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FINAL REPORT

BIOLOGICAL CONTROL OF COFFEE RUST

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1. SUMMARY

306 epiphyte fungi, 212 epiphyte bacteria and 308 mycoparasite fungi isolated from field trip collection in coffee plantations located in different climatic zones of the country, were used to carry out *in vitro* studies against coffee rust, to find any potential.

Microorganism suspension, gathered in pools were used to select those showing antagonist activity. The pools were formed by selecting the fungi at random, and bacteria according to Gram stain reaction using an incomplete block design according to Rhaghavarao-Federer model.

The methodology for *in vitro* coffee rust contamination in detached coffee leaves and coffee plants were developed.

Pools were applied in drops over young detached leaves, its effects was evaluated in three stages of disease development:a.- Inhibition of rust implantation(drops placed before inoculation with coffee rust uredospores. b.-Inhibition of sporulation (in the chlorotic spots stage in wich no uredospores can be seen. c.-Effect over full developed lesion in wich there are uredospores change in density.

The effect of microorganisms was considered as positive when results were 80 % (P), negative when results were 60% or less (N) and intermediate when results were between 60-80% (I), results refer to % od coffee leaves assayied, in wich the antagonic effect of microorganism against coffee rust was observed and expressed in percentage.

In the test of Rust Implantation Inhibition, there were neither bacteria nor fungi with positive results. In the test of Inhibition of Sporulation, a bacteria was found poritive identified as **Psudomona sp**(102). In the same disease stage, a fungus was found positive identified as **Cladosporium sp**(37).

In the stage of full developed pustule four fungi were find with possitive effect, being identified as **Paecilomyces lilacinus** (273), **Aphanicladium meliolae** (298), **Verticillium leptobactrum** (102) and **Trichoderma like verticillium** (219).; all of them were capable to colonize and cover coffee rust pustules, as a consequence density of uredospores dimished.

With the purpose of confirming results obtained with the four antagonist having effect in the stage of full developed pustule in the *in vitro* studies , assays were carried out in the greenhouse and field . In all cases applied antagonist growed with time, and coffee rust decrease. An statistycal analysis of Variance factorial of sub-samples gave comparations at 0.05 when compared to control in both cases.

According to Scanning Electron Microscopy studies carried out with the last four antagonist mentioned above, it seems that fungi **Aphanocladium meliolae** and **Phaecilomyces lilacinus** acting by penetration trhough the uredospore spicula and cause its collapso. The function action of **Verticillium leptobactrum** seems to be wrapping around rust uredospores, its whule structure seems to undergo in a marked degeneration forming physicla barries wich obstructed further development.

The fungi identified tentatively as **Trichoderma like verticillium** produce exo-1-B-C- glucosidase activity (cell wall degradation enzyme) been possible that acting by penetration through the uredospore spicula.

2 INTRODUCTION

Coffee rust is certainly the most important and economically significant disease that attacks the coffee plant.It is caused by the fungus Hemileiae vastatrix,Berk & Br. Control of the disease has relied on the use of proprietary copper fungicides. Applications of either low or high volume (10-800 L/ha) have been equal in efficacy control (Fierman and Wallis,1965;Okioga,1978;Wallis and Firman,1962)

The use of copper spray programs produce the adverse effects of field explosions of leaf miners,spider mites and increased coffee berry diseases(Fulton,1984).Its effect on the coffee berry borer, a very serious problem, in Central America (Fernandez, 1984)is not known. Up to now nobody is certain of the enviromental effect of such heavy dose applications of copper based fungicides. It has been established in samples of soils from 24 years old stands of coffee,that have been routinely treated with copper a contamination to a depth of 80 cm (Lepp et al, 1984.)

As an alternative to chemical fungicides, microbial control of plants diseases can be achieved directly through inoculation or indirectly by changing the condition prevailing in the enviroment.

Many species of rust fungi are hosts to hyperparasitic fungi (Littlefield and Heath, 1975). Verticillium lecani has been reported to growth on some races of Hemileia vastatrix (Hall 1981).

Phytopathologist of the Guatemalan Comission on Coffee Rust have observed several fungi growing on the orange coffee rust pustules,but no effort have been made to make any isolation thus,this was the main objective of the present research.

The United States of America Agency for International Develop ment contracted ICAITI to carry out a study of Biological Control of Coffee Rust(C 5-330) under terms and conditions of Grant No.PDC-0544-G-SS-5100).The objectives of the program were:

a.Isolation and characterization of mycoparasites associated with the Coffea arabica-Hemileiae vastatrix system.
b.Induction of mycoparasites in rust infected coffee plants with collected fungi strains and pure cultures form culture collections.
c.Enzymological characterization of the mycoparasitsm induced in part b.

ICAITI subcontracted the Hebrew University of Jerusalem, Faculty of Agriculture to obtain technical services set forth in the scope of work of the contract wich is enclosed in Appendix No.1 .

3. GENERAL ACTIVITIES

3.1 COLLABORATIVE EFFORTS

In order to integrate the collection of coffee infected leaves and to search for samples showing active mycoparasitism.a cooperation was initiated with entities from other countries who have carried out studies on coffee rust control.

On december 2 and 3 1986,Mr Carlos Rolz,Mrs Maria del Carmen de Arriola and Mr Roberto de León,Head and officers of the Applied Research Division of ICAITI,visited Mr Jose María Alpizar,Chief Assistant of the Coffee Department in the Ministry of Agriculture (San José,Costa Rica).The purpose of the visit was to request collaboration for the project,specifically to provide us with fungi parasites from Costa Rica coffee rust leaves. Mr Alpizar communicated their interest to collaborate in the project,and asked to Mr. Bernardo Mora and Mr Gregorio Leandro, Officer and head of the Department of Phytopathology, Ministry of Agriculture to prepare such samples.

The 4th and 5th of December, Mrs María del Carmen de Arriola and Mr Carlos Rolz,visited Mrs Cecilia Galvez, Head of the Departamento de Fitopatología of the Instituto Salvadoreño de Investigaciónes del café (ISIC),located in Santa Tecla,El Salvador.Mrs Galvez expressed her interest in the project and offered to test the isolated microorganisms from Guatemala coffee leaves,as well as those which show parasitism on coffee rust in detached coffee leaves and plants from El Salvador. Mr Emilio García, who was working at ICAITI in the project,traveled to El Salvador,and spent the week of the 9th to 13th of December 1986 at ISIC, in order to practice the inoculation techniques for coffee plants,leaves and discs infected with the rust fungi.

Contact was established with Dr Gabriel Cadena from the Centro Nacional de Investigaciónes del café,Pedro Uribe Mejía Chinchiná, Caldas,Colombia, in order to obtain from then mycoparasites to be tested. The list of microorganism they sent us can be found in the last page of table No 3.

Mrs.María del Carmen de Arriola traveled to Brazil from 9th to 14 of july 1987 in order to visit EMBRAPA (Empresa Brasileira de Pesquisa Agropecuaria) Jaguarina Sao Paulo . During his visit, she gave to Dr. Wagner Bettiol the four antagonist isolated at ICAITI to be tested. The list of microorganisms received from Brazil can be found in the last page of table No.3

The effort to obtain some mycoparasites from the Coffee Research Foundation in Africa was non satisfactory.

A research cooperation between the Guatemalan Comission on Coffee Rust Program and ICAITI was signed. A copy of it is enclosed as Appendix No 1.

From March 31 to April Mrs María del Carmen de Arriola visited Dr Ilan Chet laboratory of fungal Physiology at the Faculty of Agriculture , located in Rehovot, Israel. The purpose was to become acquinted with the techniques employed for fungal and bacteria isolation from plants. The training was completed and a series of recommendations were issued (details of which are given in 4.2 and 4.3.)

On April 16, 1986, Mrs Arriola visited Centro de Investigacao das Ferrugens do Cafeiro, Lisboa, Portugal. The main purpose to visit this center was to obtain information on **Hemileiae vastatrix** infection, spore collection and handling of infected coffee plants. Regarding the production of spores they are currently using a method wich consists on infected young coffee plants with **H. vastatrix** using spore inoculation by direct leaf to leaf contact.After its implantation, the uredospores collected in a gel capsule are stored for one month or longer at 4° C . During the infection period, the plants are kept in greenhouse without temperature control but with high relative humidity which is achieved by constant irrigation.

This center is specialized in identification of H. vastatrix strains.They are also looking for coffee hybrids that show resistance to rust fungi and have started studies on the effects of fungal elicitors and plants phytoalexins present during the pathogenic cycle in both susceptible and resistance varieties.

3.2 COORDINATION MEETINGS

A first coordination meetings was held in Rehovot , Israel in the Faculty of Agriculture with Dr.Ilan Chet. Action was taken to receive an ICAITI scientist to become acquianted with techniques for fungal collection from fields. applications on crops and isolation. The visist of Dr Ilan Chet to coordinate field trip collection of coffee infested leaves and search for samples showing active mycoparasites was programmed tentatively for December 1986.

The second coordination meeting of the project was held at ICAITI, during the week of August 11-18, 1987. The participants were Mrs Maria del Carmen de Arriola, Maria del Rosario Ricci, Fabiola de Micheo, Emilio Garcia, Carlos Rolz and Ilan Chet.

Presentation of research work currently being carried out at ICAITI was done.

A field trip for collection of samples with mycoparasites was done and the material was brought to the laboratory for isolation.

During the same period, Dr. Chet was able to visit farms and coffee plantations affected by disease at different grades of severity

During his visit to ICAITI Dr Ilan Chet presented a lecture on Biological Control of soil-born plant pathogenic fungi.

He paid a visit to the Israel Ambassador to Guatemala, Dr Azriel Gal-on, to describe the US-Israel CDR programs, Dr Ilan Chet took back to Israel three isolated fungi and coffee leaves with rust and mycoparasites to start work with these materials.

The third coordination meeting was held in Rehovot ,Israel at the UJFALESA , with Dr Ilan Chet,from 22-2-88,to 4-3-88, during this period scanning electron microscopy of isolated antagonists was carried out and results obtained were discussed; it was decided to assay the isolated microorganism by pools in order to save time.

A fourth coordination meeting was held at ICAITI, during the week of july 2-7, 1988. Greenhouse and fields experiments we re carried out with the first four isolated micrroorganism.It was decided to grow antagonists in liquid medium instead of solid medium since it presented some difficulties to handle and to prepare an homogeneous suspension.The last possible antagonist isolated was given to Dr.Ilan Chet for its identification.

4. RUST INOCULATIONS: RESEARCH ACTIVITIES, MATERIALS AND METHODS

4.1 INOCULATION OF PLANTS LEAVES

Coffee plants of the Caturra variety of 1-1.5 years of age were obtained from three sources: the greenhouse of ICAITI, El Pino Experimental Station(Roya Comission,Barberena Santa Rosa),and a nearly nursery. The plants were used to assay three different methods of inoculation.The first one was direct leaf to leaf contact, the second, spore dispersion, and the third, spraying. In all cases the use of old and damaged plant leaves was avoided: the second and the third youngest pair of leaves in the plant were employed to inoculate rust uredospores.

4.1.1.Direct leaf to leaf contact

Coffee leaves with orange rust mature lesions were cut. A portion obtained (nearly 1.5 cm. diameter)and put in contact with the underside of plant coffee leaves surface.A clip was used to fix the cut leaf portions to the entire coffee leaves.

4.1.2. Spore dispersion

Uredospores were collected from rust mature lesions of infected coffee leaves. The underside of the second and third youngest pair of leaves of each coffee plant was inoculated with these spores using a fine brush. The inoculated leaves were moistened by spraying distilled water.

4.1.3.Spray

Uniform inoculum was obtained by suspending uredospores in distilled water collected as mentioned in 4.1.2 (0.66 mg uredospores per ml) and stirred using a magnetic stirrer during 10-15 minutes.The suspension was transferred to an atomizer bottle, and the underside of coffee leaves plant surface was sprayed shaking the bottle before each application. The inoculated samples were incubated as described in 4.3.

