Population never rose again, evidencing excellent control by one thorough application of Toxaphene. The Malathion gave some leaf and fruit damage. This Malathion had been previously tested at the rate of one quart per 100 gallons of water and found satisfactory, but the concentrated solution in oil was phytotoxic. In early February, a few badly Toxaphene injured stems were found along the roads but in general phytotoxic effect was light.

Monterrey was sprayed on December 5, with one gallon 90% Toxaphene to each 10 gallons of oil. The prespray population of 5.9 caterpillars per leaf was reduced to 0.34 in 5 days, and zero in 11 days. Twenty-five days after spraying the population was still only 0.04 caterpillars per leaf. As with other treated farms, the population never rose again. Monterrey was the only farm that had a large area of the 90% Toxaphene without application of other insecticide. Stems were examined for phytotoxic effects but none were found.

During December, while sprayed farms evidenced excellent control, continuing light damage occurred in nearby farms. For example, counts of caterpillars per leaf were: Dec. 10, Los Indios 1.8; Dec. 15-20 Cobb 0.9; and Naranjo Chino 1.3 December 30. No spraying was warranted on these farms but the populations served as checks for the efficacy of the treatments in Buena Vista and Monterrey.

The first week of December, men from every farm were instructed by the Entomology Department in proper methods of surveying and identification of leaf-feeding caterpillars. These trained personnel surveyed the majority of sections of each farm at regular intervals and the results were forwarded to the Entomology Department. Farms that had high counts were immediately re-checked by the Entomologists and recommendations passed on to Dr. Thornton. This system worked well so long as the caterpillar count sheets were forwarded promptly to the Research Department.

Timing is very important in controlling the leaf-feeding caterpillars. The proper time to spray is when the infestation is beginning. All too often infestations are reported when damage is severe and the insects have gone through several generations. When populations are naturally on the decline, spraying may do more harm than good. The importance of survey is self-evident. In general, a new, rising infestation of 2.5 to 3.0 caterpillars per leaf warrants treatment. If they are not sprayed, they do considerable damage and then emerge as adults, lay more eggs and start another generation. Damage accumulates as each generation feeds, so that a continuing moderate infestation can produce considerable damage after several generations. It is better to spray immediately than to wait until much damage has occurred, and be forced to spray anyway.

Taltuzas - Tiquisate

Tests were set up in August to evaluate poison bait on a larger scale. One 32 acre section was chopped and baited with cut green bananas dusted with strychnine alkaloid at the rate of 1/3 ounce

to 1 gallon of bait. Two passes were made 3 to 4 days apart and this constituted one cycle. Before baiting, the taltuza population was estimated at 4-6 per acre. After one cycle, seven groups of mounds were counted, or in other words 7 taltuzas remained alive. This was a 90% reduction. New mounds were considered evidence of live taltuzas, because all mounds were trampled down as the baiting crews passed the section.

The cost of baiting came to $3.29 (dollars) per acre. Chopping the ground cover, the major expense, came to $2.25 per acre.

Two other sections were chopped and baited with good results. However, in all three treated sections taltuzas soon came in from adjacent sections. This pointed to the need for a well buffered plot. Late in August, the ground cover in Solola Farm was completely eaten to the ground by hornworms and armyworms. Eighty acres were baited and ten acres in the center were used for a test plot to evaluate results. Before baiting the taltuza population was estimated at 10-12 per acre with heavy damage occurring. After one cycle of poison bait only two taltuzas remained in the 10 acre plot. Three months later there had been no increase in taltuza numbers in the 10 acre plot. This was the first buffered plot and the results were considered excellent.

At the start of the field tests the bejuco prevented the rapid location of the mounds. Chopping did an excellent job of reducing ground cover but was slow work and very expensive. Other means of suppressing the bejuco were tried; these included the spraying of diesel oil, urea in water up to 300 lbs. per acre and the spraying of 2-4D. Only 2-4D was satisfactory but considered too dangerous for large scale operations.

Due to the climbing nature of the bejuco, a circular area around each mat is kept clean. It was decided to place bait around mounds in this area only as the taltuzas ultimately tunnel to the rhizome. Several sections of plantilla in Antigua Farm have been baited using this method and the results are almost as good as areas that were baited after chopping.

Another experiment is under way at present, comparing chopping versus no chopping in ratoon bananas. After the first baiting, checks showed a high per cent reduction in both plots. This test has not been going long enough to draw any definite conclusions but first indications are that it will be possible to do a good job baiting without chopping.

A survey of the soil fauna

Before application of Dieldrin to the plots in Guaruma 1, mealy bugs were always taken in the largest numbers, but after the application of insecticide, soil mites increased to such a point that they passed the mealy bugs in total numbers. In the plot that had no insecticide, mealy bugs continued to be taken in the largest numbers.

Without exception mealy bugs were always taken in greater numbers from samples close to the mat. The 6-9 inch depth always yielded the most followed by the 3-6, 9-12, and 0-3 inch depths. Samples from the inner plant area had relatively few.

Ants were usually taken in largest numbers from the 0-3 inch samples followed by the 3-6, 6-9 inch depths, with very few taken

from 9-12 inches deep. Samples close to or far from the mat had no relationship on the number taken.

Dieldrin granules temporarily reduced most arthropod forms except mealy bugs (Geococcus Coffeae and Phenacoccus Sp.) and soil mites. All forms recovered 2-3 months later except ants, which have remained reduced to date. Mealy bugs showed a slight increase over the year and soil mites greatly increased.

Dieldrin emulsion temporarily reduced all arthropods except soil mites. Two months later all forms were back to normal except symphylids. Soil mites increased greatly, mealy bugs slightly, and ants remained the same.

Mealy bugs are always taken in greatest numbers close to the mat. After 15 months arthropod numbers from the plot in Lake 1, Guaruma 3 are far below other plots. Only ants are found in normal numbers.

Phytotoxicity of insecticides
in helicopter type mixtures

Insecticide phytotoxicity depends on formulation and final use mixture as well as the technical insecticide. This was brought out strongly by the appearance of phytotoxic effects from helicopter Toxaphene application in Armuelles Division in November. Toxaphene had been phytotoxicity tested for hose spray use and had been found safe. Sixty per cent Toxaphene formulations had been used in oil spray mixtures applied by helicopter in several experimental plots early in the year. No phytotoxicity had been noted.

Early in December, development of a "helicopter type" screen for phytotoxicity was started. Simultaneously phytotoxicity testing of oil solutions of promising insecticides was begun.

Applications were made by the same paint sprayer as previously used for water base low concentration phytotoxicity evaluation. Very low pressure was used (2-1/2 to 5 lbs. per square inch) and delivery rate as low as the capability of the gun. (The goal was as large a droplet as possible, but this equipment still produced drops much smaller than from a helicopter boom. Equipment has been ordered for 1959 use that should correct this.) Although highly desirable to test effect on all ages of stems, urgency of the problem dictated testing only on 2 to 4 week-old fruit. It was found that a light application could be made from top to bottom of the stem in 9 to 11 seconds. Three stems were used as replicates for each treatment. Counts through 11 days include all stems, but by 40 to 46 days blowdown and English grade harvesting removed some of the replicates. All work was done in Guaruma 3. An indexing system was used based on severity of spotting and of confluent area damage as well as number of fingers involved as follows:

0 - Completely free of injury.

1 - Oil soak appearance (darker translucent green), no black or brown spotting.

2 - Oil soak plus a very few tiny brown or black spots on a very few fingers. Probably no damage but rather unusual speckle, flower thrips, etc. Not definite phytotoxicity.

3 - Definite phytotoxicity, as small spots and/or roughening only, and on but very few fingers.

4 - Spotting and/or roughening light, but on many fingers.

5 - Spotting severe on many fingers.

6 - Spotting beginning to become confluent, beginning to be an area effect. Severe injury.

7 - Areas on some fingers damaged, and much spot, very severe.

8 - Large black, brown or red areas plus bad spotting on most fingers.

9 - Most damage in areas, remainder spotted.

10 - All sprayed surfaces blackened, brown or red.

Not all types of injury can be fitted into any simple index system, but the above worked very well in practice. Two different experienced workers gave average indices within 0.5.

Tables 28, 29 and 30, present the 1958 treatments and include early 1959 readings. Dilution rates are what would be used in the helicopter, for example, 1) 4 means 1 quart insecticide formulation mixed with 1 gallon oil; simulating for 60% Toxaphene at rate of 1-1/2 lbs. per acre. Sigatoka oils produced an immediate oil soaked appearance, indexed at 1, or 1.5 if severe. In series, 1 Toxaphene 90 produced but slight injury within 3 weeks, but by 1-1/2 months, strong phytotoxic effects were produced, including reddening and browning, though largely restricted to spots. Sixty per cent Toxaphene formulation was much more phytotoxic, producing a rapid burning within less than a week, and most of the treated areas were red brown roughened and cracked by 1-1/2 months. The 1 lb. per acre simulated rate was almost as severe as the 1-1/2 lb. rate. Perthane caused injury more rapidly than Toxaphene 90, but at 1-1/2 months the rating was the same. The Swiss Diazinon emulsifiable formulation was extremely phytotoxic, burning all sprayed areas black within 5 days.

The second series, Table 29, was applied to determine whether the better solvents for insecticides would cause injury without the presence of insecticides.

TABLE 28

INDEX OF PHYTOTOXICITY TO FRUIT, PAINT SPRAYER
SERIES 1 APPLIED DECEMBER 11-12, 1958

Treatment	5-6 days	18 days	46 days
Texas 522 oil	1.0	1.2	1.6
Toxaphene 90, oil 1:10	2.3	3.6	5.5
Toxaphene 60 Shell, oil 1:4	7.0	7.9	8.0
Toxaphene 60 Shell, oil 1:6	5.8	7.4	8.3
Perthane R&H in 40, oil 1:4	4.2	4.9	5.4
Diazinon 20E Swiss, oil 1:4	9.0	9.4	9.3
Diazinon 20E Swiss, oil 5:7	10.0*	10.0	9.5

* Examined at a different time, this examination is 11 days post application.

TABLE 29

INDEX OF PHYTOTOXICITY TO FRUIT, PAINT SPRAYER
SERIES 2 APPLIED DECEMBER 18-20, 1958.

Treatment		4-6 days	11-13 days	40 days
Texas 522 oil		1	1.3	1.5
Conoco 70 *		1	1.3	1.7
HAN**, Conoco 70	1 lb: 1 gal	1	1.7	1.7
Xylene, Conoco 70	1 lb: 1 gal	1	2	2.5
HAN, 522	1 lb: 1 gal	1	2	2.3
Xylene, 522	1 lb: 1 gal	1	1.3	2.0
Toxaphene 60 Shell, Conoco 70	1:4	2.7	2.7	3.7
Dieldrin Coahoma 522	1:4	2	1.7	1.7

* Continental Oil Co. Mineral Oil Tech. 70

** Heavy Aromatic Naptha

A comparison of a possibly less phytotoxic oil (higher unsulfonatable residue and narrow boiling range) was included. No differences were noted between the two oils; both caused oil soak appearance. Solvent inclusion appeared to give slight increase of phytotoxicity. Dieldrin formulation was not phytotoxic.

Shell Toxaphene 60% formulation mixed with the highly refined mineral oil at a rate to simulate 1-1/2 lbs. Toxaphene per acre evidenced immediate phytotoxicity but the damage was very slight. In 40 days, this increased to only light damage. Possibly the Conoco 70 tech. mineral oil safens the Toxaphene 60. This point needs further work as it is also possible that the complete series 2 was applied during weather and soil moisture conditions not conducive to phytotoxicity. Application was during a rainy period so that the soil was very wet and cloudy cooler weather prevailed.

The third series, Table 30, was applied to determine phytotoxicity of Diazinon formulations and several other promising insecticides. Diazinon formulations were all severely phytotoxic. An immediate blackening occurred, easily seen on the worst injured stems within 2 hours of application. The U. S. 20S formulations of Diazinon was less phytotoxic than the U. S. 25E or Swiss 20E (Series 1). The E formulations are emulsifiables; the emulsifier was probably responsible for the added phytotoxicity over the 20S non-emulsifiable formulation. Either formulation diluted in water was more phytotoxic than in oil, at the same concentration. Even when diluted to hose spray concentrations (1/2 to 1 lb. Diazinon per 100 gallons of water) serious phytotoxicity resulted. In these cases spraying was continued until run-off occurred. Whenever large drops collected, black burning resulted. During subsequent growth,

large (1/2 to 3/4 inch) blackened holes developed. Texas 522 and Esso 40 oils were compared alone and as Diazinon carriers, but no definite differences were noted.

TABLE 30

Treatment	2-3 Hours	2 Days	11 Days	43 Days
Texas 522 oil	-	1.9	2.4	3.0
Esso 40 oil	-	2.0	2.0	2.3
Diazinon 20S, 522 1/2 lb Conc.	1	5.3	4	3.5
Diazinon 20S, 522 1 lb "	3.5	6.7	6	6.5
Diazinon 20S, Esso 40 1/2 lb "	-	2	2	4.5
Diazinon 20S, Esso 40 1 lb Conc.	-	8.5	7.5	8.0
Diazinon 20S, Water 1/2 lb "	7	8.5	7.8	6.3
Diazinon 20S, Water 1 lb "	8	9.4	9.2	8.9
Diazinon 25E, Water 1/2 lb "	8.5	10	10	10
Diazinon 25E, Water 1 lb "	-	10	10	10
Diazinon 25E, Water 1/2 lb 100 gal	-	5	5	5
Diazinon 25E, Water 1 lb 100 gal	-	4.7	5	5
Diazinon 20S, Water 1/2 lb 100 gal	-	3.7	3.3	3.5
Diazinon 25E, Esso 40 1/2 lb Conc.	-	9.5	8.5	9.5
Diazinon 25E, Esso 40 1 lb "	-	9	9	9.5
Triton B 1956 2% Water	-	1.3	3	1.7
Toxaphene 90, 522 1:11	-	4.7	6.7	7.5
Toxaphene 90, 522 2:15	-	4.0	6.7	8.0
Toxaphene 90, Esso 40 2:15	-	4.0	5.7	7.5
Toxaphene 60, Coahoma, 522 1:4	-	7.3	7.3	9.5
Malathion HAN, 522 1 lb	-	7.3	7.7	6.5
Sevin 36 Mull, 522 2:5	-	10.0	10.0	10.0
DDT (PBG 3), 522 2:3	-	5.0	6.3	7.5
Dieldrin Coahoma, 522 1:4	-	4.3	4.7	7.5

Diazinon phytotoxicity was an immediate effect; as the fingers enlarged, the indices in general became slightly lower. This contrasts strongly with the chlorinated hydrocarbon phytotoxic effect (i.e., Toxaphene, DDT, Perthane, Dieldrin) which worsens as the stems age.

Emulsifiers alone can cause injury as demonstrated by Triton B 1956. Where drops collected, a depressed, darkened (grey) large spot (1/2") occurred. Damage was not severe and disappeared during later growth.

The Toxaphene 90% formulation (Technical Toxaphene plus 10% Xylene) was further tested at the equivalent of 1 lb. and 1-1/2 lb per acre in both Texas 522 and Esso 40 oil. In all cases injury was evident within 2 days and progressively worsened. The Coahoma 60% Toxaphene formulation was severely phytotoxic. Technical Malathion formulated with Esso Heavy Aromatic Naptha was also severely phytotoxic. The Sevin Mull formulation was extremely phytotoxic, scorching the fingers completely black and arresting growth. Undoubtedly, the unusual solvent in this formulation is mainly responsible.

The 3 lb per gallon California Spray Chemical Corporation DDT emulsifiable caused considerable phytotoxicity, although less than the Diazinon, Toxaphene, Sevin and/or Malathion. The appearance was very different; a "poisoning" effect occurred of large areas raised, pimply and roughened but without serious discoloration during early examinations. The dark Dieldrin Coahoma formulation evidenced phytotoxicity also, but was by far the least injurious treatment.

This third series was applied during a hot, sunny period. The soil was dry and cracked. Pat of the stems were chosen as completely sun-exposed stems. These factors combined to produce an unusually severe phytotoxic

effect. The phytotoxicity of the Dieldrin and DDT formulations can only be explained on this basis as large scale helicopter applications have produced no serious phytotoxic effects. The same Dieldrin application in Series 2, produced no phytotoxic effect, and the only differences apparent were soil moisture, weather, and sun.

The urgent need for phytotoxicity information for immediate large scale field use in Panama and Costa Rica prompted testing of DDT, Toxaphene and Diazinon applied by helicopter. One acre plots were treated on December 27 in Lake 1, Guaruma 3. Treatments included:

1 - California Spray Chemical Corp. 3 lb DDT per gallon emulsifiable 2 parts to 3 parts Texas 522 oil. 1.67 gallons per acre (2 lbs. Tech. DDT per acre).

2 - Geigy Diazinon 20S (1-1/2 lbs Diazinon per gallon) 1 part to 3 parts Texas 522 oil. 1.33 gallons per acre (1/2 lb Tech. Diazinon per acre).

3 - Geigy Diazinon 20S 2 parts to 3 parts Texas 522 oil. 1.67 gallons per acre (1 lb Tech. Diazinon per acre).

4 - Coahoma 60% Toxaphene emulsifiable 1 part to 4 parts Texas 522 oil. 1.25 gallons per acre (1-1/2 lb Tech. Toxaphene per acre).

5 - Hercules 90% Toxaphene 2 parts to 15 parts Texas 522 oil. 1.13 gallons per acre (1-1/2 lb Tech. Toxaphene per acre).

As with all helicopter experiments, gallons per acre applied can only be approximated, as boom output rate is not known. Possibly rates were 20% higher than stated above. Application was poor as quantity of formulation available allowed mixing but 5 gallons or less of spray, insufficient to avoid further oil dilution in the spraying system, and insufficient to assure proper delivery on the plot area.

Stems in the plots were exposed to spray and sun by cutting off shading leaves. Results are presented in Table 31.

TABLE 31

INDEX OF PHYTOTOXICITY TO FRUIT,
HELICOPTER APPLIED DECEMBER 27, 1958

Treatment Tech. per Acre	6 Days Average	6 Days Range	17 to 21 Days Average	17 to 21 Days Range
2 lbs. DDT	0.4	0 to 1	1.8 (-)	1- to 3-
1/2 lb. Diazinon	3.0	2 to 5	3.5 (-)	3 to 5-
1 lb. Diazinon	2.5	1+ to 4	3.3 (+)	3 to 4-
1-1/2 lb Toxaphene 90	2.5 (+)	0 to 4	2.1 (+)	0 to 4-
1-1/2 lb Toxaphene 60	4.5	3 to 6	4.8	4 to 5+
No insecticide	-	-	2.0	1+ to 3-

Only the Coahoma 60% Toxaphene caused general serious injury within the 21-day observation period. Exposed stems in the hot sun were injured by 90% Toxaphene formulation and the Diazinon formulation at both rates. Most stems in the Diazinon and Toxaphene 90 plots evidenced either no injury or light injury mostly not rejectable. Toxaphene injury may develop in time. DDT caused no injury greater than that present in check areas sprayed with oil only.

Evaluation of any helicopter plot is difficult as the amount of spray applied to any particular stem is completely unknown. Application was made under conditions of hot sunny weather and very dry soil, conducive to phytotoxic effects. Stems sprayed with oil alone in regular Sigatoka control operations evidenced injuries rating from 1 to 3 on this farm at this time. As all stems received the usual oil cycles, it was difficult to separate insecticide phytotoxicity from this oil injury.

Formulations so far tested of Diazinon, Toxaphene, Sevin and Malathion are phytotoxic and cannot be used. Technical Toxaphene is phytotoxic

so that this insecticide has no promise for use at current dosage rates by oil solution. Perthane is phytotoxic but further work is needed to determine use possibility. DDT can be phytotoxic so that formulations selected and use conditions require caution. Even Dieldrin can be phytotoxic under severe test, but it is the least phytotoxic material tested.

Promising formulations of Diazinon, Sevin and Perthane will be tested in 1959. Further work will be done on solvents and carrier oils to develop specifications for forumlations to be used on bananas as high concentrates applied by helicopter. Refinements in phytotoxicity testing methods will be made, particularly use of more appropriate spray guns and nozzles (larger drops, less volume output). Preliminary trials indicate application of single drops of appropriate size by hypodermic syringe or wire loop may be superior to paint sprayer application as a test method. Phytotoxicity information will be developed on all insecticides of promise for control of banana insects and mites. Relation of phytotoxicity to soil and weather conditions, and droplet size will be studied.

Final recommendations on most promising formulae will be based on large scale helicopter insect control experiments with phytotoxicity evaluation included.

Soils and Chemistry

"Silting" as a method to improve banana lands and combat Panama Disease

This season, we analyzed a total of 420 samples of Ulúa river water for sediment content. The year was extremely dry and the usual June floods were again absent the second year in a row. One flood stage was reached in October, but the sediment content during that flood was disappointingly small. It was shown again that the early floods (i.e., May or June) are of greatest importance to our silting program, since they carry considerably more silt than the fall floods.

The Engineering Department is now assembling the data for the 1958 flood season and will report these as usual.

By arrangement with the Engineering Department in Bananaera, Guatemala, we started a study on the sediment content of the Motagua River during flooding. The samples were all taken at the Oneida Bridge at 3 ft. depth and 50 ft. from the shore. The samples were dried in Bananera and the sediments were shipped here for analyses. The samples were taken starting at a river level of 126, 3 to 4 times a day.

Figure 22 shows the river stages and corresponding sediment contents for the Motagua River during 1958. A total of 41 days the river rose above 126. The values shown in the graph are daily averages, sometimes of 4 samples, sometimes only two. However, it is clear from the figure that a close relation exists between the river stage and the sediment content. The floods can be divided into two distinct groups - Group A: the early floods through middle August; Group B: the later floods in September -

FIG. 22

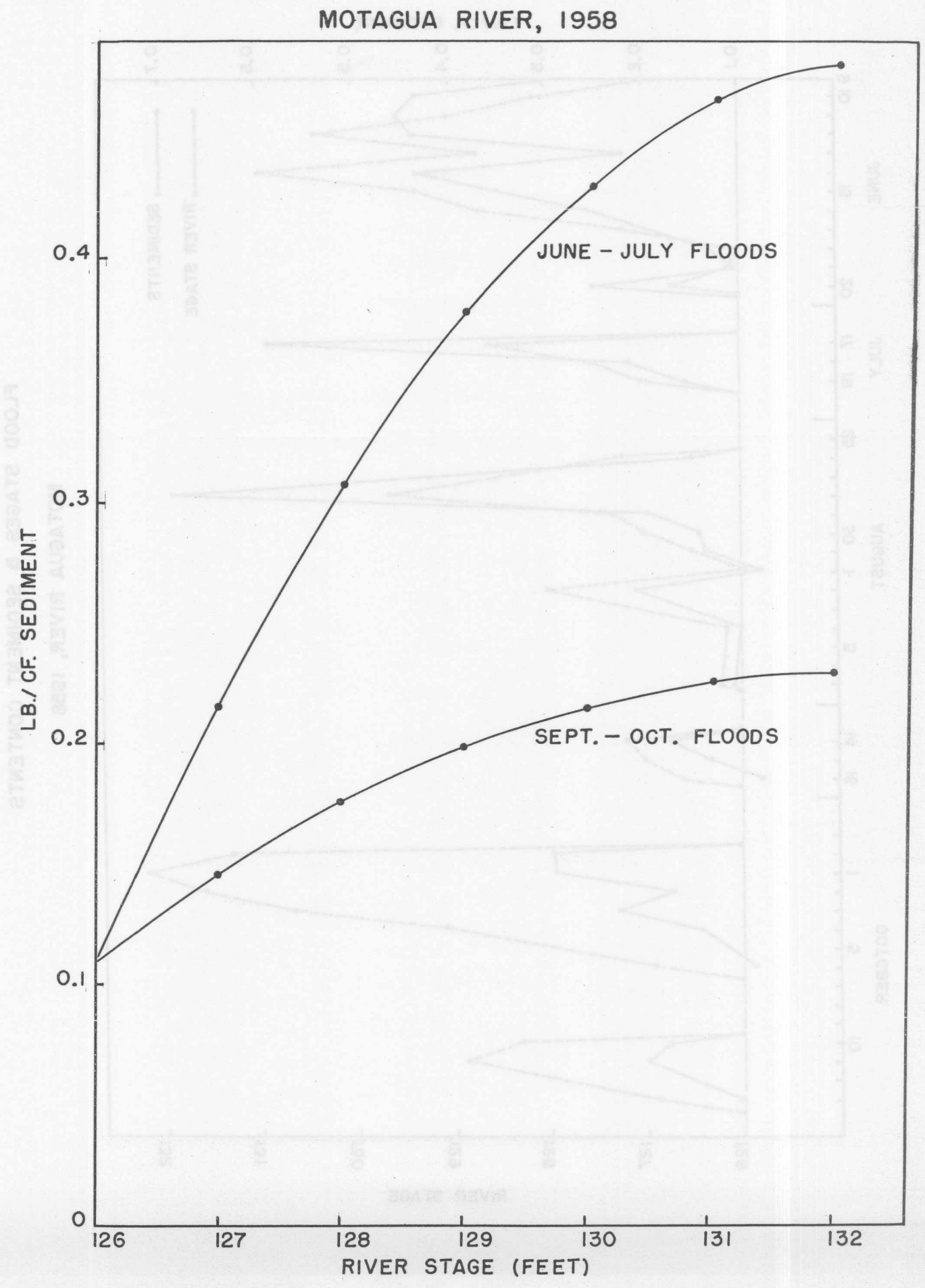
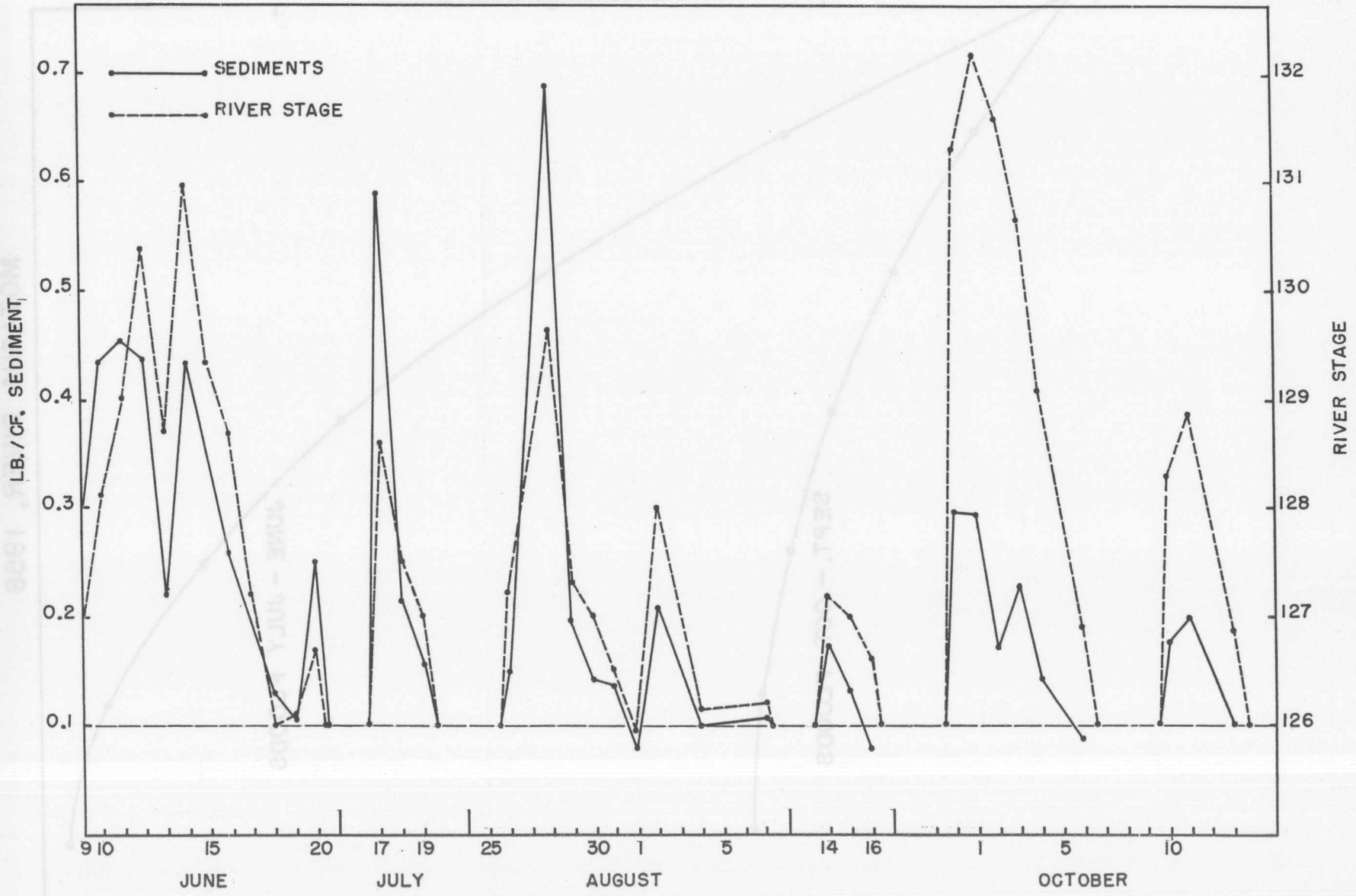

MOTAGUA RIVER, 1958
JUNE - JULY FLOODS
SEPT. - OCT. FLOODS
LB./CF. SEDIMENT
0
0.1
0.2
0.3
0.4
126
127
128
129
130
131
132
RIVER STAGE (FEET)

FIG. 23

FLOOD STAGES & SEDIMENT CONTENTS

MOTAGUA RIVER, 1958



October. In the first group the sediment contents range up to 0.7 lbs./cu. ft. (or 1.2% solid matter) with river stages that are generally below 130. The second group has stages in excess of 131, but the sediment content does not rise above 0.3 lbs./cf. This difference can be explained by the agricultural methods and the climate in the Motagua watershed. During the dry season, prior to the early floods, a great deal of land in the mountains is burned for crop preparation. When the early rains come, the land is bare and dry, and considerable erosion takes place. The later rainy season is generally preceded by more humidity and less burning, and erosion at that time is less prominent.

