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REACTION OF COCOA CLONES AND HYBRIDS TO *Ceratocystis fimbriata* AND INHERITANCE OF RESISTANCE

Thesis submitted for consideration by the Commission of the Joint Graduate Studies Program in Agricultural Sciences and Natural Resources of the University of Costa Rica and the Centro Agronómico Tropical de Investigación y Enseñanza, as a prerequisite for the degree

Magister Scientiae

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SUMMARY

The laboratory reaction of various cocoa clones to *Ceratocystis fimbriata* Ellis & Halsted was studied using the methodology described by Delgado in 1965 (Turrialba 15(4):286-289). When Delgado's results for the reaction of some selected clones could not be reproduced, various environmental factors were studied as possible sources of variation in the method. The most probable source of variation was the addition of powdered cocoa pod to the growth medium of the fungus.

A reevaluation of species of *Theobroma* and *Herrania* produced results comparable to those of Delgado.

The laboratory results for the reaction of various cocoa clones to *C. fimbriata* and Delgado's published results for these same clones did not correlate with their field mortality during 14 years.

The genetic analysis of a half-diallel cross of cocoa clones demonstrated that the general combining ability of some clones and the specific combining ability of some hybrids, have significant effects on the inheritance of survival to *C. fimbriata* attack. Also, a study of survival curves of clones and hybrids suggested the existence of additive gene effects, dominant genes for resistance and susceptibility and heterosis.

RESUMEN

Para estudiar la reacción de ciertos clones de cacao a *Ceratocystis fimbriata* Ellis & Halsted, se utilizó la metodología descrita en 1965 por Delgado (Turrialba 15(4):286-289). Al no poder reproducir los resultados esperados con clones de reacción conocida, se estudiaron diversos factores ambientales que pudieran alterar los resultados del método. La adición de polvo de mazorca de cacao al medio de cultivo artificial, demostró ser un factor importante, en este aspecto.

Sin embargo, cuando se evaluaron especies de *Theobroma* y de *Herrania*, los resultados concordaron con los de Delgado.

Las pruebas de laboratorio efectuadas en este estudio para evaluar la reacción de diversos clones a *C. fimbriata* y los resultados publicados por Delgado, no coincidieron con los datos de mortalidad de plantas en el campo, ocurridos durante los últimos 14 años.

Al realizarse el análisis genético de un dialelo parcial de clones de cacao, se demostró que la habilidad combinatoria general de algunos clones y la habilidad combinatoria específica de algunos híbridos, tienen efectos significativos sobre la herencia de la sobrevivencia al ataque de *C. fimbriata* en el campo. Además, el estudio de las curvas de sobrevivencia de clones e híbridos sugirió la existencia de efectos aditivos de genes, genes dominantes para resistencia y susceptibilidad y heterosis.

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1. INTRODUCTION

"Mal de machete", caused by *Ceratocystis fimbriata* Ellis & Halsted, is an important fungal disease of cocoa in tropical America. This pathogen has caused the greatest loss of cocoa trees in Ecuador (14), Colombia (1), Venezuela (24), and Trinidad-Tobago (39).

Chemical control of this fungus or its insect vector (20, 29, 34, 42) were inadequate. Cultural practices (1,6,24) have also been ineffective. The use of resistant varieties of cocoa is probably the most practical control method.

The most commonly used laboratory method to measure the reaction of cultivars to the pathogen was developed by Delgado and Echandi (7,8) and basically consists of cocoa branch section inoculation with the fungus. The fungus is cultivated on potato-dextrose agar (PDA) and inoculations are made using suspensions of ascospores and mycelium.

The objectives of this study were:

1) Test reproducibility of Delgado's method
2) Determine the field resistance of cocoa clones
3) Develop a method to predict heretability of *C. fimbriata* resistance in cocoa hybrids
4) Compare field mortality of cocoa clones with their laboratory reaction to *C. fimbriata*.

2. LITERATURE REVIEW

The pathogen and disease symptoms

The disease of cocoa (*Theobroma cacao L.*) known as "mal de machete" is caused by the fungus *Ceratocystis fimbriata* Ellis & Halsted, an ascomycete with a broad host range. Some species affected by this pathogen are *T.cacao*, *Crotalaria sp.*, *Coffea arabica*, *Ipomoea batatas*, *Mangifera indica*, *Hevea braziliensis*, and *Platanus racemosa* (2).

In cocoa, the insect vector, *Xyleborus ferrugineus* (F.), may disseminate the pathogen. Naundorf (28) reported that disease symptoms appeared three months after artificial inoculations of cocoa trees with *Xyleborus spp.* Of the numerous species of *Xyleborus* which attack cocoa, Saunders (33) noted that only *X.ferrugineus* attacked healthy trees and, therefore, is the principal insect vector.

Non-sterile prunning tools can also disseminate the pathogen. Malaguti (26) reduced infection incidence from 66% to 18% by sterilizing the "machetes" used in prunning.

Iton (23) described the first symptoms of an infection with *C.fimbriata* as a rapid leaf discoloration on a branch or the entire tree. Disease symptoms progress rapidly and in less than a month the leaves yellow, become necrotic, and tend to curl-up longitudinally. The dead leaves remain hanging for months after tree death (11).

The fungus grows mainly within the xylem and bark of the trunk and branches (10, 19), causing a reddish discoloration of these tissues. The fungus moves throughout the xylem tissue and can affect all parts of the tree, including the pods. The pods of an infected tree are soft. Infected xylem tissue has a distinctive odor and frequently contains tunnels bored by *Xyleborus spp.* Sawdust from *Xyleborus spp.* borings can be observed on infected tree bases. This sawdust, the bark and xylem discoloration, the soft pods, and the dead leaves attached to the branches are symptoms that differentiate this disease from other cocoa diseases.

Methods to test the reaction of cocoa *C. fimbriata*

Several methods for measuring the reaction of different cocoa clones to *C. fimbriata* are reported. Cevallos (5), Chong (6), and Domínguez (12) reported few consistent results from inoculating live branches or seedlings with *C. fimbriata*. Delgado (7) and Idrobo (21) evaluated the use of a toxin produced by the pathogen to screen cocoa seedlings for resistance. Ruiz (31, 32) developed a colorimetric method for measuring the chlorophyll destruction in the leaves of inoculated seedlings.

Capriles de Reyes (4) measured the level of chlorogenic acids in resistant and susceptible clones before and after inoculation with the pathogen. The level of chlorogenic acids, particularly gentesic acid, which has mycostatic properties, increased in the tissues of a resistant clone and decreased in the susceptible clone.

Delgado and Echandi (7, 8) adapted a method previously used by Echandi and Fernández (13) to evaluate the reaction of coffee to the same pathogen. Branch sections of clones were inoculated with suspensions of *C. fimbriata* ascospores and perithecia and mycelium growth was measured after four days.

Factors which affect the results of Delgado's method

Factors that may affect Delgado's method were studied to determine possible sources of variability. Soria and Salazar (35) noted seasonal differences in the reaction of clones. The test was most sensitive from August to November and least sensitive from May through June at Turrialba, Costa Rica. They attributed this variation to some unknown weather related physiological change in the trees. In Trinidad (40) more variation was reported when testing susceptible clones than resistant ones, indicating that the method only served for selection of very resistant clones.

Temperature affected mycelial growth and perithecial production on inoculated branch sections from resistant and susceptible clones equally (15, 40).

The physiological age of the clonal material can affect the results of Delgado's method (15, 16). New shoots growing from the trunk appear minimally more resistant than fan branches. Branch section diameter had little effect on test results (15).

Inoculum concentration may cause variation, but Espinoza (15), using various concentrations of ascopore suspension inoculations,encountered no significant differences. Culture age did not reduce inoculum viability, except when a 75-day-old culture was used (15, 16). *C.fimbriata* isolate pathogenicity may vary. *C.fimbriata* isolated from coffee is morphological identical to *C.fimbriata* from cocoa and both can be cross-inoculated (2), but each isolate grows better in its original host. *C.fimbriata* isolated from sweet potato is morphologically, but not physiologically, identical to *C.fimbriata* isolated from sycamore (18). Hybridizations between these physiological races are possible.

Basis of resistance to *C.fimbriata* in cocoa

Porer (30) observed that the variety "Nacional" of Oriunda, Ecuador, had fewer losses in the field and attributed this apparent resistance to a rapid healing of lesions.

Among the three main groupings of cocoa, resistant varieties occur in the "Forasteros", highly susceptible varieties in the "Criollos" and examples of resistance and susceptibility in the "Trinitarios" (9, 22, 25). Capriles de Reyes (4) demonstrated that the forastero 'IMC-67' contained higher concentrations of chlorogenic acids than the hybrid criollo 'OC-61' before and after inoculation with *C.fimbriata*. This characteristic may influence field resistance demonstrated by some forasteros. It has yet to be determined whether 'IMC-67' is resistant under field conditions.

Resistance to the insect vector may be a possible mechanism for resistance in cocoa (36). When *X.ferrugineus* was caged on the trunk of the clone 'SCA-12', insect mortality was significantly greater than when caged on two other clones and one hybrid.

A natural insecticide in 'SCA-12' was suspected as the cause of insect mortality.

Chong (6) inoculated six-month-old seedlings with *C. fimbriata* and reported 'SCA-6', a forastero, as a highly resistant clone. Cevallos (5) inoculated six-month-old seedlings and found that all of the crosses with 'SCA-6' were more resistant than other crosses. The clone 'SCA-6', although susceptible, showed disease symptoms much later than the other clones. Bartley (39) noted that during a seven year period 5% of ICS-1xSCA-6 had died. In contrast, Delgado (7) considered the reaction of 'SCA-6' as highly susceptible in laboratory tests.

Several studies (7, 8, 35, 41), using Delgado's method, have shown that the forasteros 'IMC-67', 'POUND-12', 'SPA-9' and the hybrid forasteros 'EET-399' and 'EET-400' are resistant. The hybrid trinitario clones 'UF-29' and 'UF-613' were field resistant after 12 years (37). However, Delgado (7) considered the clone 'UF-29' as susceptible and 'UF-613' as highly susceptible.

Field mortality of cocoa clones

Bartley, in Trinidad (39), listed the percent mortality of various clones and hybrids and noted that field loss of ICS-6 x SCA-6 seedlings were much less resistant than cuttings from this same hybrid.

Soria (37) found that *C. fimbriata* usually does not kill cocoa trees until after onset of production, with most loss occurring from seven to nine years after the trees are established.

The genetic basis of resistance

Soria and Salazar (35) suggested that susceptibility to *C. fimbriata* may be controlled by up to three independent dominant genes or by one major dominant gene pair with one or more modifiers.

They hypothesized that additional genes were involved and that these genes could intensify or diminish the effect of the major gene for susceptibility.

Bartley (39), comparing the field mortality of three clones and their hybrid progeny, suggested that susceptibility to *C. fimbriata* might be controlled by a dominant gene but gene interaction may have modified the effect of susceptibility.

3. MATERIALS AND METHODS

The procedure outlined by Delgado (7), henceforth referred to as Delgado's method, was used as a standard for laboratory tests to determine cocoa resistance to *C. fimbriata*. Four-centimeter-long sections from 1.5 cm. diameter fan branches are split to expose the xylem tissue and then inoculated with one drop of ascospore suspension (30,000 ascospores per ml. of distilled water) prepared by mixing masses of ascospores from the perithecia of the fungus grown on PDA. Ascospore masses were mixed in a small mortar and pestle, consisting of a small test tube fitted with a glass rod. Inoculated branch sections are incubated in a moist chamber for 4 days and mycelial growth and perithecia is examened with a dissecting scope, using a magnification of 16X. Arbitrary categories (0= absence of perithecia, 1= 1-5 perithecia, 2= 6-15, 3= 16 or more, and 4= completely covered with perithecia) were used to measure resistance. Mycelial growth also was considered; category 0 had no mycelial growth, 4 abundant growth and 1, 2, 3 intermediate.

Effect of light

Ten 4cm. branch sections of 'SPA-9' and 'IMC-67', resistant clones by Delgado's method, and 'UF-677', susceptible by Delgado's method were inoculated and one half of each section placed in a moist chamber under clear glass and the other half of each section placed in a dark chamber. All clonal material came from the clonal cocoa garden of CATIE, in Turrialba (climatic characteristics of Turrialba are given in Table 1A.) Inoculum was prepared from a 30 day old culture of *C. fimbriata* isolated in the clonal garden of CATIE, grown on PDA + 1% powdered cocoa pod (PDA+C), and designated TUR-1. After inoculation of all branch sections, the chambers were sealed for 4 days and then evaluated for resistance.