4.2 INOCULATION OF DETACHED LEAVES

Random samples of coffee leaves second and third youngest pair of leaves plants, Caturra variety obtained from San Fernando farm located in the department of Escuintla,(Guatemalan southern coast) wese used.The use of old and damaged leaves was avoided. Leaves were collected in the morning and stored for their transportation in moist plastic boxes containing a foam layer saturated with water. Care was taken to remove the leaves with petiole from plants. At the laboratory,the leaves were washed twice with distilled water and then inoculated using the following methods.

4.2.1 Inoculation by drops

Uniform inoculum was obtained by suspending uredospores (0.66mg/ml) in distilled cold water and stirred for ten minutes. To avoid sedimentation of uredospores the suspension was continously stirred.For the inoculation of leaves,samples containing 1 ml were taken from the suspension with a pipet. One drop of about 0.05 ml was placed over the underside of coffee leaves plants surface. Nine to ten leaves were arranged in circular plastic boxes of 35 cm diameter ,with a plastic foam in the bottom(1/4"thickness). Each leaf was inoculated with 4 to 8 drops located between the veins along the leaf. The plastic boxes were covered with plastic(6 u thikcness)

which was secured with a rubber band. They were incubated as described in 4.3

4.3 INCUBATION OF INOCULATED SAMPLES

After plant and detached leaves were inoculated with any of the above described methods, they were incubated in a dark room, at 22-23°C, with 85-90 % Relative Humidity, during 48 hours (VIP-Lab Line Serial 2274). Followed this treatment, the whole plants were transferred to a greehouse and irrigated daily during 2-3 hours.The plastic boxes containing detached leaves were transferred to room temperature under moderate light;every day the boxes were opened and the leaves moistened by spraying with distilled water. Care was taken to maintain water level in the plastic foam,but the temperature was not controlled. A group of inoculated boxes were stored under fluorescent light of 800-1000 lux,with a daily 12-hours dark period.

4.3.1 EVALUATION SYSTEM OF INDUCED RUST INFECTION IN PLANTS

The formation of mature pustules was named :M.P= Mature pustules and is defined as such when abundant uredospores production could be observed, and sporulation covers more than 50% of all chlorotic lesion.

4.3.2 EVALUATION SYSTEM OF INDUCED RUST INFECTION IN DETACHED COFFEE LEAVES

Reaction type was evaluated as follows:
S:Leaves with spots, which can be defined when chlorotic spots can be seen on leaves underside
M.P.: Mature pustule, as described in 3.2.4.

For virulence evaluation, chlorotic spots were measured from the day when spots were clearly observed and uredospores production was recorded as percentages of chlorotic lesions.

4.4 MEASUREMENT OF GERMINATION

Parallel to germination of plants and detached leaves, a evaluation of the field collected uredospores was made to establish the viability of each spore batch according to the following procedure:
Uredospores were placed in a cover glass(1.5 mg/mlwater), covered with a microslide, tranferred into a wet chamber and incubated in the dark at 25° C for 24-48 hours. After this period, spore germination count was carried out by microscopic examination (100x magnification), taking into account the number of those spores showing germinal tube.
The percentage of germination was calculated.

5.0 EPIPHYTE AND MYCOPARASITE MICROORGANISMS: COLLECTION AND IDENTIFICATION

5.1 FIELDS TRIPS FOR SAMPLE COLLECTION

With cooperation of the Guatemalan Comission Coffee Rust Control, field trips of sample collection were done. In Appendix No 2 and No 3,a list of visited farms is enclosed along with a map of Guatemala to indicate where the farms were located. Samples were taken from a parcel of land destined to epidemiological studies in wich no treatment was applied.

5.2 ISOLATION AND IDENTIFICATION

According with the general procedure for isolation of microorganisms described in the next parraph several fungi and bacteria were isolated.Each isolated microorganism was identified with a correlative number and stored under refrigeration: periodically, reinoculation was done into fresh agar slants of the proper medium with purpose of maintenance.

Sources of antagonists from coffee plantations

A-Infected leaves with **Hemileiae vastatrix.**

B-Leaves infected with **Hemileiae vastatrix** showing mycoparasitism.

C-Healthy leaves in heavy infested plants

D-Leaves with other fungus than **Hemileiae vastatrix**

E-Leaves showing mycoparasitism by other fungus besides **Hemileiae vastatrix.**

All leaves portions from A,B,C,D,or E were cuted in small particles 50-200 g from each and were placed in an erlenmeyer and 50 ml sterile water was added and shaked for one hour. Dilutions (10^{-1} to 10^{-4} were done and inoculte 0.1 ml of each dilution into three petrie dishes containing the following medium "E","Chitin" for bacteria and "MRB" and "TSM" for fungus,all sample were incubated at 30° C until good growth was obtained.

Bacteria growed in "E" or "CHITIN" were applied in Nutritive agar, incubated until desired growth obtained,the one single

colony was taked and inoculated in liquid broth , incubated at 30oC for 48 hours in a shaker at 100 rpm, centrifuge several times with sterile water to eliminate liquid medium, with this procedure posssible antagonist were ready for spraying the infected coffee plants, applied on PDA, incubated at ambient temperature until desired growth was obtained.

A mycelium portion was taken from nycoparasites growing on rust pustule, and inoculated on a PDA petri dishes and incubated at 30° C for growth (5-8 days). Another portion of mycelium was taked and inoculated on nutritive agar petri dishes and incubated at 30° C for 48 hours,isolated microorganisms were identified as described in the next parraph.

Each processed sample was identified according with the following: field collection trip number, first initial of the farm name from where the microorganism was isolated, dilution culture medium and a correlative number for bacteria or fungi.

5.3 GROWTH OF FUNGI

Isolated fungi were growed on modified TSM agar (See Table No.6), in a liter flask at room temperature in slants. After microorganisms growth , biomass was collected with 40 ml sterile water. The suspension obtained was used in assays to find any potential.

5.4 GROWTH OF BACTERIA

Isolated bacteria were growed in modified King broth (See Table No 7,in one liter erlenmeyer flask at 30°C at constant agitation of 100 rpm for 40 hours. Biomass was washed twice and resuspended in distilled water.The suspension obtained was used in assays to find potential biocontrol.

5.5 MICROORGANISM DISTRIBUTION

The microorganisms suspension obtained as described above we re gathered in pools.The pools were formed by selecting the fungi at random, using an incomplete block design according to Rhaghavarao-Federer model, and bacteria according to Gram stain reaction (see Table 8 and 9)

The pools with microorganisms suspension were used to select those showing antagonistic activity. They were applied in drops, over young coffee detached leaves, its effect was evaluated as follows:a.-Inhibition of rust implantation(drops placed before inoculation with coffee rust uredospores . b.- Inhibition of sporulation (in the chlorotic spots stages in which no uredospores can be seen).c.- Effect over full developed lesions in which there are uredospores change in density.

6.0 ANTAGONIST MICROORGANISMS

6.1 INHIBITION OF RUST IMPLANTATION

Ten detached leaves from coffee plant were placed inside each plastic box with polyurethane foam of 0.6cm thickness in the bottom. Each pool of microorganims suspensions was dripped over the leaves and then the boxes covered with plastic secured with rubber bands. After the drops dried at room temperature, the boxes were placed in a chamber at 80-85% Relative Humidity and room temperature to equilibrate moisture for 24 hr. Then the boxes transferred for two days to a room with daylight ilumination between 800-1000 LUX and 12 hr darkness periods. Finally the leaves were inoculated with rust uredospores according to the procedure described in 3.2 and stored under the conditions described above. After 25 days the production of chlorotic spots was evaluated as an evidence of positive or negative inhibition of rust implantation.

6.2 INHIBITION OF SPORULATION

During incubation the moisture level in the plastic boxes was maintained by daily spraying water over the leaves. Starting at 30 days, the production of uredospores was evaluated to determine the effect of microorganisms over the intensity of sporulation.

6.3 EFFECT OVER FULL DEVELOPED RUST LESIONS

Each pool of microorganism suspensions was applied by using drops over detached coffee leaves with full developed lesions and left at room temperature in an air conditioned room to allow the inoculated droplets to evaporate. After evaporation of the droplets, the boxes containing the leaves were placed in a dark wet chamber (80-95% R.H.) at room temperature for 48 hours. The boxes were transferred to a room under moderate light conditions (fluorescence light of 800-1000lux) with a dark period of 12 hours during 30 days. After this period the effect of inoculated microorganims was evaluated, measuring possible antagonist growth over host pustule.

6.4 IDENTIFICATION OF ANTAGONIST

Two antagonist against coffee rust were identified at the Faculty of Agriculture Rehovot, Israel. Others were identified by Dr Williams Gams at the Central Bureau voor Schimel culture in the Netherlands and the rest at ICAITI.

6.5 SCANNING ELECTRON MICROSCOPY OF ANTAGONIST

The interaction between Coffee rust (Hemileiae vastatrix) and the isolated antagonists Aphanocladium meliolae, Trichoderma like verticillium,Verticillium leptobacrum and Paecilomyces lilacinus was observed by using SEM techniques, according to Elad et al (1983).

Pieces of coffee healthy leaves, leaves infested with the pathogen along,as well as with pathogen and antagonist, were fixed in 3% glutaraldehyde in a 1 phosphate buffer (pH 7.0) amd keept in refrigaration for 3 hours. After this period samples were washed 3 times in phosphate buffer and dehydrated in acetone at different concentration from 30,50,and 70%, for 15 minutes and kept overnight in refrigeration. The next day samples were washed with 80,95, and 100% with acetone for 10 minutes of in absolute ethanol for one hour. The last step for dehidration of material was carried out in a cold chamber with vacuum 3 times for 15 minutes each.

Dried samples were coated with gold palladium in a Polaron E 50 sputter coater (Polaron Equipment Ltd, Warsdford WD 18xG) and them viewed in a scanning electron microscope (JEOL/JSM 35 L).

For antagonist alone, the folowing procedure was carried out Sterile cellophane membranes were placed on water petrie dishes 2% and 0.5 g of glucose) and the isolated antagonista was inocultated one by one and incubated at 30°C. After its growth (2-3 days) pieces of the cellophane membrane covered with the fungi were fixed in the stubs with a tape that stiks both sides and placed in a glass petrie dish. Samples were transfered to a hermetic glass chamber and placed over two beakers containing glutaraldehyde at 25% and Osmic acid at 4% in buffer phosphate respectively (pH7), and kept for 24 hours. Afterwards , samples were coated with golc palladium and then viewed in a scanning electron microscope.

6.6 EFFECT OF GERMINATION BY ANTAGONIST

Rust uredospores collected a day after, was used to prepare suspension (2.0 mg/1.5 ml) water. Besides suspension of four antagonists from masive growth collected with 40 ml steril water both were diluted to 5 ml,mixed and a drop of each was placed in a slide on a petrie dish with cotton saturated with water in the botton,then they were incubated at 25°C 24 hours. A control was carried out using rust uredospores suspension and water.

6.7 EFFECT OF RUST UREDOSPORES ON ANTAGONIST GROWTH

With the purpose of determine if antagonist can use uredospores components as carbon source, an assay was carried out using the medium in Table 14, without glucose and supplemented with chloranphenicol. Micelium of four fungi were seeded as follows:

a) medium without glucose and uredospores
b) medium with glucose (0.3%)
c) medium with uredospores without glucose, at 0.3% taken in account than uredospores cell wall makes up 20% of total spores and consist of 40% carbohydrates.

6.8 ANTAGONIST ENZYME ACTIVITY

Enzyme activity of the four isolated fungi having effect over full developed pustules were carried out as follows:
Fungal biomass was inoculated to erlenmeyer flasks (250ml) containing 30 ml medium (see Table No. 15)and incubated on a rotary shaker at 50 rpm and at 30° C
The medium was supplemented with either laminarin,glucose, methylcellulose or chitin, at concentrations of 2 mg ml-1 (see Table 16).
After the established period for fungi exponential growth, mycelia was collected by centrifugation at 1200 rpm for 15 minutes. The supernatant was filtered through filter paper Whatman No 1, and used as a crude enzyme solution for assay activity, reaction was carried out as follows:
2ml of culture filtrate in 2 ml of 0.1 M citrate buffer (pH 4.7) and 1.6 mg of soluble either laminarin, methyl celulose or chitin as substrate, followed by incubation at 40° C for one hour,the reaction was stoped by boiling.