In order to test the significance of the relation between river level and sediment content, correlation coefficients of 0.758 and 0.706 were calculated for the early and late floods respectively. In fitting curves a second degree polinomial was found to have the best fit. Figure 23 shows the calculated curves giving the relationship between sediment content and river stage. The formulas for these curves are:

Group A - Early Floods: $Y = -9.2 + 0.6X - 0.009X^2$

Group B - Late Floods: $Y = -3 + 0.2X - 0.003X^2$

Where Y is the sediment content in lbs/cu.ft. and X is the river stage 100 (i.e. at a river stage of 126 the value for X is 26).

It is clear from these curves that the sediment content of the Motagua River in flood stage reaches quantities unheard of in the Ulúa River's recorded sediment history. A rough estimate shows that the Motagua would have yielded about 1 acre foot silt per 4 cu.ft./sec. intake capacity at a design level of 127. That would mean that a 20,000 cfs

boqueron could have silted 5000 acres in one year.

One of the difficulties that appears with these high sediment contents is the high sand quantities. All sediments of the early floods graded as sandy loams (about 60% sand), except for the days 1, 2, 4, and 7 of August. The later floods were generally silty loams to clay loams. The difficulty with the high sand contents will be the design of the intake canals, since these canals will have to carry the sediment at much higher velocity than in the Honduras silting projects, to prevent dropping out of the sand. If not so designed the canals will have to be repassed after each flood season to eliminate the build-up. Another method for this might be to design a settling basin at the intake to take care of the sand, but the pity of this would be that a large part of the sediment would be wasted and the final delivery to the projects would be diminished to a small quantity. It seems reasonable that in the Motagua valley good sized cyclic silting projects, as designed at Naranjo-Chino in Honduras, would have a place. Since the silt contents run very high, reasonable assurance could be obtained that the time table for the cycles could be met. In addition, smaller boquerones can be used in such a project, and with the needed increased water velocity these would be safer than the large varieties.

It should be pointed out that these data are only for one year and as such they should not be taken as more than initial inforamtion. It will take some season more to definitely evaluate the sediment contents of the Motagua River.

For the Ulúa River sediment data of 1957 and 1958, similar tests of significance were made and no good correlation was found between river

stage and sediment content. No proper fitting curve could be calculated. It is possible that this lack of relationship is due to the extreme dryness of these last two years. In both years the usual early floods did not occur.

Soil surveys

The following is a breakdown of surveys for 1958:

HONDURAS DIVISION:

Location	Heavy clay	Light clay	Loam	Sand	Total Acreages
Ocote	181	297	423	34	935
Corozal	49	175			221
Farm 8	275	491	841	8	1615
Kele Kele	452	497	549	59	1557
Ocote		Not reported			1041
Tenampua	3790	327	483		4600
Victoria	1594	993	191		2778
West Calan	1509	731	771	28	3039
Guaruma 1		2	26		28
Campin			252		252
Copen Back	504	273	387	13	1177
Campin Lake	34	143	209		386
Mocula		68	283		351
Manacalito		52	511		563
TOTAL	8388	4049	4926	142	18,685

BANANERA DIVISION:

Location	Heavy clay	Light clay	Loam	Sand	Total Acreages
Bobos	384	1472	5425	553	7834
Media Luna		Not reported			1700
Rio Tinto		" "			4440
Aztec		" "			950
S. Motagua		" "			1000
TOTAL					15,920
GRAND TOTAL					34,605

A total of 1175 mechanical analyses and 967 pH determinations were made in this laboratory.

Soil sample preparation

A study is in progress of the methods that have been used in this laboratory for preparing soil samples for mechanical and chemical analysis. The usual system consists of: 1 - airdrying, 2 - grinding to pass a fine sieve (usually with mortar for about 5 minutes), 3 - mixing with dispersant solution in mixing machine, 4 - analysis by Bouyoucos method. The effect of grinding and mixing was checked and the results on four soils are:

TREATMENT	SOILS (figures give per cent composition)											
	Tiquisate I			Tiquisate II			Ulúa I			Chamelecon		
	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
1 min. mixing	48	46	6	54	27	19	41	50	9	45	46	9
5 min. mixing	37	46	17	52	25	23	34	50	16	41	43	16
5 min. grinding	38	42	20	50	25	25	31	46	23	36	41	23
10 min. grinding	39	42	19	50	24	26	29	45	26	34	40	26

All samples were left soaking with a dispersing solution for 24 hours to insure good dispersion. It is clear from these data that these treatments have an influence on the percentage composition found by Bouyoucos analysis, and that both grinding and mechanical mixing tend to increase the clay/sand ratio. Similar experiences with sample preparation have been reported verbally by workers from San Salvador and Nicaragua. According to these people the nature of the volcanic materials in these soils tends towards easy pulverization during grinding and mixing.

The danger of the changes due to grinding in mechanical analysis is that it may have led to errors in textural classification of our soils. Our surveyors have been trained to classify according to the mechanical analyses results. As such, it is possible that they are now classifying soils too heavy, due to repeated check backs to a faulty mechanical analysis.

More work will be done on the effects of sample preparation methods on analyses results.

Miscellaneous investigations

The laboratory made a total of 6,229 analyses during 1958. Among these were:

Soil samples	1678
Plant material	1934
Water	28
River sediments	420
Palm oil	26
Feed	2

Nitrogen uptake
in bananas

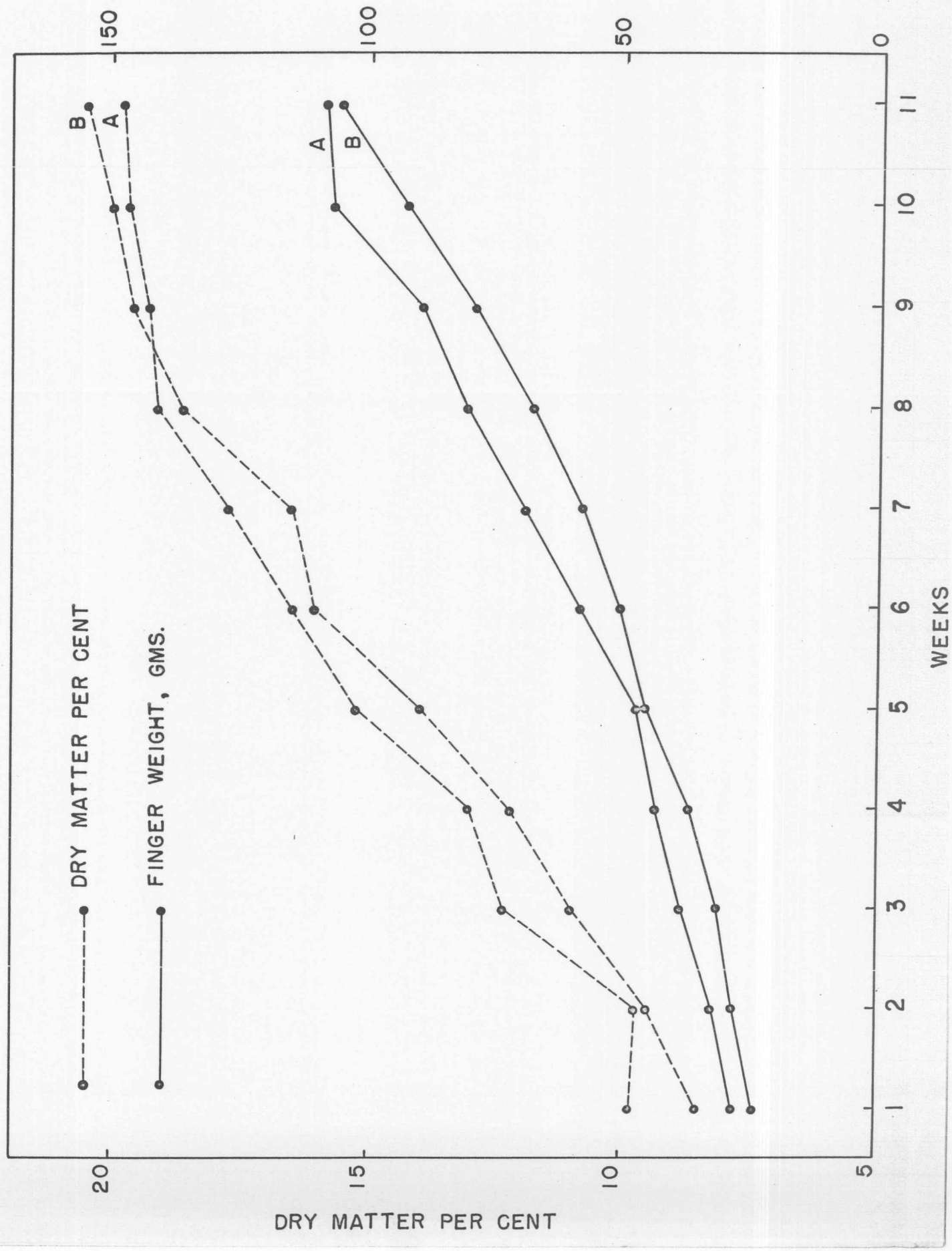
In August, a study was started on the nitrogen changes in bananas during the time from shooting to harvesting. Two series of 6 plants each were selected in Guaruma 1. The plants had received a normal farm application of urea prior to the start of the experiment. This was at the rate of about 300 lbs. N/acre/year. The age of the planting is at least 5 years. Series A received 5 lbs. $NaNO_3$ per plant just before shooting, and received in addition 1/4 lb. $NaNO_3$ every week thereafter. Series B did not receive any additional nitrogen during fruit maturing.

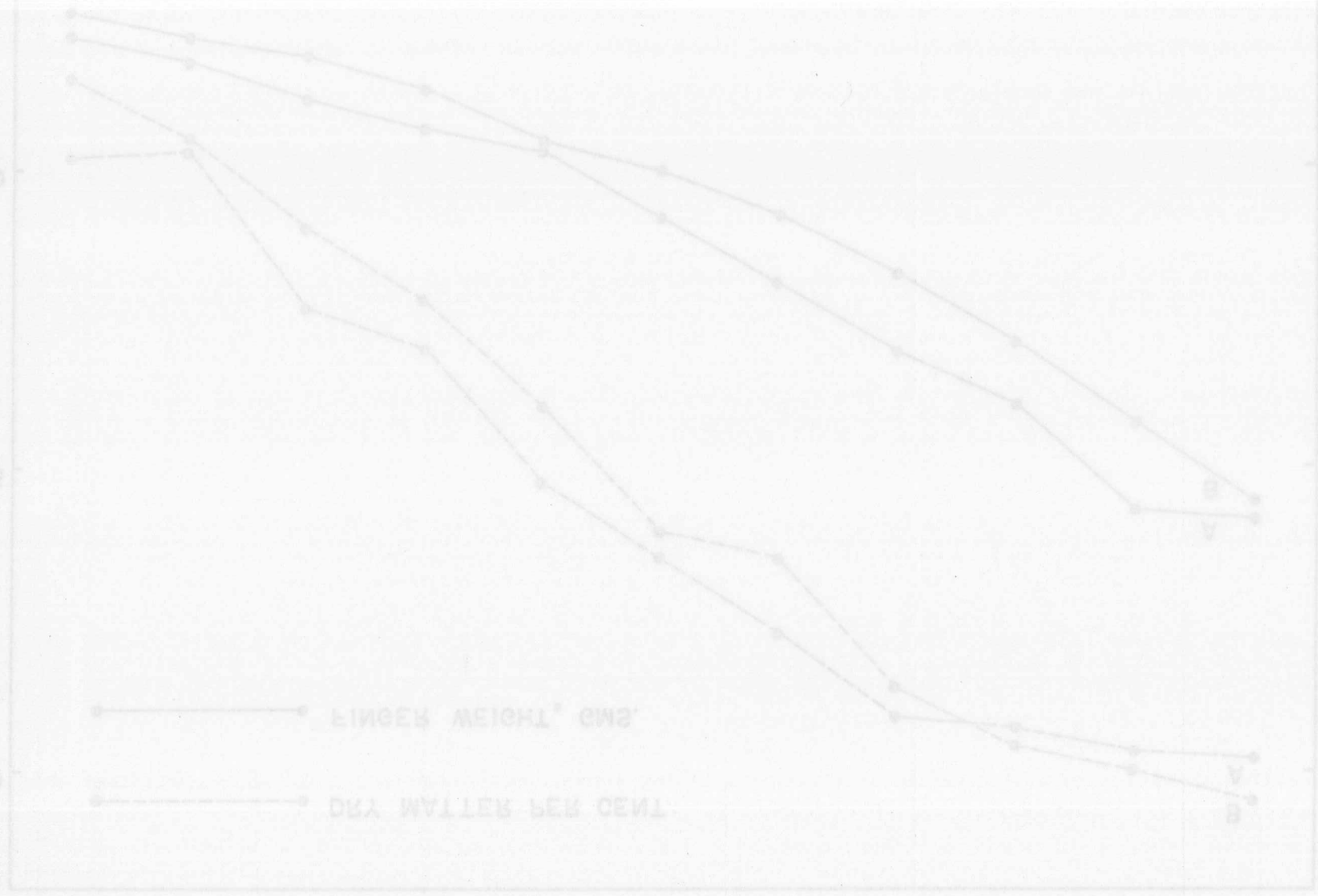
Total nitrogen was determined on the dried and ground banana samples by Kjeldahl method. The bananas were taken each week from the middle hand of the stem; one banana per stem per week from shooting to harvesting.

Figure 24 shows the nitrogen concentration (on dry weather basis) and content with maturing over the first 11 weeks of sampling, or an average of 13 weeks since shooting. The nitrogen concentration in the bananas drops continuosly during growth of the fingers, starting at about 2% around 2 weeks after shooting, down to about 0.9% around one week before harvesting. No concrete differences show between Series A and B. The variability between individual plants is quite high, especially in the rate of growth. Nitrogen concentrations are generally more uniform than nitrogen contents.

The total nitrogen content in milligrams shows a continuous increase during maturing, although the rate of increase is not equal to the increase in dry matter as shown by the drop in nitrogen percentage.

FIG. 24


FINGER WEIGHT, GMS.
150
100
50
0
DRY MATTER PER CENT
FINGER WEIGHT, GMS.
B
A
A
B
20
15
10
5
DRY MATTER PER CENT
1
2
3
4
5
6
7
8
9
10
11
WEEKS


B
A
A
B

The difference between Series A and B cannot be considered significant, since by poor luck most of the B series plants shot later than the A series. All plants that shot early (beginning August), whether Series A or B, had a faster development than all plants that shot later in August or in September. As such, it must be assumed that some climate factor has affected these values, causing the average development of Series B to be slower than that of Series A.

In November we started with complete analyses of banana plants, with the purpose of getting some up-to-date figures on the concentration of various nutrients in the plant parts. Due to a temporary shortage of equipment only one plant has been fully analyzed, and one non-fertilized plant was analyzed for nitrogen only.

Further analyses of whole banana plants will be made in the near future to acquire more information. In addition, this coming year a number of plants without fertilization will be analyzed. These will be taken from the Monte Vista agronomy experiments. Analyses will also be made of plants treated with various rates of fertilizers.

* * * * * * * * *

CLEAN SEED PROGRAM

In September 1958, a conference of Research and Production personnel was held in Boston, as a result of which the Research Department accepted the responsibility of developing a Clean Seed Program for the Company. That program was launched officially in December following a week-long conference at La Lima attended by one or more Agricultural persons from each Division, all chose by their Divisions to be responsible for the local Clean Seed Program. At this conference the

importance of clean seed and methods of detecting, eliminating, or controlling various pests that can be carried on seed were discussed in detail. The general reaction at the end of the conference was enthusiastically favorable and by year's-end, several Divisions already had the beginnings of their Clean Seed programs underway. (Various aspects of the Clean Seed program have been presented in the Research Newsletter during 1958. In Volume 6, Number 2, April 1959, an article will appear up-dating the progress of the program throughout the banana-producing Divisions.)

Infected Seed

During the year, one study was carried out at La Lima on hidden infections in seed pieces. Isolations from the outer 1/2 inch of surface of disinfected banana rhizomes incubated in glutamic acid continued to yield occasional cultures of F. oxysporum. Of 24 healthy rhizomes sampled from an area with less than 5% visibly diseased mats, 2 yielded cultures of F. oxysporum identical in appearance to F. oxysporum f cubense. One of these cultures proved to be pathogenic, inducing symptoms of Panama disease in field inoculated bananas. Although F. oxysporum f cubense is present in a small percentage of healthy-appearing rhizomes, it is not known whether such rhizomes succumb to Panama disease when planted. Studies conducted several years ago where visibly diseased rhizomes were planted indicated that as many as 20% produced healthy-appearing mats 18 months after planting.

Library

During the year 1958, 414 pamphlets, 15 microfilms and 70 books have been added to the Library and 43 journals and 3 volumes of Abaca Reports were bound.

We now have a total of 1,241 books and 648 bound scientific journals. Of these, 303 are in circulation. Books in circulation have been checked periodically and all are accounted for.

We receive 108 scientific publications, of which 20 are free and 4 come with memberships.

Photography

Photographs made total approximately 555, about equally divided between black and white and color. Black and white prints made total approximately 2000.

In addition to the usual photos made here for various departments, the following items were completed:

(May and June) - Considerable time was spent on photographs for the Pathology Booklet and Exhibit being prepared in Boston. In addition to black and white and 35 mm color, about 75 special 4 x 5 Ektachromes were made.

(July) - Twenty photos were made of Norwood Laboratory and greenhouse facilities.

(October) - Several days spent in Coto for photos of caterpillar and insect pests. (Black and white and color.)

The major new item of equipment added was a 4 x 5 View Camera, with 8-1/2" commercial Ektar lens, and a Goertz Dagor wide angle lens.

Visitors

During 1958, over 150 scientists and others interested in tropical research visited the La Lima laboratories, some for brief periods, others for extended stays. Included were Dr. W. C. Snyder, University of California (Fusarium authority), Dr. C. E. Palm, Cornell (Entomologist), Dr. J. G. Matthysse, Cornell (Entomologist on sabbatical for a year), Dr. H. H. Kramer, Purdue (Geneticist), Dr. Hugh Popenoe, University of Florida (Soil scientis), and many other leading figures in agricultural research.

Scientific Meetings

Seven Divisions of Tropical Research scientists attended the Company Fusarium Wilt Conference at Purdue University, summer, 1958, and the American Institute of Biological Sciences meetings at Indiana University, immediately following. In addition, five staff members spoke at the Annual Division Managers Conference at Tela in March, 1958, and one staff member addressed the Ministers of Finance for Central America at La Lima in June, 1958.

The Clean Seed Conference held in December, 1958, previously mentioned in this report, was the major extension conference of the year. In other extension activities, numerous staff members travelled throughout the banana-producing Divisions and to Boston in research advisory capacities.

LANCETILLA EXPERIMENTAL STATION

Tela, Honduras

During the year the work of the station, together with the timber tree acreage and the guarding of the Tela watershed area, was carried out with 43 laborers and 6 monthly employees.

The care of the watershed, formerly under the Construction Department, was turned over to the Lancetilla station. Eucalyptus deglupta was planted at a close spacing to fill in the area formerly occupied by the watchman and his manaca shack was destroyed. Boundary line fences were repaired and growing posts planted on the fence line. The watershed boundary was cleaned and new sign boards were erected in strategic locations.

The timber tree acreage was cleaned three times during the year. This work was confined to the cutting of vines, removing overtopping shade trees and the cleaning of planting lanes. One cycle of pruning was made and some thinning was done in the close spacing experiments.

In the flat land near the Golf Course and in Sections 24, 31, and 32, all bush, stumps and rocks were removed so that this area can be cheaply maintained mechanically. More of this work will be done in 1959. An additional 500 new treated pine railway ties were purchased at half price and at the end of the year were being placed in the Lancetilla railway line. The Railroad Department was also

requested to straighten the bent rails to make the track safer to use. The clerk's house was repaired and painted. Inside plumbing was installed in the office by the plant shed. The suspension bridge over the Tela River on the upper end of the property was completely rebuilt and is now in safe condition.

Plant introductions

The following plants were introduced on September 8, 1958:

Vetchia winin, Chrysalidocarpus Sp., Nephrosperma van h o utteanum, Geonoma Sp., Baikiaea plurituga, and Chamedora shiedeana, ornamental palms obtained from Fairchild Garden and growing well. Scheelia liebmanii, also an ornamental palm obtained from Fairchild Garden.

Colombian Palm (57-184), this is an unknown species from Colombia obtained through Fairchild Garden. Beautiful and ornamental.

Bengal lychee, this is a fruit tree, obtained from the Sub-Tropic Experiment Station, Miami University; growing very well.

Seedless Guava - This is a fruit tree obtained from the Florida Sub-Tropic Station.

Deplancheae tetraphylla - An ornamental tree with beautiful flowers, obtained from Sub-Tropic Experiment Station, June, 1958.

Plant distribution

	Donated	Sold	Total
Miscellaneous fruit trees	4,966	115	5,081
Economic plants	897	46	943
Ornamentals	8,120	19	8,139
Timber trees	122	-	122
Totals	14,105	180	14,285
Miscellaneous seeds *	432	-	432
Miscellaneous fruit *	-	8,100	8,100
Totals	432	8,100	8,532

* Pounds

Visitors

A total of 159 visitors were shown around the station during 1958.

CHANGUINOLA RESEARCH STATION

Almirante, Panama

Green manure observation nursery

Observation plots were set up to determine which species of green manures grew well under Changuinola conditions and were suitable for further study in association with nematodes.

Soybean - Avoyelles and O-Too-Tan varieties were a total failure due to attacks by the Diabrotica beetle. Many of the plants died before seeding. Frequent insecticide applications were necessary to keep attacks under reasonable control.

Crotalaria spectabilis - This species is not suitable for Changuinola. The plant flowers early and forms very little leaf. Fairly attractive to the Diabrotica beetle. This Crotalaria species was tried out on many localities in Changuinola but proved a poor grower everywhere. Nematode resistant.

Crotalaria retusa - A very good variety. Grows well and carries an excellent canopy of leaves. Very slightly attractive to the beetle. Pods damaged by a borer.

Crotalaria juncea - Rather similar to C. retusa. Slightly attractive to the beetle. Pods damaged by borer.

Crotalaria anagyroides - The best of the Crotalarias tested. Plants grow fast and carry a heavy canopy of leaf. Almost completely immune to the Diabrotica beetle. Pods attacked by borer. Immune to nematode attack.

Crotalaria usaramoensis - Poor type. Spindly branches and little foliage. Nematode resistant. Apparently not very suitable for Changuinola conditions.

Crotalaria incana and C. pumilla - Seed failed to germinate.

Crotalaria brownei - Grows well. Carries good cover of foliage. Spindly upper branches. Pods severely damaged by a borer.

Crotalaria guatamalciensis - Not a very good type. Foliage sparse. Seeds profusely. Pods lightly attractive to a pod borer. Very susceptible to a spider mite.

Crotalaria clarkei - A medium sized green manure crop. Leaves defoliate easily. Did not flower.

Crotalaria agathifolia - A medium sized plant. Forms a fairly heavy canopy of foliage but branches are spindly and easily broken by winds. Flowered, but seed did not set.

Crotalaria grahamiana - In the field the plants were fairly poor with a light canopy of leaf. Growth was good in cement tanks and the plants carried an excellent crop of leaf. Pods severely damaged by a borer.

Crotalaria semperflorens - A slow grower. Leaf badly damaged by caterpillars. Did not flower.

Crotalaria mucronata - In field sowing the seed failed to germinate. In cement tanks growth was good and plants carried heavy foliage. Severely attacked by a pod borer.

Crotalaria breviflora - Poor type. Spindly branches and very little foliage. Did not set seed.

Crotalaria intermedia - Spindly branches. Foliage cover very light. Pod borer damage was very severe.

Tephrosia vogelii - Almost completely immune to the Diabrotica beetle. Grows well and carries an excellent cover of foliage. Very susceptible to Radopholus similis and to root-knot nematodes.

Tephrosia candida - Very similar to T. vogelii but small leaf. Also susceptible to R. similis and root-knot nematodes.

Velvet Bean - Made excellent growth and is a good cover. Is very attractive to the Diabrotica beetle.

Pueraria phaseoloides - At first growth was slow but later grew fast and formed an excellent cover. Twines over and strangles everything it comes in contact with. Take about 6 months to give good cover sufficiently thick to keep down weeds.

Calapagonium muconoides - An excellent ground cover which forms a complete mat in around 2 months from planting in rows 3 feet apart. However, it is very attractive to the Diabrotica beetle. Roots along runner and has a tendency to climb. Seeds profusely.

Stylosanthes gracilis - Commonly known as stylo or brazilian lucerne. Only a few seed from the consignment received from Ceylon germinated. These were grown in cement tanks. An excellent type which promises to be a good ground cover. Unlike many other ground covers S. gracilis does not climb. It forms a thick mat which grows out 3-4 feet from the center rooted stem. Another advantage is that the low-spreading shoots do not root at the nodes enabling the thick mat to be rolled back easily, like a carpet. This plant has not flowered but cuttings root easily. Cuttings have been established in baskets for further work and observation on nematodes resistance, etc.

Centrosema pubescens - Not a good cover. Grows slowly and is choked with weed growth.