Effect of temperature

Mycelium growth and perithecia production on two clones, was determined at 10C, 20C, 24C (in incubators) and 25± 4C (laboratory temperature).

The branch sections were placed in petri dishes with moist filter paper. Inoculum was prepared from 30-day-old culture of isolate TUR-1 grown on PDA+C. The clones tested were 'SPA-9' and 'UF-677' considered resistant and susceptible, respectively, by Delgado.

Effect of inoculum concentration

A concentrated ascospore suspension was prepared and measured with a hemicytometer, then diluted to various concentrations and used in 4 trials of varying concentrations, but with an over-all range of 10 to 3,150,000 ascospores per ml. of suspension. The source of inoculum was a 30-day-old culture of TUR-1 grown on PDA+C. The clones tested were 'IMC-67' and 'ICS-1', considered resistant and susceptible, respectively, by Delgado.

Pathogenicity of different isolates of *C. fimbriata*

The pathogenicity of 8 isolates from various infected cocoa trees were compared (Table 3A). Two isolates were from Turrialba and 6 were from the La Lola Experimental Cocoa Farm (Table 2A is a summary of the climatic characteristics of La Lola). Since the two areas are at least 60Km. apart, with a complete absence of cocoa plantations between them, it was expected that these isolates would show some differences in pathogenicity.

Fifteen clones, representing resistant, moderately resistant, and susceptible material were selected from the list of clones evaluated by Delgado. These clones were then inoculated in the laboratory with suspensions of ascospores from the 9 isolates grown on PDA+C. The concentration of all suspensions was approximately 30,000 ascospores per ml.

Evaluation of species of *Herrania* and *Theobroma*

Herrania and *Theobroma* species, from the clonal garden of CATIE, previously tested by Delgado, were inoculated following Delgado's method. The inoculum source was isolate LL-2 grown on PDA+C.

Evaluation of cocoa clones

A reevaluation of 49 of the 50 clones evaluated by Delgado was made using his procedure. The results were compared with Delgado's original data. Isolate LL-2, grown on PDA+C, was used.

Effect of powdered cocoa pod in the growth medium

Branch sections from five different clones (including susceptible and resistant) were separated into two groups. One group was inoculated with an ascospore suspension from a 14-day old culture of isolate LL-2 grown on PDA + 1% dry powdered cocoa pod (prepared from a mature pod of clone 'UF-677'). The other group was inoculated with an ascospore suspension originating from a 14-day-old colony of the same isolate, grown on PDA alone. All other steps in Delgado's procedure were unchanged.

Field mortality of cocoa clones and their hybrid progeny

Field mortality data from Experiment La Lola No.26 established in 1966 at La Lola Experimental Farm, Limon Province, Costa Rica were used to estimate the reaction of six clones and their hybrid progeny to *C. fimbriata*. Delgado classified clones 'SPA-9', 'IMC-67', and 'POUND-12' as highly resistant and clones 'ICS-1' and 'ICS-45' as highly susceptible. Clone 'UF-613', although classified by Delgado as highly susceptible, showed tolerance to the disease in the field. Diallel crosses between these six clones were attempted, but due to the auto-incompatibility of the first three clones, autofecundations and many crosses could not be obtained.

Hybrid trees from other experiments at La Lola were used in retrocrosses to produce triple hybrids, but the incompatibility inherent in many of the crosses limited the amount of crosses obtained. The three hybrid trees used were T9B1P6A6 (POUND-12 x UF-613), T3BA6 (ICS-1 x IMC-67) and T3BA21 (ICS-1 x IMC-67).

The experimental design consisted of 4 blocks of 36 plots each, with the plots randomly distributed in a 6 x 6 simple square lattice.

All plots contained 15 trees planted at a distance of 3 x 3 m.

In addition to the 6 clones planted as rooted cuttings and the 29 crosses planted as seedlings, each block contained a plot of the hybrid ICS-1 x SCA-6 as a control. This is susceptible in the field.

Yield and tree mortality were recorded bi-weekly, beginning in the fifth year of the experiment. The first trees died after the iniciation of observations, but causes of death were not recorded. To compensate for this uncertainty, mortality causes were determined for eight months, beginning in February, 1979. For purposes of this study, the cause of tree death will be attributed to *C. fimbriata*.

Dates of tree death, average monthly maximum-minimum temperature and total monthly precipitation were recorded for the 13 1/2 year duration of this experiment. These environmental variables were compared with the monthly tree mortality.

Additional mortality data were collected from the plots of 10 clones in Experiment La Lola No.19 in La Lola Experimental Cocoa Farm, established in February of 1965. This experiment was designed to furnish information on the yield of cocoa clones and hybrids.

Due to several aspects of the design, this experiment cannot easily be used in a genetic study of resistance or in comparison with Experiment No.26. This experiment was planted a year earlier than Experiment No.26. The planting distance of trees was 2 x 2 m. versus 3 x 3 m. for Experiment No.26. During the first few years after establishment, dead trees were replaced with the hybrid SCA-6 x ICS-1. In view of the above limitations of the experiment, mortality data was used only from clones: 'IMC-67', 'SCA-6', 'POUND-12', 'UF-12', 'UF-29', 'UF-613', 'UF-654', 'UF-667', 'UF-668', 'UF-677' and 'ICS-1'. Mortality incidence of these clones and for the six clones from Experiment No.26 were compared with laboratory measurements of resistance.

4. RESULTS AND DISCUSSION

LABORATORY RESULTS

Effect of the absence or presence of light

Light apparently affects the laboratory measurement of clonal reaction to *C. fimbriata* (Table 1). Differences between clones, light and darkness, and the interaction of those variables was significant (Table 4A). Delgado did not mention control of light, but although light may stimulate the growth of mycelium and the production of perithecia, its presence or absence probably did not cause the differences between the reactions measured in this study and the reactions measured by Delgado. Delgado considered 'SPA-9' and 'IMC-67' as highly resistant (less than 1.00), but in the present study, these two clones reacted susceptibly under both light and dark conditions. The clone 'UF-677', considered highly susceptible by Delgado (more than 3.00), was just susceptible.

Effect of temperature

Temperature markedly affects perithecia production and mycelium growth (Table 2). No fungal growth occurred at 10°C and growth was impeded, but not halted, at 20°C. The other two temperatures did not affect fungal growth.

Differences between reactions of the two clones, at the four temperatures, were minimal. There was no significant difference ($\alpha = 0.05$) between the two clones, although there was an interaction between clones and temperature (Table 5A), indicating that different clones react differently to temperature variations.

However, the difference between Delgado's results for these two clones and the results obtained in this study cannot be explained as resulting from an effect of temperature. Other workers (15, 40) also have reported that the method is not affected by temperature, except to accelerate or slow down fungal growth on all clones more or less equally.

Table 1. Effect of light on the number of perithecia and amount of mycelium of *C. fimbriata* formed on detached branches of three cocoa clones. Turrialba, Costa Rica, 1980.

Clone	Light	
	Absence	Presence
SPA-9	2.40[1]/	3.00
IMC-67	3.05	3.40
UF-677	2.75	2.70
Average	2.73	3.03

1/ Average between perithecia and mycelium of 10 repetition per treatment, expressed in units from Delgado's scale (7).

Table 2. Effect of temperature on the number of perithecia and amount of mycelium of *C. fimbriata* formed on detached branches of two cocoa clones. Turrialba, Costa Rica, 1980.

Clone	Temperature (C)			
	10	20	24	25±4
SPA-9	0.00[1]/	1.90	3.30	3.00
UF-677	0.00	1.30	3.40	3.60
Average	0.00	1.60	3.35	3.30

1/ Average between perithecia and mycelium of 5 repetitions per treatment, expressed in units from Delgado's scale (7).

Effect of inoculum concentration

Inoculum concentration had little effect on the reaction of two clones (Table 3). Change in fungal growth was observed for the 1000 ascospores/ml concentration. This variation could be attributed to a lack of ascospores in some of the drops of inoculum placed on the branch sections. This was evidenced by either no growth or abundant growth when the concentration was low. The minimum concentration should be 5000 ascospores/ml. Delgado used a concentration of 15,000 ascospores/ml.

Effect of the isolate of *C. fimbriata*

Isolate LL-1 was significantly different from all other isolates except LL-3 (Tables 4 and 6A). Isolate LL-3 was different from isolates TUR-2 and LL-6. These differences cannot explain the reaction of susceptibility manifested by the previously resistant clones used in this experiment. If the pathogenicity of the isolate of *C. fimbriata* used by Delgado in Turrialba in 1964 was different from the pathogenicity of isolates used in this study, then one might conclude that during the 15 years since that study, the pathogenicity of *C. fimbriata* and its possible races has increased. Delgado's isolate no longer exists and it cannot be determined whether the pathogenicity of his isolate was less than the isolate used in this study.

Evaluation of the resistance of species of *Herrania* and *Theobroma*

Reevaluation of the species tested by Delgado indicates that the reaction of these species has not changed substantially (Table 5). The four species of *Theobroma* that Delgado considered resistant are still resistant. The addition of cocoa pod to the growth medium of the fungus does not affect the reaction of these species to *C. fimbriata*. The observed differences, could be attributed to interpretation of the scale used to measure resistance. A variation 0.5 between the same readings of two workers is not uncommon.

Table 3. Effect of the inoculum concentration of *C. fimbriata* on the number of perithecia and the amount of mycelium formed on detached branches of two cocoa clones. Turrialba, Costa Rica, 1980.

Concentration Ascospores/ml X10	Trial							
	A1/		B2/		C2/		D2/	
	IMC-67	ICS-1	IMC-67	ICS-1	IMC-67	ICS-1	IMC-67	ICS-1
3150	3.40[3]/	4.00						
1570	3.70	3.90						
600	3.10	3.50						
100			3.60	3.55				
60	3.00	3.40						
30	2.70	3.20	3.70	3.12				
15	2.60	3.20	3.60	3.15	3.70	3.55		
10							2.70	3.00
5			3.50	2.95	3.50	3.45		
1			0.00	0.00			2.70	2.78
0.5			3.20	2.80	3.20	3.20		
0.1							0.80	0.60
0.01							0.65	0.20

1/ Average of perithecia and mycelium in 5 repetitions per concentration

2/ Average of perithecia and mycelium in 10 repetitions per concentration

3/ Values expressed in units from Delgado's scale (7).

Table 4. Effect of the isolate of *C. fimbriata* on the number of perithecia and amount of mycelium formed on detached branches of 15 cocoa clones. Turrialba, Costa Rica, 1980.