6.9 ANTAGONIST GROWTH CURVES

Growth curves were carried out for each antagonist,in order to establish time in hours of exponential growth (those having effect over full developed pustules) on Table No 14 the medium used for fungi growth can be found, procedure was carried out as follows: Fungus biomass was seedeed into erlenmeyer flasks (250 ml) containing 30 ml medium (see Table 13) and incubated on rotary shaker at 250 rpm and at 30° C.

Periodically samples were taken out to analyze sugar and biomass. On figures 5-8 growth curves can be found,from these results the corresponding time to the exponential phase of the fungi growth was obtained and used for enzyme activity determination studied as a function of possible relationship in the interaction between the identified antagonists and the host. Inoculum concentration was $2x10^6$ for Aphanocladium meliolae,$1x10^6$ for Trichoderma like verticillium, $0.9x10^6$ for Paecilomyces lilacinus and for Verticilium leptobactrum 2.5 $x10^6$ spores by ml.

7.0 ANALYSIS OF CHLOROPHYLL CONTENT IN COFFEE LEAVES

During rust infection chlorotic spots can be observed on leaf surface, samples were taken at different incubation times and treated as follows:
Chlorotic leaf portion was removed , finelly cutted and weighted (0.25 g). Transferred into a morter and macerated with 10 ml of a solution of acetone (85 %),little at a time and grinding until tissue was finely grinded . Mixture was transferred into a funnel and filtered under vacuum, residual washed tissue treated with more acetone and grinded again.The filter was washed as mentioned before. The procedure was repeated until the tissue is devoid of any green, and washings are colorless. Gathered filters were made volume and readings of chlorophyll content in a spectrophotometer at 660 nm optical density.(AOAC,1975) modified. Employing the method mentioned above. samples of 10 healthy and infected leaf portions were analysed during disease development.

8.0 ANTAGONIST MICROORGANISMSM GREEHOUSE AND FIELD EXPERIMENTS

8.1 GREENHOUSE EXPERIMENTS

With the purpose of confirming results obtained with anatagonists that were positive in the _in vitro_ studies,assays were carried out in greenhouse conditions.
The underside of the 2o and 3o youngest pair of leaves of four Caturra coffee plant was inoculated with uredospores using a fine brush, inoculated plants were moistened by spraying distilled water, and incubated in a dark room at 22-23° C. with 85-90% R.H during 48 hours. After mentioned treatment , the whole plant were transferred to a greenhouse and irrigated daily during 2-3 hours. 50-60 days after inoculation, when pustules were full developed, the four antagonist suspention was applied by spray, four plants was used as control without antagonist application, and four each antagonist. Inoculum concentration was Trichoderma like verticillium 8.2×10^{7}, Paecilomyces lilacinus 1.1×10^{7}, Aphanocladium meliolae 4.96×10^{8}, and Verticillium leptobactrum 2.6×10^{6}, spores per ml. Weekly lectures were carried out measuring coffee rust infection and antagonist growth.

8.2 FIELD EXPERIMENTS

16 coffee trees selected at random and infected with coffee rust (Hemileiae vastatrix) from the San Isidro Farm located in Retalhuleu,Guatemala were sprayed with conidial solutions of the four identified antagonists at the following concentration:Verticillium leptobactrum 2.6×10^{6},Phaecilomices lilacinus 11×10^{6},Verticillium like trichoderma8.2×10^{7},and Aphanocladium meliolae 4.96×10^{8}.

Four bandoles or the second and third youngest pair of four trees of Caturra variety were used to spray each isolated antagnonist. The degree of coffee rust infection and the effect of applied antagonist before its sprayed was measured weekly.

9.0-MICROORGANISMS FROM OTHERS COUNTRIES

Twelve fungi were received from the CENTRO NACIONAL DE INVESTIGACIONES DEL CAFE, PEDRO URIBE MEJIA, CHINCHINA CALDAS COLOMBIA, the list can be found in the last page of Table No 4. Besides,the microorganism from Brazil , received from EMBRAPA (EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA, 13-820, JAGUARIUNA, SAO PAULO, BRAZIL, and two fungi from Costa Rica, DEPARTAMENTO DE FITOPATOLOGIA, DIRECCION DE INVESTIGACIONS AGRICOLAS., Apdo 10.094, San Jose Costa Rica They were studied for its possible antagonic effect over full developed rust lesions stages.

10.0 RUST INOCULATION: RESULTS AND DISCUSION

10.1 RUST INDUCED INFECTION IN COFFEE PLANTS

According with results it was established that the best method for plant infectation was one described in 3.1.2 (spore dispersion),with this method a longer leaf surface can be embraced. As can be seen in Table No.1, 2065 plants (28 lots) were incubated for rust induction. With the chosen method, in the last group of inoculated plants, a 94% of infection was obtained.

10.2 RUST INDUCED INFECTION IN DETACHED LEAVES

In Table No.2. results obtained in 20 lots of detached coffee leaves inoculated by the best method established, mentioned before are presented, it can be seen that lot No.20 showed a 100% induced infection.

10.3 INDUCED RUST EVOLUTION

In Appendix No.5 several pictures are included, showing the rust evolution over detached coffee leaves. In Appendix No.6 some mycoparasites at different stages of invasion can be seen.

10.4 EVALUATION OF VIRULENCE OF INDUCED RUST IN DETACHED COFFEE LEAVES

Taking into account measurements of chlorotic spots diameter carried out during disease assessment (27,29, 33 and 36 days after inoculation) statystical analysis was carried out and showed a probability of 26% that treatment means were equal: this means that chlorotic spot increase with time up to 30-40 days after inoculation. In graphs No.1,2,and 3, histograms of

diameter distribution among several chlorotic spots can be found. Statystical analysis showed a probability of 99.90% that uredospores production increased with time. In graphs 4 and 5, histograms of uredospores production against time can be found.

11.0 EPIPHYTE AND MYCOPARASITE MICROORGANISMS: RESULTS AND DISCUSSION

From 22 visited farms, 306 epiphyte fungi and 212 epiphyte bacteria were isolated and identified according to the field trip number , first initial of the farm name from where the microorganism was isolated , the dilution, culture medium and a correlative number. From 19 visited farms 308 mycoparasites fungi were isolated and identified as described above.

Microorganisms and mycoparasites isolated following the procedure described in 3.8 and 3.9, were used in a screening looking for antagonic effect over coffee rust. In Table 3 and 4, list of isolated fungi and bacteria are presented, classified in ordinal number by isolated order and on Table No5, a list of isolated mycoparasites, and also a list of identification indication from where samples were collected.

The antagonic effect of the bacteria pools during the three stages of rust development on detached coffee leaves can be found in Table 10, and in Table 11 for the effect of the fungi.

Results refer to % of coffee leaves assayied, in which the antagonic effect of microorganism against coffee rust was observed and expressed in percentage.

The effect of microorganims was considered as positive when results were 80% or more(P); and negative when results were 60% or less(N), and intermediate when results were between 60 and 80%(I).

In the test of rust implantation inhibitionm , there were neither bacteria nor fungi with positive results. In the test of inhibition of sporulation , when results were overlapped in the respective diagram (Table 9), a bacteria was found in group K with positive effect of 83% in the pool 91 and 80% in the pool 98, it was identified as number 102 (**Pseudomonas sp**). In the same disease stage, a fungus was find with 80% effectivity in the pool 54. and 90 % in the poll 60 (Table 11), when results were overlapped in the respective diagram (Group H), it was established as a fungus

identified with number 37 (Cladosporium sp) see Table 8.

In the stage of full developed pustule, it was found a fungus with 84% effectivity in the pool 4 and 90% in the pool 8, being identified as number 102, Group A , table 8 (Trichoderma like verticillium).

In the same disease stage, a second fungus was identified as positive with 82% effectivity in the pool 30, and 83% in the pool 35, being identified in the correspondent diagram group D, table No 8, as number 219.(Verticillium leptobactrum).

A third fungus was found positive,with 80% effectivity in the pool 39,and 80% in the pool 46, being identified as number 273.(Paecilomyces lilacinus) in the group E,(Table 8) A fourth fungus was found showing 85% of effectivity in the pool 80 and 80% in the pool 84, being identified in the group J, as 298.(Aphanocladium meliolae) (Table 8).

This four fungi showed antagonic activity against coffee rust by covering the pustules within time and reducing the density of uredospores.

In figure No 1-4, results of virulence of the four mycoparasite fungi over coffee rust can be found, recorded as percentage covering uredospores surface, against total time during its in vitro assay over detached coffee leaves. On Appendix No 6,some pictures of mentioned mycoparasites can be seen.

In the three stages studied,several microorganism were found with intermediate activity such as bacteria No.11 and fungus No.40; in the stage of implantation inhibition bacteria No.65 and No 38,fungi No.28 and No 75; and bacteria No.65 and No.38 in the effect over full developed pustule.

12.0 ANTAGONISTS MICROORGANISMS: RESULTS AND DISCUSSION

12.1 IDENTIFICATION

Two isolated possible antagonist against coffee rust were identified at UJFALESA.
Fungi No. 273 was identified as Paecilomyces lilacinus(16-P-C-5-PDA) and fungi No.298 as Aphanocladium meliolae (20-CH-C-5-PDA).

Fungi No. 102 and No 219 were sent to Dr. William Gams at the Central Bureau voor Schimel culture in the Netherlands, fungi No 219 was identified as Verticillium leptobactrum (12-ST-C-5-PDA) and fungi No102 could not be identified properly, tentatively was identified as Trichoderma like verticillium (6-BUA-C-5-MRB).See table No. 13.

Fungi No 37 was identified in ICAITI as **Cladosporium** sp(2-R-B-3) and bacteria 102, as **Pseudomona** sp.(4-V-A-4-CH)

12.2 SCANNING ELECTRON MICROSCOPY OF ANTAGONIST

The interaction between Coffee rust (Hemileiae vastatrix) and the isolated antagonist **Aphanocladium meliolae, Trichoderma like verticillium, Verticillium leptobactrum** and **Paecilomyces lilacinus** was observed by using SEM techniques, according to Elad et al (1983) as it was mentioned before, results can be found on Appendix No.7.

On figure No.1 infection of a coffee leaf with **Hemileiae vastatrix** can be seen, mycellium that grows intercellullarly sending branches through the mesophyll and showing plenty of well visible haustoria, uredospori sori protrude like a bouquet through the stomata giving the characteristic yellow color in the leaf underside. Besides hiphae and conidia at an initial stage of antagonist **Paecilomyces lilacinus** can be seen.
On figure No.2 coffee rust emerged from leaf stomatas is invaded by mentioned antagonist and its mycelium can be seen extensively (figure No.3).

On figure No. 4 internal hyphae branch of antagonist **Aphanoclacium meliolae** can be recognised inside a urediniospore of **Hemileiae vastatrix**, the mycoparasite grew closely adpressed to the host spore surface between the warts.

On figure No. 5, hiphae of **Trichoderma like verticillium** surrounded coffee rust uredospores with possible penetration hiphae of mycoparasite grew in the strongly warted convex surface of rust urediniospores.

Figure No.6 show the same antagonist hiphae tip projecting its hiphae to the rust spicula, possible penetration place.

On figure No.7 hiphae of **Verticillium leptobactrum** are starting its sorrounding coffee rust uredospora. Figure No.8 and 9 shows a masive invassion of the mentioned antagonist, the function action seems to be wrapping around rust uredospore, its whole structure seems to undergo a marked degeneration forming physical barries wich obstructed further development, the production of a massive polysaccharide like a matrix which cover the rust and apparently kills it.