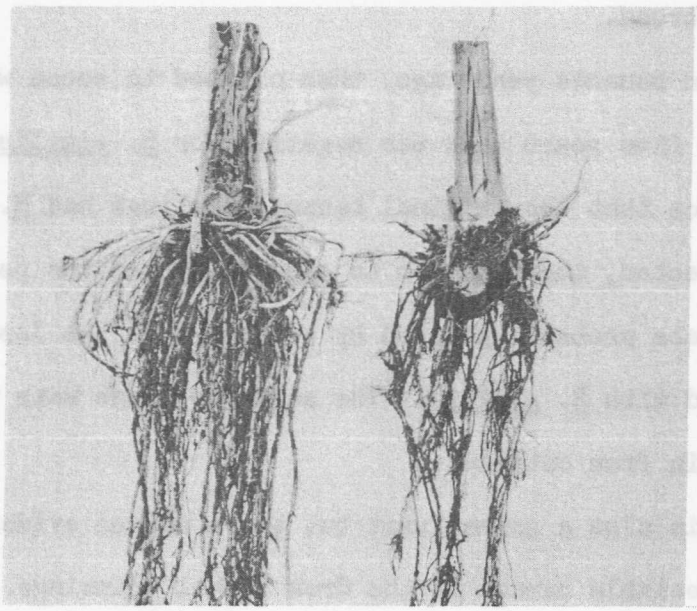
Cadjanus cadjan (pigeon pea)- A very good grower. Susceptible to R. similis.

Relation of nematodes
to Panama Disease

In pot experiments an association of Radopholus similis or Meloidogyne incognita acrita, two common nematode pests of Gros Michel bananas in areas of severe wilt disease, and an inoculum of 14 million Fusarium oxysporum f cubense spores to a square inch of soil was not a pre-requisite to wilt disease. However, wilt disease symptoms were aggravated when the fungus was associated with R. similis and, to a lesser degree, with M. incognita acrita. The time interval between inoculation with the fungus and the appearance of severe wilt disease symptoms was considerably shortened when the fungus inoculum was added to plants already severely infected with R. similis. R. similis infection resulted in severe root destruction and mutilation (Fig. 25). The mean number of roots on plants growing for 19 weeks in the presence of R. similis was only one-fifth that of plants grown in nematode-free soil. M. incognita acrita infection caused marked stunting of the plants during the first 13 weeks of growth and, though this measurable difference was maintained throughout the duration of the experiments, it was not so apparent at an examination after 19 weeks. (For full details of this research, see LOOS, Clive A. Symptom expression of the Gros Michel banana in the presence of Radopholus similis (Cobb, 1893) Thorne, 1949 and Meloidogyne incognita acrita Chitwood, 1949. Proc. Helm. Soc. Wash. 26 (1): - 1959.)

FIGURE 25

Root systems of R. similis infected (right) and nematode-free check (left) plants. Plants 19 weeks old.



The prevalence and distribution of root-knot, Radopholus and other nematodes on new and future plantings

Radopholus similis, the burrowing nematode, is of widespread occurrence through Changuinola banana plantings.

Examination of virgin land (land not previously in bananas) failed to reveal the presence of R. similis. From those observations it must be assumed that the pest is not indigenous to the Changuinola area but was imported from abroad.

An area which was in bananas years ago, then planted to cocoa which was abandoned about five years back was negative for R. similis. There was no evidence that the original banana plantings had R. similis, or if they were infected, the rotation to cocoa destroyed the pest. Occasional banana mats probably planted by squatters in the last 5-6 years, were infected with R. similis. The seed for those mats were apparently brought in from outside.

Root-knot nematode is also a common pest but there was no evidence that it causes appreciable damage to the Gros Michel plantings. Two species of root-knot nematodes, Meloidogyne incognita acrita and M. arenaria were recognized on Gros Michel and Cocos.

Hoplolaimus, Rotylenchus, Helicotylenchus, Tylenchorhynchus, Paratylenchus, Criconemoides, Hemicycliophora, Xiphinema and Trichodorus, all ectoparasites, were recovered from soil around banana roots.

Radopholus similis causes lesions on the roots and rhizomes. (Figs. 26 - 27 - 28.) The lesion extends through the depth of the root cortex and often girdles the root causing it to die-back to the lesion. The nematode enters the rhizome through the roots (Fig. 29).

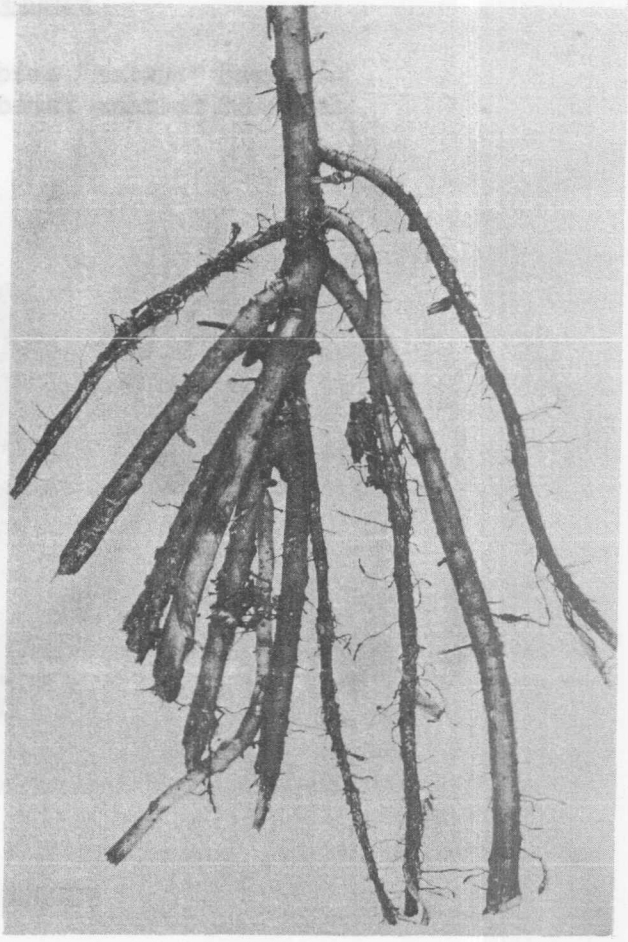


FIGURE 26

Gros Michel roots infected with Radopholus similis. Note the original infection which killed the root and stimulated new root formation above the injury. The new roots are also infected.

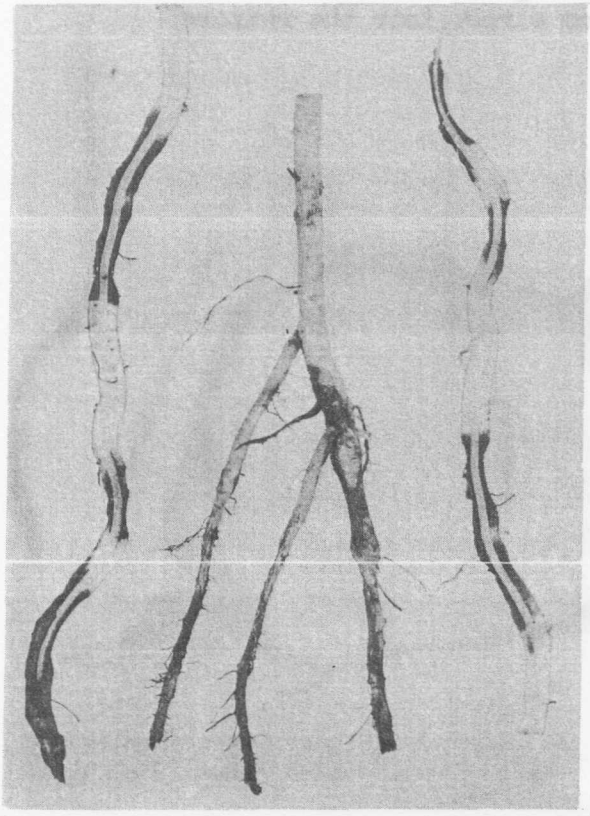


FIGURE 27

R. similis infected roots. Two of the roots are split to show discolored cortical tissues of the infected area.

FIGURE 28

A pared "button" seed plant rhizome. Outer skin of rhizome pared to show R. similis lesions.

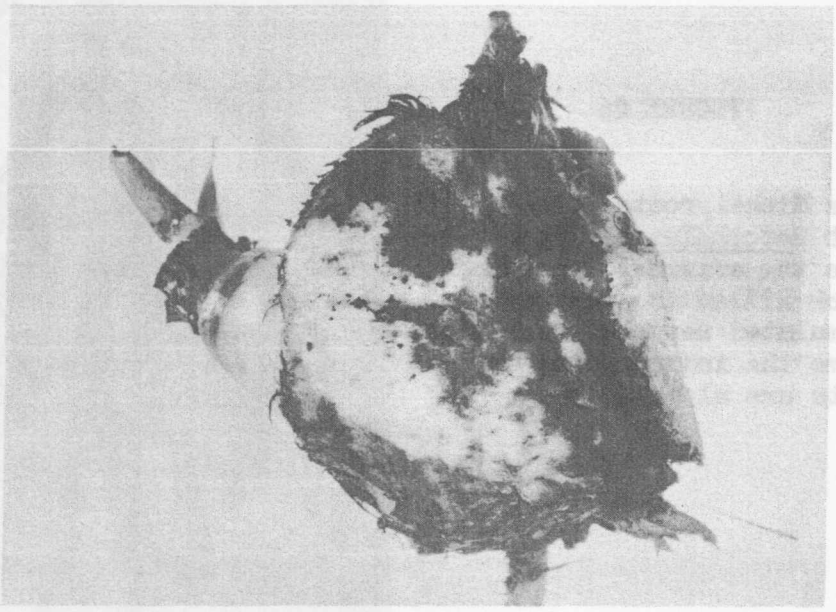
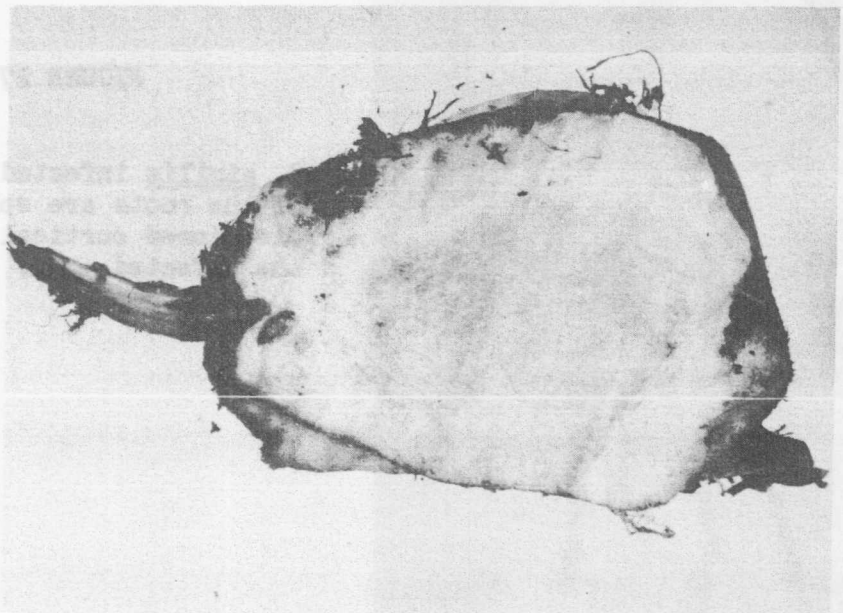


FIGURE 29

A split rhizome showing the extension of the nematode lesion from a root into the rhizome cortical tissue.



Flood-fallowing for at least six months destroys R. similis.

Soil examinations alone, for determining the presence of R. similis is of doubtful value. R. similis is an endoparasite and is in the soil as a migrant from one root to another for only a short period. The presence of root-knot larvae in soil washings is also of doubtful diagnostic value as the pest may have developed on one of the weeds grown in association with bananas. Roots and rhizomes are satisfactory material for examination.

R. similis infections are more general on the rhizomes and the roots close to the plant bowl, though infections some distance from the rhizomes also occur. Infections with root-knot are more generally away from the bowl especially on roots growing through the surface mat of decaying vegetation.

R. similis is a sluggish nematode. The Baerman funnel technique for extracting nematodes is therefore not very satisfactory. Material should be washed after maceration through a series of graded sieves and residues left on the 200 and smaller mesh sieves should be examined under a microscope.

Attacks of R. similis, beside inducing plant debility, weaken the plant anchorage causing it to tip over easily under light wind pressure or fruit weight.

It is recommended that the movement of seed fron one Division to another or between farms in Divisions should be controlled, in accordance with the Clean Seed program.

Establishment of a nematode
and Panama free nursery

Root-knot (Meloidogyne) burrowing nematode (Radopholus similis) and certain ectoparasites (Helicotylenchus, Rotylenchus, Hoplolaimus, Paratylenchus) found in association with bananas in Panama, are parasites on the banana roots.

The trimming of roots flush with the rhizomes eliminates the root-knot and the ectoparasitic nematodes. Although root-trimming may reduce the burrowing nematode populations on seed planting pieces, that operation is of minor importance as large colonies exist in lesions formed in the cortical tissues of the seed rhizome.

Lesions containing the burrowing nematode are formed in "button" seed, sword sucker and maidenhead rhizomes, and on the rhizomes of bullheads. Any type of seed is, therefore, liable to be a carrier of Radopholus similis contaminations to new planting sites.

The burrowing nematode infection in the cortical tissues of the rhizome is confined to the area of lesioned tissue only. If the rhizome is pared of that lesioned area the nematode is completely eliminated from the seed piece.

The burrowing root-knot and ectoparasitic nematodes associated with bananas are killed if they are brought in direct contact for 5 minutes with water at 55° C.

Sprouting from the heart or central bud of "button" seed rhizomes or sword sucker rhizomes is not impaired if the seed is immersed for 5 minutes in water at 55°C. However, small seed from which heart sprouting is encouraged is very susceptible to a bacterial rhizome rot.

ectoparasite: that lives on the surface of the host.

The incidence of rhizome rot ranged from nearly 60% when small seed was used to only 2% when large maidenheads were planted. Plants grown from small seed succumb to rhizome rot earlier than those from larger seed.

Large maidenhead rhizomes, trimmed of all roots and pared of lesions, centrally split and with the central heart of the cabbage removed, develop sprouts from 2-3 weeks after planting. All external buds and eyes are removed on paring. New growth takes place from buds situated deep in the cabbage tissues. The degree and rapidity of recovery depend on the severity of paring. Seed which carry deep nematode lesions necessitating extensive and deep paring may take 6 weeks to sprout or, though roots may form, there is no bud stimulation.

The heavy paring necessary to remove Radopholus lesions in seed from farm plantations causes reduction in the percentage of sprouting. The percentage of successful takes on a commercial scale planting, using seed which had to be deeply pared due to Radopholus infection is about 50% using large split maidenheads, under Changuinola conditions. Heavy paring of the rhizomes of sword sucker seed did not impare sprouting from the heart of the cut-back pseudostem. In both damp saw-dust and in soil the seed sprouted and in under 6 weeks carried opened leaves. To avoid the probability of carrying contaminants the original experiments were done with pared seeds which were dipped into water at 55°C for 5 minutes.

In Table 32 are the results of the failures in plantings with pared Gros Michel sword sucker and maidenhead rhizomes taken 3 months after planting. Those failures are cases of rhizome rot from date of planting to date of examination.

TABLE 32

Results of plantings with pared Gros Michel sword sucker rhizomes. Growth was from the central heart of the cut-back pseudostem. In addition to paring the seed was hot water treated for 5 minutes at 55°C. Recordings were made 3 months after planting.

Weight range of trimmed seed	Number of seed planted	Failures	
		Number	Percentage
Under 1 lb.	198	111	56%
1 lb. - 2 lbs.	598	275	46
2 lbs.- 3 lbs.	246	69	28
3 lbs.- 4 lbs.	100	12	12
4 lbs.- 6 lbs.	93	2	2

Project 6
plantings

In January we commenced the planting of Lakes 20, 21 and part of 24 of Farm 65 with split, pared seed from Farm 62. The seed was heavily infected with Radopholus and necessitated deep trimming to eliminate it. The seed was not dipped or hot water treated.

Successful takes were in the region of 45-60%. This necessitated the resupplying of vacancies on more than one occasion. Finally a stand was established and in August a number of the plants commenced to flower.

No extensive work was undertaken on the preparation of heavily trimmed seed for commercial planting as it was not considered an economic proposition. Further there was little purpose in such an undertaking as the area under trimmed seed plantings would supply large numbers of nematode-free seed for expansion purposes.

It was eventually decided that the nematode-free seed should be dug up and used for the establishment of a seedbed for the California and 5B plantings.

The lakes were examined for nematode infection with the following results:

Area of Pared Seed Plantings	Mats examined	Mats infected with Radopholus	% infected mats
Lake 20 Gros Michel	1391	78	6%
Lake 20 Cocos	746	49	7
Lake 21 Gros Michel	208	7	3
Lake 24 (Part) Gros Michel	207	2	1
Regular Farm Plantings			
Lake 22 Gros Michel	25	21	84%
Lake 22 Cocos	25	23	92
Lake 23	25	20	80
Lake 24 (Part)	85	76	89

Bordeaux and Nemagon dips

Split, heavily pared maidenheads from Project 5A were dipped in Bordeaux and Nemagon. The trimming table was washed with 5% formaldehyde before paring of seed.

	Sprouted
A. Dipped 1 minute Bordeaux 10-10-50. Seed buried in soil.	49%
B. Not dipped. Seed buried in soil.	29%
C. Dipped 1 minute in Bordeaux 10-10-50. Seed covered with banana leaves.	49%
D. Not dipped. Seed covered with banana leaves	42%

	Sprouted
E. Dipped in Nemagon 1/250 concentration. (Nemagon Emul. Conc. used) buried in soil	45%
F. Dipped in Nemagon 1/250. Covered with banana leaves.	45%
G. Dipped in Nemagon 1/1000 concentration. Buried in soil.	46%
H. Dipped in Nemagon 1/1000 concentration. Covered with banana leaves.	48%

To ascertain whether formalin, used on the trimming table and on the knives, may cause reduction in sprouting, washed maidenhead rhizomes were pared and split on tables which were previously well washed with water. The knives were sterilized and washed with water. The prepared seed was treated as follows:

	Sprouted
A. Not dipped. Buried in soil.	42%
B. Dipped in Bordeaux 10-10-50. Buried in soil.	46%
C. Not dipped. Placed on ground and covered with banana leaves.	38%
D. Bordeaux 10-10-50. Placed on ground and covered with banana leaves.	58%

The formalin cleaned table or the formalin dipped knives had little effect on germination of well matured maidenhead seed. Dips or methods of germination did not materially increase sprouting. The degree of paring is probably the deciding factor.

Life histories and host range of
parasitic nematodes in banana fields

Radopholus similis larvae and females, at any stage of development, can move in and out of banana roots.

The male spear is small and degenerate compared with that of the female.

Although the males may move in the lesioned tissues of the plant, they are unable to re-enter a root.

Copulation and fertilization of the female takes place in the plant tissues. A single fertilization is sufficient for the normal life of a female.

Generally, unfertilized females do not lay eggs though an occasional unfertilized female may lay a very few eggs which hatch normally. In such a case the pre-egg laying period of the female is considerably prolonged.

If a gravid or a mature female is removed from the plant tissues egg-laying ceases in under 48 hours. During the first 24 hours of removal from the plant tissues there is little cessation in the rhythm of egg-laying but in the second day the number of eggs laid is considerably reduced. From the third day onwards egg-laying ceases completely.

Active females may be kept alive for several weeks in a small spot of water, provided the water is changed every 2 or 3 days. Females who cease egg-laying in water, over a period of several days, commence to re-lay in under 48 hours when they are reintroduced into a plant root. The mechanism of egg-laying is definitely tied up with the obligate parasitic environment of a host plant tissue.

A female may lay up to seven eggs a day. Egg-laying continues for over three weeks. The average eggs laid per day is around four.

Eggs laid in or transferred to water hatch normally. There is no evidence of delayed hatching outside the plant tissues. On the

contrary eggs laid in water hatch faster than those laid in Tephrosia candida roots.

Incubation period in water is five days and seven days in a root, at room temperature around 85°F.

The emerged second stage larva (the first moult is accomplished in the egg) does not grow outside the plant tissue. The hatched larvae are very active. Fat contents in its body are dissipated in 3-4 days indicating that entry into a host must be accomplished up to a few days from hatching. Larvae hatch from eggs laid in the plant tissue develop normally.

The larval period in Tephrosia candida roots, is 10 days. There is a pre-egg-laying period in the adult of two days.

The life history, egg to egg, is 19 days.

The method used for the study of the life history was that developed by Gadd and Loos for the study of Pratylenchus coffeae. Individual felames for egg-laying studies, etc., are placed in a small spot of water on a microscope slide. A Tephrosia candida seedling with a radicle about an inch long is placed on the glass so that the root-tip rests in the water spot. Sand which has passed through a 60 mesh sieve is added slowly over the root-tip and water-spot and when sufficient is added to cover the root tip it is dampened and the slide placed for 48 hours in a 100% humidity chamber. At completion of the 48 hours the seedling is removed from the chamber, washed of adhering sand, and planted in sterilized sand. By this method the female nematode or larva enters the root immediately behind the root-tip. A faint discoloration can be observed at the infection point 3 to 4 days later. A typical brown lesion develops in 10 days when the colony,

raised from the single female, feeds on and destroys the root cells. Accurate estimation of the contents in the lesion is made by macerating the root in a mixture containing equal parts of 10% chromic and 10% nitric acids for about 30 minutes. Air is extracted from the root cells, while the specimen is still in the macerating fluid. This is important as otherwise air bubbles obscure observation of eggs and small larvae. After maceration the root is washed in water for about an hour and then placed on a glass slide. A few drops of 10% caustic potash are added over the root and a cover glass placed over it. Light pressure with a needle on the cover glass flattens the root and permits easy examination and counting of the population in the lesion, under the low power of a microscope.

Red Rust Thrips

Damage to fruits by the thrip Chaetanaphothrips orchidii has been of relative small importance since the control practice of fruit spraying with Dieldrin was developed in 1954. Here in the Bocas Division this procedure was modified to include spraying both the fruit and the pseudostem, which is also attacked by the thrips.

During the latter part of 1957, the entire Division was placed under oil spray by helicopter for Sigatoka control, thus eliminating the permanent pipe system on which the ground spray operation was dependent. Therefore, all experiments for thrips control from that time on were designed to evaluate the helicopter as a means of dispersing insecticides. Attempts were made to use the abandoned systems on old farms and also to lay temporary 3/4" pipe lines on the new farms that did not have an installation. Both operations proved to be time-consuming and expensive.

The area selected for the first experiment was three pilot plantings of 5.7, 4.9, and 4.8 acres located in Project 5. These plots were planted prior to the farms in this area to observe the incidence of Panama disease. They contained mature banana plants with grade fruit. The standard emulsifiable Dieldrin concentrate (1.5 lbs. of actual Dieldrin per gallon) was mixed with Orchard oil at the rate of one part of the concentrate to two parts of oil. This mixture was applied by the helicopter during the regular Sigatoka cycle, using the standard nozzles. Approximately one half gallon (.75 lbs. actual) Dieldrin and one gallon of oil was deposited per acre. A second application was made in 10-days using the same formulation, making a total of 1.5 lbs. of actual Dieldrin applied for the two applications. No phytotoxicity was noted and only a temporary reduction in ant activity was observed.

Thrip counts before treatment on tagged plants are shown in Table 33.

TABLE 33

COUNTS ON FRUIT. ADULTS AND NYMPHS

Plot No.						Average
1	6-11	7-30	5-20	10-46	0-0	2.6 - 13.4
	4-20	1-7	3-0	0-0	0-0	
2	2-10	0-15	0-0	0-0	2-27	.9 - 9.5
	2-23	0-4	0-0	0-8	3-8	
3	0-0	0-0	2-5	0-0	0-3	.8 - 5.9
	5-39	0-5	1-3	0-4	0-0	

COUNTS ON PSEUDOSTEMS. ADULTS AND NYMPHS

1	0-0	30-40	12-10	15-20	0-0	7.1 - 10.0
	0-0	3-0	0-0	3-10	9-20	
2	2-0	2-0	0-0	0-0	0-0	.4 - 0
3	0-0	0-0	2-4	1-11	0-0	1.3 - 8.9
	5-69	3-4	2-1	0-0	0-0	

Five days following the first application the counts on the tagged plants in all plots had fallen to 0-0 except on one stem of fruit in Plot 1 which remained the same (1-7) as before treatment. Further counts made five days after the second application showed 0-0 counts on all of the tagged plants.

These encouraging results led to a large scale field trial on Farms 52 and 54. These farms were sprayed in the same manner using the same formulation as was used in the experiment. As a control, temporary 3/4" pipe lines were laid in one plot which was hose sprayed with Dieldrin emulsion at the usual rate of 1 quart of the concentrate per 100 gallons of water. Table 34 shows average thrips counts before and after treatment.

TABLE 34

	Average Thrips Counts Before Treatment	Average Thrips Counts After Treatment
Area 4	3.60/3.62	0/0
Area 5	3.32/6.26	.06/.10
Area 6	1.10/2.24	0/0
Area 10	23.30/5.50	0/0
Area 11	3.18/ .92	0/0
Area 12	7.54/2.34	0/0

Although these experiments show conclusively that effective thrips control can be achieved with the helicopter, additional tests are needed to determine the minimum amount of Dieldrin needed.

Leaf-feeding caterpillars

During the year a severe outbreak of the leaf-feeding caterpillar, Ceramidia butleri, occurred in most farm areas. Although parasitization of both eggs and larva aided in suppressing the population build-up, some farms became infested to the extent that chemical control measures were deemed necessary.

Farm 61 was selected as an area in which to evaluate the helicopter as a means of combating this pest. Table 35 shows average caterpillars per leaf before and after treatment.

TABLE 35

AVERAGE CATERPILLARS PER LEAF BEFORE AND AFTER TREATMENT WITH DIELDRIN APPLIED BY HELICOPTER

	LAKE 1 .37 lbs. Dieldrin/A	LAKE 2 Control	LAKE 3 .75 lbs. Dieldrin/A
Before treatment	5.3	5.4	7.7
2 days after treatment	2.1	5.5	0.4
7 days after treatment	0.8	5.3	0.0

The same 1.5 lbs. Dieldrin emulsifiable concentrate was used as in the thrips control experiments.

Following this experiment, several badly infested farms were treated with Dieldrin at the lesser dosage. Control was satisfactory.

SEVILLA RESEARCH LABORATORY

Santa Marta, Colombia

After the termination of the large, heavily wind-damaged agronomy experiments at the close of 1957, a period of reorientation followed for the Sevilla Station. The administration and supervision of the experimental area had been the primary objective and had required the full attention of superintendent and assistant. Comparatively few problems concerning pests and diseases had been encountered. At the beginning of 1958, however, conditions had changed. A new course for the activities of the Station was indicated as a consequence of new developments, the most important of which were:

A. The realization that the Fusarium wilt infection in the Rio Frio District was more widespread than was originally assumed.

B. A suspected virus condition which made its appearance in the Sevilla District.

C. The change from Bordeaux to oil spray, which necessitated increased vigilance for disturbances in the ecological balance.

Apart from giving attention to these problems the Station carried on fertilizer trials in observation plots for mixed fertilizer and for different levels of nitrogen. At the request of the Management a preliminary selection of seed trees was made in the oil palm plantings and advice was given on seedbed and nursery care. The "fetish palm" or idolatrica-type was studied and a synopsis prepared of literature on the subject. Disorders and abnormalities in bananas were checked.

Potential evapotranspiration was introduced as a means to estimate water requirements and an attempt at the determination of water retention capacities of Colombia soils is underway.

Fusarium wilt

It was not until June 1957, that Dr. R. H. Stover positively identified the suspected condition of a few mats in Martiana Farm, Rio Frio District, as Fusarium oxysporum f cubense.