CLONE	Fungus Isolate															
	TUR-2		TUR-4		LL-1		LL-2		LL-3		LL-4		LL-5		LL-6	
	I*	II+	I	II	I	II	I	II	I	II	I	II	I	II	I	II
UF-242	3.0	3.2	3.0	3.6	2.5	3.2	3.0	3.3	2.9	3.8	3.3	3.3	3.0	3.1	3.4	3.3
R-101	3.4	3.8	3.5	3.3	3.0	3.3	3.1	3.2	2.9	3.1	3.2	3.2	3.1	3.6	3.5	3.7
APA-5	3.8	3.4	3.6	3.7	2.8	3.0	3.5	3.7	3.2	3.7	3.3	3.4	3.2	3.4	3.1	3.8
R-105	3.3	3.1	3.5	3.0	3.1	3.0	3.0	3.1	2.1	3.2	3.2	3.1	3.2	3.6	3.4	3.0
SPA-10	3.5	3.2	2.8	3.2	2.4	3.0	2.7	3.1	2.5	3.3	2.7	3.2	2.7	3.2	2.9	3.5
POUND-12	3.4	3.9	3.1	3.7	3.1	3.8	3.6	3.6	3.7	3.5	3.6	4.0	3.6	3.5	3.4	3.7
UF-221	3.1	3.4	3.3	2.8	3.0	3.2	3.4	3.1	3.0	3.1	3.4	3.2	3.2	3.5	3.0	3.5
IMC-67	3.6	3.6	3.2	3.3	2.9	2.8	3.3	3.0	2.9	3.1	3.4	3.3	3.2	3.2	3.2	3.4
UF-667	3.4	3.9	3.3	3.7	3.1	3.9	3.3	3.8	3.4	4.0	3.2	3.4	3.2	3.4	3.1	3.4
SPA-7	3.6	3.5	3.1	3.4	3.1	3.5	3.5	3.4	2.9	3.6	3.1	3.7	3.2	3.6	3.4	3.7
UF-668	3.4	3.0	3.3	3.0	2.7	2.8	3.8	3.0	3.3	2.9	3.4	3.4	3.1	3.6	3.2	3.6
R-48	3.8	2.8	3.8	2.6	3.8	2.9	3.8	3.1	3.8	3.2	3.8	3.3	3.8	3.7	3.7	3.1
SPA-9	3.7	3.7	3.6	3.9	2.9	3.6	3.2	3.7	3.1	3.5	3.2	3.7	3.3	3.9	3.4	3.7
ICS-45	3.1	3.5	3.1	3.5	2.8	3.0	3.2	3.2	2.8	3.2	3.2	3.8	2.8	3.5	2.9	4.0
SPA-12	3.7	3.8	3.5	3.4	3.1	3.8	3.3	3.5	3.3	3.3	3.4	3.8	3.3	4.0	3.4	3.9
$\bar{X}$	3.5	3.5	3.3	3.3	2.9	3.3	3.3	3.3	3.0	3.4	3.3	3.4	3.2	3.5	3.3	3.5
$\bar{\bar{X}}$	3.5c†		3.3bc		3.1a		3.3bc		3.2ab		3.3bc		3.3bc		3.4c	

* Average of perithecia and mycelium in 10 repetitions per clone (Trial I)
\+ Average of perithecia and mycelium in 5 repetitions per clone (Trial I)
† Test of Duncan

If the pathogenicity of *C. fimbriata* has changed, this change has not greatly affected the measurement at the level of species of *Herrania* and *Theobroma*.

Evaluation of reaction of clones

The susceptible reaction of all 49 clones tested, conflicts with Delgado's original data that rated 3 as resistant (less than 1.000) and 10 as tolerant (1.000 - 2.500) (Table 6). The powdered cocoa pod in the growth medium of the fungus could be the source of this variation, significant at α=0.05 (Table 8A).

A pathogenicity difference between the isolate used in these determinations and the isolate used by Delgado, could be the source variation. The reaction of species (Table 5) and the results of the test of isolate (Table 4) make this unlikely.

Effect of the growth medium on laboratory resistance

To determine the effect of the addition of cocoa pod to the growth medium of the fungus, eight isolates of *C. fimbriata* were transferred to PDA. Only isolate LL-2 grew in this medium and mycelial growth was reduced. Perithecia production was less than in a culture containing cocoa pod.

The only observable difference between the LL-2 culture and the other cultures was the presence of a small piece of PDA+C, which had been transferred along with the fungus. It appeared that the isolates needed a trace of cocoa pod to grow in PDA, and that the amount present in this piece of PDA+C was sufficient to stimulate fungal growth.

Ascospore suspensions were prepared from this culture and from another LL-2 culture of the same age, but grown on PDA+C. Branch sections from five clones were inoculated with the ascospore suspensions and the results presented in Table 7. There is no significant difference at α = 0.05 between the two suspensions used in the inoculations (Table 9A).

Table 5. Reevaluation of resistance of species of *Theobroma* and *Herrania* using inoculum from a colony of *C. fimbriata* grown on PDA + 1% cocoa pod. Turrialba, Costa Rica, 1980.

Species	Average of perithecia and mycelium	
	PDA + 1% cocoa 1/	PDA 2/
Theobroma angustifolia	0.300	0.037
Th. mammosa	0.400	0.042
Th. simiarum x *Th. mammosa*	0.367	0.177
Th. mammosa x *Th. simiarum*	0.433	0.332
Th. speciosa	3.665	1.117
Th. grandiflora	0.365	1.882
Th. subincana	2.265	1.357
Th. Simiarum	3.100	2.187
Th. bicolor	3.765	2.567
Th. microcarpa	3.833	3.137
Th. cacao var. *Pentagona amarilla*	3.565	3.860
Th. cacao var. *Pentagona roja*	3.965	3.570
Herrania nicterodendron	3.333	2.917
H. albiflora	4.000	2.930
H. balaoensis	3.833	3.165
H. cuatrecasana	3.468	3.222
H. nitida	4.000	3.637
H. purpurea	3.598	3.667
H. humbratica	3.735	3.737
Average	2.736	2.292

1/ Average of 15 repetitions per species

2/ Average of 36 repetitions per species from Delgado (7)

Table 6. Reevaluation of the reaction of 49 cocoa clones using inoculum from a colony of *C. fimbriata* grown on PDA+1% cocoa pod (PDA+C), Turrialba, Costa Rica, 1980.

Clone	Ave. of perith.and mycel. PDA+C[1]/	PDA[2]/	Clone	Ave. of perith.and mycel. PDA+C[1]/	PDA[2]/
SPA-9	3.944	0.430	APA-5	3.500	3.625
POUND-12	3.111	0.515	R-2	4.000	3.652
IMC-67	3.527	0.597	SPA-5	3.667	3.747
OATONCO	3.805	1.945	UF-654	3.583	3.765
IAL-93	3.580	2.025	MATINA	2.945	3.777
SPA-11	3.888	2.237	ICS-6	3.833	3.817
SPA-10	3.694	2.277	UF-613	3.250	3.820
SPA-12	3.833	2.375	UF-672	3.110	3.875
SPA-7	3.333	2.417	UF-221	3.527	3.890
UF-273	3.194	2.470	UF-10	3.861	3.930
SIC-6	3.889	2.512	ICS-95	4.000	3.940
R-101	3.583	2.722	R-48	3.416	3.957
UF-242	3.805	2.735	UF-296	3.194	3.970
R-105	3.277	2.860	UF-11	3.027	3.985
SIC-2	3.694	3.277	UF-677	3.750	3.985
R-117	3.916	3.277	SCA-12	3.028	4.000
R-41	3.472	3.290	UF-650	3.361	4.000
SIC-28	3.389	3.302	SCA-6	3.528	4.000
LAFI-7	4.000	3.317	ICS-1	3.691	4.000
UF-29	3.278	3.320	UF-168	3.694	4.000
APA-4	3.333	3.387	UF-668	3.888	4.000
UF-12	3.666	3.472	UF-676	3.888	4.000
R-9	3.861	3.485	UF-667	3.944	4.000
R-52	3.027	3.540	ICS-45	3.944	4.000
R-19	3.472	3.610			

1/ Average of 18 repetitions per clone

2/ Average of 36 repetitions per clone from Delgado (7)

Table 7. Effect of the growth medium on the pathogenicity of *C. fimbriata* inoculated on detached branches of 5 cocoa clones. Turrialba, Costa Rica, 1980.

	Culture Medium	
CLONES	PDA	PDA + 1% COCOA
IMC-67	2.90[1]/	3.20
SPA-9	3.90	3.60
EET-399	2.80	2.90
SCA-6	3.10	3.10
ICS-1	2.90	3.18
Average	3.12	3.18

1/ Average of perithecia and mycelium in 5 repetitions per clone, Isolate L1-2.

A difference in the nutritional requirements between these isolates and the isolates used by Delgado (7) and other workers (15, 40, 41) probably exists, since their isolates grew on PDA without an addition of cocoa pod. Montes de Oca (27) inoculated some of the clones tested by Delgado with *C. fimbriata* isolated and grown on PDA+C and encountered variations. By counting the number of perithecia per clone, he recorded differences between clones which were small, but significant. However, Soria and Salazar (35) duplicated Delgado's results using a culture of *C. fimbriata* grown on PDA + 1% cocoa stem.

To determine whether isolate pathogenicity was the source of variation, a culture of isolate L1-2 was sent to Dr. Carmen Suárez for testing in Pichilingue Experimental Station in Ecuador. When she inoculated branch sections from clones: 'IMC-67', 'POUND-12' and 'ICS-1', as well as tree #5715, with suspensions of ascospores and mycelium from isolate LL-2 grown on PDA+C and from an isolate of Pichilingue grown on PDA, there were differences between the reactions of the two isolates.

Isolate LL-2, inoculated on clone IMC-67, produced perithecia and mycelium, but growth was less than that presented by the same isolate inoculated on the other clones. In comparison, the inoculations with the isolate from Pichilingue continued to give results comparable to Delgado's original data. Dr. Suárez suggested that this difference between the two isolates might arise from the fact that isolate LL-2 was isolated on PDA+C and the other was isolated on PDA[1]/.

Perhaps at the time of isolation, a strain of the fungus was selected for growth on PDA+C and another strain was selected for growth on PDA. These two strains would then be physiologically, but not morphologically different. Soria and Salazar's data (35) could be explained by the fact that their culture of *C. fimbriata* was isolated on PDA and later grown on PDA+C[2]/ . Apparently the fungus isolated on PDA can also grow on PDA+C. The differences between the reactions of clones as measured in this study, and the reactions noted by Delgado could be due to a physiological difference between isolates.

FIELD RESULTS

Field mortality in Experiment La Lola No.26

The validity of field data is based on the assumption that *C. fimbriata* caused the death of all cocoa trees, although direct determinations of the cause of death for many of the trees is lacking. This assumption was made because:

1. Dying cocoa trees with symptoms of *C. fimbriata* were observed in all the blocks of the experiment (see Figures 1A and 2A).
2. Of the 11 cocoa trees which died during the 8 month period of observation, all of them had symptoms of *C. fimbriata*.

1/ Suárez, C. personal communication, Pichilingue Experimental Station, Ecuador, 1979.

2/ Salazar, G., personal communication, CATIE, Turrialba, Costa Rica, 1979.

3. *Roselinia sp.*, a fungal pathogen which kills cocoa trees, has never been observed in this experiment.
4. Die-back, a physiologically caused disease of cocoa, has not been observed in this experiment.
5. Shade trees, planted amongst the cocoa, can fall and cause the death of cocoa trees. In this experiment, no shade trees have fallen.

The percent mortality of the clones and hybrids in Experiment La Lola No.26 ranges from 1.67 to 96.67 (Table 10A). The variation between plots of the same clone or hybrid was minimal.

Genetic analysis of percentage survival of cocoa clones and hybrids

The use of percent age data in a statistical analysis often has theoretical objections, since such data does not have a normal distribution. Percentages can be transformed, with the aid of various methods, to data which approximates a normal distribution. However, when the data originates from populations which contain the same number of individuals, then such percentages are real numbers which have a normal distribution and do not require transformation.

A genetic analysis of the clones and hybrids in Experiment La Lola No.26 is complicated because many of the crosses in the originally planned diallel are missing. The crosses, needed to complete the diallel, are impossible to obtain due to incompatibility.

Enough crosses exist to construct a half-diallel (Table 8) using the data from 10 of the 29 hybrids in the experiment. The clone 'SPA-9' was excluded for lack of crosses with the other five clones.

Table 11A contains the analysis of variance for the 10 cross diallel. The variation between blocks is not significant and there is no significant interaction between blocks and crosses at the 0.05 level.

Variation between crosses is significant at $\alpha = 0.05$.

Three important measurements calculated in genetic analysis are: average combining ability (ACA), general combining ability (GCA), and specific combining ability (SCA).

Table 8. Percent survival of 10 single crosses of a half diallel cross between 5 clones. Data from Experiment La Lola #26, La Lola Experimental Cocoa Farm, Limón, Costa Rica, 1980.

	IMC-67	POUND-12	ICS-1	ICS-45	Average combining ability of clones
UF-613	.8833	.7000	.5167	.2000	0.5750
IMC-67		.6000	.8500	.7833	0.7791
POUND-12			.8500	.2500	0.6000
ICS-1				.0333	0.5625
ICS-45					0.3166

Average survival of 10 crosses: 0.5667

The ACA of a clone is the average performance of the clone, as calculated by the average performance of all the hybrids in the diallel in which that clone was a parent. The clone 'IMC-67' has the highest ACA for survival and 'ICS-1' the lowest (Table 8). The ACA does no- reflect the variability in the performance of a clone in a series of crosses.

The GCA of a clone represents the average performance of a clone in a series of crosses. It also gives an indication of the consistency of that performance and estimates the additive effect of genes. GCA is expressed as a positive deviation from the mean performance of all the crosses in the diallel.