The effect and presence of the mentioned antagonist can easily compared with a non infected uredospore (figure No.10).

12.3 EFFECT OF GERMINATION BY ANTAGONISTS

Results can be found on Table No 17, as can be seen there were not a great decrease in percentage of germination caused by four antagonists.

12.4 EVALUATION OF ENZYME ANTAGONISTS

The production of cell wall degradation enzymes was studied as a function of possible relationship in the interation between the identified antagonist and the host. Enzymatic activity of the four isolated fungi having effect over full developed pustules was studied.

On table No.16 the results of enzyme activity can be found. Fungi **Paecilomyces lilacinus** and **Trichoderma like verticillium** showed **exo-1-B-C** glucosidase activity. (Laminarase activity) ,in both using laminarin and glucose as carbon sources, corresponding to especific activities of 1.45 and 0.53 umoles of glucose per miligram of protein. The rest of fungi showed negigible activities in all cases.

12.5 EFFECT OF RUST UREDOSPORES ON ANTAGONIST GROWTH

Obtained results can be found on Table No. 19, the growth of the four fungis expressed in biomass (g/100 ml DW). As can be seen better results were obtained when medium was supplemented with uredospores than when it contains glucose as carbon source. It show us that antagonists use in someway the cell wall carbohydrates for its growth.

13.0 EFFECT OF Hemileiae vastatrix ON CHLOROPHYLL CONTENT OF LEAVES

As a visible effect of the development of interaction of the **Hemiliae vastatrix** in the underside of coffee leaves, chlorotic spots can be seen after 10-14 days of disease implantation with time chlorotic spots increasse and can clear visible.Employing the method mentioned above,samples of 10 healty and infected leaf portions were analysed during diseases development. Obtained results can be found on Figure No.9, as can be seen due to disease, there is an effect over chlorotic content in the affected leaves parts.

14.0 MICROORGANISMS: GREENHOUSE AND FIELD EXPERIMENTS

14.1 GREENHOUSE EXPERIMENTS

With the purpose of confirming results obtained with antagonists that were positive in the in vitro studies assays were carried out in the greenhouse.

Weekly lectures were carried out measuring coffee rust infection and antagonist growth.

In all cases applied antagonist growed with time, and coffee rust decrease. An statystical analysis of Variance factorial of sub-samples gave comparations significant al 0.05 when compared to control.

On figure No 10,11,12,13, obtained results can be found,in the four mycoparasites studied, antagonists growed with time and coffee rust decreased. An statystical analysis of Variance factorial of sub samples gave comparation significant at the 0.05, when compared to control.

14.2 FIELD EXPERIMENTS

On figuresNo. 14,15,16, and 17, obtained results can be found, in the four mycoparasites studied, antagonists growed with time and coffee rust decreased. An statystical analysis of Variance factorial of sub samples gave comparation significant at the 0.05, when compared to control.

15.0 MICROORGANISM FROM OTHER COUNTRIES

All microorganisms from other countries were studied for its possible antagonic activity in the effect over full developod rust lesions stage (see Table No.12) none of them gave positive results.

16.0 CONCLUSIONS

A **Pseudomona sp** and a fungi **Cladosporium sp** with antagonic activity capable to inhibit multiplication of the rust uredospores were found.

Antagonic activity was found in four fungi; all of them were capable to colonize and cover coffee rust pustules as a consequence density of uredospores dimished.

It seems that fungi **Aphanocladium meliolae** and **Paecilomyces lilacinus**, acting by penetration through the uredospore spicula, cause its collapso.Verticillium leptobactrum seems to wrapping around uredospores causing a marked degeneration. Trichoderma like verticillium produce exO-1-B-C glucosidase activity been possible taht acting by penetration through the uredospore spicula.

There was not inhibitory action from any isolated fungi and bacteria over coffee rust implantation; besides, no bacteria had effect over full developed pustule and fungi affected uredospores production.

Some of the isolated microorganisms showed and intermedia antagonic activity inhibition with the stage of sporulation and with the effect over full developed lesions.

17. RECOMMENDATIONS

To verify the effect of identified antagonist on man, the animals , the plants and in the enviromental.

To establish the optimum dosage for coffee rust,seasonality and frecuency of application.

To develop proper technology for big scale production, needed for its application into the coffee fields.

To study the effect of the different funguicides used for dissease contol against antagonist, in order to determine the possibility of combined pest control.

18. PROJECT EXPENDITURE

INSTITUTO CENTROAMERICANO DE INVESTIGACION
Y TECNOLOGIA INDUSTRIAL - ICAITI
DESTRUCTION OF AFLATOXINS IN CORN AND NUCLEIC
ACIDS IN YEAST-CORN MIXTURES
PROJECT Nr. AID 936-5542-G-3066-00
ICAITI Nr. 83/4.6.1800

	Budget	Prior Period's Expenditures	This Period	Total Acumulated	Available
SALARIES	68 848.00	69 375.73	345.60	69 721.33	(873.33)
TRAVEL	1 700.00	1 824.14	-	1 824.14	(124.14)
EQUIPMENT, MATERIALS & SUPPLIES					
Materials & Reagents	8 700.00	7 242.48	284.21	7 526.69	1 173.31
Glassware & Supplies	9 000.00	10 821.99	-	10 821.99	(1 821.99)
Technical Books	1 400.00	1 721.24	-	1 721.24	(321.24)
Technical equipment	24 900.00	25 707.95	-	25 707.95	(807.95)
Total Equipment, Materials & Supplies	44 000.00	45 493.66	284.21	45 777.87	1.777.87 (1 493.66
OTHER					
Photocopies	400.00	87.95	108.06	196.01	203.99
Communications	1 152.00	190.25	56.35	246.60	905.40
Subscriptions	600.00	124.63	-	124.63	475.37
Various - other	1 400.00	183.30	-	183.30	1 216.70
Total Other	3 552.00	586.13	164.41	750.54	2 801.46
TOTAL US$	118 100.00	117 279.66	794.22	118 073.88	26.12

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19.0 Appendix, graphics and figures

APPENDIX 1

INSTITUTO CENTROAMERICANO DE
INVESTIGACION Y TECNOLOGIA INDUSTRIAL
(ICAITI)

COSTA RICA
EL SALVADOR
GUATEMALA
HONDURAS
NICARAGUA

CENTRAL AMERICAN RESEARCH INSTITUTE FOR INDUSTRIAL TECHNOLOGY
Avenida La Reforma 4-47, Zona 10
GUATEMALA, C. A.

APARTADO POSTAL 1552
Cable: ICAITI
Telex: 5312-ICAITI-GU
TELEFONOS: 310431 y 317456

INSTITUTO CENTROAMERICANO DE I
INDUSTRIAL, C
- 7 JUL. 1985
No. 5406 Hora

CONVENIO DE INTENCIONES CONJUNTAS Y COOPERACION MUTUA ENTRE
INSTITUTO CENTROAMERICANO DE INVESTIGACION Y TECNOLOGIA INDUSTRIAL

Y

COMISION MEXICO-GUATEMALA PARA LA PREVENCION Y
CONTROL DE LA ROYA DEL CAFETO

PARA ACCION
1 IA
PARA INFORMACION
1 QW
2
3
4
5

C O N S I D E R A N D O:

Que la Comisión México-Guatemala para la Prevención y Control de la Roya del Cafeto, es una organización internacional, con presupuesto propio y autonomía operacional; que fue creada por los Gobiernos de México y Guatemala, para contrarrestar el peligro que para la caficultura nacional, tiene la enfermedad conocida como Roya del Cafeto, causada por **Hemileia vastatrix**, Berk y Brown, y que asimismo la Comisión Roya está implementando programas de Investigación, Capacitación y Asistencia Técnica, con el fin de mantener una caficultura que permita obtener un beneficio económico y un significativo aporte al bienestar social, ampliando no sólo el mercado interno del país, sino a la vez contribuyendo a disminuir la alta vulnerabilidad económica que en el Sector Externo, presenta Guatemala.

C O N S I D E R A N D O:

Que los recursos disponibles en Comisión Roya, para el Programa de Investigación son limitados, es indispensable que se busque la cooperación de otras entidades como el Instituto Centroamericano de Investigación y Tecnología Industrial, para lograr la realización de programas conjuntos que permitan aprovechar al máximo los conocimientos tecnológicos, recursos humanos, patrimoniales y financieros que redundan en un mayor beneficio, para la caficultura y agricultura del país. El propósito fundamental de ICAITI y COMISION ROYA,

dustrial, para lograr la realización de programas conjuntos que permitan aprovechar al máximo los conocimientos tecnológicos, recursos humanos, patrimoniales y financieros que redundan en un mayor beneficio, para la caficultura y agricultura del país. El propósito fundamental de ICAITI y COMISION ROYA, que motiva la creación de este convenio, es favorecer al conglomerado socioeconómico que gira alrededor del cultivo del café, para que estas dos instituciones aprovechen al máximo sus recursos comunes y lograr en su meta final, un impacto en la caficultura.

P O R T A N T O:

Con base en lo considerado, las partes:

I. Se comprometen a ejecutar las acciones conjuntas necesarias, para lograr los objetivos siguientes:

1. Elaborar estudios de parasitismo micótico, con énfasis en Roya del Cafeto.

2. Estudios de Control biológico de Hemileia vastatrix, Berk & Br.

3. La utilización conjunta de recursos, para beneficio mútuo, como objetivo de mejoramiento de la agricultura y caficultura del país.

Corresponde a Comisión Roya:

1. Permitir a ICAITI la utilización del invernadero o de otras instalaciones localizadas en el Centro de Capacitación e Investigación El Pino,

así como proporcionar asistencia técnica, para la realización de actividades relacionadas con los proyectos a elaborar.

2. Colaborar a través de su personal, con los términos de ICAITI, para la solución conjunta de problemas específicos que requieran de esta adición de esfuerzos.

3. Proporcionar áreas de construcción de futuros laboratorios, dentro del Centro El Pino.

4. Cooperar en la realización de las actividades y aportaciones de recursos necesarios, de acuerdo a las condiciones prevalecientes en función de cumplimiento de acuerdos específicos que la implementación de este proyecto implique y que previamente hayan sido considerados pertinentes para ambas instituciones.

III. Corresponde a ICAITI:

1. Buscar los recursos financieros para lograr que los programas y estudios que se puedan poner en marcha, incluyendo materiales, mano de obra y mejoras en las instalaciones que los proyectos amparados por este Convenio demanden.

2. Efectuar los trabajos de laboratorio, que requieran la elaboración de los proyectos que se aprueban, siempre que se cuenta con financiamiento para ello.

3. Multiplicar los organismos que en el campo se observen, como potenciales para efectuar el control Biológico.

4. Cooperar en la realización de las actividades y aportaciones de recursos necesarios, de acuerdo a las condiciones prevalecientes en función de dar cumplimiento a acuerdos específicos que la implementación de este proyecto, implique y que previamente hayan sido considerados pertinentes por ambas instituciones.

IV. Ambas instituciones deben aprobar los proyectos de trabajo que incluirán objetivos generales y específicos, meotodologías, planes de ejecución. Cualquier otro aspecto no considerado en el presente Convenio, será resuelto de mutuo acuerdo.

V. Los resultados obtenidos, producto de las acciones conjuntas desarrolladas directa o indirectamente, bajo el amparo de este convenio, serán publicados por científicos de ambas entidades, que hayan participado en su obtención.

VI. El Presente Convenio, tiene una duración de dos años a partir de la fecha de su firma y se prorrogará automáticamente por un lapso igual, a menos que una de las partes exprese en forma escrita a la otra, el deseo de finalizarlo, por lo menos con seis meses de anticipación al período en que el Convenio podría finalizar. La finalización de este Convenio, no debe ser motivo de obstáculo o interrupción para actividades que previamente se estén desarrollando, al amparo del mismo.