A quiet search for centers of infection was commenced to ascertain the extent of the disease without unduly disturbing the owners of the farms concerned. At the close of 1957, a total of 700 diseased mats had been found in 13 farms, 100 cases had been marked with numbered stakes to keep case histories and 12 healthy seed had been planted in a heavily infected section at Enana Farm.

Now, at the close of 1958, we know of roughly 2000 cases scattered in 30 farms. A survey of cases shows that although the infection is generally found along the irrigation ditch, the cogedero Enano, there is some infection in farms not within this general area. We have no way of knowing whether the cases we find are new or recurrent infections. Several owners have grown suspicious of the disease and chop out infected plants. This makes a proper estimate extremely difficult.

We have been fortunate in that no severe blowdowns have hit the area. O the twelve healthy replants, the first one came down with Fusarium wilt after six months, 50% was showing disease symptoms after eight months and after one year all replants had contracted the disease. Originally 100 case histories were kept, but 21 markers were lost leaving us with 79 mats under observation. The case histories have

been kept since January 1958. The results so far are:

	April	May	Sept.	Nov.
Percentage healthy after initial infection	67.1	64.5	30.4	21.5
No healthy regrowth and/or recurring infection	32.9	35.5	69.6	78.5

After one year of observation 24 out of 79 plants have shot fruit. This is 30.4%, which can be divided as follows:

Harvested	6.3 per cent
Plant diseased while hanging	11.4 " "
Still hanging	12.7 " "

The practical implication of these figures is that after one year 80% of the cases has proven to be totally unproductive. In many instances there is only feeble regrowth and 17.7% has not shown any sign of life for three months or more.

All cases observed show the syndrome of the non-yellowing type of Fusarium wilt. Whenever there was suspicion of the yellowing type, diseased tissue was sent to La Lima. Only the cultivar associated with the non-yellowing type was found.

In March 1958, a government sponsored commission of plant pathologists, headed by Dr. K. L. Skiles, spent three days in Sevilla to check our findings of Fusarium wilt.

Los Angeles Disorder

The Los Angeles Disorder is a suspected virus condition first observed in and named after Los Angeles Farm in the Sevilla District. The condition was first described by the undersigned and subsequently investigated by Drs. N. C. Thornton, V. C. Dunlap and R. C. Bullock.

The condition disappeared during the rainy season but showed up again as soon as dry weather set in. In May, all suspected mats were dug up and diced, with the exception of a few moderate cases in nearby Peralonso Farm. None of the replants in the formerly infected area have shown the condition so far. The Peralonso mats are developing normally.

Five of the worst cases from Los Angeles were dug up and the bullheads planted in an irrigated pasture at the Patuca cattle farm. Development has been slow but no virus symptoms are present.

Fusarium Stalk Rot

As was to be expected after a season of heavy and repeated wind damage, a certain amount of stalk heart rot is prevalent in our plantings. Most plants are coming back slowly, a few are beyond recovery. Those are cut out during normal pruning cycles.

Caterpillar damage

The change from Bordeaux to oil spray for Sigatoka control so far has not caused any sudden increases in banana pests as in some other Divisions. In the course of the year routine collections were made of the limacodid and brassolid caterpillars. The limacodid leaf-feeding caterpillar (saddle-back) appeared in number only in the oil palm plantings. The attack was contained by natural means. A very active pupal parasite was observed ovipositing in the clusters of cocoons at the leaf bases. This dipterous pupal parasite was reared in the laboratory and sent to La Lima for identification.

The brassolid Opsiphanes was rarely found, chiefly in the oil palms and often heavily parasitized. Two different wasps attack the insect, one during the larval, the other during the pupal stage.

The pupal parasites were reared in the laboratory and taken to La Lima for identification. A few Caligo butterflies have been observed, but so far not a single forked caterpillar has been reported. Light but not abnormal lead damage is caused by geometrid and Ceramidia larvae. Insect life in general does not seem to have increased during the year.

Banana stalk borer incidence is light. The moths have become a rare insect in Colombian plantations. In the course of the year routine checks were made for banana root borers. As in previous years not a single specimen was found.

Fertilizer experiments

The comparison plots for nitrogen and mixed fertilizer were carried on during the year. The plots were put in the beginning of 1957. Nitrogen is applied at five levels, from 100 to 500 pounds per acre. The treatments for the other type of experiment are: N, P, K, NP, NK, NPK. The levels are 400 lbs. of nitrogen, 300 lbs. P_2O_5, 1000 lbs. K_2O, for single treatments as well as combinations. There are seven experiments of each type.

In spite of the considerable amount of fertilizer applied, no consistent and/or clearly-defined differences have shown between the treatments. All plots suffered wind damage which makes appraisal even more difficult. At present there is a considerable amount of fruit hanging in the experimental plots, and some biometrical work is underway to determine whether we can glean some information from

Farm	Date	Total Caterpillars	Total Pupae	Percentage of Parasitized pupae
Papayos	Jan.-58	12	54	79.6
	Mar.-58	3	25	96.0
	May -58	17	16	100.0
San Francisco	May -57	228	767	86.9
	July-57	37	153	94.7
	Sept.57	86	278	86.6
	Nov.-57	4	24	100.0
Sangay	May-57	367	253	53.1
	July-57	81	870	92.5
	Sept-57	87	497	92.4
	Nov.-57	6	139	97.1
	Jan.-58	1	43	100.0
	Mar.-58	4	8	100.0
Esperanza	May -57	186	851	92.0
	July-57	23	394	95.9
	Sept-57	46	199	90.9
	Nov.-57	7	66	96.9
	Jan.-58	2	17	100.0

We have tried the same insecticides, at the same rates, that we used on Caligo but none has been able to control Opsiphanes.

Ceramidia control

Two species of Ceramidia has been found in Tenguel: C. viridis and C. caurensis; and right now they are responsible for most of the damage caused by leaf-feeding caterpillars in Tenguel.

There are two natural enemies attacking Ceramidia: The first is a Tachinid fly, smaller than the one that attacks Caligo, and the second is a black wasp; both of them parasitize on the pupae. Unfortunately there are not very many of these parasites; consequently the percentage of parasitism on Ceramidia is very low.

For controlling Ceramidia we have tried Toxaphene, Dieldrin, Malathion, Perthane, Spray oil.

Dieldrin and Toxaphene have proved to be the best. At the moment, we are using Dieldrin which is being applied by means of knapsack sprayers at a rate of 1 pound active per acre in two applications 20-days apart. (The first applications were made at 10-days interval, but experience has proved that 20-days work better.)

One application only has been tried versus two; in one instance one application only worked well in plantilla farms, (short plants with good coverage by moto-blo sprayers) giving about 3 months control; in the other instance one application was tried in farms in maintenance, (tall plants with only partial application to leaf surface) but did not work, giving only one month control.

Two Dieldrin applications on farms in maintenance brought control of Ceramidia for 3 or 4 months.

All these applications have been done using low volume knapsack sprayers and the insecticide applied as water emulsion.

Sibine caterpillars

Saddle-back caterpillars had a very rapid increase, mainly in San Francisco Farm after we sprayed Dieldrin to control Ceramidia.

Damage in San Francisco was very high from May to August. In August

a fungus attacked the caterpillars killing over 90% of them. This is a white, powdery fungus that covers the whole body of the larva; the caterpillar remains stuck to the leaves for a long time. The chart below shows an idea of the virulence of this fungus.

Date	Total Caterpillars	Parasitized	Per cent Parasitized
August 13-58	539	205	38%
" 19-58	285	168	58
" 27-58	158	143	90
Sept. 22-58	73	59	80

As we can see in the chart, in little over a month this very high population of Sibine caterpillars was almost wiped out.

We have used Dieldrin, Toxaphene, Malathion and Perthane trying to control Saddle-back; so far we know that Dieldrin does not have any toxic effect on Sibine caterpillars. About the effect of the other insecticides on Saddle-back caterpillar we cannot comment, since at the same time that we were making the experiment, the parasitic fungus attacked and killed most of the caterpillars from the treated and check plots; so we were unable to collect any data from this experiment.

SCIENTIFIC PUBLICATIONS

STOVER, R. H. 1958. Studies on Fusarium wilt of bananas. II. Some factors influencing survival and saprophytic multiplication of F. oxysporum f cubense in soil. CAN. JOURN. BOT. 36: 311-324.

STOVER, R. H. 1958. Studies on Fusarium wilt of bananas. III. Influence of soil fungitoxins on behavior of F. oxysporum f cubense in soil extracts and diffusates. CAN. JOURN. BOT. 36: 439-453.

STOVER, R. H. and S. R. FREIBERG. 1958. Effect of carbon dioxide on multiplication of Fusarium in soil. NATURE 181: 788-789.

STOVER, R. H. and Max J. FIELDING. 1958. Nematodes associated with root injury of Musa species in Honduran banana soils. PIANT DISEASE REPORTER 42: 8, 938-940.

SEQUEIRA, Luis. 1958. Bacterial wilt of bananas: Dissemination of the pathogen and control of the disease. PHYTOPATHOLOGY 48: 2, 64-69.

TIMONIN, M. I. 1958. Scaptocoris talpa on roots of banana and other plants in Honduras. FAO PIANT PROTECTION BULLETIN 6: 5.

BUDDENHAGEN, Ivan W. 1958. Induced mutations and variability in Phytophthora cactorum. THE AMERICAN JOURN. OF BOTANY 45: 5.

SEQUEIRA, Luis, Taylor A. STEEVES, Margaret W. STEEVES and Joseph M. RIEDHART. 1958. Role of root injury in Panama Disease infections. NATURE 182: 309.

STOVER, R. H. 1959. Studies on Fusarium wilt of bananas. IV. Clonal differentiation among wild type isolates of F. oxysporum f cubense. CAN. JOURN. BOTANY (In press)

STOVER, R. H. 1959. Bacterial rhizome rot of bananas. PHYTOPATHOLOGY (In press)

PAGE, O. T. 1959. Observations on water economy of Fusarium-infected banana plants. PHYTOPATHOLOGY (In press)

PAGE, O. T. 1959. Fusaric acid in banana plants infected with Fusarium oxysporum f cubense. PHYTOPATHOLOGY. (In press)

BUTLER, Alfred F. 1959. Fertilizer experiments with the Gros Michel banana. TROPICAL AGRICULTURE (In press)

SEQUEIRA, Luis, BUDDENHAGEN, Ivan. 1958. Disinfectants and tool disinfection for prevention of spread of bacterial wilt of bananas. PLANT DISEASE REPORTER (In press)

LOOS, Clive A. 1958. Symptom expression of Fusarium wilt disease of the Gros Michel banana in the presence of Radopholus similis (Cobb, 1893) Thorne, 1949 and Meloidogyne incognita acrita Chitwood, 1949.

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PART 2
SUMMARY

Pathology

Investigations on Moko Disease carried out during this year have established the following points:

Pseudomonas solanacearum exists in Coto as two different strains. The B or banana strain attacks bananas and can also attack Heliconia species. The T or tomato strain attacks tomatoes and eights species of local weeds, but it does not attack bananas. In plowed areas, where the T-strain was prevalent, many weeds wilted but bananas did not develop Moko disease.

The addition of tyrosine to culture media provides a good basis for the identification of strains of P. solanacearum pathogenic to bananas. Most T-strain isolates produce a dark pigment on tyrosine media, B-strain isolates produce no such pigment.

In detached banana leaf tissue, P. solanacearum disappeared within a period of decomposition of only eight days and it is clear that this organism can survive in detached host tissue for a period of a few days only. In soils stored for 5 1/2 months, the Moko organism could be detected only in soils stored at 75-100% humidity levels. Other tests indicate that, in the absence of host plants, the Moko organism will not survive in soils maintained at 65% w.h.c. for six months.

Banana plants did not develop Moko disease when heavy suspensions of Pseudomonas solanacearum were added to the surface of the soil. However, when the roots were injured by cutting at the time the soil was inoculated, 100% infection was generally obtained. Similarly, when the Moko organism was applied to intact roots, or roots with shallow injuries, no infection occurred, but disease resulted in every case when the vascular system was exposed.

Laboratory and field tests have shown that formaldehyde is the most practical material for tool disinfection. The use of a scabbard of 10 per cent formaldehyde in which two machetes can be immersed is a simple and satisfactory means of eliminating Moko disease transmission by machetes. Of 18 other chemicals tested, some were effective at lower concentrations than was formaldehyde but were inactivated by banana sap or soil.

Chloropicrin, applied to soil in cylinders at the rate of 8 c.c. per square foot, effectively eliminated Pseudomonas solanacearum from the soil.

In extensive fallowing experiments in the field, the presence of a cover crop (kudzu) appears to reduce spread of Moko disease. These results have not been fully confirmed.

Continued investigations on Panama disease have revealed that: Urea, when applied at levels as low as 1 and 3/4 tons per acre, is an extremely effective fungicide against Fusarium oxysporum f. cubense. Soils treated with high amounts of urea retain a strong toxicity towards the Panama disease fungus for periods as long as five months.

Intensive laboratory investigations on the nature of the toxic effect of urea-treated soils indicated that the buildup in nitrites is directly correlated with the toxicity of these soils to the Panama and Moko disease organisms. Complete eradication of the fungus can be obtained at nitrite-N levels slightly over 200 ppm. When nitrogen fertilizers were applied in amounts adjusted to supply identical amounts of nitrogen, neither ammonium nor nitrate nitrogen were toxic to Fusarium, but urea and sodium nitrite were highly toxic. Rates of urea as high as 7 tons per acre were used in field experiments established to determine the practical possibilities of utilizing this material for disease control.

In field experiments, a one-year rotation with velvet beans, sorghum, and sugar cane as green manure treatments indicated favorable effects on Panama disease control only from the sugar cane treatment. The tremendous increase in the numbers of yeasts that occurs within a few hours after incorporation of sugar cane may be important in the Fusarium - depressing effect of cane soils. Permanent crop rotation experiments have been established with good results to date.

There was no correlation between chlamydospore survival and "resistance" of Armuelles soils to Panama disease. Other important factors affecting survival of the fungus in the soil are the presence of sugars exuded from wounded banana roots and the presence of certain chytrids which stimulate Fusarium sporulation and growth.

A considerable number of systemic fungicides have been found to be completely ineffective for Panama disease control.

Preliminary results from field plots comparing oil spray vs. Bordeaux spray for Sigatoka control indicate no apparent differences in plant growth under the two treatments. The information to date indicates no significant difference between the number of leaves on the plants in the oil plots and the number in the Bordeaux plots. Soil factors obscure any possible differences in plant height under the two treatments.

In tests carried out to obtain clean seed it was found that hot-water treatments that button seed can withstand do not eradicate either Fusarium or Pseudomonas located internally in the buttons. By careful selection of seed and heat treatment to eliminate superficial parasites, it has been possible to establish a nucleus of disease-free Gros Michel seed. Similar efforts are being made to obtain a source of clean Cocos seed for the immediate use of the Golfito Division.

Entomology

The entomological activities of this Station during 1958 have been largely confined to studying life histories and devising methods of control of the various species of leaf-feeding caterpillars attacking bananas.

The banana leaf-feeding caterpillar Ceramidia butleri requires about 35 days for development from egg to adult emergence. The larva passes through 6 to 7 instars. Dieldrin proved to be more effective than other insecticides in the control of this caterpillar, but gave relatively poor results when used on other leaf-feeding caterpillars, such as Sibine and Caligo species. Toxaphene was generally superior

over a larger range of caterpillars than either dieldrin, DDT, or malathion.

A laboratory method was devised to study the effectiveness of insecticide - oil mixtures applied on leaf disks. With this method, rates equivalent to those applied by helicopter in the field were obtained and it was possible to screen a large number of chemicals as possible insecticides for control of the major leaf pests of bananas.

Good control of bacterial tip rot of bananas was obtained by fruit bagging with polyethylene.

SPECIAL SUPPLEMENTARY REPORT

April 15, 1959

Special attention is called to preliminary reports from Coto Research Station where Pseudomonas solanacearum has been found attacking a species of Heliconia (platanillo) in extensive areas of virgin woodland now scheduled for planting or already planted in the Golfito Division of Costa Rica. These new findings have serious implications and create an entirely new situation regarding Moko disease in virgin areas.

On March 31, 1959, inspection of an area in Farm 54, Golfito Division, where five possible cases of Moko disease had been reported, confirmed presence of the disease and simultaneously revealed Pseudomonas solanacearum infection of several Heliconia plants in the immediate vicinity. These plants appeared normal except for a slight browning and distortion of the center leaf. Examination of the rhizome, however, revealed a soft watery rot, and all isolations from this tissue yielded colonies of P. solanacearum of the banana type described in our 1958 Annual Report.

Pathogenicity tests do not yet allow definite conclusions regarding ability of the Heliconia isolates to attack bananas. However, inoculated Gros Michel plants showed symptoms of wilt within 7 days. Agricultural personnel are reporting Moko disease in the same areas where infected Heliconia plants were found which is indicative of the close correlation between the two diseases.

Studies to date indicate that the banana type of P. solanacearum is present in virgin woodland areas of the Coto Valley, where the organism attacks native Heliconias. Due to the wide diversity of isolates obtained from Heliconias, they may be a bridging host between susceptible solanaceous weeds and the banana plant. Work is now in progress to explore this possibility.

Surveys of Heliconia disease are now planned at all farms which have been scheduled for future development.

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PATHOLOGY

Moko Disease Investigations

A. Studies on the biology of Pseudomonas solanacearum

1. Host range

a) General

Intensive laboratory and field studies carried out during this year indicate clearly that Pseudomonas solanacearum exists locally as two different strains. The B or banana strain attacks bananas and can also attack Heliconia species. The T or tomato strain attacks tomatoes and eight species of local weeds, including one Heliconia species, but it does not attack bananas. Both strains occur together in some Moko abandonments.

All isolates obtained from tomato, tobacco and potato from different parts of the world were similar to the T-strain in that they were unable to attack bananas.

Some banana isolates can attack certain weeds as well as tomatoes under artificial inoculation conditions; but no B-strain isolate has yet been obtained from weeds wilting naturally in the field (Heliconia excepted).

Other than Heliconia, the solanaceous weed Physalis angulata is the most likely candidate host for the B-strain, since it has wilted when planted in soil to which the B-strain was added.

No Heliconia growing in the virgin jungle has ever been found to be attacked by Pseudomonas solanacearum. The few diseased Heliconia plants found in Moko abandonments indicate that the role of Heliconia in maintaining the Moko organism is either very small, or is obscure.

Most of the other weed hosts are transitional species which invade newly plowed fields or disturbed areas along roads, etc. They are soon supplanted in the natural succession, but where they are attacked by Pseudomonas, as in some plow-fallow Moko abandonments, they undoubtedly serve to build up the population of the T-strain in the soil.

The relationship between the T- and B-strains is unknown at present. However, the experiments carried out to date show no correlation between the presence of the T-strain and banana wilt.

At the present time there is a moderate amount of evidence against and no evidence for the presence of the B-strain in the virgin areas at Coto.

On the other hand the T-strain has been isolated from two symptomless Eclipta alba plants growing approximately one half a kilometer from bananas, and above them, in a hilly area. This area was taken out of virgin jungle in 1957 and converted to a pasture. It is unknown if traffic in the area, or possible insect transmission, were responsible for the presence of the organism there. However, this remains as the first evidence for the presence of the T-strain in a virgin area. Many other isolations from weeds there and in other virgin areas have all been negative.

B- and T-strain isolates can be separated by visually comparing their colonial morphology on a tetrazolium medium.

Similarly, there is a fairly good correlation between the T-strains and the ability to turn a tyrosine medium brown. No B-strain isolate has the ability to turn the tyrosine medium brown whereas most (but not all) of the T-strain isolates are able to turn the medium brown. In addition, the T-strain isolates from weeds at Coto differ from the isolates tested from other parts of the world in their inability to survive on PDA medium.

B-strain isolates differ in their virulence to bananas (as well as to tomatoes), in their colonial morphology, and in other respects. Two types are clearly separable on the basis of symptom expression in bananas. One is designated the "normal-type", the other is designated the "distortion-type". Normal-type isolates cause typical Moko disease symptoms, with yellowing and collapse of the leaves. Distortion-type isolates, which are rare, cause distortion and stunting symptoms quite unlike the typical Moko syndrome (Fig. 1) and do not kill the plants. Some of the normal-type isolates also possess the ability to cause lateral bending and breakage of the plant (Figure 2). In addition, the normal type isolates vary in their ability to cause rapid death of the host. Some kill the host within 7-10 days, others take one month or longer.

Some T-strain isolates, especially those from Eclipta alba at Coto, can cause slight distortion symptoms on banana, similar but less severe than those caused by the distortion-type B-strain isolates. In addition these T-type isolates can live for a considerable time in bananas (up to $2\frac{1}{2}$ months, the longest time tested) but appear to be unable to move freely or proliferate in the bananas as do the B-strain bacteria. The long association with the internal environment of the banana plant has so far not resulted in the appearance of a pathogenic B-strain mutant from the T-strain isolates.

Pandok beurem, a true seeded banana, is susceptible to both the B- and T-strains of Pseudomonas solanacearum, through both root and pseudostem inoculations (Figure 3). Pandok should therefore be an excellent indicator host plant to use to prove whether or not P. solanacearum is present in virgin areas. Balbisiana, another true seeded Musa, is susceptible only to the B-strain.

Banana varieties Valery, Lacatan, Congo, Tumoc, Giant Fig, Cavendish, Vimama, and the so called Phillipine banana, were susceptible to B-strain P. solanacearum when inoculated through the pseudostem.

B-strain isolates mutate readily to at least two other morphologically different colony types. The mutant colonies are small and red on tetrazolium, quite different from the large white fluidal colonies of the original isolations. Mutation seems to be unidirectional from the fluidal type to the small red types. One of the mutant types produces no symptoms when inoculated into bananas. The other mutant produces distortion-type symptoms similar to those from

Figure 1A. Distortion-type Moko symptoms, typical of a few B-strain isolates. 45 days after inoculation.

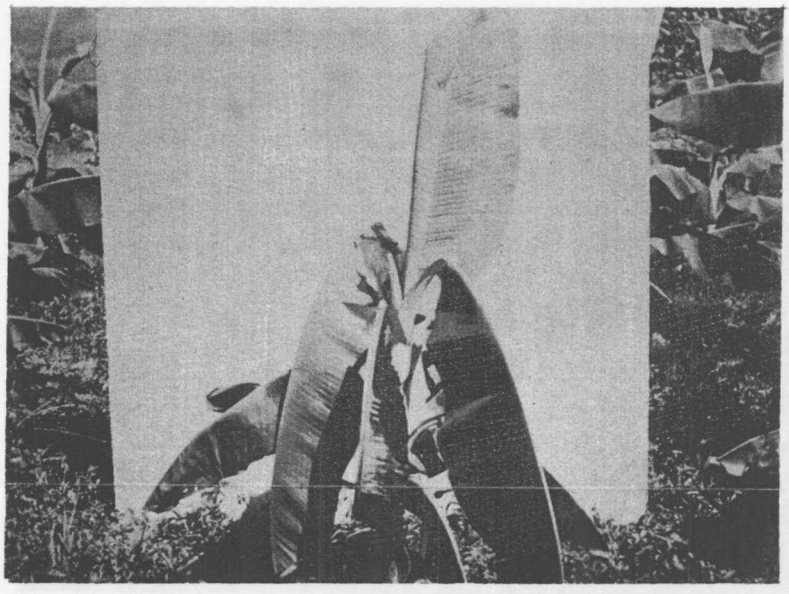


Figure 1B. Normal-type Moko symptoms, typical of the majority of Moko isolates; 14 days after inoculation.

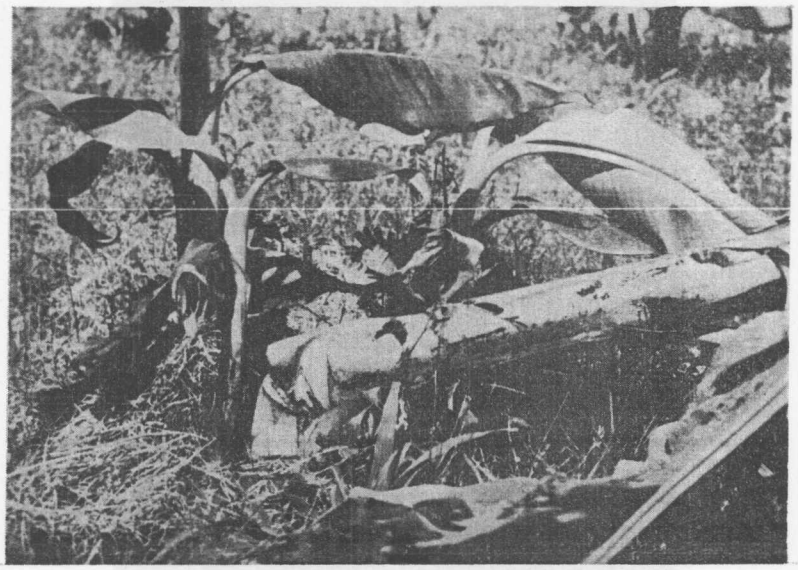
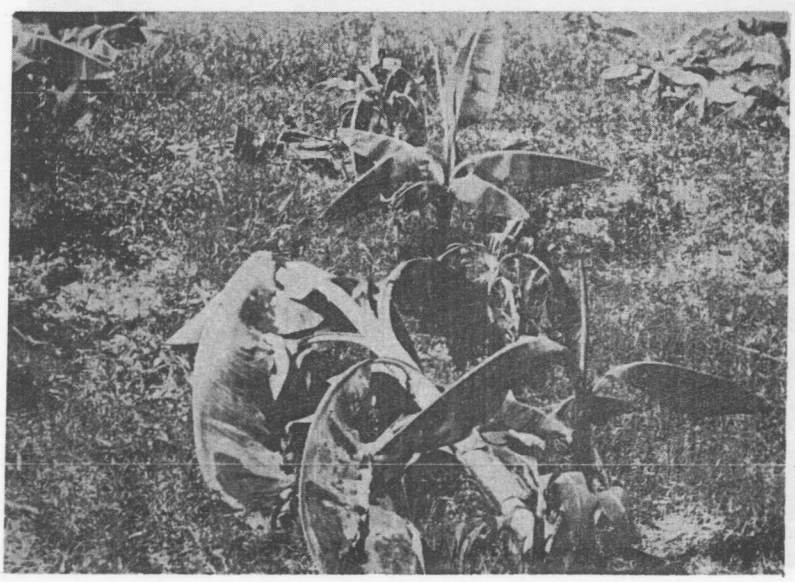


Figure 2. Bending and breaking of plant, typical following inoculation with certain Moko isolates. 14 days after inoculation.



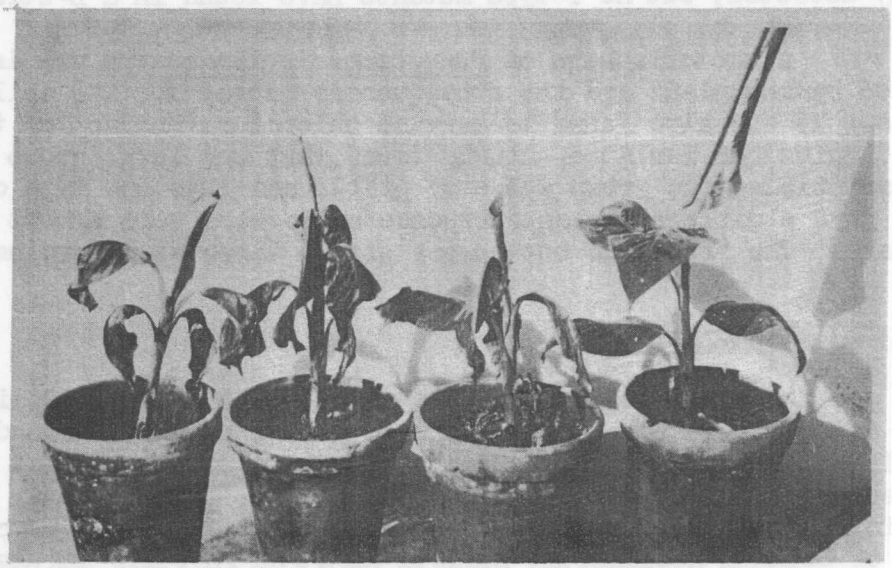


Figure 3. Pandok seedlings dying after stem inoculation with *P. solanacearum* Left to right: B-strain, B-strain, T-strain, Control, 16 days after inoculation.