The clone 'IMC-67' has the highest positive GCA and 'ICS-45' the most negative (Table 9).

The calculation of the SCA of each cross requires a measurement of variability within each plot.

Since the percent survival of trees for each plot cannot be used to measure this variability, the percent survival of the 5 trees in each of 3 rows per plot was used. This gives 3 individual measurements per plot and 12 values per cross.

Table 9. General combining abilities of the clones used in a half-diallel cross from Experiment La Lola No.26, La Lola Experimental Cocoa Farm, Limon, Costa Rica, 1980.

Clone:	IMC-67	POUND-12	UF-613	ICS-1	ICS-45
GCA :	0.2798	0.0468	0.0135	-0.0089	-0.3312

The SCA of a cross represents the deviation of its performance from the performance as predicted from parental GCA's or the additive effect of genes. The SCA indicates the effect of a dominant gene or genes in the hybrid. The SCA also indicates the presence of positive or negative heterosis, which is the interaction of dominant genes in the hybrid (17).

The hybrids IMC-67 x ICS-45 and POUND-12 x ICS-1 have the largest positive SCA's (Table 10), and therefore demonstrate the most effect of dominant genes and positive heterosis for resistance to *C. fimbriata*.

Table 10. Specific combining abilities of the hybrids of a half-diallel cross from Experiment La Lola No.26, La Lola Experimental Cocoa Farm, Limón, Costa Rica, 1980.

	IMC-67	POUND-12	ICS-1	ICS-45
UF-613	0.0248	0.0748	-0.0525	-0.0472
IMC-67		-0.2915	-0.0028	0.2695
POUND-12			0.2472	-0.0305
ICS-1				-0.1918

IMC-67 x POUND-12 and ICS-1 x ICS-45 have the largest negative SCA's, and therefore demonstrate the most effect of dominant genes for susceptibility and negative heterosis for resistance to *C. fimbriata*.

The mean value of a hybrid is the sum of the parental GCA's, plus its SCA. The mean value represents the deviation of the hybrid's survival from the mean survival (0.5667) of all the hybrids of the diallel.

The hybrids UF-613 x IMC-67, POUND-12 x ICS-1 and IMC-67 x ICS-1 have the highest survival values of the diallel (Table 11). The hybrids ICS-1 x ICS-45, UF-613 x ICS-45 and POUND-12 x ICS-45 have the lowest survival values.

TAble 11. Mean values for survival of the hybrids of a half-diallel cross from Experiment La Lola No.26, La Lola Experimental Cocoa Farm, Limon, Costa Rica, 1980.

	IMC-67	POUND-12	ICS-1	ICS-45
UF-613	0.3181	0.1351	-0.0479	-0.3649
IMC-67		0.0351	0.2681	0.2181
POUND-12			0.2851	-0.3149
ICS-1				-0.5319

The effect of the GCA and the SCA are both significant at $\alpha = 0.05$ (Table 12A). This suggests that a breeding program designed to improve the resistance of cocoa to *C. fimbriata* can be based on the use of clone with good GCA's (such as 'IMC-67'; to produce specific hybrids with good SCA's and high survival values.

Components of phenotypic variance

The overall phenotypic variance (V_P) of the survival values of the diallel crosses can be partitioned into its sources or components: the additive genetic variance (V_A), the dominance variance (V_D), the environmental variance (V_E), and the interaction variance (V_I).

The interaction variance arises from an interaction between the V_A and the V_D and is considered by Falconer (17) to be very small and therefore can be ignored. Calculated values for the other components of phenotypic variance are:

$$V_A = 0.0596 \pm 0.0582$$

$$V_D = 0.0492 \pm 0.0286$$

$$V_E = 0.0478 \pm 0.0075$$

$$V_P = 0.1566$$

The first two components have large standard errors, suggesting that the number of crosses used to calculate these components was small. Also, the use of clones, which are not homozygous inbred lines, introduces a variable which probably affects the accuracy of the estimates. This variable arises from the genetic variation between gametes produced by a heterozygous clone.

These three components of the phenotypic variance can be used to estimate the heritability (h^2) of the clones used in the diallel. Heritability is the ratio between the genetic variance (V_G) and the phenotypic variance or the proportion of the variability within a population caused by gene action and not environmental effects. The V_G is the sum of all the components of V_P minus V_E and is equal to 0.1088, disregarding standard errors. Therefore:

$$h^2 = V_G/V_P = 0.1088/0.1566 = 0.6948$$

or 69.48% of the phenotypic variance of the hybrids of the diallel is due to gene action and 30.52% is due to the environment which might be explained by the fact that individual tree death is determined by both the genes of resistance of the tree and by the environmental effect on the expression of these genes. The environmental effect on the pathogen, which could possibly determine how often it comes in contact with the tree, probably is more important.

When the environmental component of the phenotypic variance is large, the trait being measured probably is being coded for by a "large" number of genes. With many genes involved, the environment has more chance to effect a given trait by altering the expression of these genes. When only one gene pair is coding for a trait, the genes can only express themselves or not, depending upon the environment. This gives rise to very little variation. When many genes are involved, as appears to be the case for resistance to *C. fimbriata* in cocoa, the trait has a large range of variability due in part to the effect of the environment.

Since the remainder of the phenotypic variance is almost equally determined by additive variance and dominance variance, very little improvement in resistance can be expected by crossing resistant clone with high yielding clone of low resistance because the additive effect of genes represents only 38.06% of the variation in resistance. Clones with dominant genes plus genes with an additive effect should be used to improve the resistance of cocoa to *C. fimbriata*. This would first require the determination of the genes which code for resistance, which apparently has never been done.

Survival curves for clones and hybrids

The recorded date of death of trees in each plot of each clone and hybrid was used to calculate the average survival per year of the 14 years (6 months in the nursery plus 13 1/2 years in the field) since Experiment La Lola No.26 was started. No deaths occurred until the fifth year of the experiment.

The average number of trees surviving for the four plots of each clone or hybrid (Table 13A) was used to calculate regressions using the linear model: $Y = b_0 + b_1X + b_2X^2 + b_3X^3$ Y is the number of surviving trees in a plot of 15 years, X is the number of years since the experiment was started and the b's (Table 14A) are coefficients. The coefficients of the equation determine the slope of the survival curve for each clone or hybrid.

This model was used since it has the highest R^2 values of the linear models tested for this data. The large R^2 values in all of the regressions (Table 14A) indicate that the linear model has a good fit for the data.

The predicted survival per year of the clones and hybrids was graphed (Figures 1 through 4). The predicted number of trees alive at the start of the experiment may be more less than the actual 15 trees present and some of the curves predict that the number of trees actually increase in time, but those are only effects of the linear model. These curves serve to compare the survival between clones and their hybrid progeny and do not represent exactly the actual survival data.

The survival curves of the hybrids IMC-67 x ICS-1, ICS-1 x IMC-67 and their clonal parents (Figure 1a) indicate that the two hybrid populations are very similar, therefore there appears to be no cytoplasmic effect on resistance in the clones. These hybrids appear to demonstrate heterosis for resistance to *C. fimbriata* since their survival rate is greater than their clonal parents. However, the small SCA of IMC-67 x ICS-1 (Table 10), does not support this assumption. The resistance of these hybrids is due to the additive effect of genes. The survival curves of 'IMC-67' and 'ICS-1' do not accurately reflect their GCA's (Table 9).

The survival curve of the hybrid IMC-67 x ICS-45 (Figure 1b) demonstrate that this hybrid also has more resistance than its clonal parents. However, the resistance of this hybrid is less than the hybrids formed from 'IMC-67' and 'ICS-1'. This lower resistance is due to an additive effect of genes, since 'ICS-45' has a larger negative GCA than 'ICS-1'. Also the large positive SCA of this hybrid demonstrates the action of a dominant gene or genes for resistance in 'IMC-67'.

There appears to be maternal effects in the hybrids POUND-12 x ICS-1 and ICS-1 x POUND-12, since there is a difference of resistance between them (Figure 1c). When 'ICS-1' is pollinated with 'POUND-12' the hybrid is less resistant than when 'POUND-12'

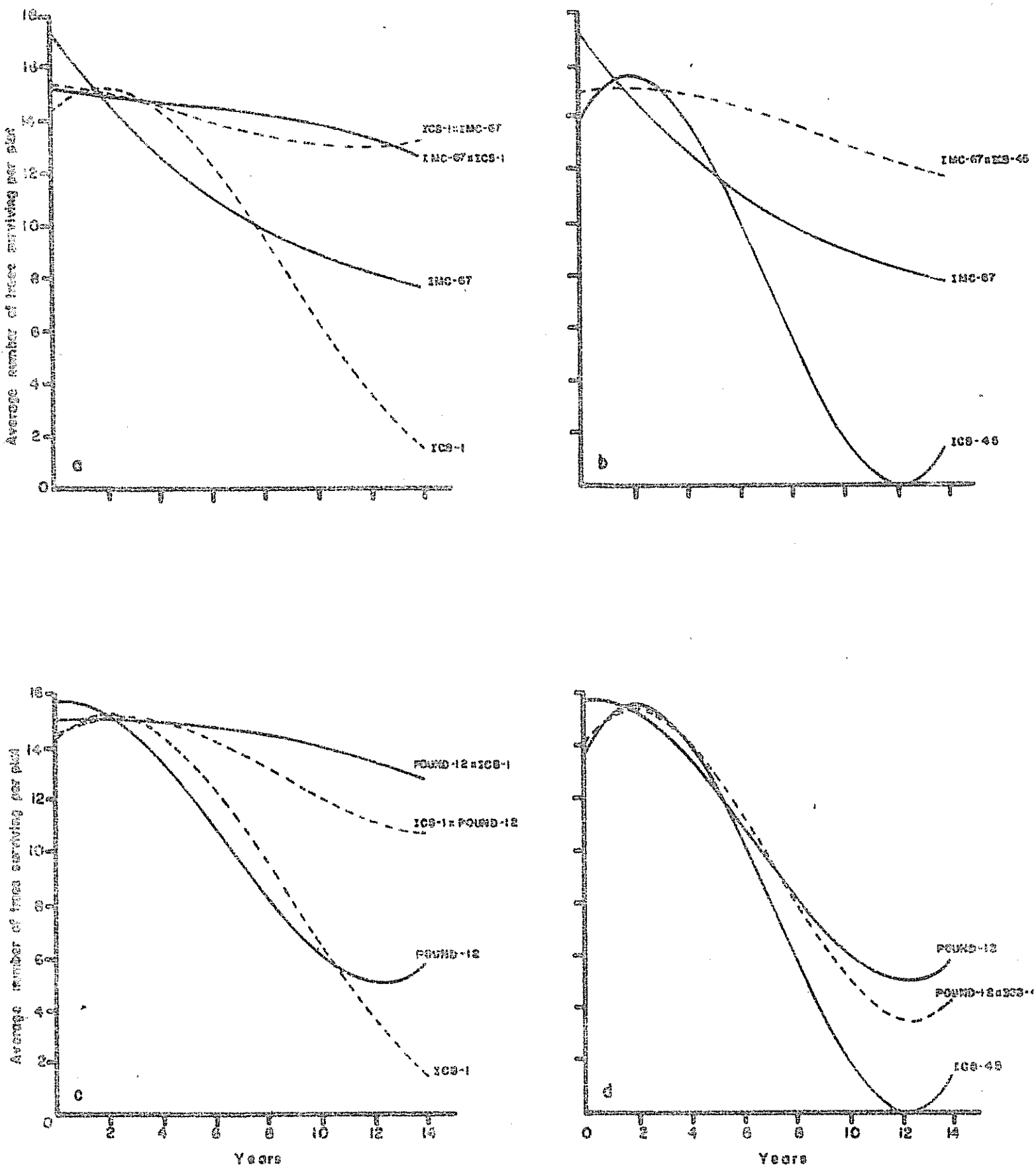

Fig. 1 Mean survival rate of cocoa clones and their hybrid progeny as related to age of trees. Curves calculated using 15 trees per plot with 4 repetitions (Linear model $Y = b_0 + b_1 X + b_2 X^2 + b_3 X^3$)

is pollinated with 'ICS-1'. This difference in resistance is similar to the difference between the clonal parents. These hybrids possess more resistance than their parents. The large SCA of POUND-12 x ICS-1 reflects this heterosis.