VII. El Presente Convenio, entrará en vigor formalmente de manera inmediata después de haber sido firmado.

Suscrito en la Ciudad de Guatemala, a los 7 días del mes de junio de mil novecientos ochenta y seis. En dos ejemplares en español, ambos igualmente válidos en la representación de la Institución, aceptamos, ratificamos y confirmamos el presente Convenio.

DIRECTOR GENERAL ICAITI

JEFE EJECUTIVO COMISION ROYA

APPENDIX 2 AND 3

APPENDIX 2

FIELD SAMPLE COLLECTION

FIRST STAGE

Altitude under 300 meters below sea level (zones 3.6.9).Rainfull 0-2500, 2500-3800, 3800 mm and more respectively.

FARM	DATE	ZONE	IDENTIFICATION NUMBER
"EL EDEN " Taxisco, Santa Rosa	2/2/87	3	1
"PALMIRA" Santa Lucía Cotz. Escuintla	5/3/87	6	9
"OFELIA" Malacatán, San Marcos	19/2/87	9	5

SECOND STAGE

Altitude under 300-700 meters below sea level (Zones 2,5,8). Rainfull 0-25--,2500-3800 mm or more respectively

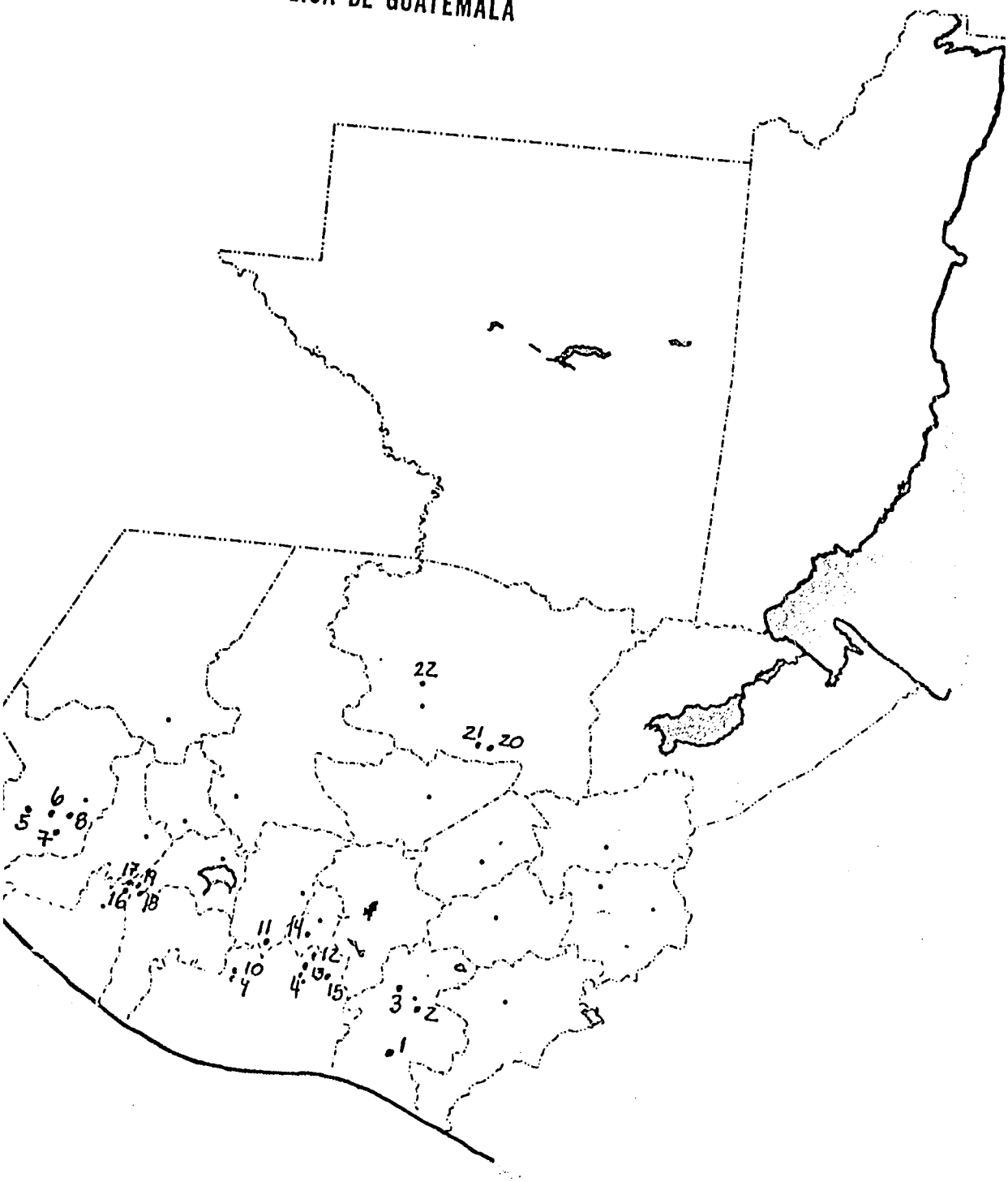
"CONCEPCION" Cuilapa. Santa Rosa	2/2/87	2	2
"EL RODEO" San Marcos	19/2/87	5	6
"SAN ANTONIO LA PAZ Santa Lucía, Cotz. Escuintla	5/3/87	5	10
"SABANA GRANDE" El Rodeo,Escuintla	19/3/87	5	12
"PACHILHA",Tucurú Alta Verapaz	23/4/87	5	20
"SAN IGNACIO" San Felipe, Reu	2/4/87	8	16
"LOS ENCUENTROS" San Felipe, Reu	2/4/87	8	17

THIRD STAGE

Altitude under 700 meters below sea level (zones 1,4,7).
Rainfall from 0-2500-3800 mm or more respectibely.

FARM	DATE	ZONE	IDENTIFICATION NUMBER
"PASTORIA,Barberena Sanra Rosa	2/2/87	1	3
"CAFETILLO,Alotenango Sacatepequez	2/4/87	1	14
"BELLA VISTA",Palín Escuintla	2/4/87	1	15
"CHIMOTE" San Pedro Carchá, Coban	23/4/87	1	21
"SANTA BARBARA" Yepocapa, Chimaltenango			
"EL EDEN"Tumbador San Marcos	2/2/87	4	4
"CUCHIL"Tumbador Alta Verapaz	23/4/87	4	22
"PARIS" Pueblo Nuevo Suchitepéquez	2/4/87	7	19
"LORENA" San Rafael,Pie de la Cuesta San Marcos	19/2/87	7	8
"EL VOLCAN" Escuintla	19/3/87	7	13

MAPA DE LA REPUBLICA DE GUATEMALA



APPENDIX 3

FIELD SAMPLE MYCOPARASIT COLLECTION

FIRST STAGE

Altitude under 300 meters below sea level(zones 3,6,9).
Rainfall 0-2500,2500-3800 mm or more respectively.

FARM	DATE	ZONE	IDENTIFICATION NUMBER
"PALMIRA" Santa Lucia Cotz. Escuintla	12/8/87	6	2

SECOND STAGE

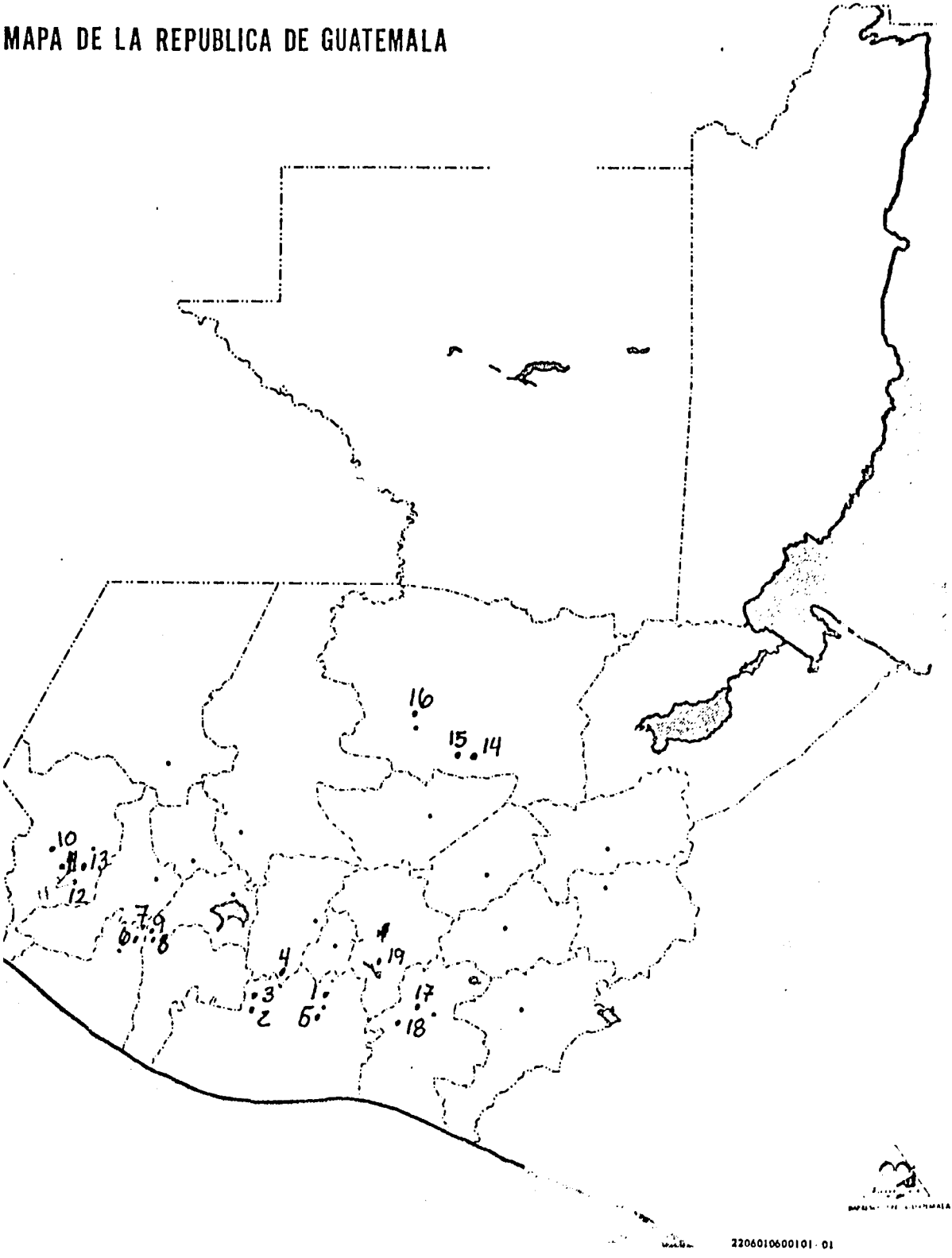
Altitude from 300-700 meters below sea level,(zones2,5,8)
Rainfuall 0-2500-3800 and 3800 or more respectively.

FARM	DATE	ZONE	IDENTIFICATION NUMBER
"SAN ANTONIO LA PAZ" Santa Lucia Cotz. Escuintla	7/9/87	5	3
"SAN IGNACIO,San Felipe Rue.	22/9/87	8	6
"LOS ENCUENTROS" San Felipe, Reu.	22/9/87	8	7
"EL RODEO",El RODEO San Marcos	23/9/87	5	11
"FACHILHA,El Rodeo San Marcos	29/9/87	5	14
"SANTA TERESA,Tucuru Alta Verapaz"	29/9/87	5	15

THIRD STAGE
Altitude above 700 meters below sea level (zones 1,4,7).
Rainfall 0-2500-3900 and 3800 or more respectively.