"distortion-type" fluidal isolates, or to those from some T-strain isolates from Eclipta alba.

Chloromycetin can be used to select T-strain cells from mixed population of T- and B-strain cells. This provides a tool to search for T-strain cells in a large population of B-strain cells. This is possible because of the lesser sensitivity of the T-strain to chloromycetin, as was initially demonstrated by A. Kelman. B-strain chloromycetin-resistant mutants have been readily detected; but no T-type mutants have found in a B-strain.

A bacteriophage of Pseudomonas solanacearum was isolated from a diseased banana plant and was subsequently tested for its ability to type strains. It was also found to survive chloroform extraction treatment. When tested against 46 isolates, it was found that one large group of isolates was not susceptible, one group was susceptible and gave one type of plaque response, others gave a different plaque response and others were almost eradicated by the phage. The reactions cut across strain lines as determined by host response.

b) Field studies

An examination of a Moko abandonment in Farm 49, in May 1958, two months after it had been plowed, showed that a complete ground cover had been established. Transects showed that the most abundant species were grasses, followed by Trema micrantha, Solanum nodiflorum, Eclipta alba, Solanum torvum, two unindentified species, and Physalis angulata. A high percentage of the plants of Solanum nodiflorum, Eclipta alba and Physalis angulata were wilting from attack by the T-strain of P. solanacearum. A few plants of Solanum torvum were similarly affected.

The less abundant species present were Momordica sp. Cyperus sp. Unknown A., Amaranthus spinosus, Portulaca oleraceae, Physalis pubescens, Heliconia sp., tomato, Sida rhombifolia, Ochroma lagopus, Lantana sp., Ceiba pentandra, Phyllanthus niruri, Pousettia sp., Chamaesyce nutants, C. hirta, Fleura aestuans, Cecropia sp., Calathea sp., Heliotropium sp., and Tournefortia angustiflora. Of this group the following were affected by the T-strain P. solanacearum: Unkown A, Physalis pubescens, Heliconia sp., tomato, and Tournefortia angustiflora. Heliconia was also found to be affected by the B-strain of P. solanacearum.

The relative susceptibility of these hosts, based on the percent wilting in the Moko abandonment in Farm 49, is presented in Table 1.

Table 1. Relative susceptibility of nine local plants to natural infection by T-strain *P. solanacearum*

Plant Species	Susceptibility rating*
Solanum nodiflorum	HS
Physalis angulata	HS
Eclipta alba	MS
Tomato	MS
Unkown A	LS
Tournefortia angustiflora	LS
Solanum torvum	VLS
Physalis pubescens	VLS
Heliconia sp.	No rating - insufficient plants

*HS - High susceptibility - over 50% wilting,
MS - Moderate susceptibility,
LS - Low susceptibility,
VLS- Very low susceptibility - less than 1% wilting.

c) Cross inoculations

A large number of isolates were tested by inoculating young banana plants via the pseudostem. Ten to fifteen plants 2-3 feet tall were inoculated per isolate with 1 c.c. of bacterial suspension by hypodermic syringe.

Pooled data from a number of inoculations are presented in Table 2.

Table 2. Disease incidence of Gros Michel banana plants following pseudostem inoculations with different isolates of P. solanacearum.

No. of isolates	Source	Morphological type	Disease Incidence*
10	Bananas, Coto	B	100
2	Bananas, Coto	B	Distortion
3	Bananas, Honduras	B	100
1	Bananas, Honduras	different	100
1	Plantain, Coto	B	100
4	Bananas, Panama	B	100
2	Heliconia, Coto	B	100
1	Heliconia, Coto	B	Distortion
1	Banana, Coto	Mutant A	0
1	Banana, Coto	Mutant B	Distortion
2	P. angulata, Coto	B	100
14	Physalis angulata, Coto	T	0
1	Physalis pubescens, Coto	T	0
5	Solanum nodiflorum, Coto	T	0
1	Tomato, Coto	T	0
1	Tomato, Coto	T	10**
1	S. torvum, Coto	T	0
1	Tobacco, Panama	T	0
1	Tomato, Trinidad	T	0
2	Eclipta alba, Coto	T	0***
1	Unknown A. Coto	T	0
2	Solanum "ornamental", Coto	T	0
1	Heliconia, Coto	T	0
5	Tomato, U.S.A.	T	0
1	Tobacco, U.S.A.	T	0
1	Potato, Israel	Different	0

*Per cent of plants showing external wilt symptoms from 20-44 days after inoculation.

**One plant showed slight wilt symptoms; the reisolate was inoculated on bananas again and caused no symptoms.

***Eclipta alba isolates from virgin area produced most severe distortion symptoms of all T-strain isolates.

These data clearly support the hypothesis that different pathogenic strains occur and that the presence of bacterial wilt in other plants may have nothing to do with danger for subsequent banana culture. These data however pose some interesting questions. Can the distortion producing T-strain isolates from Eclipta alba mutate to the distortion producing B-strain which subsequently might evolve into the normal-type virulent banana strain? Or can the B-strain mutate to the T-strain? If neither occurs how did both B- and T-strain isolates come to be present in some Moko abandonments?

So far the T-strain has been found in weeds in 4 different Moko

abandonments at Coto, as well as in a pasture surrounded by bananas, and in a pasture recently made in an elevated virgin area.

The T-strain has strikingly never been found in weeds present in a large Moko abandonment in Farm 51. It likewise was not found from abandonments in Pajuil Farm, Laurel District; nor in a virgin area in Coto projected for Farms 56 and 57. In both of the latter areas a concerted effort was made to detect wilted Eclipta alba plants growing there naturally.

Other interesting questions are: How do the two strains differ, making one unable to grow and proliferate in the banana, and the other encountering an ideal environment there? Similarly, how and why does the banana manage to limit the growth and development of the one strain and not of the other?

B-strain and T-strain isolates have been tested by inoculating not only pseudostem, but also rhizome and roots of bananas. Roots were inoculated by pouring a suspension of bacteria onto the soil in the root area of potted plants, and cutting the roots at the same time. Results have been the same in all cases - wilt results no matter where the inoculation site with the B-strain and no disease results from any site with the T-strain.

A limited number of inoculations have been made on Marglobe tomatoes with B- and T-type isolates. The T-type isolates from weed hosts at Coto are highly pathogenic to tomatoes, in some cases appearing more virulent than isolates from tomatoes in North Caroline. B-strain isolates from bananas are in general less pathogenic than T-type isolates. However, wilting and death can follow both stem and root inoculation with some banana isolates. Similarly, Physalis angulata plants have wilted following soil infestation with high levels of the banana strain. Datura stramonium also is differentially susceptible to the 2 strains.

d) Correlations between weed- and banana- wilt.

(1) In a plow-fallow Moko abandonment in Farm 49 where there was a high incidence of wilting weeds, 100 bananas were planted in four separated plots. Seeds of Solanum nodiflorum and Physalis angulata were also planted among the bananas. Within 2 months many hundreds of these weeds were wilting immediately around the banana plants. Only T-strain isolates were recovered from these weeds. Within 3 months the majority of the weeds had wilted. At this time the roots of the banana were cut; even with root cutting, the bananas did not become diseased. After 7 months, when the experiment was terminated no bananas had become diseased.

(2) In a garden near the laboratory where tomatoes had wilted with bacterial wilt, rows of bananas were planted adjacent to 2 rows of tomatoes. The majority of the tomatoes wilted from bacterial wilt. Only the T-strain was isolated from these tomatoes. The roots of the banana plants were cut 3 months after planting and after 7 months, when the experiment was terminated, no bananas had become diseased.

(3) In a large plowed abandonment in Farm 51 a search for weeds that were wilting was unsuccessful. This was surprising since the same weeds a short distance away in Farm 49 were wilting abundantly. Four rows of mixed tomatoes, Solanum nodiflorum and Physalis angulata were established beside

young banana plants which were part of another experiment. None of the weeds wilted even though bananas wilted in adjacent rows. However, due to the extreme difficulty of establishing vigorous solanaceous plants in the rainy season, this test was not considered to be enterily satisfactory. Soil from around dying banana plants was placed in cans in which Physalis angulata was planted. None of these plants have wilted in the short period the test has run. This experiment is not yet complete.

To summarize: These 3 simple experiments indicate that there may be no direct correlation between weed- and banana- wilt.

2. Survival of the Moko organism

An understanding of the problem of survival of the Moko bacterium in soil and in host tissue is of fundamental importance in planning control of the disease. As with other root diseases, little is known about the ability of the Moko bacterium to survive under a variety of conditions. Just how the organism survives in the soil and how survival is affected by soil pH, moisture texture, other microorganisms, etc. is purely a matter of speculation. The paucity of our knowledge on this aspect of Moko disease is merely the reflection of the inherent difficulties of estimating bacterial populations in the soil and the lack of laboratory methods to select out the pathogenic forms of Pseudomonas solanacearum from the complex bacterial flora of the soil.

During this year, efforts have been made to attack the problem of survival of the Moko bacterium from various angles. Laboratory, greenhouse, and field studies have been initiated and methods have been developed which have been of considerable usefulness in the estimation of population levels of the bacterium.

a) Methods for the determination of survival of the Moko bacterium

Several laboratory methods have been developed as an aid in determining population levels of Pseudomonas solanacearum in the soil and in host tissue. The most useful technique consists of direct isolation on Kelman's tetrazolium chloride medium. Population levels can be estimated by appropriate plate dilution procedures. In our method, 1/10thc.c. of a highly diluted sample from soil or host tissue is spread evenly over the surface of the agar with a sterile glass rod. The typical distinctive appearance of the pathogenic types of Pseudomonas solanacearum on Kelman's medium makes colony counting a relatively simple procedure. Kelman's technique is by far the most useful tool that we have at our disposal in population studies of the Moko bacterium.

The selective qualities of Kelman's medium have been improved considerably through the addition of L-Tyrosine. During an investigation of possible selective media for the isolation of Pseudomonas solanacearum from soil, it was determined that banana isolates (139 and F80) do not produce pigment when grown in a tyrosine medium (.1%) whereas a tomato isolate (K77) readily produces a dark pigment when grown in the same medium (Fig. 4). Therefore, the addition of tyrosine to Kelman's medium readily provides a basis not only for the identification of P. solanacearum but for the determination of strains pathogenic to bananas as well.

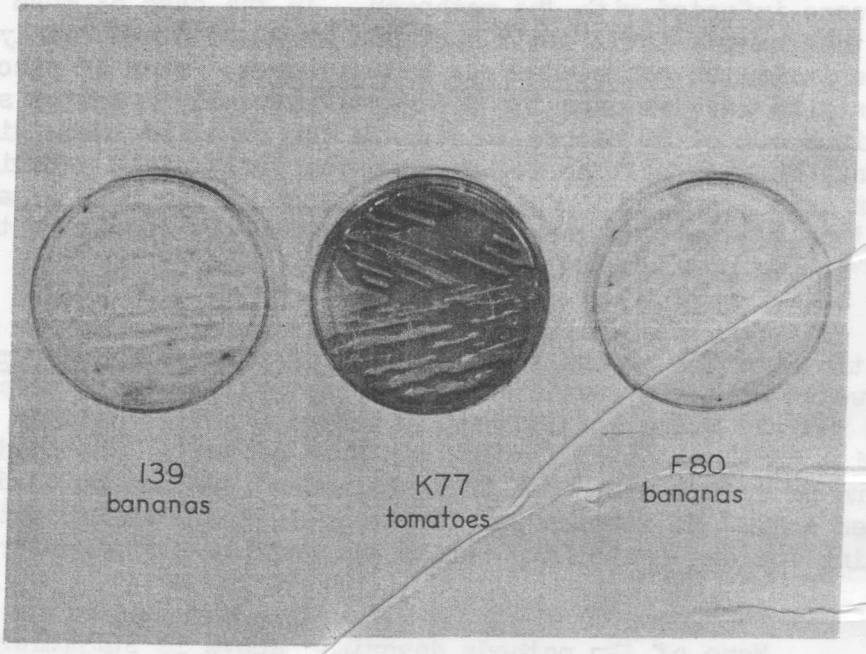


Figure 4. Pigment formation in a tyrosine medium by *Pseudomonas solanacearum* isolates from bananas and tomatoes.

Through preliminary tests with artificially and naturally infested soils, it was determined that serial dilution techniques on a selective medium are useful only in cases of heavily infected soils. At low population levels, the sampling problems involved are such that it is not practical to attempt direct isolation of the organism. Therefore, baiting techniques and seedling susceptibility tests were investigated as possible tools for the determination of low population levels. Baiting techniques usually consist of exposing parts of susceptible plants to infested soils, where these plant parts can become infected with the pathogen. In the case of Moko disease, it was found that banana fruit stalk sections provided a moderately successful method for the isolation of Pseudomonas solanacearum. When 6" pieces of freshly cut fruit stalks were exposed for 5 days to naturally infested soils, it was possible in 3 out of 20 pieces to isolate the organism from discolored vascular bundles. Whereas baiting techniques with fruit stalk material appear to be of help in determining the presence or absence of the Moko organism in the soil, the method was not considered to be sufficiently sensitive to allow estimations of the actual populations of the pathogen.

Tomato seedlings, of a variety locally known as California, are susceptible to the banana strain of Pseudomonas solanacearum during the first three weeks of their development. Susceptibility tests were run with this tomato variety grown in artifically infested soil. Wilting occurred only at bacterial populations of 7×10^6 per gram of soil. The need for such a high inoculum potential indicates that the method can not be used for naturally infested soil. It is possible that seedlings of other plants would be more promising for susceptibility trials and work is now in progress along these lines.

None of the methods described above is sufficiently sensitive to allow a proper estimation of low population levels of Pseudomonas solanacearum in the soil. In the final analysis, the banana plant itself provides the only accurate method to detect the presence of the Moko bacterium in the soil. In greenhouse tests, symptoms of Moko disease appeared on young banana plants grown in pots from button-seed planted in naturally infested soil, two weeks after the roots were cut. Since the uninjured root system of a banana plant is resistant to penetration by the Moko bacterium, it is necessary in all cases to injure the roots extensively. Until more practical methods are found the latter remains the only sensitive procedure to detect Pseudomonas solanacearum in the soil.

b) Survival of the Moko bacterium in host tissue

Since banana plants attacked by Moko disease are usually cut down and diced into small pieces with machetes, it seemed important to determine if diseased banana tissue could remain infective for long periods. Uniformly infected material was obtained by cutting a long strip of a diseased leaf sheath into small rectangular pieces of equal weight (1.3 gr.). The leaf pieces were placed on moist paper toweling inside standard Petri dishes. At daily intervals, four leaf samples were removed, disintegrated for 1 minute in a Virtis "23" Homogenizer, and the resulting suspension was plated out, after considerable dilution, on Kelman's TZC medium. The results of population counts made during an incubation period of 8 days are shown in the following table:

Table 3. Survival of Pseudomonas solanacearum in Host Tissue.

Incubation	Colonies per gr. of tissue ($\times 10^7$)
Initial	385
24 hours	1,043
48 hours	4,310
72 hours	2,233
120 hours	1,012
144 hours	80
168 hours	40
192 hours	0

The evidence obtained indicates that the numbers of Moko bacteria per gram of detached tissue increase significantly during the first 48 hours of decomposition. Thereafter, a rapid decline in the population of Pseudomonas occurs so that the pathogen cannot be detected after a period of decomposition of only eight days. The disappearance of the Moko bacterium is correlated with the development of extremely high populations of saprophytic bacteria which render a soft, pulpy consistency to the leaf tissue. Within the limits of our method of isolation, it would appear that the Moko bacterium can survive in detached banana tissue for a period of a few days only.

c) Influence of soil moisture on survival of the Moko bacterium

Since the Moko bacterium cannot survive for extended periods in infected host tissue, it must be able to survive in the soil, competing with other soil microorganisms for available nutrients. Soil conditions, therefore, must determine to a great extent the degree of survival of the Moko bacterium. Soil moisture, in particular, plays an important role in limiting or favoring survival of the bacterium. When soils were stored for 5½ months and maintained at 25, 50, 75, and 100% of their water-holding capacity, the Moko organism could be recovered from buried slides only from soils stored at the 75 and 100% humidity levels. The bacterium was abundant only at the 75% level. The results obtained corroborate results previously reported regarding the extreme sensitivity of the Moko bacterium to dryness.

Even under favorable soil moisture conditions, the Moko organism does not appear capable of survival for very extended periods. Soil contained in large tin cans was inoculated with a heavy bacterial suspension from 48-hour cultures of a pathogenic strain of Pseudomonas solanacearum isolated from bananas. To inoculate the soil, each can was watered with 500 c.c. of a solution containing at least 600,000 bacterial cells per c.c. The soil moisture was adjusted to 65% w.h.c. and the cans were covered with plastic to prevent loss of water. At regular intervals, survival of the bacterium was checked by isolation on TZC medium and by the simple procedure of planting a banana button seed in each can. At the three-month storage level, the Moko organisms could no longer be detected by isolation on TZC agar, but the soils were highly infectious as shown by the development of wilt symptoms on banana plants grown

in the cans. At the six-month level, symptoms of Moko disease did not appear on bananas planted in the test soils, even after the root systems were repeatedly injured. The results indicate that, in the absence of host plants, the Moko organism will not survive in soils maintained at 65% w.h.c. for six months.

d) Survival of the Moko bacterium in urea-amended soils

Because of the practical possibilities of urea applications for Panama disease control, as described in previous sections of this report, tests were carried out with the purpose of determining the effect of urea-amendment on survival of Pseudomonas solanacearum in the soil. Samples of soils treated with 7 tons of urea per acre were weighed into sterile culture tubes and the tubes were inoculated with a heavy suspension of Moko bacteria. Untreated soils were also inoculated. Isolations made after one week indicated that the Moko organism was not present in urea-amended soils whereas it could be easily obtained from the controls.

With the purpose of determining the nature of this toxic effect of urea-treated soils on Pseudomonas, the toxicity of: 1. An impurity of technical urea (biuret) and 2. An intermediate product of urea metabolism (ammonium carbamate) was studied in culture. Biuret was found to produce inhibition of bacterial growth only at concentrations over 1,000 ppm. Ammonium carbamate was not toxic to Pseudomonas at concentrations as high as 10,000 ppm. It was concluded that these materials could not account for the toxicity of urea-treated soils.

Since work reported in another section of this report indicated that the toxicity of urea-treated soils was due to the accumulation of nitrites, the sensitivity of the Moko bacterium to this form of nitrogen was determined in liquid culture. Tubes of peptone-beef extract medium were modified by the addition of sterile solutions of sodium nitrite. The tubes were then inoculated with Pseudomonas solanacearum and growth of the organism was checked within 24 hours. The following results were obtained:

Table 4. Toxicity of Sodium Nitrite to Pseudomonas solanacearum.

$NaNO_2$ Concentration	Growth in tubes after 24 hours			
400 ppm	+	+	+	+
500 ppm	+	+	+	+
600 ppm	+(*)	+(*)	+(*)	+(*)
700 ppm	-	-	-	-
800 ppm	-	-	-	-

*Very limited growth

The results indicate that the Moko bacterium is very sensitive to the presence of nitrites in the medium. Growth is completely inhibited at concentration of nitrites easily attained in soils treated with heavy dressings of urea. Thus, nitrites appear to be responsible for the toxic action of urea-treated soils on Pseudomonas solanacearum.

e) Survival of the Moko bacterium under various cropping sequences

Because crop rotation procedures are known to be of value in the control of many root diseases and survival of the Moko bacterium in the soil appears to be dependent on the presence of susceptible host plants, it seemed important to determine the effect of rotation practices on survival of this bacterium. Fifty concrete cylinders, 24" in diameter, were used in this rotation work. The cylinders were inserted in the ground so that only 2 inches protruded over the surface of the soil. The soil contained within each cylinder was heavily inoculated with a pathogenic strain of the Moko bacterium and 10 cylinders planted to each of the following crops:

1.	Bananas	- Highly susceptible
2.	Tomatoes	- Lightly susceptible
3.	Mimosa invisa	- Not susceptible
4.	Sorghum	- Not susceptible
5.	Bare fallow	- ----

As expected, the first two crops have wilted continuously in the inoculated soil. Good crops of sorghum and Mimosa invisa are being obtained. It is planned to plant all tanks to bananas after a period of six months so that the effects of the various rotation schemes can be determined.

3. Penetration of the Host

a) Importance of root injury

On the basis of preliminary experiments it was reported earlier that root injury played a significant role in infection of banana plants by the Moko bacterium. More recent experiments complement and confirm these results.

No infection occurs when heavy suspensions of Pseudomonas solanacearum are added to the surface of the soil. When, however, the roots are injured by cutting at the time the soil is inoculated, 100% infection is generally obtained (Table 5). The infection, furthermore, can be traced only through the stele of the cut roots. The non-injured roots, on diseased plants in early stages of infection, showed no discoloration.

Table 5. Relationship of root injury and Moko disease

Treatment	No. of plants	No. of Diseased plants
Moko organism - cut roots	5	5
Moko organism - non-cut roots	6	0*
No Moko organism - cut roots	5	0

*Two roots showed slight discoloration. This was believed to result from late infection through wounds of unknown causes.

The results of the experiment clearly indicate that the organism is a wound parasite.

In other experiments, conducted on plants grown in glass front boxes and on plants grown in vermiculite, similar results have been obtained.

It was shown that the organism would enter the root only where the vascular tissue was exposed. When heavy suspensions of the Moko organism were applied to intact roots, or roots with shallow injuries no infection occurred. However, when heavy suspensions were applied to roots with vascular tissue exposed infection readily occurred. It is interesting to note here that these results are similar to those obtained by Drs. L. Sequeira and T. Steeves in the case of penetration of the banana plant by Fusarium oxysporum f. cubense. It appears that this pathogen, likewise, lacks the ability of attack living cells. The results are in agreement with those of a number of other workers.

Another experiment was designed in order to make a preliminary study of the survival of the organism in the soil and to show the significance of root injury in permitting the entrance of the pathogen into the plant. This was accomplished by injuring the root systems of banana plants at various periods of time after having added the Moko organism to the soil. All plants were grown in plastic pots. The soil was inoculated with material from freshly cut suckers. The virulence of the added pathogen as well as that of the recovered pathogen was checked by the colony appearance on tetrazolium medium. Bacterial counts were made using standard dilution techniques. Only virulent colonies were counted. The results of this experiment are shown in Table 6.

Table 6. Relationship between soil inoculation and root injury.

Soil Inoculated Date	Roots Injured Date	Disease Incidence*
		0/0
	September 22	0/0
September 22	September 22	5/5
September 22	September 25	4/5
September 22	September 29	0/0
September 22	October 4	0/0
September 22	October 16	2/5**

*Number showing symptoms over number inoculated
**Two plants probably contracted disease through natural injury.

For clarity the significant results will be listed and discussed in order.

(1) A wound, either artifically inflicted or natural, is necessary to permit the entrance of the pathogen into the plant. This condition is borne out by the fact that only those plants, with the exception of two, which had their roots cut exhibited external and internal Moko disease symptoms. It is assumed that the two exceptions contacted the disease through natural injuries since they exhibited Moko symptoms before the roots were artifically wounded. These results are in perfect agreement with those found previously in this laboratory as well as those found by other workers.

(2) The population of the organism once within the plant reaches astronomical figures. Bacterial counts indicated that approximately 69,600,000,000 virulent cells were added per pot. Although this figure appears relatively high it is insignificant when compared to the number of virulent cells that can be recovered from a diseased plant. Approximately 123,250,000,000 virulent cells were isolated from only 4.5 g. of diseased tissue.

(3) Once within the banana plant the organism distributes itself over the entire plant. In the present experiment the roots were inoculated only on one side of the plant. The organism, however, could be isolated from the roots on the opposite side of the plant as well as from the rhizome and the pseudostem.

(4) An injured, diseased root might well provide the source of inoculum for a healthy root.

(5) In general the organism is a poor competitor and the population does not remain high in soil free from host plants. In the present experiment the population fell off so rapidly that the organism could not be isolated from infected soil after 5 days. Furthermore it can be assumed that the population in the soil declined since the number of diseased plants decreased as the interval of time increased between the application of the organism to the soil and the time the roots were injured.

(6) The rate of symptom expression appears dependent upon severity of inoculation. In Table 6 it can be noted that, as the time interval lenghthened between application of the organism and injury to the roots, the rate of symptom expression decreased.

Another experiment was conducted during the past year designed to study the mode of entry of Pseudomonas solanacearum into the banana plant - utilizing a plant grown in nutrient solution. (Fig. 5). After the appearance of a well developed root system the Moko organism was added to the nutrient solution. The virulence of the organism as well as the population was checked by its appearance on TZC medium as suggested by Dr. Kelman.

The plant, with a supposedly uninjured root system, failed to exhibit any Moko disease symptoms after growing in the presence of the organism for a period of one month. When, however, the roots were finally cut the plant exhibited external and internal Moko disease symptoms within 15 days. This

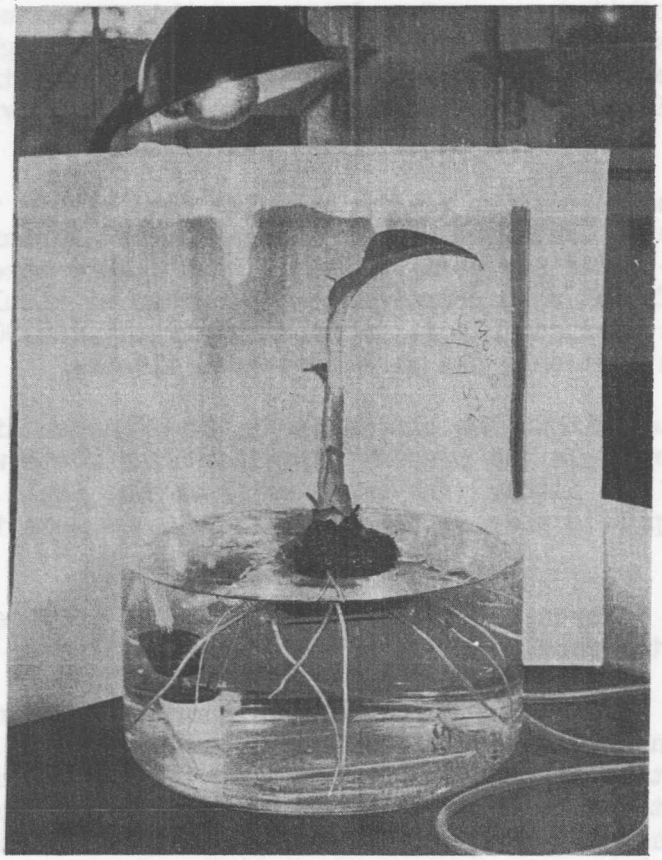


Figure 5. Banana plant growing in nutrient solution. Note well developed root system used for Moko penetration studies.

technique should be extremely promising in studying the mode of entrance of not only Pseudomonas solanacearum but also of Fusarium oxysporum f. cubense. The importance of studying this aspect, of course, lies in its significance in the underground spread of these two diseases.

b) Inoculum potential

(1) Pseudostem inoculation - An experiment was carried out to determine the number of bacteria necessary to induce Moko disease, and to see if symptom expression differed with inoculum levels. Results would indicate how many bacteria would need to be brought to a healthy sucker by a contaminated machete to induce disease.