The resistance of POUND-12 x ICS-45 lies between the resistances of the parental clones (Figure 1d) and demonstrates the additive effect of genes. The small SCA of this hybrid does not eliminate the possibility that the effect of a dominant gene for resistance in 'POUND-12' is canceled by the effect of a dominant gene for susceptibility in 'ICS-45'.

There may be a paternal effect in the hybrid ICS-1 x UF-613 and UF-613 x ICS-1, since the resistance of each hybrid more closely resembles the resistance of the clone used for pollination (Figure 2a). There appears to be an additive effect of genes since the resistance of the hybrids lies between the resistance of the clonal parents and the SCA of UF-613 x ICS-1 is small.

The resistance of the hybrid UF-613 x ICS-45 (Figure 2b) more closely resembles the resistance of 'ICS-45' than 'UF-613'. This is an additive effect of genes and reflects the large negative GCA of 'ICS-45'. The negative SCA of this hybrid, although small, may indicate the action of a dominant gene for susceptibility in 'ICS-45'.

The resistances of the hybrids SPA-9 x ICS-1 and its reciprocal cross are almost identical (Figure 2c), indicating lack of cytoplasmic gene effects. A dominant gene for resistance in 'SPA-9' is evidenced by the hybrid's resistance being more similar to 'SPA-9' than 'ICS-1'.

Some additive gene effect is probable because the hybrids are less resistant than 'SPA-9' and are therefore affected by the low resistance of 'ICS-1'.

ICS-1 x ICS-45, a cross between two susceptible clones, is also very susceptible (Figure 2d). 'ICS-45' probably has a dominant gene for susceptibility, since the hybrid has a large negative SCA and its survival curve resembles the survival curve of 'ICS-45'.

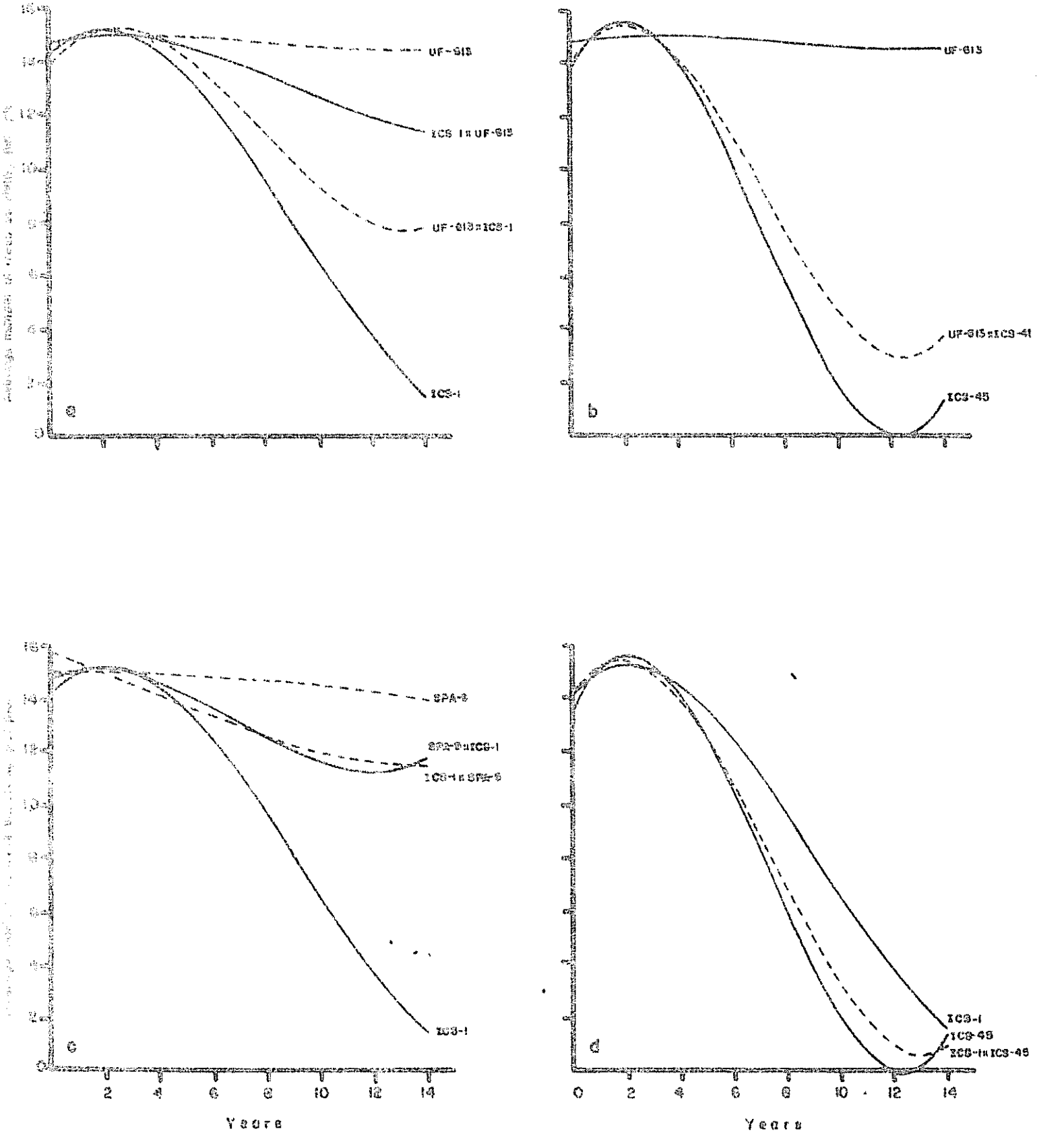

Fig. 8 Mean survival rate of cocoa clones and their hybrid progeny as related to age of trees. Curves calculated using 15 trees per plot with 4 repetitions (Linear model $Y = b_0 + b_1 X + b_2 X^2 + b_3 X^3$)

Figure 3a represents the survival curves of the crosses formed by self pollinating the clones 'ICS-1' and 'ICS-45'. The resistance of ICS-1 x ICS-1 is similar to the resistance of the clone 'ICS-1'. This suggests that during the recombination of genes, no new combinations are formed which differ from the resistance of 'ICS-1'. If this is true, then 'ICS-1' is mostly homozygous for its genes of resistance.

In contrast, when 'ICS-45' is self pollinated, the cross contains individuals which are less susceptible than their clonal parents. Perhaps during recombination of genes, some individuals do not receive a dominant gene for susceptibility. This would only be true if 'ICS-45' is heterozygous for a dominant gene for susceptibility.

The hybrids formed from the crossing of 'UF-613' and 'IMC-67' (Figure 3b) are very resistant, but not as resistant as the clone 'UF-613'. Since the two hybrids have almost identical resistance, there are no apparent maternal or paternal effects from the clones. The small SCA of UF-613 x IMC-67 indicates an additive effect of genes.

The most resistant hybrids are formed by crossing 'UF-613' with 'SPA--' (Figure 3c). These clones are also the most resistant in the field and since the crosses between them are equally resistant, it appears that they may have homozygous genes of resistance. The resistance of these hybrids could also be explained by the presence of a homozygous dominant gene for resistance in one of the clonal parents.

The presence of this dominant gene would compensate for any recessive gene for susceptibility also present in the hybrids. Since 'UF-613' doesn't show any effect of a homozygous dominant gene when crossed with 'IMC-67' (Figure 3b), 'SPA-9' probably contributes the dominant gene for resistance.

The retrocrosses of 'IMC-67' and 'ICS-1' with two trees (T3BA21 and T3BA6) of the hybrid ICS-1 x IMC-67 are plotted in Figure 3d. T3BA21 appears more resistant than T3BA6 since ICS-1 x T3BA21 is more resistant than ICS-1 x T3BA6.

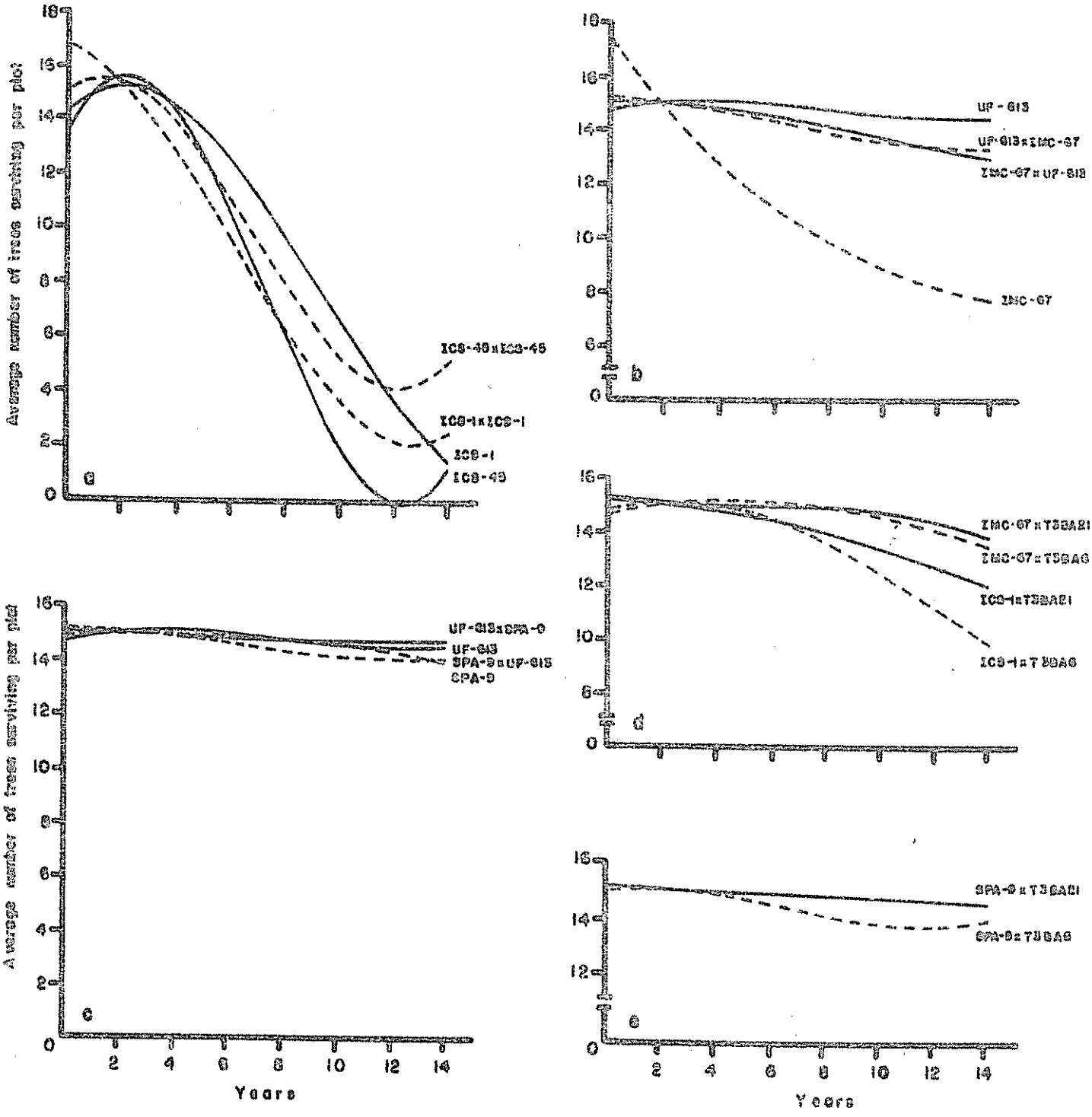

Fig. 3 Mean survival rate of cocoa clones and their hybrid progeny as related to age of trees. Curves calculated using 16 trees per plot with 4 repetitions (Linear model $Y = b_0 + b_1 X + b_2 X^2 + b_3 X^3$)

The difference in resistance between IMC-67 x T3BA21 and IMC-67 x T3BA21 and IMC-67 x T3BA6 is small, but T3BA21 appears more resistant than T3BA6. These curves show the additive effect of genes since the retrocrosses with 'ICS-1', which has a negative GCA, are more susceptible than the retrocrosses with 'IMC-67', which has a positive GCA.

The survival curves for 'SPA-9' crossed with T3BA21 and T3BA6 (Figure 3e) also demonstrate that T3BA21 is more resistant than T3BA6, but the difference is small. The similarity of these curves to the survival curve of 'SPA-9' (Figure 3c) suggests that 'SPA-9' has a dominant gene for resistance or that few individuals with homozygous recessive genes for susceptibility are formed during gene recombination.