FARM	DATE	ZONE	IDENTIFICATION NUMBER
"EL VOLCAN" Escuintla	12/8/87	7	1
"STA. BARBARA, San Pedro Yepocapa,Chimaltenango	7/9/87	7	4
"SAN FERNANDO" Escuintla	10/9/87	4	5
"PARIS",Pueblo Nuevo Suchitepequez	22/9/87	7	8
"PATIO DE BOLAS" Pueblo Nuevo Suchitepequez	22/9/87	7	9
"EDEN",Tumbador San Marcos	23/9/87	4	12
"LORENA"San Rafael Pie de la Cuesta San Marcos	23/9/87	4	13
"CHIMOTE" San Pedro Carchá, Alta Verapaz	29/9/87	4	16
"PASTORIA",Barberena Santa Rosa	5/10/87	1	17
"LAS MARINAS"Pueblo Nuevo Vinas, Santa Rosa	5/10/87	1	18
"SAN JOSE LA LAGUNA" Villa Canales,Guatemala	5/10/87	1	19
"LAS ILUSIONES"San Pablo San Marcos	23/9/87	5	10

MAPA DE LA REPUBLICA DE GUATEMALA



APPENDIX 4

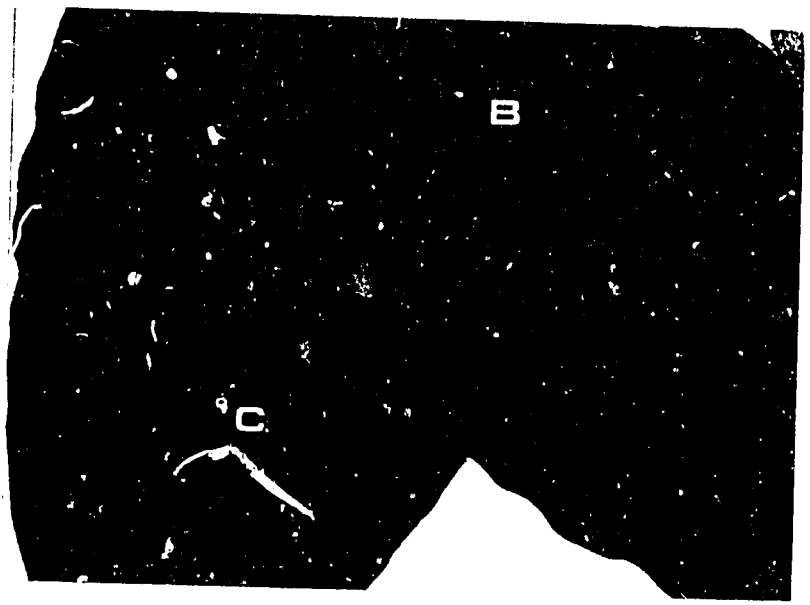
RUST EVOLUTION OVER DETACHED COFFEE LEAVES

RUST INDUCED EVOLUTION

A = 13 - 18 DAYS A. I.

A. I. = AFTER INOCULATION

RUST INDUCED EVOLUTION

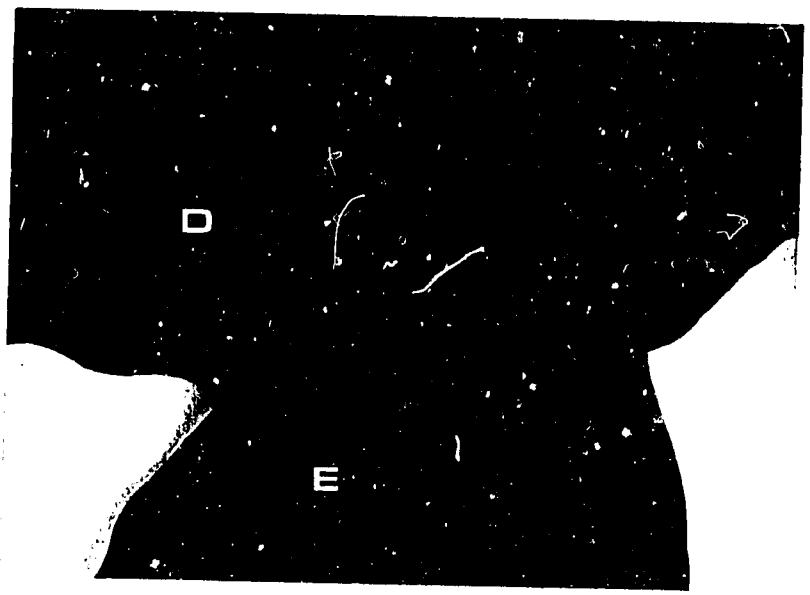


B = 25 - 28 DAYS A. I.

C = 30 - 32 DAYS A. I.

A. I. = AFTER INOCULATION

RUST INDUCED EVOLUTION

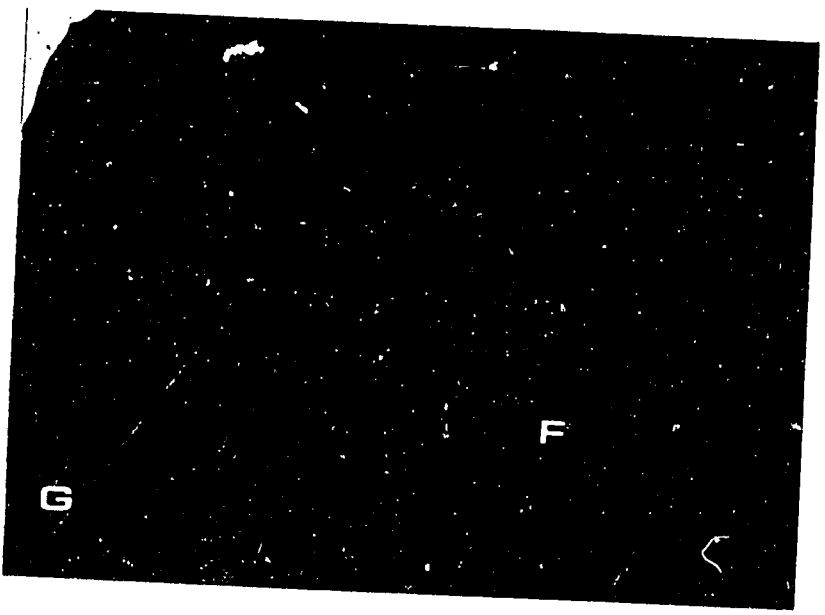


D = 38 - 40 DAYS A. I.

E = 35 - 38 DAYS A. I.

A. I. = AFTER INOCULATION

RUST INDUCED EVOLUTION

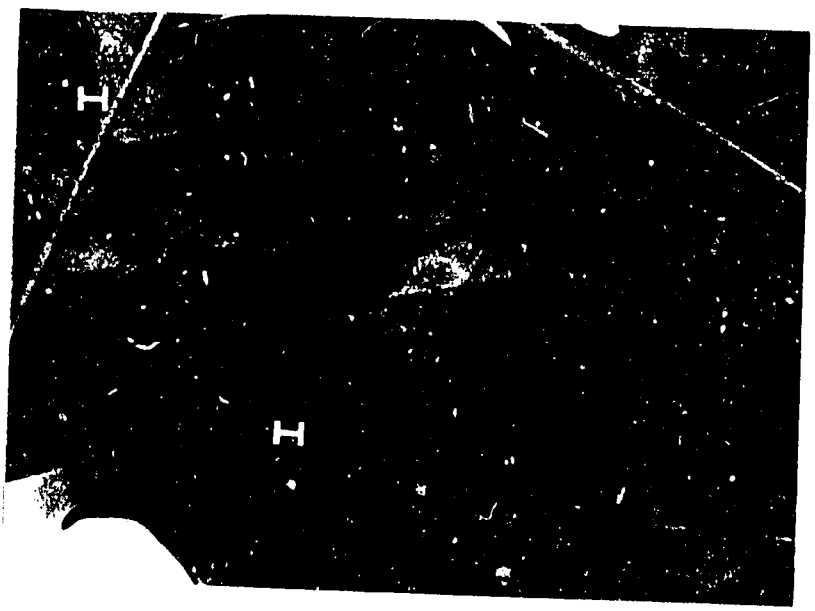


F = 42 - 45 DAYS A. I.

G = 45 - 50 DAYS A. I.

A. I. = AFTER INOCULATION

RUST INDUCED EVOLUTION



H = 55 - 60 DAYS A. I.

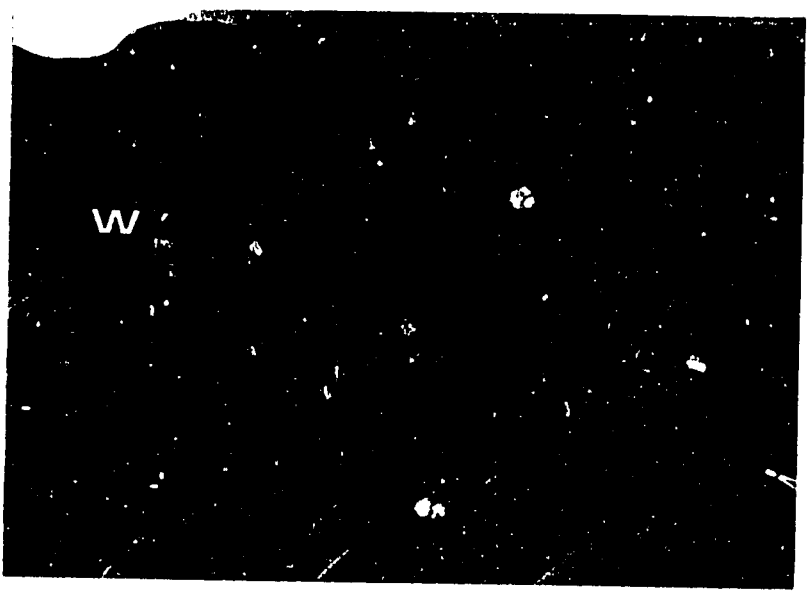
A. I. = AFTER INOCULATION

APPENDIX 5

SOME MYCOPARASITES AT DIFFERENT STAGES OF INVASION

Paecilomyces lilacinus, over rust infected coffee leaves

Trichoderma like verticillium, Aphanocladium meliolae and Verticillium leptobactrum over rust infected coffee leaves.