Serially diluted bacterial suspension were prepared so that 1 c.c. for the 8 inoculum levels would contain respectively: 1) 14; 2) 140; 3) 1,400; 4) 14,000; 5) 140,000; 6) 1,400,000; 7) 14,000,000; 8) 140,000,000 bacteria. One c.c. was injected into the pseudostems of each of 15 plants that were 3-4' tall. Results are presented in table 7.

Table 7. Disease incidence following pseudostem inoculation with various inoculum levels of bacteria.

Treatment	Disease incidence after* 14 days	42 days
1) 14/c.c.	0/15	0/15
2) 140/c.c.	5/15	7/15
3) 1,400/c.c.	7/15	15/51
4) 14,000/c.c.	9/15	15/15
5) 140,000/c.c.	10/15	15/15
6) 1,400,000/c.c.	14/15**	15/15
7) 14,000,000/c.c.	15/15**	15/15
8) 140,000,000/c.c.	15/15**	15/15
9) Control	0/15	0/15

*Number showing symptoms over number inoculated

**After 10 days only plants in these treatments showed symptoms.

It is interesting that no disease resulted with 14 bacteria per plant and with 140 bacteria, disease resulted in only 45 per cent of the cases. With higher levels, however, disease resulted 100 per cent of the time. At 10 days symptoms were apparent only at the highest 3 inoculum levels. In general, plants at these higher levels died more quickly than at lower inoculum levels.

(2) Root or soil inoculation - Soil in 5 gallon cans containing bananas was infested with Moko bacteria at levels of approximately 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, per can. Roots were not wounded artificially. No disease resulted at the lower levels of 10^5, 10^6, and 10^7 bacteria per can. Disease resulted at levels of 10^8, 10^9, 10^{10}, and 10^{11} in 60, 90, 100 and 90 per cent of the replicates respectively.

This showed that a high inoculum level in the soil is necessary before disease results. This was interesting especially since the soil for this experiment was obtained from a banana plantation and should have contained all the insects, nematodes, etc. which might normally create the necessary wounding for infection to occur. This also indicates that soil inoculum potentials must be given consideration in other experimental work or generalizations.

In another experiment, where 500 million bacteria of the tomato or banana strain were applied per plot, disease resulted only with the combination of the B-strain and artificial root wounding. After 60 days both the T- and B-strain were still viable in the soil as was demonstrated by direct streaking on a tetrazolium medium.

4. Nutritional requirements of P. solanacearum

Investigations have recently been initiated to study the nutritional requirements of this organism. The results of one experiment on the inlfuence of C-N sources are shown in Table 8.

Table 8. Growth of P. solanacearum on various C-N sources

Basal medium +	Growth
Glutamic acid	+ + + +
Glutamine	+ + + +
Aspartic acid	+ + + +
Asparagin	+ + + +
Glucose	+
Sucrose	+
Glucose + KNO_3	+ + + +
Sucrose + KNO_3	+ + + +
Glucose + Urea	+ + + +
Sucrose + Urea	+ + + +
Glucose + $NaNO_2$	+
Sucrose + $NaNO_2$	+

It can be seen from these results that the organism can readily utilize several amino acids and related compounds as sole sources of carbon and nitrogen. It can also be seen that the organism is capable of growing on Glucose or Sucrose with nitrate nitrogen or urea as a nitrogen source.

In these experiments it appeared that certain of the substances tested favored the establishment of specific variants. This aspect will be investigated more throughly.

B. Studies on the Control of Moko Disease

1. Disinfectants and methods of disinfection in relation to Moko disease control

Evidence from observations made in the field by production personnel indicated that the methods of tool disinfection used in the farms were not very efficient. The problem was referred to this Department and a thorough investigation was made, in the laboratory and in the field, of all possible weak points in the current methods of disinfection.

a) Laboratory tests

Disinfectants were tested in the laboratory against an isolate of Pseudomonas solanacearum from banana by introducing 0.1 ml of a 48-60 hour nutrient broth bacterial culture into 10 ml of the chemical appropriately diluted in distilled water. The tube was then agitated and one loop of the solution was transferred to nutrient broth after 1/2, 1 and 5 minutes. The nutrient broth tubes were checked for turbidity after 48 and 60 hours. The more effective chemicals were then tested in the presence of 10 per cent (w/w) sterile soil or 10 per cent (v/v) sterile banana sap. Banana sap was collected aseptically from severed pseudostems and mixed with the diluted chemical solution.

Eighteen chemicals were tested for bactericidal action against Pseudomonas solanacearum, and are ranked in order of effectiveness in Table 9. Five of the chemicals tested were completely bactericidal in 30 seconds at concentrations as low as 100 ppm active ingredient. One of these was an organic mercury compound (Ceresan M), one an antibiotic (Aureomycin), two were quaternary ammonium compounds (Hyamine 3500, and Experimental R-2731), and one contained calcium hypochlorite (Lo-Bax-W). Formaldehyde, the chemical in current use as a machete disinfectant, was bactericidal only at a concentration of 10,000 ppm or higher.

Table 9. Bactericidal effectiveness of different chemicals against Pseudomonas solanacearum

Chemical	Parts per million (active ingredient)																	
	100			500			1000			5000			10,000			50,000		
	Minutes[a]			Minutes			Minutes			Minutes			Minutes			Minutes		
	½	1	5	½	1	5	½	1	5	½	1	5	½	1	5	½	1	5
CERESAN M	-	-	-															
AUREOMYCIN	-	-	-															
HYAMINE 3500	-	-	-															
EXPERIMENTAL QUATERNARY	-	-	-	-	-	-												
LO-BAX-W	-	-	-	-	-	-												
HYAMINE 2389	+	+	-	-	-	-												
HYAMINE 2744	+	+	-	-	-	-												
CYPREX	+	+	+	-	-	-												
HYAMINE 1622	+	+	-	+	-	-												
$KMNO_4$	+	+	+	+	+	-				-	-	-						
SEMESAN	+	+	+				+	-	-									
FORMALDEHYDE	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-			
LYSOL	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-			
PHENOL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-			
OMADINE DISULFIDE[b]	+	+	+				+	+	+									
OMADINE ZINC[b]	+	+	+				+	+	+									
DITHANE D-14													+	+	+	-	-	-
VANCIDE 51							+	+	+				+	+	+	+	-	-

[a]Growth (+) or no growth (-) after 48 hours in nutrient broth, following exposures to chemical for 30 seconds, 1 minute and 5 minutes.

[b]Ranking uncertain.

Cyprex, Vancide 51, and three of the more effective chemicals were compared with formaldehyde for bactericidal action in the presence of soil or banana sap (Table 10). The effectivenss of each of these chemicals except formaldehyde was markedly reduced by banana sap. Soil reduced the effectiveness of all except formaldehyde and Lo-Bax-W.

Table 10. Effect of soil or banana sap on bactericidal action of various chemicals.

Chemical		Alone			Plus sap[a]			Plus soil[a]		
		Minutes			Minutes			Minutes		
		½	1	5	½	1	5	½	1	5
Hyamine 3500,	1000 ppm	-	-	-[b]	+	+	+	+	+	+
	2000 ppm	-	-	-	-	-	-	-	-	-
Exp. Quat. R-2731	200 ppm	-	-	-	+	+	+	+	+	+
	500 ppm	-	-	-	+	+	+	+	+	+
Lo-Bax-W	200 ppm	-	-	-	+	+	+	-	-	-
	500 ppm	-	-	-	+	+	+	-	-	-
Cyprex	100 ppm	+	+	-	+	+	+	+	+	+
	500 ppm	-	-	-	+	+	+	+	+	+
Vancide 51	50,000 ppm	+	-	-	+	+	+	+	+	+
Formaldehyde	5,000 ppm	+	+	-	+	+	-	+	+	-
	10,000 ppm	-	-	-	-	-	-	-	-	-

[a]Chemical solution contained ten per cent by volume of sterile banana sap, or ten per cent by weight of sterile soil.

[b]Growth (+) or no growth (-) after 48 hours in nutrient broth, following exposure to chemical for 30 seconds, 1 minute and 5 minutes.

b) Field tests

In field tests designed to determine the effectiveness of various methods of machete disinfection, the machete was first contaminated by drawing the blade through a diseased sucker so that at least 5 inches were covered with the bacteria-containing sap. Three different methods were then used to bring the machete into contact with formaldehyde (Figure 6): (1) The "scabbard immersion" method consisted of immersing a machete in a metal scabbard containing 2 liters of the disinfectant. (2) The "sponge-pail" method consisted of wiping the machete twice with a sponge that had been soaked in a pail of formaldehyde

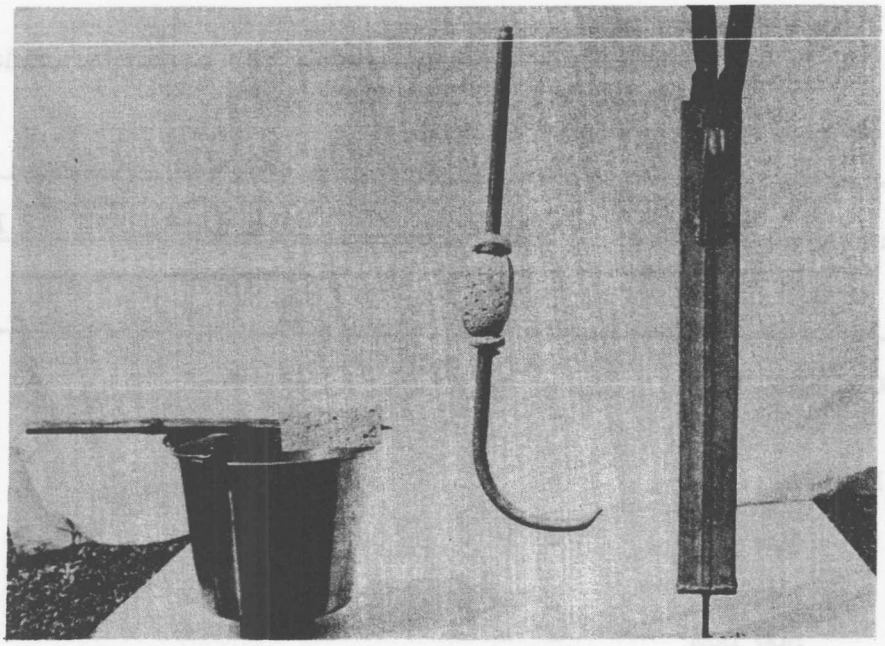


Figure 6. Tools used for machete disinfection trials: sponge-pail, gancho, and scabbard.

solution. (3) The "gancho" method consisted of wiping the machete across a sponge attached to a hollow aluminum tube containing formaldehyde. After disinfecting for a definite time interval one healthy sucker was cut.

Disease readings were taken after 3 weeks by cutting the suckers and checking for the discolored vascular strands characteristic of Moko disease. Many of the suckers were stunted and exhibited blackening of the outer leaf sheaths (Figure 7).

In the first laboratory screening many chemicals were found to be more effective than formaldehyde at equal concentrations. However the effectiveness of several of these chemicals was reduced in the presence of banana sap. Other candidate chemicals were considered to be too toxic to man or too expensive for field use. In addition to formaldehyde, Hyamine 3500 was considered to merit a field test (Table 11).

Table 11. Disease incidence following machete disinfection by immersion in Hyamine 3500.

Chemical	Concentration Active ingredient	Disease incidence (per cent) Immersed 10 sec.	Immersed 30 sec.
Hyamine 3500	1000 ppm	40	26
Hyamine 3500	2000 ppm	24	0
Hyamine 3500	5000 ppm	14	0
Control (no disinfectant)		80	

Machete immersion in 2000 ppm of Hyamine 3500 for 30 seconds eliminated disease transmission; but immersion for only 10 seconds was not completely effective even at 5000 ppm. Since Hyamine 3500* was not very effective at 10 seconds, formaldehyde* was considered to remain the most promising candidate disinfectant for use in banana pruning.

c) Disinfection methods with formaldehyde

Examination of the gancho, a tool that has been in current use, showed that the sponge was soon occluded by banana sap, and the flow of formaldehyde to the sponge was often erratic. Other more simple tools, such as a bamboo section containing formaldehyde with a piece of burlap protruding through a slit had similar drawbacks. Experiments showed that rapid wiping of a machete over an only partially wet sponge was not sufficient to disinfect the blade.

*Hyamine 3500 costs approximately 8.5¢ per gallon at 10,000 ppm. Formaldehyde costs approximately 12¢ per gallon at 5 per cent.

Figure 7. Regrowth of banana suckers 3 weeks after cutting with a machete contaminated with *P. solanacearum* (left), and a disinfected machete (right).

A comparison was made of the efficiency of the gancho and scabbard-immersion methods at two formaldehyde concentrations (Table 12).

Table 12. Disease incidence following machete disinfection by two methods at two formaldehyde concentrations.

Method[a]	Formaldehyde concentration	Disease incidence (per cent)
Scabbard immersion	5%	0
Gancho	5%	0
Scabbard immersion	1.3%	36
Gancho	1.3%	44
Control (no disinfection)		95

[a]Time from complete coverage of machete by solution to cutting test sucker was 30 seconds.

It is interesting that although a 1 per cent solution was effective in killing the bacteria in laboratory tests, a 1.3 per cent solution allowed up to 44 per cent disease transmission in the field test.

Although no transmission occurred with the gancho method at a concentration of 5 per cent, the gancho sponge was becoming occluded with banana sap by the end of the experiment and it was difficult to obtain good coverage of the machete blade.

Formaldehyde was then tested at two concentrations and various time intervals by the scabbard method and by the sponge-pail method (Table 13).

Table 13. The effect of disinfection time and formaldehyde concentration on machete disinfection by two methods.

Method	Formaldehyde concentration	Time in seconds[a]	Disease incidence (per cent)
Scabbard	5%	10	2
Scabbard	5%	20	2
Scabbard	5%	30	0
Sponge-pail	5%	10	4
Sponge-pail	5%	20	4
Sponge-pail	5%	30	0
Scabbard	10%	10	0
Sponge-pail	10%	10	0
Control (no disinfectant)			95

[a]Time from complete coverage of machete by solution to cutting test sucker.

Thirty seconds were required to insure complete disinfection at a concentration of 5 per cent. Only 10 seconds were required for complete disinfection at a concentration of 10 per cent.

d) Conclusions

The use of a scabbard of 10 per cent formaldehyde in which 2 machetes can be immersed is a simple and satisfactory means of eliminating Moko disease transmission by machetes. When the machete is immersed in 10 per cent formaldehyde for 10 seconds or more, no transmission occurs. If the alternate use of 2 machetes lengthens disinfecting time to 30 seconds, 5 per cent formaldehyde would be equally effective.

Other previously recommended methods of disinfecting machetes, and lower formaldehyde levels were not so effective in eliminating disease transmission.

Of 18 other chemicals tested, some were effective at lower concentrations than was formaldehyde, but they have other less desirable characteristics.

Field tests indicate that with some chemicals, concentrations which are effective in laboratory screening tests are not effective in the field, and much higher concentrations must be used.

Banana sap and soil interfere with the effectiveness of the bactericidal action of some of the chemicals.

e) Recommendations

Machete disinfection for the prevention of spread of Moko disease should be carried out by the use of a scabbard containing 2 machetes in 10 per cent formaldehyde. The machetes should be used alternately so that each may be immersed at least 10 seconds after use on one mat only. (Approximately 10 per cent formaldehyde is prepared from 37 per cent commercial formalin by mixing 1 part formalin with 3 parts water).

Previously recommended disinfection methods and concentrations should be supplanted by the present recommendations.

Machete disinfection should be carried out on clean farms that are adjacent to Moko-diseased farms.

Since machetes do not transmit Moko if scabbard immersion is properly carried out, "jerk-pruning" is neither superior nor inferior so far as disease transmission is concerned. However, at edges of Moko abandonments, where jerk-pruning might throw infested soil on the exposed plant surface, scabbard-disinfected machetes are probably to be preferred.

2. Effectiveness of Chloropicrin in the Control of Moko Disease

Chloropicrin, a highly effective soil fungicide, has proven effective in reducing losses to bacterial wilt in North Carolina and in Japan. It

seemed desirable, therefore, to test the effectiveness of this material against the Moko organism.

Fifty concrete cylinders 2 feet in diameter were used in the fumigation work. They were buried in soil, supplied with sufficient drainage, and isolated from contamination by covering inter-cylinder soil with wood shavings. The soil in each cylinder already contained the tomato type Pseudomonas solanacearum, but was inoculated also with 2 gallons of liquid obtained by mixing chopped infected banana pseudostems in water. Chloropicrin (Larvacide) was then introduced by a pippette at a 6 inch depth at rates of 2, 4 and 8 c.c. per square foot at 9-10 inch centers. Ten cylinders were used per replicate and they were covered immediately after treatment with plastic to prevent escape of the gas. Bananas and seed of Physalis angulata were planted two weeks after applying the chloropicrin.

No bananas in treated cylinders had become diseased after 4 months (Table 14). Roots of each plant were cut and after 6 months bananas in 2 treated tanks had become diseased.

This indicates that the lower dosages reduced the Pseudomonas population to a sufficiently low level so that natural infection did not occur, but infection was still possible following artificial root wounding.

Table 14. Disease incidence of bananas in chloropicrin-treated soil cylinders.

Treatment	Disease incidence	
	After 4 months	After 6 months with roots cut
Soil infested; no chemical	100	100
Soil infested; 2 c.c./sq.ft.	0	10
Soil infested; 4 c.c./sq.ft.=	0	10
Soil infested; 8 c.c./sq.ft.	0	0
Soil not infested; no chemical	0	0

Results with Physalis angulata were interesting in that after 4 months, some plants were diseased that were growing in soil that had been treated at the lower dosages. However the strain of the bacterium in the wilted plants was the banana strain only where no chemical had been applied (Table 15).

Table 15. Disease incidence on Physalis angulata in chloropicrin-treated soil cylinders.

Treatment	Disease incidence*	Number of times each strain was recovered	
		Banana	Tomato
Soil infested; no chemical	50	2	7
Soil infested; 2 c.c./sq.ft.	30	0	7
Soil infested; 4 c.c./sq/ft/	10	0	3
Soil infested; 8 c.c./sq.ft.	0	-	-
Soil not infested; no chemical	40	0	6

*Per cent of cylinders with one or more wilted plants within 4 months.

In summary, chloropicrin applied to soil in cylinders at the rate of 8c.c. per square foot effectively eliminated Pseudomonas solanacearum from the soil, since neither bananas nor Physalis angulata became diseased when grown after soil treatment (Figure 8).

Chloropicrin applied at 2 and 4 c.c. per square foot reduced disease incidence in bananas from 100 per cent in the control to 10 per cent in the treated cylinders. Disease appeared in the bananas in the treated cylinders only after the roots were cut.

Although expensive, chloropicrin may offer promise in spot treating infested soil around isolated individual Moko cases appearing in new areas.

3. Seed Treatment with Aureomycin

If banana button seed come in contact with Moko bacteria, proliferation of the pathogen usually results in death of the buttons. An experiment was conducted to demonstrate the importance of allowing the cut surfaces of button seeds to heal over before planting. Button seed were brought into the laboratory and allowed to dry for two days. The surfaces were then cut and allowed to heal over for various periods of time. Before being planted they were dipped into a suspension of Moko bacteria obtained from a pseudostem. The results are shown in Table 16.

Table 16. Influence of healing time on germination of inoculated button seed.

Length of healing time	Germination*
5 days	20/20
3 days	17/20
2 days	17/20
1 day	14/20
0	2/20

*Number germinated after three weeks over number planted.

Disease resulted even after 3 days had elapsed after cutting, indicating the importance of a chemical seed dip in farm practices.

In another experiment, banana buttons were cut, then immediately dipped in a suspension of Pseudomonas solanacearum, then after 1, 5, or 60 minutes were dipped in Aureomycin hydrochloride (100 or 500 ppm) for 1½ minutes. After 32 days, disease data were obtained (Table 17).

Figure 8. Bananas growing after soil treatment with chloropicrin (left) and no treatment (right). Dosage was 8 c.c. per square foot; photographed after 4 months.

Table 17. Effect of Aureomycin seed dip on subsequent Moko disease incidence.

Treatment of button seed	Disease incidence per cent
Moko only	60
Moko - 1 min. - Aureo 500	0
Moko - 1 min. - Aureo 100	5
Moko - 5 min. - Aureo 500	10
Moko - 5 min. - Aureo 100	30
Moko -60 min. - Aureo 500	50
Moko -60 min. - Aureo 100	60
No treatment	0

It was interesting that with only 1 minute elapsing between inoculation and the aureomycin 500 dip, no disease resulted; but at other treatments, disease resulted, increasingly with lesser aureomycin or more time between inoculation and treatment. Although no practical use is evident from the dosages used, it may be profitable to consider higher aureomycin levels or longer dips.

4. Fallowing for Moko Disease Control

Areas abandoned for Moko disease cannot be replanted successfully without a fallowing period. This period has been estimated to be of at least two years, but there is no information from a well-replicated field experiment as to the actual fallowing period required or how this requirement could be modified by the use of a cover crop. Therefore, an extensive field experiment, involving a total of 20 acres, was established in an area gradually abandoned for Moko disease over a period of four years. The experimental area was divided into twenty one-acre plots, with the purpose of replating areas selected at random, at six month intervals ranging from 0 to 24 months fallowing. Each plot was split in two sections to allow the introduction of another factor, a cover crop. Kudzu was selected as a non-susceptible cover crop and planted in half-plots selected at random. Therefore, the experiment had a simple split-plot randomized block design.

The results from the first plantings, which have now been established for seven months, indicate that Moko disease appeared rapidly in plots where no fallowing period was allowed. The results as follows

	Ave. Incidence of Moko disease (%)
Weed fallow	25.5
Cover crop fallow	8.7

There is a suggestion that the presence of kudzu reduced spread of the disease, but the differences are not statistically significant as yet. Information from the 6-month fallowing level, which has now been planted, should indicate whether or not the effect from kudzu is truly significant.

Information presented in our Annual Report for 1957 indicated that flood fallowing procedures seemed promising as a control measure for Moko disease. Due to limitations on pumping equipment, information was available only from one area which was flooded for a period of three months. Since information from a non-replicated experiment cannot be taken without reservations, it seemed important to establish a more elaborate field experiment with the purpose of determining the actual effects of flood fallowing on survival of the Moko bacterium.

Construction of eight one-half acre lakes has now been completed. The lake areas were planted to bananas with the purpose of providing a uniform inoculum, through artificial infection, before the plots are flooded. It is intended to carry out two treatments: 1) continuous flooding for four months, and 2) flood-dry-flood procedures for a similar period of four months. Two half-acre plots outside the lake areas have been planted to serve as controls.

Panama Disease Investigations

A. Organic Amendments in Relation to Panama Disease Control

1. Influence of Organic Amendments on Survival of Fusarium oxysporum f. cubense in the Soil

Information published in previous Annual Reports gave ample evidence that green manure treatments adversely affected the survival of Fusarium oxysporum f. cubense in the soil. It was established that certain leguminous plants, such as Crotalaria and kudzu, and grasses such as sorghum and sugar cane, effectively reduced the population of this fungus. Further, the beneficial effects of some of these plants could be enhanced by drying the material before incorporating into the soil, by the addition of nitrogen fertilizer and by the addition of lime.

Because of possible errors in the estimation of Fusarium populations in the case of chemical adjuncts to green manures, it was felt that more extensive tests should be made to confirm the results obtained in these preliminary experiments. The present account incorporates information obtained from laboratory tests and from field plots on the use of cover crops and chemical adjuncts for Fusarium control. A considerable portion of this work deals with the use of urea as an amendment and studies designed to determine the nature of the fungicidal effect of urea incorporation.

a) Influence of sugar cane and chemical adjuncts

As in previous experiments, these studies were carried out with soils contained in large (24") concrete tanks. The materials under investigation were added to the tanks at the desired level and thoroughly mixed to a depth of six inches. The sugar cane treatment consisted of six pounds of finely chopped material per tank, a rate roughly equivalent to 42 tons per acre. Lime (Calcium hydroxide) and urea were added at 1, 2, and 5 lbs. per tank and calcium cyanamid at 2, 4, and 10 lbs. per tank, as a single treatment or in combination with sugar cane. In the present series of tests a total of 32 tanks were used and of these a sufficient number received no soil amendments to serve as controls.

Fusarium spore populations were followed by the standard buried slide technique. Slides bearing drops of a plain agar suspension of Fusarium spores were buried in the amended soils and recovered a biweekly intervals. The soil covering the agar drops was washed out carefully with a gentle water stream, the drops removed, placed in sterile water and disintegrated in a Waring blender. The resulting suspension was diluted and planted in Martin's agar. The results of isolations made during a period of inoculation of 16 weeks are shown in the following tables.

Table 18 A. Influence of various soil treatments on Fusarium spore population

Treatment	Fusarium spore populations: % reduction 16th week	Spores/cc. (thousands) av. 8 samples	Average pH.
Sugar cane	90.3	55.8	5.9
Sugar cane and plastic	77.6	97.7	5.7
Sugar cane and lime (1 lb.)	60.1	188.2	7.8
Sugar cane and lime (2 lbs.)	86.3	136.6	7.9
Sugar cane and lime (5 lbs.)	99.8	72.1	8.5
Lime (1 lb.)	0.0	219.1	7.9
Lime (2 lbs.)	0.0	139.5	8.1
Lime (5 lbs.)	100.0	1.0	8.6
Control	0.0	202.9	5.9

Table 18 B. Influence of various soil treatments on Fusarium spore populations

Treatment	Fusarium spore population: % reduction 16th week	Spores/cc. (thousands) av. 8 samples	Average pH.
Sugar cane	90.3	55.8	5.9
Sugar cane and urea (1 lb.)	86.0	3.0	7.4
Sugar cane and urea (2 lbs.)	100.0	0.2	7.7
Sugar cane and urea (5 lbs.)	100.0	0.3	8.0
Urea (1 lb.)	99.1	0.6	7.7
Urea (2 lbs.)	99.5	0.1	7.9
Urea (5 lbs.)	100.0	0.1	8.3
Control	0.0	202.9	5.9

Table 18 C. Influence of various soil treatments on Fusarium spore populations

Treatment	Fusarium spore population: % reduction 16th week	Spores/cc. (thousands) av. 8 samples	Average pH.
Sugar cane	90.3	55.8	5.9
Sugar cane and cyanamid (2 lb.)	79.9	89.9	7.7
Sugar cane and cyanamid (4 lb.)	100.0	20.3	8.5
Sugar cane and cyanamid (10 lb.)	100.0	5.4	8.5
Cyanamid (2 lb.)	82.5	93.1	7.9
Cyanamid (4 lb.)	99.5	0.2	8.4
Cyanamid (10 lb.)	99.5	0.3	9.5
Control	0.0	202.9	5.9

The results obtained suggest the following conclusions:

1. As in all previous tests, a significant reduction in the Fusarium population was obtained in soils ameded with sugar cane.

2. Almost complete eradication of the fungus was noted in all cases when urea was used, either alone or in combination with sugar cane.

3. A beneficial effect of lime was noted only at the highest level used, i.e. 5 lbs. per tank, roughly equivalent to 35 tons per acre. In tests previously reported, a significant drop in spore population was obtained at levels as low as 1 lb. per tank. This discrepancy may be explained in terms of differences in the pH levels obtained.

4. Calcium cyanamid caused a very significant drop in the Fusarium spore population, particularly when applied at high rates, i.e. 4 and 10 lbs. per tank.

5. Low rates of lime and calcium cyanamid interfered with the Fusarium depressing effect of sugar cane.

6. The use of a black polyethylene cover did not improve the effect of sugar cane as a green manure treatment for Fusarium control.