The resistance similarities of the triple hybrids POUND-12 x T3BA6 (Figure 4a) and ICS-1 x T3BA6 (Figure 3d) suggests that 'POUND-12' and 'ICS-1' contribute the same resistance to these hybrids. The similarity is also expressed in their survival curves (Figure 1c).

POUND-12 x IMC-67 and the reciprocal cross have greater survival than their clonal parents (Figure 4a) indicating the presence of hybrid vigor. However, the large negative SCA of IMC-67 x POUND-12 is an indication of negative heterosis. The resistance predicted by the sum of parental GCA's exceeds the actual resistance of the hybrid.

The survival curves of the triple hybrids UF-613 x T9B1P6A6 (POUND-12 x UF-613) and ICS-1 x T9B1P6A6 are plotted in Figure 4b. The survival curve of UF-613 x POUND-12 was included for comparisons since the reciprocal cross POUND-12 x UF-613 was not made. UF-613 x T9B1P6A6 is more resistant than UF-613 x POUND-12 and ICS-1 x T9B1P6A6 is less resistant. This suggests additive gene effects on the part of 'UF-613' and 'ICS-1' which have positive and negative GCA's, respectively.

The survival curve of ICS-1 x SCA-6 is plotted in Figure 4c. Plots of the clone 'SCA-6' were not included in this experiment

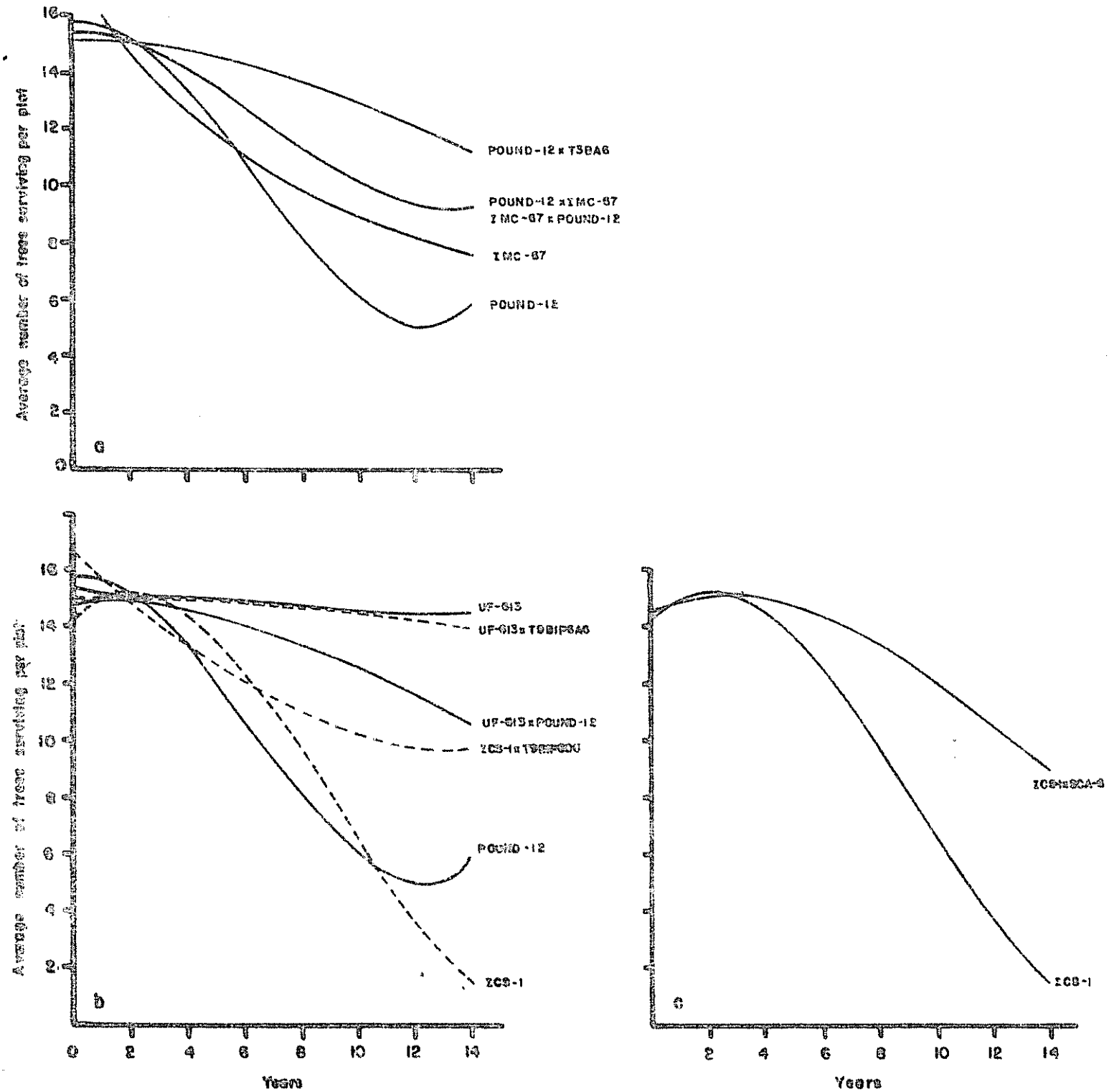

Fig. 4 Mean survival rate of cocoa clones and their hybrid progeny as related to age of trees. Curves calculated using 15 trees per plot with 4 repetitions (Linear model $Y=b_0+b_1X+b_2X^2+b_3X^3$)

so it is impossible to determine whether this hybrid is more, or less resistant than this clone. Bartley (39) observed this first seven years after planting and reported a 5% tree loss. This same hybrid, in Experiment La Lola No.26, had a 6.7% tree loss in the first seven years after planting, but lost an additional 31.7% during the next seven years. This confirms Soria's (37) observation that the greatest loss of cocoa trees to *C. fimbriata* begins from seven to nine years after planting.

Correlation of mortality with environmental parameters

The number of trees dying per month in Experiment La Lola No.26 (Figure 5) was related to total monthly precipitation and average maximum and minimum temperature. There is no correlation between monthly mortality and these environmental factors (Table 15A). If tree death occurrs two to three months after infection with *C. fimbriata*, the highest correlation between mortality and these factors would occurr two to three month before. However, there is no correlation for up to five months before death. This lack of correlation is perhaps due to the fact that dates of inicial tree infection were not determined. Present evidence suggests that precipitation and temperature do not have any effect on the incidence of the disease.

Correlation of field mortality and laboratory resistance

Field mortality of 10 clones, along with their laboratory reaction to *C. fimbriata* (grown on 2 different growth mediums) is presented in Table 12. The coefficients of correlation for this data (Table 17A) are low and no correlation is apparent.

The factor being measured by Delgado's method, perhaps is not an important component of resistance in cocoa. The genetic analysis and the survival curves of clones and hybrids suggest that several genes are responsible for resistance to *C. fimbriata*. If each gene controls a separate characteristic for resistance, then a loboratory test that only measures one of these characters could not determine a clone's actual resistance and would therefore show little correlation with field resistance. The lack of correlation between field data and Delgado's method may indicate that the method does not measure resistance to *C. fimbriata* in cocoa.

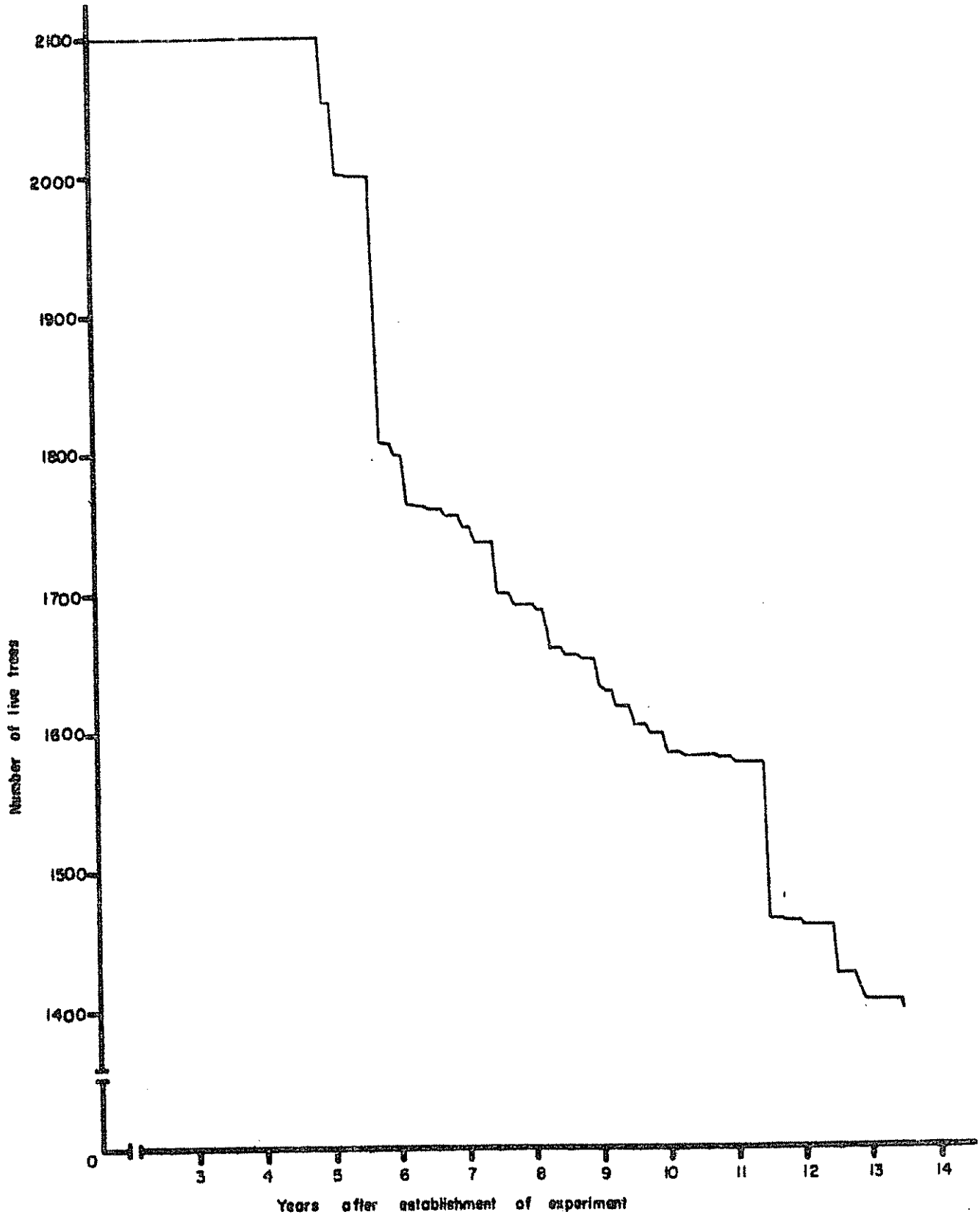

Fig. 5 Acumulative mortality of cocoa trees during 13.5 years in experiment La Lola #26, La Lola Experimental Cocoa Farm, Limon, Costa Rica, 1980

Table 12. Correlation between field mortality and laboratory resistance of 13 cocoa clones inoculated with ascospore suspensions from colonies of *C. fimbriata* grown on PDA and PDA + 1% cocoa pod(PDA+ C). Turrialba, Costa Rica, 1980.

Clone	% Mortality[1/]	Average of perithecia and mycelium	
		PDA[2/]	PDA+C[3/]
UF-29	2.344	3.320	3.278
SPA-9	6.667	0.430	3.944
UF-613	7.917	3.820	3.250
SCA-6	10.937	4.000	3.528
UF-12	18.750	3.472	3.666
IMC-67	20.583	0.597	3.527
POUND-12	39.286	0.515	3.111
UF-668	48.437	4.000	3.888
UF-667	53.125	4.000	3.944
UF-654	65.625	3.765	3.583
UF-677	73.437	3.985	3.750
ICS-1	86.667	4.000	3.691
ICS-45	96.667	4.000	3.944

1/ Data from Experiments La Lola #19 and #26

2/ Average of 36 repetitions per clone from Delgado(7)

3/ Average of 18 repetitions per clone

5. CONCLUSIONS

1. Light, temperature, and inoculum concentration were not determined to be a source of variation in Delgado's method.

2. Differences between isolates of *C. fimbriata* are not the source of variation in Delgado's method.

3. The addition of 1% powdered cocoa pod to the growth medium of the fungus, could not be shown to be the source of variation in Delgado's method. However, only one of the 9 isolates tested grew on the growth medium not containing cocoa pod, and growth was greatly reduced. Delgado's isolate (which grew on PDA without cocoa pod) probably had a different nutritional requirement than the isolates used in this study and this difference is probably the source of variation in Delgado's method.