APPENDIX 6

SCANNING ELECTRON MICROSCOPY OF ANTAGONIST

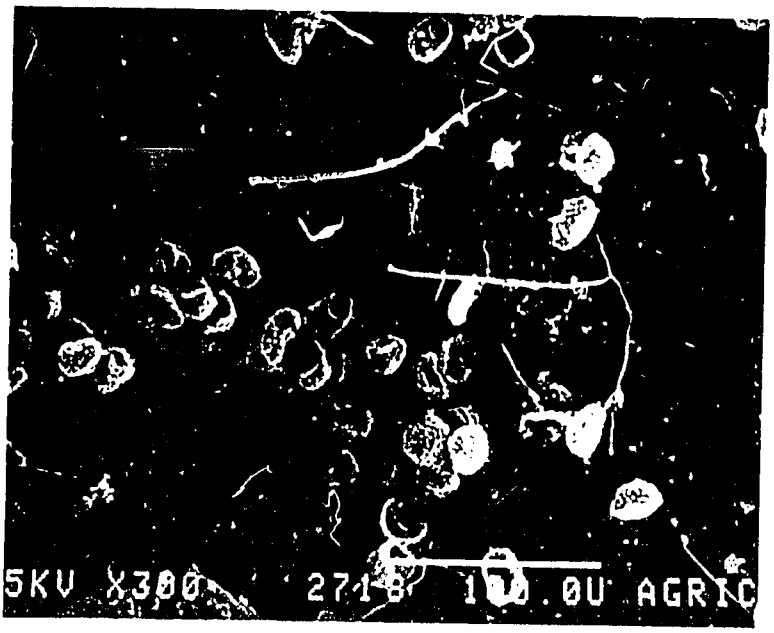


FIGURE 1

FIGURE 2

FIGURE 3

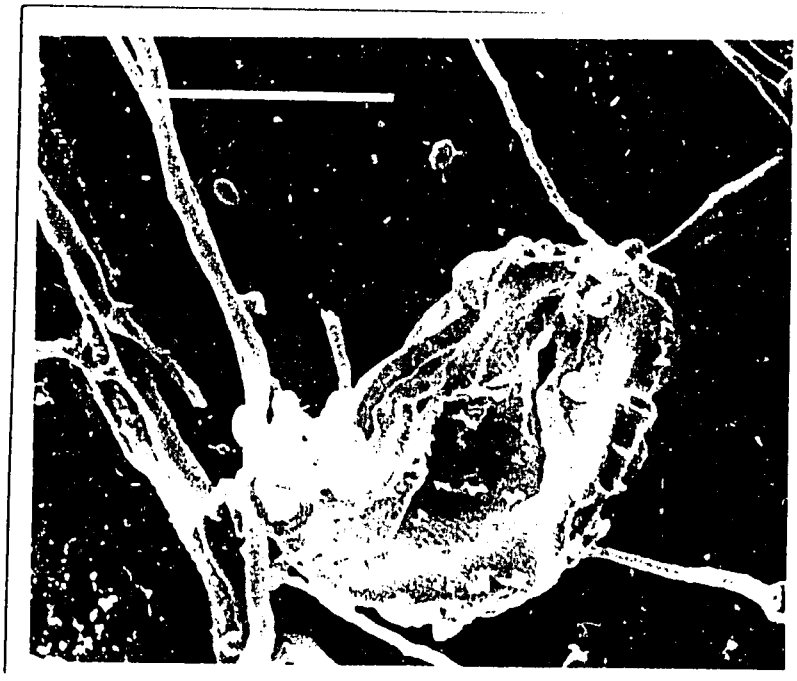


FIGURE 4

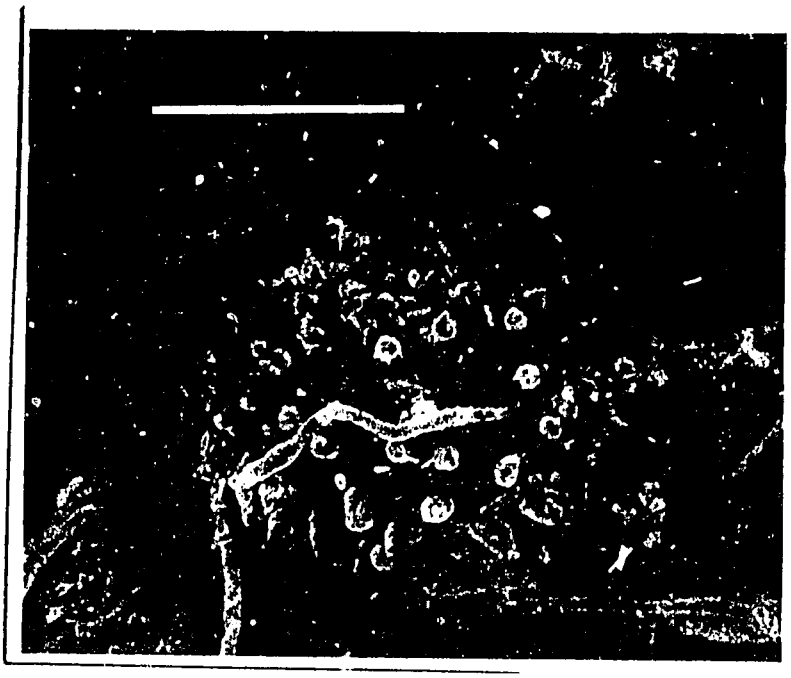


FIGURE 5

FIGURE 6

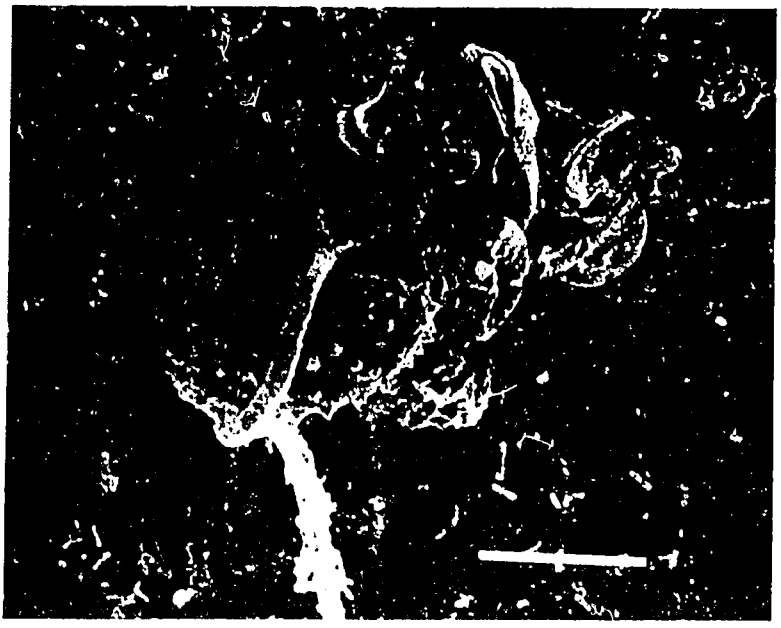


FIGURE 7

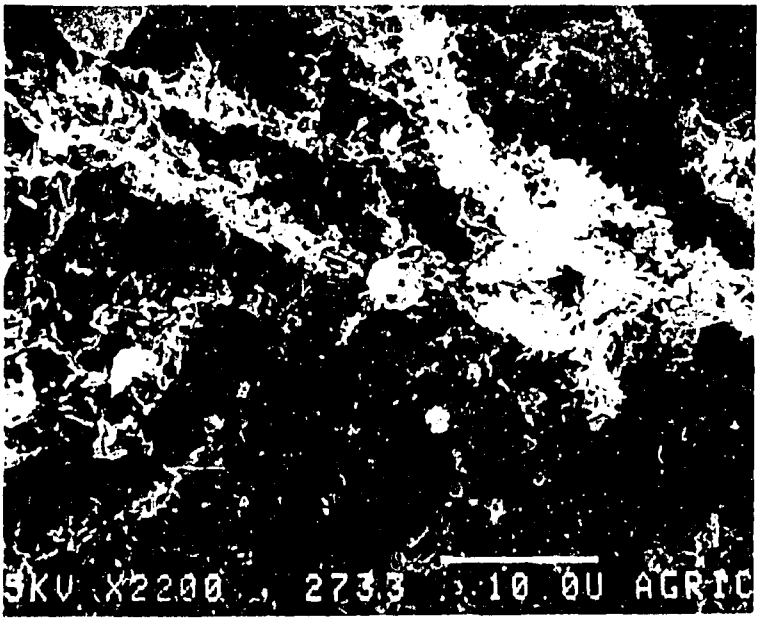


FIGURE 8

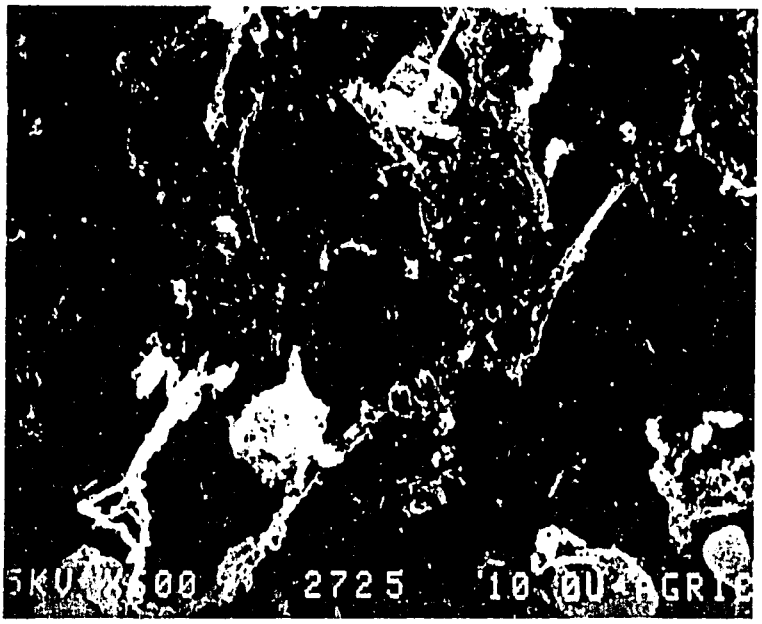


FIGURE 9

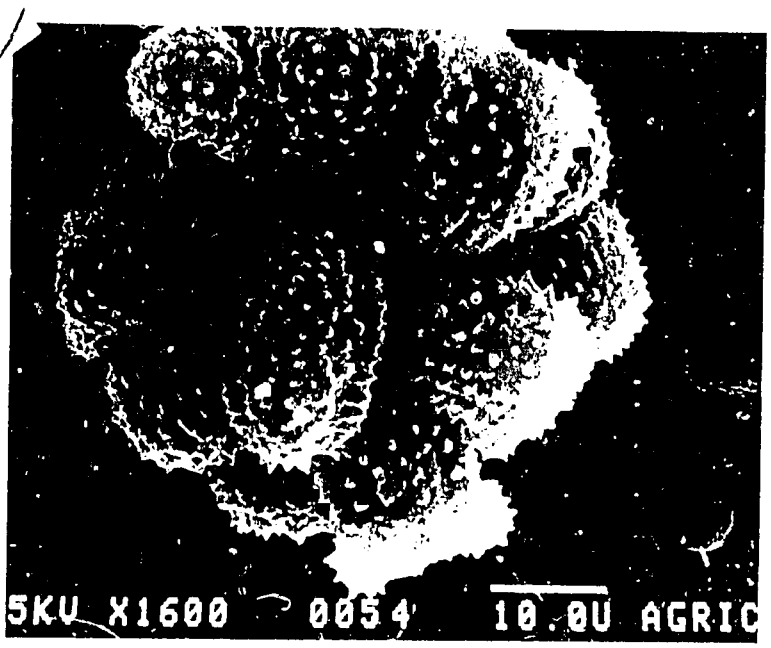


FIGURE 10

APPENDIX 6

SCANNING ELECTRON MICROSCOPY OF ANTAGONIST

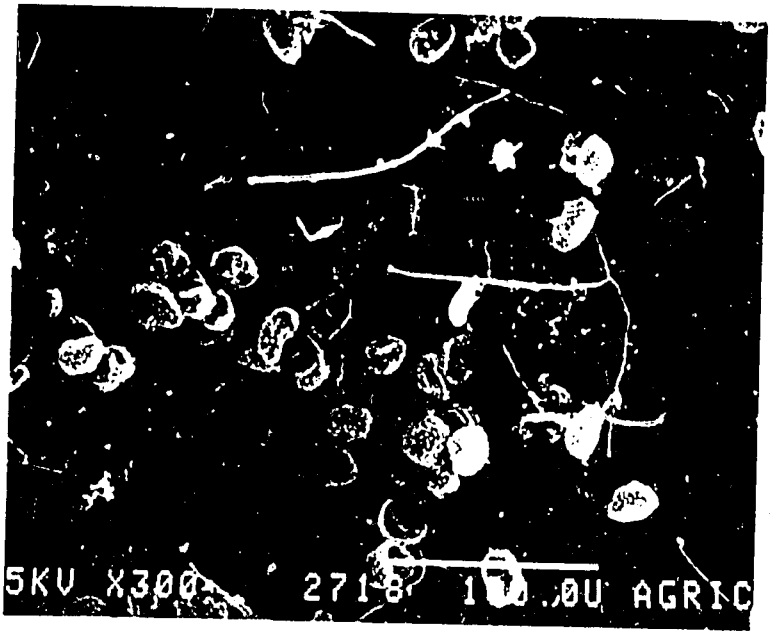


FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

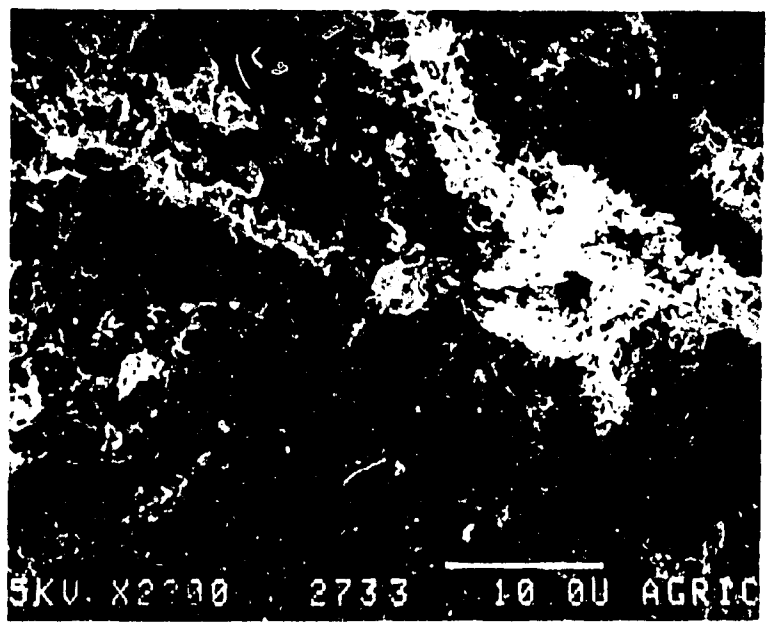


FIGURE 8

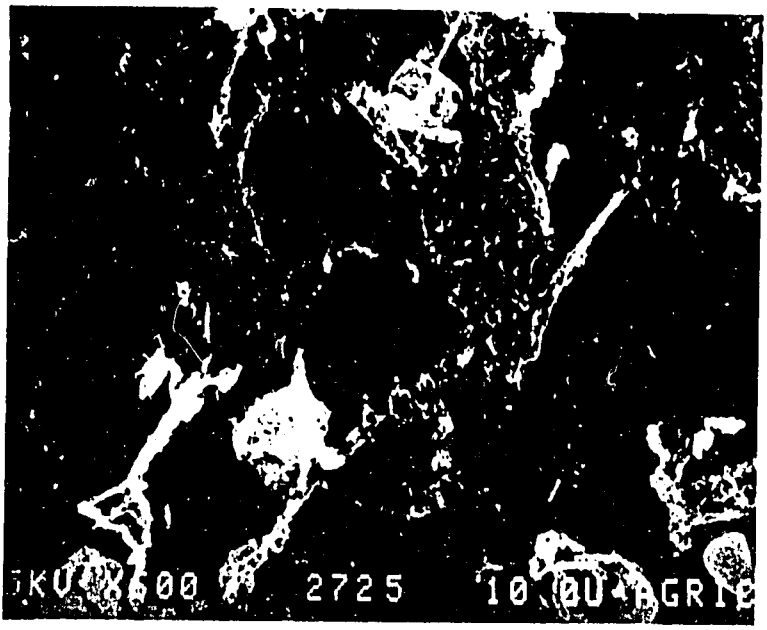


FIGURE 9

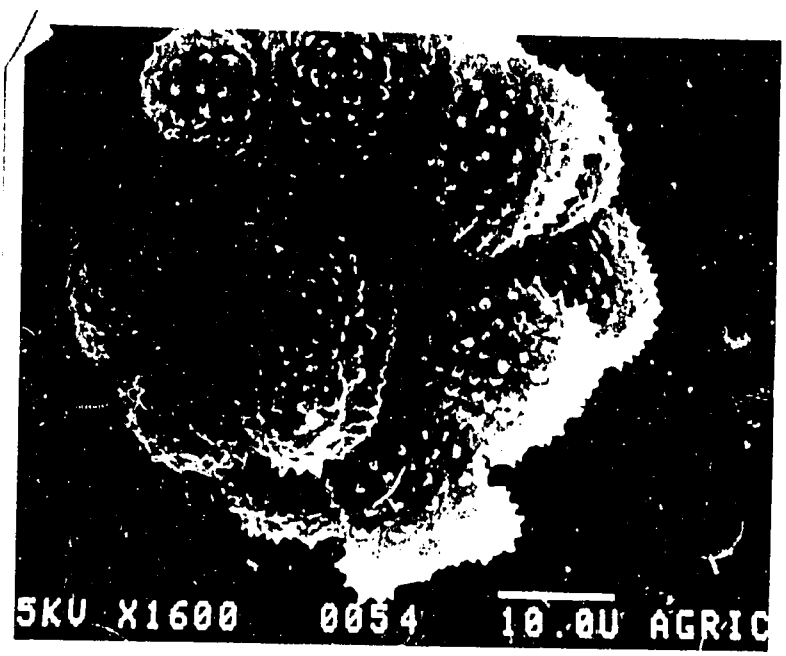


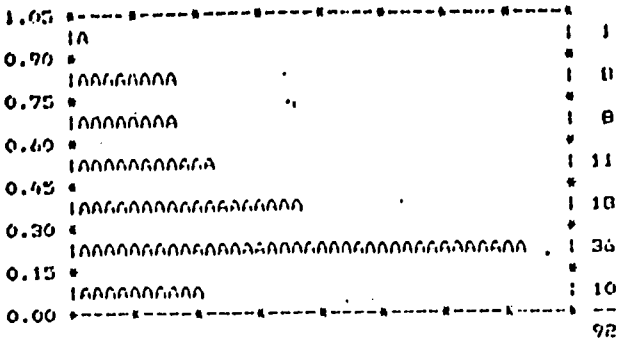
FIGURE 10

VIRULENCE OF INDUCED RUST IN DETACHED COFFEE LEAVES

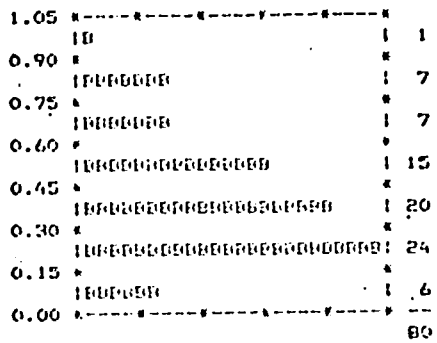
DIAMETER DISTRIBUTION AMONG SEVERAL CHLOROTIC SPOTS

GRAPS 1, 2 & 3

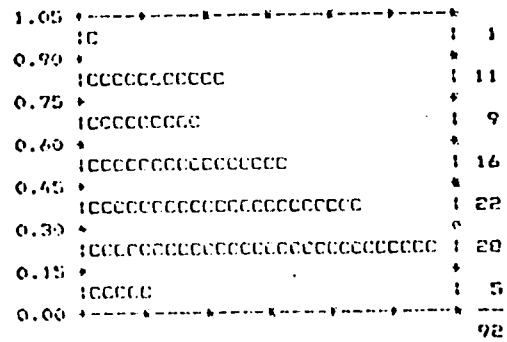
GRAPH 1



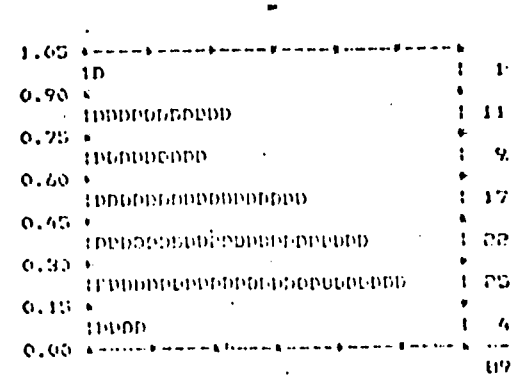
B



C



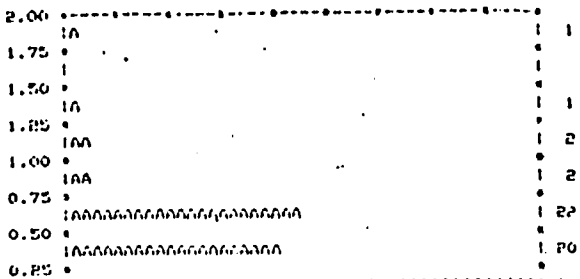
D



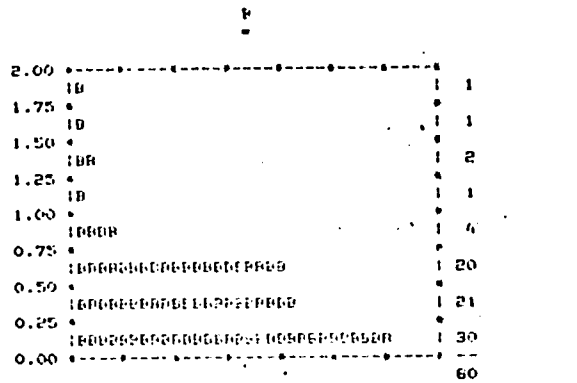
DAYS AFTER INOCULATION

A = 27 C = 33
B = 29 D = 36

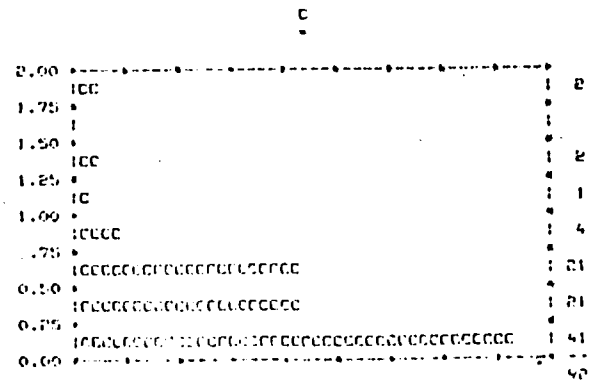
A


2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
1
1
2
2
22
20
44
92

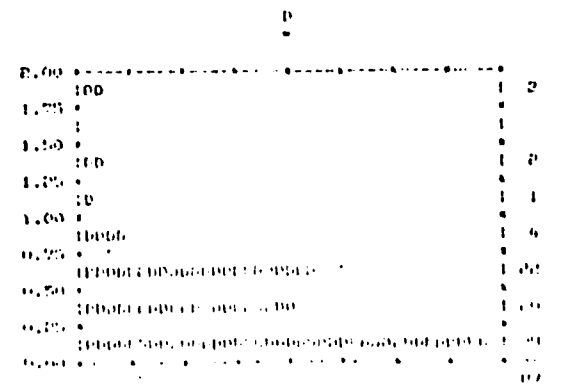
B


2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
1
1
2
1
4
20
21
30
60

C


2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
2
2
1
4
21
21
41
90

D


2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
2
2
1
4

A

```
1.40 *-----*-----*-----*
     |A                |  1
1.20 *                 *