The most significant result of this series of tests is the full confirmation that urea, when applied at levels as low as 1 lb. per tank (7 tons per acre), is an extremely effective fungicide. This material, therefore, appeared to merit forther investigation as a possible organic soil amendment for Panama disease control.

b. Influence of various levels of urea

Information presented in the preceding section indicated the need for tests to determine the lowest possible dosage of urea that could be used in

the field for control of the Panama disease fungus. For this purpose, Coto clay-loam soil contained in 24-inch concrete tanks was treated with urea at 5 different levels. The chemical was mixed with the soil to a depth of six inches, and each treatment was replicated four times. Fusarium populations were followed during a 48-day period after urea was applied. Sampling methods previously described were used. The results obtained are summarized in the following table:

Table 19. Influence of various levels of Urea on survival of Fusarium oxysporum f. cubense

Urea level	Fusarium populations (1/1000) days					
	10	17	24	31	41	48
1. 1 3/4 tons per acre	292.8	2.1	0.2	0.2	0.0	0.0
2. 3.5 tons per acre	236.5	1.6	0.0	0.0	0.0	0.0
3. 7 tons per acre	75.0	0.1	0.0	0.0	0.0	0.0
4. 14 tons per acre	4.0	0.0	0.0	0.0	0.0	0.0
5. 35 tons per acre	0.0	0.0	0.0	0.0	0.0	0.0
6. Control	314.4	351.4	263.4	234.0	226.3	247.4

It is clear from the data presented above that Fusarium spores were killed within 41 days at all levels of urea used. At exceedingly high rates (i.e. 35 tons/acre) the fungus was completely eliminated within ten days after the chemical was applied, but at lower rates elimination of the fungus was more gradual. It would appear from these tests that the level of 1 3/4 tons per acre should be the lowest practical rate to be used in field work.

c) Residual fungicidal effect in soils treated with high levels of urea

At the completion of the experiment described in the preceding section, when it was assumed that most of the urea applied had been decomposed, slides containing Fusarium spores were again introduced into the various tanks and survival of the fungus was determined after an incubation period of one week by standard methods. The following results were obtained:

Table 20. Residual toxicity of urea-treated soils seven weeks after application

Initial urea level	Fusarium populations (1/1000) 1 week incubation
1. 1 3/4 tons per acre	364.0
2. 3.5 tons per acre	412.0
3. 7 tons per acre	45.0**
4. 14 tons per acre	1.5**
5. 35 tons per acre	4.5**
6. Control	379.0

$L.S.D._{05} = 63.4$

$L.S.D._{01} = 86.9$

It is evident that a strong residual fungicidal effect was present in soils treated with high amounts of urea, even though more than 7 weeks had elapsed since the chemical was applied.

In order to determine if the soils treated with high rates of urea would retain their toxicity towards Fusarium for periods longer than 7 weeks, soil samples were collected and stored in beakers in the laboratory. Fusarium spores were introduced into these soils at frequent intervals and survival of the fungus was determined by standard methods. Toxicity of soils treated with urea at 14 and 35 tons per acre was still evident five months after application of the chemical. At this time, a statistical analysis of the data showed that differences in Fusarium survival were just within the required levels for significance.

Evidence for the extremely long residual effect of heavy dressing of urea was also obtained from another source. Soil samples obtained from field plots treated with various levels of urea, in a well-replicated randomized block design, were brought to the laboratory and toxicity towards Fusarium spores determined by the standard buried slide method. At the time toxicity tests were carried out, four months had elapsed since urea was applied. The results obtained are shown in the following table:

Table 21. Residual toxicity of urea-treated soils. Four months after application

Urea level	Fusarium population (1/1000) 21 day incubation
1. 1 3/4 tons per acre	487.6
2. 3 1/2 tons per acre	116.0**
3. 7 tons per acre	43.2**
4. Control	487.8

$L.S.D._{05} = 113.0$

$L.S.D._{01} = 150.3$

It is evident from the data presented above that a fungicidal effect was present in samples from field plots treated with high amounts of urea four months previously. We may conclude from all the available evidence that the toxic factor present in urea-treated soils is either extremely stable or it is being maintained at high levels by slow decomposition of high amounts of urea. Because of the practical possibilities for control of the Panama disease fungus through the application of high amounts of urea it seemed important to attempt to determine the course of toxic factor production in amended soils and the nature of the toxic effect.

d) Nature of the fungicidal effect of urea-treated soils

Urea is subject in the soil to various changes brought about by microorganisms. Since urea by itself does have fungicidal properties, it follows that the toxicity of this material to Fusarium spores in the soil must be due to either; 1) the toxicity of decomposition products or 2) the unfavorable activities of organisms involved in this decomposition.

Fusarium oxysporum f. cubense will grow well in culture media containing up to 20,000 ppm of urea and it will hydrolyze and utilize this material as a nitrogen source. Growth does not stop until the ammonia liberated in the process of hydrolysis accumulates in the plates at extremely high concentrations. Under these conditions, ammonia is merely a fungistatic agent and does not cause death of the spores even after very prolonged exposures.

Therefore, it seemed unlikely that urea, or its products of decomposition and concomitant changes in soil pH, could result by themselves in the highly fungicidal effects noted in urea treated soils and described in previous sections. This is further substantiated by the fact that soils saturated with ammonium hydroxide solutions, at concentration as high as 16,000 ppm, will not eliminate Fusarium spores in the soil (Annual Report, 1956). In addition,

ammonium carbamate, a known intermediate in the hydrolysis of urea, was found to cause only slight inhibition of the fungus at concentrations as high as 10,000 ppm.

Urea is hydrolyzed in the soil, with the formation of ammonia, by a large number of microorganisms as well as by specific groups of bacteria. These bacteria are mainly of the Urobacilli group and they increase tremendously in numbers upon addition of urea to soil. Therefore, the inhibitory effects of urea could be the result of direct antagonism of Urobacilli and related forms towards Fusarium oxysporum f. cubense. To test this possibility, urea-hydrolyzing bacteria from Coto clay-loam soils were isolated on selective media. These bacteria were grouped into seven different types, all capable of hydrolyzing urea very rapidly. The activities of these bacteria against Fusarium oxysporum f. cubense were determined in plate culture from parallel streakings of the fungus and the test organism. Only one of the bacteria isolates was found to be slightly antagonistic against Fusarium and it was concluded that Urobacilli could not be responsible for the toxic effects of urea-amended soils.

Commercial urea contains an appreciable amount of biuret, a compound known to be relatively toxic to plants. Since greenhouse and field tests previously described had been carried out with commercial urea, it was inferred that biuret contamination could be responsible for the toxicity of urea- treated soils. However, tests carried out in plate culture indicated only slight inhibition of Fusarium at concentrations of 10,000 ppm. and it was concluded that biuret can not account for the toxicity of treated soils.

Having discarded the possibilities that the process of urea decomposition in the soil, the resulting products of urea decomposition, or biuret contamination, could play a significant role in the toxic effects of urea amendment, attention was then directed to the process of nitrification. In this process, ammonia liberated upon decomposition of urea is rapidly oxidized to nitrites and then to nitrates by specific groups of soil bacteria. It seemed important, therefore, to establish correlations between the course of nitrification and the appearance of factors toxic to Fusarium oxysporum f. cubense.

Coto clay-loam soil was weighed in 100 gr. portions into small aluminum cans containing a microscope slide bearing agar drops of a heavy suspension of Fusarium spores. The soils were watered with a solution adjusted to supply .5 grams of urea per 100 gr. of soil. The cans were weighed and constant weight was maintained by replacing at frequent intervals the water lost by evaporation. At specified intervals during a total incubation period of 25 days, samples were removed and the soils leached with IN potassium chloride solution. Nitrification was followed by colorimetric determination of the levels of ammonia, nitrites, and nitrates. Ammonia was determined by Nesslerization; nitrites were estimated with alpha-naphthylamine and sulfanilic acid. Nitrates were estimated by reduction to nitrites with zinc dust after elimination of nitrites with sodium azide. Fusarium population levels were determined by standard plating techniques, as previously described. The results obtained are shown in the following table:

Table 22. Transformation of nitrogen and toxicity in urea-amended soils.

Days	Nitrogen Source (ppm) Ammonia Treated	Ammonia Check	Nitrites Treated	Nitrites Check	Nitrates Treated	Nitrates Check	Fusarium populations 1/1000 Treated	Fusarium populations 1/1000 Check
3 days	245.0	0.0	4.5	0.3	350.1	349.1	318.0	448.3
6 days	1171.7	0.0	91.3	0.5	246.5	232.3	211.7	499.2
13 days	44.5	0.0	751.7	0.1	411.0	181.0	74.4	485.3
19 days	19.7	0.0	718.0	0.1	672.0	192.5	0.0	441.7
25 days	13.3	0.0	670.0	0.1	606.0	235.0	0.0	594.5

The data presented above gives ample evidence that the toxicity of urea-amended soils is correlated with the accumulation of nitrites. The deleterious effects of nitrites on protoplasm are well known and it is clear from the table above that eradication of the fungus occurred when nitrite concentrations of 700 ppm. were obtained. The nitrate levels obtained are not considered toxic to microorganisms and it is unlikely that nitrates play a role in the toxicity of these soils to Fusarium.

Having established the fact that nitrite accumulation is responsible for the fungicidal effects of urea-treated soils, it seemed important to determine the actual levels of nitrite-nitrogen that are effective against Fusarium oxysporum f. cubense in the soil. Following procedures previously described, soils were weighed into small cans, watered with a solution of sodium nitrite adjusted to supply nitrite-N levels from 203 to 1220 ppm, and survival of the fungus determined by serial dilution techniques. The following results were obtained:

Table 23. Influence of nitrite-N on survival of Fusarium oxysporum f. cubense in the soil.

Nitrite-N conc.	Fusarium populations (1/1000) Incubation: 20 days
203 ppm	1.7
405 ppm	0.0
810 ppm	0.0
1220 ppm	0.0
Control	465.8

The results obtained indicate that almost complete eradication of the fungus can be obtained at nitrite-N levels slightly over 200 ppm., if the spores are exposed to this concentration in the soil for a period of 20 days. Since nitrite-N levels considerably higher than 200 ppm are obtained in soils

treated with high amounts of urea, the evidence supports previous conclusions regarding the predominant role of nitrites in the toxicity of urea-treated soils.

Further evidence on the toxicity of nitrites was obtained using pure culture methods. When sodium nitrite was added to standard potato - dextrose culture medium, amounts in excess of 300 ppm - N were found to inhibit the growth of Fusarium in this medium. Due to the fact that nitrite solutions are relatively unstable, particularly in culture media containing high amounts of organic matter, the nitrite concentrations found to be effective in pure culture are higher than those found to be operative in the soil.

e) Influence of nitrogen fertilizers

Evidence for the extreme toxicity of nitrites in the soil has been obtained from another source. Considering that decomposition of urea in the soils results in the accumulation of ammonia, which is readily oxidized to nitrites and then to nitrates, it seemed important to compare the toxicity of sources of these three forms of nitrogen. Soil contained in small cans was watered with solutions containing urea, potassium nitrate, sodium nitrite, and ammonium sulfate - adjusted to supply identical amounts of nitrogen. Survival of Fusarium oxysporum f. cubense in these soils was determined by methods previously described, after an incubation period of 20 days. The results are summarized in the following table:

Table 24. Influence of nitrogen fertilizers on survival of Fusarium oxysporum f. cubense in the soil

Nitrogen Source	Amount N added	Fusarium populations (1/1000) Incubation: 20 days
1. Urea	1870 ppm	10.1
2. Potassium nitrate	1870 ppm	456.0
3. Sodium nitrite	1870 ppm	0.0
4. Ammonium sulfate	1870 ppm	458.0
5. Control	1870 ppm	429.5

$L.S.D._{05} = 55.9$

$L.S.D._{01} = 74.4$

The data presented above indicates that neither ammonium nor nitrate nitrogen are toxic to Fusarium at the concentrations used. As expected, significant differences in survival of the fungus were obtained only with urea and nitrite nitrogen, a fact which immediately suggests that nitrites are responsible for the toxic effect in both cases.

f) Phytotoxicity of urea

The high fungicidal effects of heavy dressigns of urea suggested the possibility of utilizing this material for control of Panama disease in the field. Since nitrites are known to be highly phytotoxic it seemed desirable to investigate the effect of heavy applications of urea to the soil on subsequent growth of bananas.

Soil contained in large (24") concrete tanks was treated with three levels of urea known to give complete eradication of Fusarium oxysporum f. cubense. A randomized block design, with twelve replicates of each treatment, was used. The urea was allowed to decompose for one month and at the end of this period the tanks were planted to bananas. Growth measurements were taken after the plants had grown for 1 1/2 months. The results are shown in the following table:

Table 25. Banana growth in urea-amended soils

Urea level	Average height in cm.	% mortality
3 1/2 tons per acre	30.0	0.0
7 tons per acre	21.8	8.3
14 tons per acre	18.4	50.0
Control	31.9	5.5

It is evident from the data presented above that urea at a rate of 14 tons per acre causes high mortality and severe stunting of banana plants. Some phytotoxicity was present at the rate of 7 tons per acre, but no deleterious effects were noted at the rate of 3½ tons. Therefore, soils treated with urea at levels higher than 3½ tons per acre should not be planted to bananas within one month after application of the chemical.

g) Urea applications for control of Panama disease in the field

Because of the practical possibilities for control of Panama disease through the application of high amounts of urea, a field experiment was established utilizing 3 rates known to be effective under laboratory conditions. The chemical was evenly distributed by means of a fertilizer spreader and rototilled to a depth of six inches. The plots were 1/2 acre in size, arranged in a randomized block design involving four replications of each treatment.

The following treatments were carried out:

1. Urea at 1 3/4 tons per acre
2. Urea at 3 1/2 tons per acre
3. Urea at 7 tons per acre
4. Control

The plots were planted to bananas three months after application of urea, so as to avoid the phytotoxic effects indicated in the previous section. Wide strips were left between plots to avoid unfavorable border effects. At

this time, Panama disease has not appeared in sufficient numbers to allow a proper evaluation of the treatments.

2. Field Trials for Control of Panama Disease with Green-manure Treatments

Ample evidence has been obtained in the laboratory concerning the favorable effects of certain cover crop plants, when incorporated into the soil, in reducing the spore population of Fusarium oxysporum. Details of laboratory screening work were given in our Annual Report for 1957. It seemed important to establish field trials with the purpose of determining the practical possibilities of using green manure treatments for control of Panama disease.

Plots located in an area recently abandonded for Panama disease were planted to sorghum, velvet beans, and sugar cane. Each treatment was replicated three times, in a randomized block design. Details of the experimental procedures were given in a previous report and it is only necessary to state that during a period of one year the cover crops were turned under as they reached maturity. At the end of this rotation period, the plant materials were allowed to decompose in the soil for three months before planting to bananas. Disease surveys were carried out at monthly intervals. The following table summarizes the information obtained during the first nine months:

Table 26. Green manure treatments for Panama disease control

Cover crop	# Crops turned under	Tonnage	Incidence of disease
			%
Velvet beans	2	50 tons/acre	70.8
Sorghum	3	80 tons/acre	62.0
Sugar cane	1	80 tons/acre	40.8**
Control	-	-----	83.4

$L.S.D._{05} = 26.1$

$L.S.D._{01} = 30.6$

The results obtained indicate that the sugar cane treatment resulted in a significant reduction in the incidence of Panama disease. These results support conclusions reached from laboratory evidence regarding the beneficial effects of sugar cane as a green manure treatment. Velvet beans, at the tonnage used, were completely ineffective. Sorghum appeared to be partially effective, but the difference in disease incidence with the control plots was not statistically significant. It is interesting to note that these field results closely parallel the results obtained in laboratory screening work regarding the effectiveness of the plants used, as reported in our Annual Report for 1957.

It is evident from the results obtained that the incidence of Panama disease was high enough to preclude any practical application of this method of control. In addition, nitrogen-deficiency symptoms were apparent in the plants growing in the cane plots and this deficiency may have affected the results considerably. The indications are, however, that higher tonnages of cane and longer fallowing periods would be necessary in order to obtain economic returns with this control system.

3. Sugar Cane Decomposition in Relation to Control of Panama Disease

a) Numbers of microorganisms

A considerable amount of evidence has accumulated, from laboratory and field experiments, regarding the Fusarium - depressing effects of sugar-cane amended soils. The nature of this toxic effect, however, is still a matter of conjecture. Information presented in previous reports indicated that the stimulation of resistant spore germination and the subsequent activities of soil microorganisms appeared to be involved in the reduction of spore survival in cane-amended soils. The microbiological changes in these soils are, therefore, of considerable importance and efforts were made to study these changes under controlled conditions.

Coto clay-loam soil was weighed into glass beakers and the beakers placed in a water bath at 32°C ± .5. A number of these soil samples were amended with 10% by weight of finely chopped sugar cane stalk material. The beakers were covered with parafilm to prevent loss of water by evaporation. Changes in numbers of microorganisms were followed by means of dilution techniques on appropriate selective media. Isolations were made after 48 hours and thereafter at weekly intervals. The average population levels from samples obtained throughout a 34-day period are shown in the following table:

Table 27. Numbers of microorganisms in sugar-cane amended soils.

Type	Numbers of microorganisms (Thousands per gram)	
	Sugar-cane soil	Control
Bacteria	3,704.6	530.8
Actinomycetes	26.6	105.1
Fil. fungi	16.5	35.6
Yeasts	1,733.8	0.7

Information obtained from individual samples used to obtain the average data shown above, permits the following tentative conclusions:

(1) Within 48 hours after addition of sugar cane, there is a ten-fold increase in the number of soil bacteria. Bacterial population levels drop

sharply within the next few days, but the overall number remain higher than in the check soils even after 34 days.

(2) The most strikin effect resulting from the incorporation of sugar cane to soil is the tremendous increase in the number of yeasts that occurs within a few hours. The highest level is reached within 10 days and a gradual decrease in numbers occurs thereafter.

(3) The population of actinomycetes and filamentous fungi is drastically reduced within a few days after the incorporation of cane. However, the numbers of fungi begin to increase within 3 weeks and within 34 days they reach the normal level in the soil. Results from previous tests indicate that filamentous fungi take over after bacteria and yeasts have utilized the readily available carbohydrates.

b) Activity of antagonistic fungi from cane soils

The importance of fungi in the latter stages of cane decomposition and the well-known antagonistic properties of some of these organisms against Fusarium oxysporum f. cubense in culture, led to investigations on the possible activities of representative members of this group in normal soil. Fungi isolated from sugar-cane soils were screened for antagonism against the Panama disease fungus in culture and those showing marked activity were selected for soil tests. Spore suspensions were preared from 10-day old cultures of the following fungi: Trichoderma viride, Aspergillus niger, Fusarium solani, Fusarium moniliforme, and a species of Penicillium. These spore suspensions were added to Coto clay loam soil contained in glass beakers and survival of Fusarium oxysporum f. cubense in these soils was determined by the standard buried slide technique. A statistical analysis of the data obtained indicates that none of the added fungi effectively reduced the population of Fusarium oxysporum f. cubense in the soil. These results agree entirely with reports from many sources regarding attempted control of root disease organisms through the addition of antagonistic fungi or bacteria to soil.

c) Activity of soil extracts from cane soils

The increased microbiological activity in the soil resulting from the incorporation of sugar cane, as previously described, suggested that materials directly inhibitory to Fusarium could be released in amended soils by microorganisms favored by the presence of large quantities of readily available carbohydrates.

Duplicate samples of inoculated normal and cane-amended (10%) soils were incubated in a constant temperature water bath at 32°C for two months. At the end of this period, Fusarium spores could not be recovered from the amended soils whereas extremely high numbers were still present in the control soils. Five-hundred gram samples of these soils were placed in a constant perfusion apparatus and 50 c.c. of distilled water was constantly circulated through the soil. After a 24-hour percolation, the extracts were evaporated under vacuum to a 10 c.c. volume. One-half of the total volume of each extract was autoclaved and the other half was filtered through a bacterial filter. The activity of these autoclaved and nonautoclaved extracts against Fusarium oxysporum f. cubense was then determined in plate culture. No definite inhibitory effects were noted

with any of the extracts and there were no apparent differences between extracts from normal or from amended soils.

4. Permanent Crop Rotation for Panama Control

The permanent nature of banana cultivation allows a constant build-up of root disease organisms in the soil. A rotation scheme that does not maintain bananas in any particular location for long periods should prevent disease spread to a great extent. Therefore, a reclaimed area which had previously been abandoned for Panama and Moko disease was selected for a permanent row crop rotation project, merely with the purpose of determining if this type of cultural practice was feasible on a production scale. The project was initiated in June, 1957 with two five-acre blocks planted to Cocos bananas in widely separated rows (24' x 6'). The spaces between rows were planted to kudzu as a ground cover. It was intended to rotate bananas and kudzu at yearly intervals.

Excellent growth of bananas was obtained during the first eighteen months of this project (Fig. 9). The planting distances used allowed excellent light distribution and no special competition problems were apparent. The plants in the experimental area produced fruit well ahead of the surrounding Gros Michel bananas planted at the same time. Most of the plants produced their inflorescence towards the eastern side of the plots so that harvesting was greatly facilitated. The fruit obtained was not of good quality due to severe drought during the early part of 1958 and extensive nematode damage. The fruit weights obtained, however, were considerable higher than those of Gros Michel plants located outside the experimental area (81.6 vs. 70.7 lbs.).

The original rows of bananas were allowed to produce plantilla and first ratoon crops. As soon as the plantilla crop was harvested, new rows of bananas were planted between the original rows and the latter destroyed after the ratoon crop was harvested. The incidence of root diseases in this rotation experiment during the first eighteen months was as follows:

	Old rows	New rows
Panama disease	10.3%	2.5%
Moko disease	0.6%	0.1%

The high incidence of Panama disease during the first part of this project should allow a good evaluation of this control system. It is hoped that the incidence of disease in the new rows will not increase during the next twelve months to a point beyond that obtained in the first stage of this experiment.

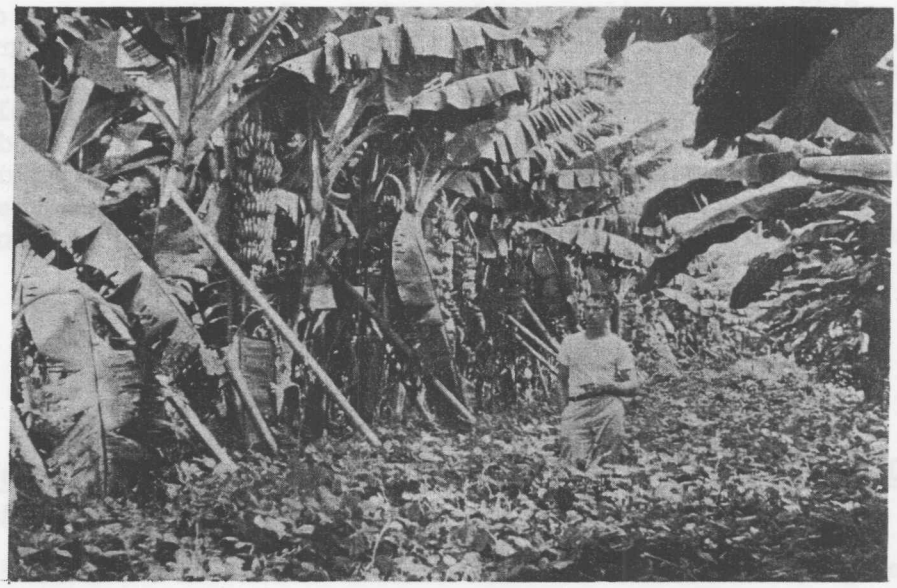


Figure 9. Permanent crop rotation project. Note excellent growth of bananas, planted at 24' X 6'. Ground cover is tropical kudzu.

B. Influence of Certain Soil Factors on Survival of Fusarium Spores

1. Chlamydospore Survival in Resistant and Susceptible Soils

Information included in our Annual Report for 1957 suggested that certain resistant soils from Armuelles Division exerted a significant depressing effect on the survival of introduced Fusarium spores. Because these tests were carried out with a limited number of soils, it seemed desirable to compare a side variety of Armuelles soils with "susceptible" soils from the Coto area..

Soils representing a wide variety of conditions were collected from farms Bogomani and Zapatero in Armuelles Division. These farms are approximately 30 years old and they have solid stands of bananas, in spite of the fact that Panama disease has been present in the area for many years. Samples were also collected from a Panama disease abandonment and from a virgin woodland area in the Coto District. A mixture of Fusarium chlamydospores and conidia was introduced into these soils and survival of the fungus. after an incubation of two months, was determined by standard techniques. The results obtained are summarized in the following table:

Table 28. Survival of Fusarium oxysporum f. cubense spores in various soils.

Soil	Clasification	Fusarium spores per c.c. (Thousands)
Bogomani, Sect. 24	Resistant	52.5**
Bogomani, Sect. 33	Resistant	562.5
Bogomani, Sect. 48	Resistant	413.5
Zapatero, Sect, 31	Resistant	821.5
Zapatero, Sect. 21	Resistant	473.6
Zapatero, Sect. 24	Resistant	484.0
Farm 53 (virgin soil)	?	832.5
Farm 44 (abandonment)	Susceptible	579.5

$L.S.D._{05} = 197.5$

$L.S.D._{01} = 262.7$

A statistical analysis shows that survival of the fungus was significantly lower in resistant soils only in one instance (Bogomani, Sect. 24). It is clear that there is no correlation between longevity of the farm and "resistance" as determined under laboratory conditions. Previous results were based only on two samples from resistant areas and survival in these soils justified the conclusion that they were not favorable for survival of the fungus. The present series of tests indicate that inhibitory factors are not present in all "resistant" soils and in fact, that these factors are the exception rather then the rule for soils from Bogomani and Zapatero farms in Armuelles.

2. Influence of Sugars Exuded from Wounded Banana Roots

In conjunction with previous studies dealing with the influence of amino acids on germination, growth, and sporulation on Fusarium oxysporum f. cubense it was decided to investigate the effect of carbohydrates exuded from the cut surfaces of banana roots using similar techniques. Chromatograms were spotted with material directly from roots or rhizome tissue. In the case of rhizome tissue the chromatograms were spotted from cylinders of tissue cut with a small cork borer. The chromatograms were resolved in n-Bu-OH, HOAc, H_2O or phenol. Ammoniacal $AgNO_3$, triphenyltetrazolium chloride, aromatic amine salts and p-anisidine KCl were used for the detection of sugars. Unfortunately, reagents for testing the presence of phosphorilated sugars were unavailable.

Glucose, fructose and sucrose were the only sugars found using the preceding technique. Glucose and sucrose appeared in the highest concentration, fructose in the least. The concentrations of these sugars varied greatly. In cases where a great deal of light was available to the plant they were high, and in cases where the light was limited they were extremely low. (The light factor was considered significant because of the close planting in normal practice and because of the extreme cloudiness during rainy seasons).

The sugars appeared in almost equal concentrations in the cortex and stele of the rhizome; in the root, however, the highest concentration was always found in the stele. This appears significant in view of the fact that the organism apparently only enters the root where stelar tissue has been exposed.

The influence of these sugars on the germination of spores of Fusarium oxysporum f. cubense was investigated utilizing the technique described in the previous report on amino acids. Discs were cut from suspected areas of resolved chromatograms and placed on seeded glass slides. Control discs were treated similarly.

The results showed that the three sugars detected stimulated germination, growth and sporulation of spores of Fusarium oxysporum f. cubense. Since the results were obvious they were not subject to statistical analysis.

3. Influence of Chytrids

In studying the nature of stimulatory substances present in banana roots, one of the methods used was to apply to seeded glass slides, filter paper discs upon which substances from the cut root surfaces were allowed to diffuse. In order to determine whether the stimulation occurred under normal conditions the glass slides with discs in place were buried in soil. Upon termination of the experiment the slides were removed from the soil, carefully washed, and examined under the microscope.