4. The results of the reevaluation of the species of *Herrania* and *Theobroma* , previously evaluated by Delgado, demonstrate that the reaction of these species has not changed substantially.

5. The results of the reevaluation of clones demonstrate a significant difference from the results obtained by Delgado. All clones had reactions characteristic of susceptibility.

6. The results of the genetic analysis of a half diallel cross demonstrate that the general combining abilities of clones, and the special combining abilities of crosses, both have significant effects. These measurements should therefore be used as a basis for selection of clones and hybrids, to increase the resistance of cocoa to *C. fimbriata*.

7. The phenotypic variance, for survival of 10 cocoa hybrids, is 38.06% due to additive gene effects, 31.42% due to dominant gene effects, and 30.52% due to environmental effects.

8. The clones 'IMC-67' and 'SPA-9' probably have dominant genes for resistance to *C. fimbriata*.

9. The clone 'ICS-45' probably has a heterozygous dominant gene for susceptibility to *C. fimbriata*.

10. The clone 'ICS-1' probably has a homozygous dominant gene for susceptibility to *C. fimbriata*.

11. No correlation was shown between average monthly precipitation, maximum-minimum temperature, and incidence of death due to *C. fimbriata*.

12. No correlation was shown between field mortality of clones and their laboratory reaction to *C. fimbriata*.

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APPENDIX

Table 1A. Geographic position and climatic characteristics of Turrialba, Costa Rica.

Geographic Position

Altitude: 602 meters above sea level

Latitude: 9°53´North

Longitude:83°39´West

Climatic Characteristics[1/]

Mean annual temperature: 22.2C

Mean annual precipitation: 2,673mm

Average relative humidity: 87.4%

Average daily solar radiation: 423.72cal/cm^2/day

Average daily evaporation: 3.99mm

Life zone: Premontane wet tropical forest

1/ Average of more than 14 years

Table 2A. Geographic position and climatic characteristics of La Lola Experimental Cocoa Farm, Limon, Costa Rica.

Geographic Position

Altitude: 40 meters above sea level

Latitude: 10°06´North

Longitude: 83°23´West

Climatic Characteristics[1/]

Mean annual temperature: 24.9C

Mean annual precipitation: 3,666mm

Average relative humidity[2/]: 87.0%

Average daily solar radiation: 279.20cal/cm^2/day

Average daily evaporation[3/]: 1.78mm

Life zone: Lowland wet tropical forest

1/ Average of more than 16 years

2/ Average for Limon

3/ Average for 1979,Limon

Table 3A. List of isolates of *Ceratocystis fimbriata*

Isolate No.	Date Isolated	Place of Origin	Cocoa Clone
TUR-2	4 Oct 1978	CATIE, Turrialba	ICS-53
TUR-4	27 Jun 1979	La Universidad Turrialba	?
LL-1	16 Jan 1979	La Lola, Limon	Open Pollen
LL-2	17 Jan 1979	" " "	" "
LL-3	" " "	" " "	UF-667
LL-4	" " "	" " "	UF-676
LL-5	" " "	" " "	"
LL-6	" " "	" " "	"

All isolates grown on PDA+1% cocoa pod

Table 4A. Analysis of variance for effect of light

Source of Variation	d.f.	MS	*α=0.05
Repetitions	9	0.178	
Treatments	5	1.178*	
Clones	2	1.754*	
Light	1	1.350*	
Clones x Light	2	0.538*	
Error	45	0.146	

Table 5A. Analysis of variance for effect of temperature

Source of Variation	d.f.	MS	*α=0.05
Repetitions	4	0.042	
Treatments	5	4.335*	
Temperature	2	9.925*	
Clones	1	0.008	
Temp x Clones	2	0.908*	
Error	20	0.127	

Table 6A. Analysis of variance for effect of isolate

Source of Variation	d.f.	MS	*α=0.05
Isolates	7	0.383*	
Error	232	0.103	

Table 7A. Analysis of variance for species inoculated with suspensions from PDA, PDA + cocoa pod

Source of Variation	d.f.	MS	*α = 0.05
Repetitions(species)	18	3.839*	
Treatments(PDA, PDA+C)	1	1.879*	
Error	18	0.307	

Table 8A. Analysis of variance for clones inoculated with suspensions from PDA, PDA + cocoa pod

Source of Variation	d.f.	MS	*α=0.05
Repetitions(clones)	48	0.472	
Treatments(PDA, PDA+C)	1	2.636*	
Error	48	0.496	

Table 9A. Analysis of variance for clones inoculated with suspensions from PDA, PDA + cocoa pod

Source of Variation	d.f.	MS	*α=0.05
Repetitions(clones)	4	0.243*	
Treatments(PDA, PDA+C)	1	0.009	
Error	4	o.o27	

Figure 1A. Arrangement of plots and location of dead cocoa trees in repetitions I and III of Experiment La Lola No. 26, La Lola Experimental Cocoa Farm, Limon, Costa Rica, 1980.

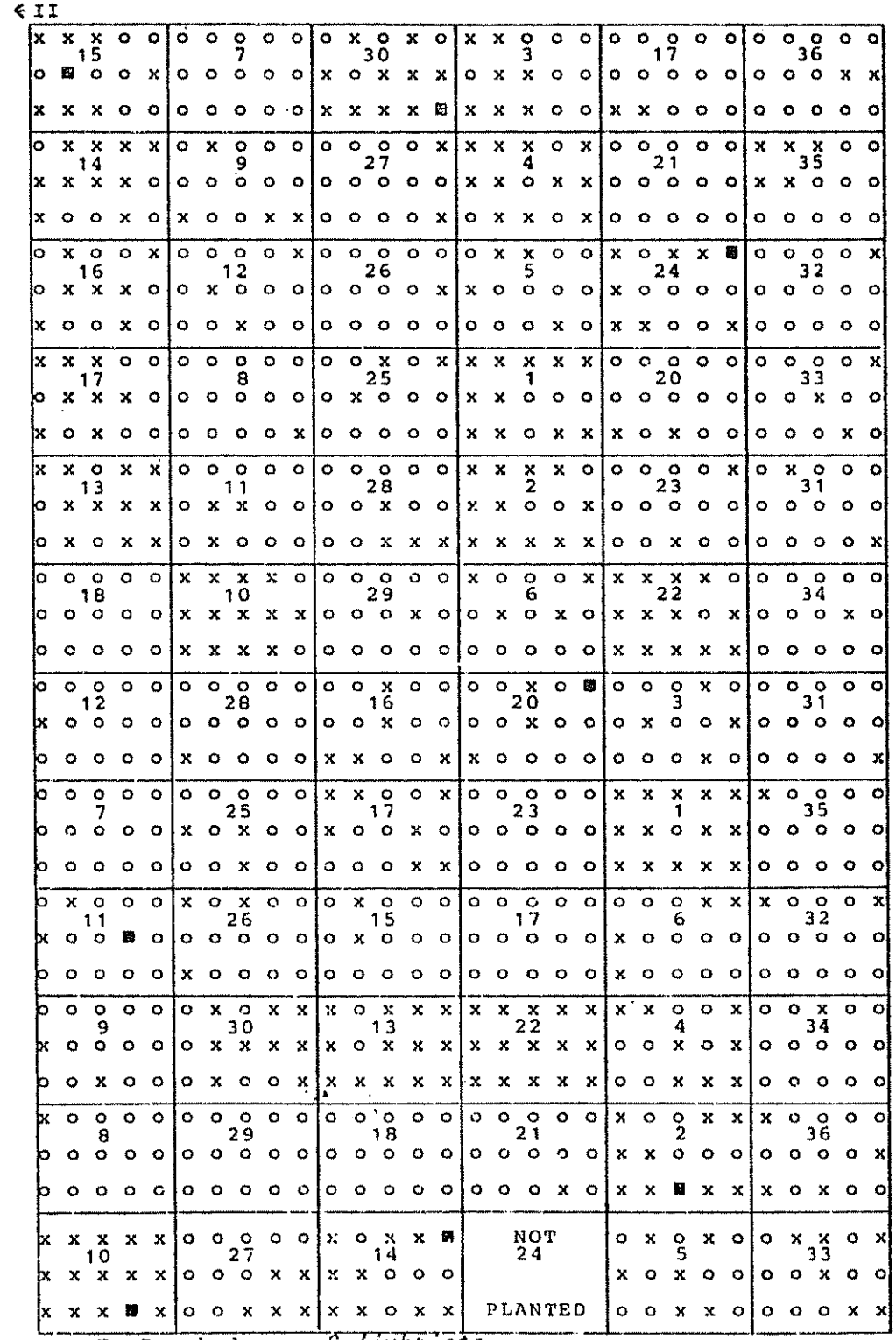

Figure 2A. Arrangement of plots and location of dead cocoa trees in repetitions II and IV of Experiment La Lola No. 26, La Lola Experimental Cocoa Farm, Limon, Costa Rica, 1980.

I →

x x x x x 30 x o o x x x o o x x	x x x x x 10 x x x x x x x x x x	o o o o o 33 o o ■ o o o o o ■ x	o o o x o 19 o o o o o o o o o x	x o o x o 23 o o x o o o o o o o	o x o o x 26 o x o o x o o o o o
x o x o o 6 x o x o o o o o o o	o o o x x 34 o o o x o o o o o o	x o x x x 27 o o x x x x o x o o	x x x x x 1 x x x x x x o x o o	o o o o o 35 o o o o o o o o o o	o o o o o 32 x o o o o o o o x o
NOT 24 PLANTED	x x x x x 22 x x x x x x x x x x	o x x x o 15 o x o o o x x o x o	o o o o o 31 o o o o o o o o o x	o o o o o 5 o o x o o o o o o o	o o o o o 8 o o o o o o x o o o
x o o o x 12 o o o o x o o o o o	o o o o o 16 x x o o o x o o x o	o x o o o 21 o o o o o o o o o o	o o o o o 7 x o o o o o o o x o	x o o o x 11 o o x o o x o o o o	o x o o o 20 x x x x x x o x x o
o o o o o 18 o o o o o o o o x o	o o o o o 28 o o o o o o o o o o	o o o o o 9 o o o o x o o x o o	x o x o x 13 x x x x x x x x x x	o o x o x 17 x x x x x o o o o o	x x x x x 2 x x x x x x x o x x
x o o x o 36 o o o o x o o o o o	o o x x x 4 o o x x x o o x x x	x o x x o 3 x o x x x o o o x o	o x x o x 25 o o o o o o x x x x	o o o o o 29 o o o o o o o o o o	o x x x x 14 x x x x x x x x o o
x x x x o 2 x x x x x o x x x o	o o o o o 18 o o o x o o o o o o	x o o o o 34 o o o o x o o x o o	o x o o x 27 o x o o o o o x x x	o o o x o 31 o o o o o o o o o o	o o o o o 5 o o o o o o x o o x
o o x o o 26 o o o o o o o o o o	o x o o o 6 x o o o o o o o o o	x x x x x 22 x x x x x x x x x x	o x x o o 33 o x o o o o x x o o	x x x x x 13 x x x x x o o x o o	x o o x x 17 x o o x x x o x o o
o o o o o 8 o x o o o o o o o o	NOT 30 PLANTED	x x x o x 4 x x x x x x x x x x	o o x o o 9 o o o o o o o o o ■	x x x x x 1 x o x x x x x x o x	o o x o o 35 o o o x o o o o o o
o o x x x 20 o o o o o x o o x o	NOT 24 PLANTED	o o x o o 16 x o x x x o o o x x	o o o o o 21 o o o o o o o o o o	o o o o o 19 o o o o o o o o o o	o o o o o 29 o o o o o o o o o o
x x x x o 14 x x x x x x x x x o	x x o o x 12 o o o o x o x o o o	x x x x x 10 x x x x x x x x x x	x o o o o 15 o o o o o o o o o o	o o o o o 25 o x o o x o o o x x	o x o x x 11 x o x o o x o o o o
o o o o o 32 x o o x o o o o o o	o o o o o 36 x x x o o x x o o o	o o o o o 28 o o o o o o o o o o	o o o o x 3 x o x o o o x x o o	o o o x o 7 o o o o x o o o o o	o o o o o 23 o o o x o o o o o o

IV

II

III →

N- Death due to *C. fimbriata*
x- Cause of death not noted
o- Live tree

Table 10A. Mortality of trees per plot and % mortality of clones and hybrids, Experiment La Lola No. 26, La Lola Experimental Cocoa Farm, Limon, Costa Rica, 1979.

Plot No.	Clone or Hybrid	Blocks: I	II	III	IV	%Mort.
24	IMC-67	7	-	-	-	46.67
9	IMC-67 x ICS-1	4	2	2	1	15.00
5	IMC-67 x ICS-45	4	1	6	2	21.67
3	IMC-67 x POUND-12	7	8	4	5	40.00
35	IMC-67 x UF-613	5	0	1	2	13.33
28	IMC-67 x T3BA21	4	0	1	0	8.33
23	IMC-67 x T3BA6	2	3	0	1	10.00
13	ICS-1	12	13	13	14	86.67
34	ICS-1 x IMC-67	1	3	1	3	13.33
1	ICS-1 x ICS-1	11	15	14	13	88.33
10	ICS-1 x ICS-45	13	15	15	15	96.67
25	ICS-1 x POUND-12	3	7	3	4	28.33
6	ICS-1 x UF-613	4	4	4	2	23.33
33	ICS-1 x SPA-9	3	2	6	4	25.00
27	ICS-1 x T9B1P6A6	2	9	5	6	36.67
12	ICS-1 x T3BA21	3	3	1	5	20.00
20	ICS-1 x T3BA6	2	9	4	5	33.33
4	ICS-45 x ICS-45	11	9	8	14	70.00
30	POUND-12	10	11	9	-	66.67
26	POUND-12 x ICS-1	1	4	3	1	15.00
14	POUND-12 x ICS-45	10	12	10	13	75.00
11	POUND-12 x T3BA6	3	4	2	6	25.00
22	ICS-45	13	15	15	15	96.67
18	UF-613	0	1	0	1	3.33
32	UF-613 x IMC-67	1	2	2	2	11.67
17	UF-613 x ICS-1	8	7	7	7	48.33
2	UF-613 x ICS-45	13	13	10	12	80.00
15	UF-613 x POUND-12	8	7	2	1	30.00
29	UF-613 x SPA-9	1	0	0	0	1.67
8	UF-613 x T9B1P6A6	1	1	1	1	6.67
19	SPA-9	2	2	0	0	6.67
36	SPA-9 x ICS-1	2	3	4	5	23.33
7	SPA-9 x UF-613	0	2	0	2	6.67
21	SPA-9 x T3BA21	0	1	1	0	3.33
31	SPA-9 x T3BA6	2	1	1	1	8.33
16	ICS-1 x SCA-6	7	4	5	7	38.33

Table 11A. Analysis of variance of half diallel cross

Source of Variation	d.f.	MS	*α=0.05
Blocks	3	0.062	
Crosses	9	1.072*	
Blocks x Crosses	27	0.052	
Within Plots(error)	80	0.048	

Table 12A. Analysis of variance of GCA and SCA

Source of Variation	d.f.	MS	*α=0.05
GCA	4	0.142**	
SCA	5	0.053*	
Error	80	0.004	

Table 13A. Average number of trees surviving per year per plot in Experiment La Lola No. 26.

Clone or Hybrid	Years: 0-4	5	6	7	8	9	10	11	12	13	14
IMC-67	15.0	10.0	10.0	10.0	10.0	9.0	9.0	9.0	9.0	8.0	7.0
IMC-67 x ICS-1	15.0	14.5	14.2	14.2	14.2	14.2	14.0	14.0	13.0	12.7	12.7
IMC-67 x ICS-45	15.0	15.0	14.0	14.0	13.7	13.2	13.0	13.0	12.2	11.7	11.7
IMC-67 x POUND-12	15.0	14.5	11.5	11.2	11.0	10.7	10.2	10.2	9.7	9.0	9.0
IMC-67 x UF-613	15.0	14.7	14.2	14.2	14.0	13.7	13.7	13.7	13.5	13.0	13.0
IMC-67 x T3BA21	15.0	15.0	14.7	14.7	14.7	14.7	14.7	14.7	14.2	13.7	13.7
IMC-67 x T3BA6	15.0	15.0	15.0	15.0	15.0	14.5	14.5	14.5	13.7	13.5	13.5
ICS-1	15.0	15.0	10.5	10.0	10.0	9.2	6.2	6.2	2.5	2.0	2.0
ICS-1 x IMC-67	15.0	14.7	13.2	13.2	13.2	13.2	13.2	13.2	13.0	13.0	13.0
ICS-1 x ICS-1	15.0	13.7	5.7	5.5	5.0	4.7	4.7	4.7	3.0	1.7	1.7
ICS-1 x ICS-45	15.0	15.0	8.0	7.5	7.0	4.5	3.2	3.0	1.2	0.5	0.5
ICS-1 x POUND-12	15.0	15.0	14.0	13.7	13.2	12.2	12.0	12.0	11.0	10.7	10.7
ICS-1 x UF-613	15.0	15.0	14.2	13.7	13.2	13.2	13.0	12.5	11.7	11.5	11.5
ICS-1 x SPA-9	15.0	13.7	12.7	12.5	12.2	12.2	12.2	12.2	11.7	11.5	11.2
ICS-1 x T9B1P6A6	15.0	12.0	11.0	10.7	10.7	10.7	10.7	10.7	9.7	9.5	9.5
ICS-1 x T3BA21	15.0	15.0	14.2	13.5	13.5	13.5	13.5	13.5	12.5	12.0	12.0
ICS-1 x T3BA6	15.0	15.0	14.2	14.0	13.5	12.7	12.5	12.5	10.5	10.2	10.0
ICS-45 x ICS-45	15.0	15.0	8.5	7.7	7.0	6.2	5.7	5.7	4.7	4.5	4.5
POUND-12	15.0	14.7	8.0	7.7	7.7	7.3	6.3	6.3	6.0	5.0	5.0
POUND-12 x ICS-1	15.0	15.0	15.0	14.2	14.2	14.0	14.0	14.0	13.7	12.7	12.7
POUND-12 x ICS-45	15.0	13.5	10.5	9.7	7.5	6.2	4.7	4.7	4.0	3.7	3.7
POUND-12 x T3BA6	15.0	15.0	13.7	13.7	13.5	13.2	13.0	13.0	12.2	11.2	11.2
ICS-45	15.0	15.0	9.0	7.0	4.7	3.2	2.2	1.5	0.7	0.5	0.5
UF-613	15.0	15.0	15.0	15.0	15.0	14.5	14.5	14.5	14.5	14.5	14.5
UF-613 x IMC-67	15.0	14.5	14.0	13.7	13.7	13.7	13.7	13.7	13.2	13.2	13.2
UF-613 x ICS-1	15.0	15.0	13.7	12.5	10.5	10.0	9.5	9.5	7.7	7.7	7.7
UF-613 x ICS-45	15.0	14.7	11.0	9.2	6.2	5.2	5.0	5.0	3.5	3.2	3.0
UF-613 x POUND-12	15.0	14.7	13.7	13.0	13.0	13.0	12.7	12.7	11.7	10.7	10.5
UF-613 x SPA-9	15.0	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7
UF-613 x T9B1P6A6	15.0	15.0	14.7	14.7	14.7	14.5	14.5	14.5	14.2	14.0	14.0
SPA-9	15.0	15.0	14.7	14.7	14.7	14.5	14.5	14.5	14.5	14.0	14.0
SPA-9 x ICS-1	15.0	14.7	13.2	13.0	12.2	11.7	11.7	11.7	11.5	11.5	11.5
SPA-9 x UF-613	15.0	15.0	14.2	14.2	14.2	14.2	14.2	14.2	14.0	14.0	14.0
SPA-9 x T3BA21	15.0	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.5	14.5	14.5
SPA-9 x T3BA6	15.0	14.7	14.2	14.2	14.0	13.7	13.7	13.7	13.7	13.7	13.7
ICS-1 x SCA-6	15.0	15.0	14.5	13.7	13.2	12.5	12.2	12.0	10.0	9.2	9.2

Table 14A. Coefficients for the linear equation $Y = b_o + b_1X + b_2X^2 + b_3X^3$, survival curves, Experiment La Lola No. 26

Y = No. of trees surviving X = No. of years since establishment

Clone or Hybrid	Coefficients				R^2 %
	b_o	b_1	b_2	b_3	
IMC-67	17.367	-1.414	0.069	-0.001	86.840
IMC-67 x ICS-1	15.260	-0.169	0.015	-0.001	92.875
IMC-67 x ICS-45	14.867	0.209	-0.061	0.002	97.442
IMC-67 x POUND-12	15.412	0.066	-0.119	0.006	93.087
IMC-67 x UF-613	15.107	-0.003	-0.022	0.001	96.113
IMC-67 x T3BA21	15.173	-0.137	0.025	-0.002	91.289
IMC-67 x T3BA6	14.825	0.098	-0.010	0.000	94.155
ICS-1	14.283	0.996	-0.279	0.010	96.377
ICS-1 x IMC-67	15.339	-0.085	-0.038	0.002	85.993
ICS-1 x ICS-1	16.741	-0.388	-0.210	0.012	89.024
ICS-1 x ICS-45	14.284	1.409	-0.460	0.021	95.971
ICS-1 x POUND-12	14.451	0.590	-0.137	0.005	98.468
ICS-1 x UF-613	14.719	0.343	-0.087	0.003	97.991
ICS-1 x SPA-9	15.732	-0.335	-0.019	0.001	91.990
ICS-1 x T9B1P6A6	16.575	-0.867	0.014	0.001	88.379
ICS-1 x T3BA21	15.140	0.002	-0.027	0.001	92.293
ICS-1 x T3BA6	14.634	0.377	-0.082	0.002	97.400
ICS-45 x ICS-45	15.083	0.761	-0.348	0.017	92.381
POUND-12	15.760	0.176	-0.242	0.013	89.762
POUND-12 x ICS-1	15.014	0.036	-0.013	0.000	92.827
POUND-12 x ICS-45	14.070	1.421	-0.432	0.020	98.814
POUND-12 x T3BA6	15.139	0.004	-0.028	0.001	95.358
ICS-45	13.534	2.150	-0.619	0.029	97.111
UF-613	14.771	0.194	-0.036	0.001	85.285
UF-613 x IMC-67	15.278	-0.108	-0.016	0.001	91.795
UF-613 x ICS-1	13.853	1.249	-0.293	0.012	97.802
UF-613 x ICS-45	13.862	1.669	-0.477	0.022	96.846
UF-613 X POUND-12	15.385	-0.156	-0.010	0.000	94.488
UF-613 x SPA-9	15.115	-0.068	0.002	0.000	80.805
UF-613 x T9B1P6A6	14.990	0.029	-0.009	0.000	96.325
SPA-9	15.042	-0.011	-0.003	0.000	92.618
SPA-9 x ICS-1	14.786	0.437	-0.146	0.007	96.346
SPA-9 x UF-613	15.116	-0.016	-0.018	0.001	85.570
SPA-9 x T3BA21	15.123	-0.082	0.007	0.000	85.503
SPA-9 x T3BA6	14.978	0.119	-0.049	0.003	96.420
ICS-1 x SCA-6	14.514	0.477	-0.098	0.002	97.829

Table 15A. Matrixes of correlation for mortality, precipitation, and maximum-minimum temperature.

	Precipitation	Maximum Temp.	Minimum Temp.
	Mortality	Mortality	Mortality
Month of death	0.3362	-0.1773	0.0309
1 month before	-0.1430	-0.0134	-0.0587
2 months before	-0.0398	0.0517	0.0015
3 months before	0.2152	0.0823	0.1463
4 months before	0.0431	0.0984	0.2139
5 months before	-0.0755	0.2308	0.2281

Table 16A. Matrixes of correlation for percent mortality of clones, PDA, PDA+C

	Mortality	PDA	PDA+C
Mortality	1.000		
PDA	0.4064	1.000	
PDA+C	0.4495	0.2552	1.0000