Areas were observed on the slides where germination, growth and production of chlamydosphores were greatly stimulated. A closer examination of these same areas revealed hyphae radiating from these central areas. The hyphae proved to be rhizoids of Chytrids - the thalli of which were embedded in the discs.

Whether the stimulation of Fusarium oxysporum f. cubense resulted from production of gas(es), nutrients, or other substances produced by the Chytrid, the bacteria associated with the Chytrid or both is unknown.

C. Screening Systemic Fungicides for Control of Panama Disease

The use of materials having systemic activity against the Panama disease fungus when they are absorbed by the roots or foliage of the banana plant could afford practical control of the disease. It seemed important, therefore, to establish methods to screen chemicals with possible chemotherapeutic properties. Due to lack of proper greenhouse facilities, the method adopted consisted of spraying various selected chemicals on young banana plants growing in heavily infested soil in the field. The materials were sprayed at various levels, in a simple latin-square design involving a total of 28 plants per treatment. The materials were applied at weekly intervals for a period of three months and, at the end of this period, disease counts were made based on external and internal symptoms of Panama disease. The method provided a simple and efficient means for testing systemic protectants. As an example, the following table summarizes the results obtain with DL-Phenylalanine and Phenylthiourea, two chemicals known to have systemic activity in other plants.

Table 29. Systemic activity of phenylthiourea and phenylalanine.

Treatment	Conc.	Incidence of Panama Disease (%)
A. DL - phenylalanine	500 ppm	28.5
B. DL - phenylalanine	100 ppm	28.5
C. DL - phenylalanine	50 ppm	21.4
D. Phenylthiourea	500 ppm	28.5
E. Phenylthiourea	100 ppm	7.9
F. Phenylthiourea	50 ppm	7.1
G. Control	--	17.9

It can be concluded from the data presented above that none of the treatments was effective in the control of Panama disease. Since severe phytotoxic effects, consisting of stunting and splitting of leaf sheaths, was observed at the 500 ppm. levels of the two materials used, tests at higher concentrations were not feasible.

The method described above has been used to screen organic mercurials selected for possible systemic activity, as follows:

	Range
1. Ethyl mercury p-toluene sulfonanilide	76-308 ppm.
2. Ethyl mercury tetrahydro endomethano hexachlorophthalimide	31-1000 ppm.

These materials were found to be completely ineffective for control of Panama disease and because of high phytotoxicity, tests at higher dosages were not considered. Other materials are now under investigation.

Sigatoka Disease Investigations

Research at the Coto Station on Sigatoka disease has been limited to field studies comparing control with aerial oil spray and standard Bordeaux mixture hose spray. Although oil application has come into widespread use, few experiments have been conducted to determine the effects of oil on banana plant growth. It was thought that any differences in plant growth between Bordeaux and oil sprayed bananas would show up readily in young plantilla.

1. General

The experimental area consists of 12 paired plots in Potrero C and D of Farm 45. Six plots are 216 by 680 feet and six are 216 by 492 feet. The plots are situated in two rows of six and are receiving alternately oil and Bordeaux. The bananas are planted 6 feet apart in rows 24 feet apart with 10 rows per plot. The smaller plots have 820 plants, the larger 1,100. Each plot is bordered by a large drainage ditch, running parallel with the length of the plot. The plants in the plots on the south side were severely stunted due to poor drainage and cross drains were added.

Work was initiated in March 1958 and planting was carried out from May 21 to June 10. Kudzu was planted between the rows several weeks before planting the bananas. Split bullhead and large sucker seed were obtained from the seedbed in Farm 54 making careful Moko surveys.

The first 4 applications of oil were made with a Motoblo, applying approximately 2-2½ gallons per acre. Spraying was started on August 28, on a 21 day cycle. The last 2 applications were with a helicopter, applying approximately 1¼-1½ gallons per acre. The plots are sprayed with 3 helicopter passes 60 feet apart, per plot, covering the control rows with the same dosage as is normally applied in the field, but slightering the edge or buffer rows.

Drift has been checked at each application, and although there is drift, if application is in early morning the 6 central Bordeaux rows remain free of visible oil droplets. The plan is to take data from the central 6 rows of each plot.

At the present time the plants are shooting or have shot; tagging is being carried out and the following data are being recorded: 1) treatment, 2) plot, 3) row, 4) date when shot, 5) height when shot, 6) number of leaves when shot, 7) number of hands. The following data will be added when the fruit is harvested; 8) date when cut, 9) fruit weight, 10) number of leaves when cut. In addition, heights of the plants were taken in October 5, 6, 7 and December 15, 19 and are presented in Tables 30 and 31.

Table 30. Mean heights and coefficients of variation of plants in oil spray and Bordeuax plots, October 5, 6, 7, 1958.

	Treatment			
	Oil		Bordeaux	
Plot	Height	C.V.*	Height	C.V.
3	4.86	38.6	5.21	34.5
4	6.8	21.5	4.7	33.8
5	7.5	16.6	6.5	24.0
6	7.5	22.4	6.8	17.9

*68% level of security

Table 31. Mean heights of plants in oil and Bordeaux plots, December 15, 19, 1958.

	Treatment			
	Oil		Bordeaux	
Plot	Height	C.V.	Height	C.V.
3	9.4	-	10.0	-
4	10.9	-	9.5	-
5	12.3	-	11.5	-
6	11.2	-	11.6	-

Poor drainage and scalping of the soil initially stunted severely the plants in oil and Bordeaux plots 1, 2, and 3 and in Bordeaux plot 4. At the present, plants in oil and Bordeaux plots 3 are growing well and are comparable. Bordeaux plot 4 is considerably poorer than oil plot 4. Plots 1 and 2 of both treatments remain poor.

2. Preliminary Data, Oil v. Bordeaux

One-hundred plants in each of 8 plots were checked for number of leaves, and results are presented in Table 32.

Table 32. A comparison of mean leaf number per plant in oil and Bordeaux plots, December 1, 1958.

	Mean No. of leaves per plant	
Plot	Oil	Bordeaux
3	12.54	12.49
4	12.26	12.37
5	11.58	12.35
6	12.16	12.04
Total Average	12.13	12.31

Although there averaged 0.18 more leaves per plant in the Bordeaux treatment, this was due mostly to the low average (11.58) in oil plot 5. Oil plot 5 was hit more severely by a blowdown on December 13, than were the other plots. One-hundred of the surviving plants were checked but these probably had some leaf-loss due to the wind. Leaf counts on plants that had shot showed an average of 12.3 for 266 plants under Bordeaux as compared with 12.4 for 403 plants under oil. By the time all leaf counts were taken, all of the leaves on the plants had been subjected to the respective spray treatment for their life.

3. Blowdown

On December 13, 1,583 plants were doubled by the wind. A check of the plants showed that in each plot pair more plants were doubled in the oil than in the Bordeaux treatment (Table 33).

Table 33. A comparison of blowdown loss in oil and Bordeaux plots.

	Blowdown Loss	
Plot	Oil	Bordeaux
4	204	37
5	393	196
6	296	84

At first it was believed that the difference possibly was due to the oil treatment. A map of the distribution of the blowdown plants was made (available upon request) and it was clear that other than the edge oil plot which took the brunt of the wind, oil plot 5 was especially hard hit. A comparison of plant heights taken shorthly before the blowdown revealed that the plants in this plot were the tallest, and therefore presumably the weakest.

In conclusion, the results to date indicate that there is no significant difference between the number of leaves on the plants in the oil plots and the number in the Bordeaux plots. Although the average height of the plants varies from plot to plot, soil factors obscure any possible effects of the oil spray.

Miscellaneous Investigations

1. Hot Water Treatment for Banana Seed

Intensive investigations were carried out during the early part of this year on the possible use of hot water treatment as a therapeutic agent for diseased seed.

a) Experimental results

Approximately 20 per cent of buttons obtained from Panama-diseased plants contained discolored strands from which Fusarium oxysporum f. cubense was isolated. Fusarium was recovered from infected buttons after they were heated directly in water at 65°C for 10 minutes or at 60°C for 15 minutes. Fusarium was also recovered in a few cases from buttons wrapped in polyethylene plastic and immersed in water at 65°C for 30 or 45 minutes.

Ninety-five per cent of buttons cut from Moko-diseased plants contained discoloration due to Pseudomonas solanacearum. Pseudomonas was recovered by isolation and inoculation from buttons wrapped in plastic and immersed in water at 60°C for 30 minutes, 65°C for 30 minutes, and in one case from a button immersed at 60°C for 45 minutes.

A heat treatment of 65°C for 5 minutes was not sufficient to eliminate Moko from seed that had been in contact with the bacteria for as short a time as 2 minutes immediately before heating.

Buttons wrapped in plastic were immersed in water for various treatments. At 60°C for 45 minutes, survival was 11 per cent; at 65°C for 30 minutes, 10 per cent; at 65°C for 45 minutes, zero per cent.

b) Conclusions

Hot water treatments that button-seed can withstand do not eradicate either Fusarium or Pseudomonas located internally in the buttons.

Buttons can survive hot water immersion of 65°C for 5 or 10 minutes or of 70°C for 5 minutes, and at these treatments parasitic organisms present superficially only are killed.

Hot water immersion is not effective for the Moko pathogen even when the seed has been in contact with the bacterium for only a few minutes.

Button-seeds obtained from Moko diseased plants usually carry the Moko pathogen internally. Button-seeds from Panama-diseased plants can contain the Panama Fusarium internally.

Buttons hot water treated at 65°C for 5 minute appear to "germinate" better and initially to grow more rapidly than do non-heat-treated buttons.

c) Recommendations

Button-seed used to establish a nucleus disease-free seed bed can be heat treated in water of 65°C for 5 or 10 minutes to eliminate superficial parasites other than the Moko bacterium.

Before heating, the seed should be washed and root trimmed with a disinfected knife; the pseudostem should be cut to approximately 6 inches. After heating, the pseudostem should be cut with a disinfected knife so that the leaf bases are very short. At this time the area around the growing point will turn black or pink if the button has been killed by the heat treatment. Such buttons can be discarded and the remainder should be placed in the sun to dry and harden for one day. The buttons should be planted so that they are covered by only ½-1 inch of soil.

2. A Program for Certified Seed

a) Objectives

Following the procedures outlined in a recent article in the Research Extension Newsletter, considerable efforts have been made during this year to establish a nucleus of disease-free banana plants which could be propagated under rigorous conditions of care.

The objectives of the disease-free seedbed undertaking, which was initiated in April 1958, were threefold:

(1) To see if it were possible to establish a small nucleus of completely disease-free plants, using carefully selected and heat-treated button seed. (500 plants established from buttons heated at 65°C for 5 or 10 minutes).

(2) To see if the Moko bacterium was present in the particular virgin area selected for the seedbed site (an isolated well drained area in Farm 55).

(3) To provide an initial source of propagation material for the Agriculture Department, so that when the anticipated feasibility of disease-free seed was realized, a source would be ready.

The results to date indicate complete success on all 3 counts.

(1) No disease has occurred, indicating that the seed did not carry pathogens.

(2) No disease has resulted even though root-wounding was practiced to more readily detect possible pathogens in the soil. This indicates that at least in this particular virgin area the Moko pathogen was not initially there.

(3) Propagation of the suckers is now being carried out to provide Agriculture with a seed source of the Gros Michel variety.

b) Discussion

The promising results mentioned above are results to date - how much disease may appear tomorrow, next month or 6 months from now is not predicted by the present record. The status of the various pathogens with regard to this seedbed is as follows:

(1) Moko disease - The seedbed was set-up mainly with Moko in mind. The fact that no Moko has appeared would indicate quite conclusively that the seed was not carrying this pathogen. The absence of the Moko bacterium in the soil is also indicated, but the presence of very low numbers of a potential Moko pathogen is not completely precluded. As propagation ensues, with the necessary increased traffic, the possibility of bringing Moko in from the outside, increases. The roots have been wounded purposely and also in removing suckers in November - sufficient time for Moko to have appeared from root infection.

(2) Panama disease - Since Panama disease appears more slowly, the chances of the fungus being in that virgin soil or in the seed are greater than for the Moko bacterium. However, considering the seed source and the care used, it is not believed that seed carried the Panama fungus. The possibility of its presence in low amount in the virgin area still remains.

(3) Nematodes - Spot checks were recently made in the seedbed and neither Radopholus nor root-knot nematodes were detected. Considering the seed source, and experience with nearby farms, one would not expect Radopholus to appear. Root-knot, however, is common in nearby areas and might reasonably be expected to appear, from an initially low and undetected level.

In addition to the successful establishment of a disease-free seedbed of Gros Michel bananas, as described above, efforts have been made more recently to establish a nucleus of disease - free Cocos bananas. Heat-treated Cocos button-seed were planted in cans containing sterile soil and placed in a virgin woodland area. The seeds were planted in cans to prevent loss of the entire lot, should disease appear at any time during a period of observation of three months. The procedure has been highly successful and the results to date indicate that at least 500 nematode and disease-free Cocos plants will be available for propagation.

Another important aspect of our efforts to obtain clean seed has been the establishment of a Cocos seed-bed for the immediate requirements of the Division. Following recommendations given by this Department, over 2000 large, peeled, heat-treated seed were planted in a virgin woodland area. The nematode and disease-free condition of these plants will be established shortly and, if approved for propagation, the Division will have a good source of Cocos seed for their immediate needs.

DIVISION OF TROPICAL RESEARCH
COTO RESEARCH STATION

ENTOMOLOGY

Leaf-feeding Caterpillars

The entomological activities of this Station during 1958 have been largely confined to studying life histories and methods of control of the various species of leaf-feeding caterpillars attacking bananas. For reasons not yet fully explained, the Panama and Costa Rica Divisions experienced destructive outbreaks of a number of lepidopterous species that although previously known to be pestiferous, had never reached such devastating proportions. It is thought that the change over from Bordeaux to oil spray for Sigatoka control is the principal factor that brought about these outbreaks. Bordeaux is known to act as a repelent to some caterpillars, and this action undoubtedly aided in preventing or supressing population build-ups.

Biological Investigations

The life history of Ceramidia butleri, the predominant leaf-feeding species, was completed during the year. This caterpillar requires about 35 days for development from egg deposition to adult emergence.

The eggs of the moth are deposited on the underside of banana leaves where they hatch in 5 to 6 days. Soon after emerging from the egg the young larva consumes the egg shell and then begins feeding on the leaf. The entire larval life, 18 to 23 days, is spent on the under surface of the leaf where the larva passes through 6 to 7 instars. The duration of the last instar is the longest and during this period the larva consumes more food than during all of the preceding stages. Pupation takes place on the under surface of the same leaf on which the larval life is passed and averages 9 days. The adult is a medium sized, bluish-black moth that is very active during the day.

The entomogenous bacterium, Bacillus thuringiensis was tested for the control of Caligo oileus, Ceramidia butleri, and Sibine apicalis both in laboratory and field experiments.

The results of the tests are shown in Table 1.

Table 1.

Laboratory tests with Bacillus thuringiensis on Caligo, Ceramidi and Sibine Larvae

Insect	Dosage	Cumulative Percent Mortality (Days after test commenced)			
		1	2	3	4
Caligo	1 gm./gal.	0.0	12.5	29.2	37.5
	2 gms./gal.	40.0	52.0	70.0	79.1
Ceramidia	1 gm./gal.	12.0	75.0	95.7	100.0
	2 gms./gal.	12.0	72.0	92.0	100.0
Sibine	1 gm./gal.	0.0	0.0	12.0	92.0
	2 gms./gal.	4.0	29.2	58.3	95.8

Two field experiments, one applying this pathogen mixed in water at the rate of 1 oz. of the wettable spore powder per acre and the other with 1 pound of the material mixed in orchard oil per acre, were performed. The helicopter was used for applying the material in both trials. Neither of these treatments significantly reduced the Caligo populations on which they were tested.

Chemical Control Investigations

Dieldrin, toxaphene, DDT and malathion were evaluated in field experiments for the control of leaf-feeding caterpillars. All of the applications were made by helicopter with the insecticides mixed in orchard oil. The results of these tests showed toxaphene to be generally superior over a larger range of caterpillars than either dieldrin, DDT, or malathion. However, dieldrin proved to be more effective than the others in controlling C. butleri, but gave relatively poor results when used on Sibine sp. or Caligo. DDT was found to be superior for the control of Sibine sp., but would not adequately control Caligo. Malathion was inferior to any of the insecticides tested.

A series of laboratory screening tests were performed during the latter half of the year in which all of the principal insecticides available were tested against Ceramidia, Caligo and Sibine caterpillars.

During the course of these investigations a new method of applying insecticide-oil mixtures at field rates to banana leaf disks was developed.

This method consists offloating .02 ml. of the insecticidal solution (the equivalent dosage of one gallon per acre) on water in a glass crystalization dish. Banana leaf disks of the same diameter as the dish were placed top side down on the resultant oil film. Retention by the leaf of the inseciticide-oil mixture was 90.6% (average of 13 colorimetric tests as shown in Table 2).

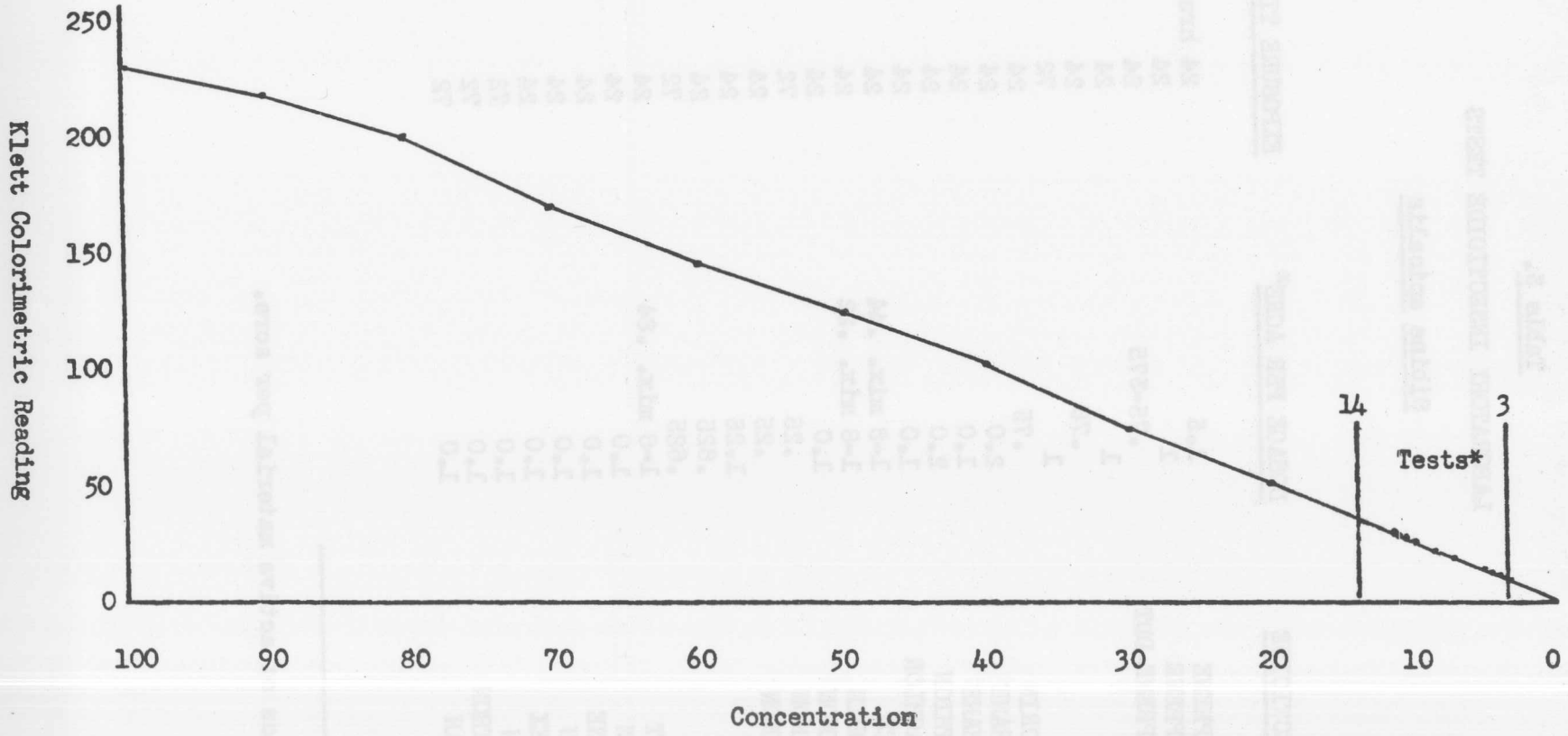
Application of insecticides in this manner give an even, consistent residue on the leaf surface that can be replicated with accuracy.

Following treatment, the leaf disks were placed, treated side out, in 800 ml. beakers. The bottoms of these beaker cages were covered with small disks cut out from larger treated disks, placed treated side down. An untreated leaf disk was placed under the treated one to keep it out of contact of water which was added to keep the leaf in a more succulent condition. Treated leaves in these cages remained attractive to caterpillars for several days.

Field collected caterpillars of uniform size were then placed in the cages and allowed to feed for 24 hours on the treated leaf. Following this period, mortality counts were made and the leaves changed for untreated ones. Observations were continued for 72 hours. The results of these tests, as presented in tables 3, 4 and 5 show DDT, diazinon, rothane and aldrin to be most effective for controlling Sibine. Toxaphene, diazinon and a combination of toxaphene and DDT were superior to others when tested against Caligo. Ceramidia was easily killed by a large number of insecticides of which toxaphene, DDT, dieldrin, perthane, heptachlor, diazinon, sevin, ethion, thiodan, and rothane gave 100 percent kills.

Table 2.

Colorimetric Curve of Oil Plus Brilliant Blue Aniline Dye.
Comparison of Density to Concentration.



* Material remaining in crystalization dish after treatment of leaf disk.

Table 3.

LABORATORY INSECTICIDE TESTS

Sibine apicalis

INSECTICIDE	DOSAGE PER ACRE*	EXPOSURE TIME	PER CENT DEAD OR MORIBUND
TOXAPHENE	1.5	24 hrs.	77.1
TOXAPHENE	1	24	74.8
TOXAPHENE-DDT	.75-375	24	80.0
DDT	1	24	94.5
DDT	.75	24	83.0
DDT	1	72	100.0
DIELDRIN	.75	24	60.0
PERTHANE	2.0	24	60.0
PERTHANE	1.0	24	30.0
MALATHION	2.0	24	60.0
HEPTACHLOR	1.0	24	70.0
NILATE	1-8 mix. .44	24	20.0
PHOSDRIN	1-8 mix. .22	24	28.0
DIAZINON	1.0	24	96.0
DIAZINON	.25	72	100.0
DIAZINON	.25	24	100.0
SEVIN	1.25	24	98.0
SEVIN	.625	24	93.0
SEVIN	.625	72	80.0
THIMET	1-8 mix. .84	24	24.0
KORLAN	1.0	24	68.0
ROTHANE	1.0	24	100.0
ALDRIN	1.0	24	100.0
PHOSTEX	1.0	24	0.0
ETHION	1.0	72	47.6
DECAPTHON	1.0	72	76.3
THIODAN	1.0	72	80.5

* Pounds of active material per acre.

Table 4.

LABORATORY INSECTICIDE TESTS

Caligo oileus

INSECTICIDE	DOSAGE PER ACRE*	EXPOSURE TIME	PERCCENT DEAD OR MORIBUND
TOXAPHENE	1.0	24 hrs.	100.0
TOXAPHENE	.50	24	88.0
TOXAPHENE-DDT	.75-.375	24	95.8
DDT	1.0	24	46.0
DDT	.50	24	58.0
DIELDRIN	.187	24	51.4
PERTHANE	1.0	24	92.0
DIAZINON	.50	24	100.0
SEVIN	.625	24	84.0
MALATHION	1.0	24	68.0
ROTHANE	1.0	24	40.8

* Pounds of active material per acre.

Table 5.

LABORATORY INSECTICIDE TESTS

Ceramidia butleri

INSECTICIDE	DOSAGE PER ACRE*	EXPOSURE TIME	PER CENT DEAD OR MORIBUND
TOXAPHENE	1.0	24 hrs.	93.5
TOXAPHENE	.25	24	100.0
TOXAPHENE-DDT	.75-.375	24	83.3
DDT	1.0	24	100.0
DIELDRIN	.375	72	100.0
DIELDRIN	.187	24	95.7
DIELDRIN	.0935	24	71.3
PERTHANE	1.0	24	100.0
PERTHANE	0.5	24	100.0
PERTHANE	.50	24	80.0
MALATHION	1.0	24	68.5
HEPTACHLOR	.50	24	100.0
NILATE	.44	24	68.0
PHOSDRIN	.22	24	80.0
DIAZINON	1.0	24	100.0
DIAZINON	.5	72	100.0
SEVIN	1.25	24	100.0
SEVIN	.625	72	100.0
PHOSTEX	1.0	72	15.0
PHOSTEX	1.0	24	5.0
ETHION	1.0	72	100.0
ETHION	.25	24	50.0
THIODAN	1.0	72	100.0
ROTHANE	1.0	72	100.0

* Pounds of active material per acre.

Peel Scarring Beetle

A small chrysomelid beetle (Allochroma sp.) appeared in several farms during October and November. Control measures using .75 pounds of dieldrin per acre applied by helicopter were satisfactory. Larvae of this beetle have been found feeding on the roots of Gamalote grass (Paspalum fasciculatum) which is probably its primary natural host.

Insect Transmission of Moko Disease

To date no insects have been proven to be important in the transmission of Moko disease from one banana plant to another. Laboratory experiments in which large number of Metamasius sericeus were allowed to feed over night between two pieces of Moko infected pseudostem and then removed and caged on the surface of freshly cut suckers were performed. Under these artificial conditions, only 20% of the suckers showed disease symptoms 21 days after treatment.

A small area on Farm 51 was chosen for a field experiment to substantiate the laboratory findings. In this experiment borer traps made of Moko infected pseudostem disks were placed in the area for collection of M. sericeus. At the time of release of the collected beetles 213 suckers were cut and marked. 18 days later these suckers were examined and all were found to be free of disease. Similar tests with Cosmopolites sordidus and Rhynchorophorus palmarum also showed that these beetles were not important in the spread of Moko disease.

Banana Stalk Borer

Although the population of this insect has continued to decline throughout most areas, efforts were made to find a chemical control method to use should it become a serious problem again.

Chlordane, cryolite, DDT, dieldrin, endrin, liquid soap, malathion, mobisol 2522, nicotine sulfate, oprex No. 3, prorex No. 1, rotenone, vegetable oil, and DNOC have shown little promise as ovicides against eggs of the stalk borer. DNOC was effective in laboratory tests, but in the field trials proved to be ineffective in reducing the viability of borer eggs.

Transmission of Bacterial Tiprot of Bananas

Various species of insects have been observed visiting the flower ends of the young banana fingers. In trying to determine if these insects are important in transmitting tip-rot, an effort was made to prevent their coming in contact with the young banana fingers by means of non-perforated polyethylene bags.

During October and November of 1957, 309 of these bags were placed on the young fruit before the young fingers were exposed beneath the brackets. At the same time, 300 plants were marked as controls. During January and February these fruits were checked for the presence of tip-rot. As shown in Table 6, disease incidence was reduced considerably by the bagging treatment.

It is not recommended however, that this method be used, as bagging with the non-perforated bags results in burned and disfigured fruit.

Table 6.

Comparison of Disease Incidence
Between Bagged and Unbagged Fruit

	No. of stems with tip-rot	% stems with tip-rot	No. stems disease free	% stems disease free
Bagged	14	9%	146	91%
Unbagged	165	56%	127	44%