     |                 |
1.00 *                 *
     |                 |
0.80 *                 *
     |AAAA             |  4
0.60 *                 *
     |A                |  1
0.40 *                 *
     |AAAAAA           |  6
     *                 *
```

B

```
1.40 *-----*-----*-----*
     |B                |  1
1.20 *                 *
     |                 |
1.00 *                 *
     |B                |  1
0.80 *                 *
     |BBB              |  3
0.60 *                 *
     |BB               |  2
0.40 *                 *
     |BBBBBBB          |  7
0.20 *                 *
     |BBBB             |  4
0.00 *-----*-----*-----* --
                        18
```

C

```
1.60 *-----*-----*-----*
     |C                |  1
1.40 *                 *
     |                 |
1.20 *                 *
     |CC               |  2
1.00 *                 *
     |CC               |  2
0.80 *                 *
     |CCC              |  3
0.60 *                 *
     |CCCCCC           |  6
0.40 *                 *
     |CC               |  2
0.20 *                 *
     |CC               |  2
0.00 *-----*-----*-----* --
                        10
```

D

```
1.60 *-----*-----*-----*
     |D                |  1
1.40 *                 *
     |                 |
1.20 *                 *
     |DD               |  2
1.00 *                 *
     |D                |  1
0.80 *                 *
     |DDDD             |  4
0.60 *                 *
     |DDDDD            |  5
0.40 *                 *
     |DDDD             |  4
0.20 *                 *
     |D                |  1
0.00 *-----*-----*-----* --
                        10
```

UREDOSPORES PRODUCTION

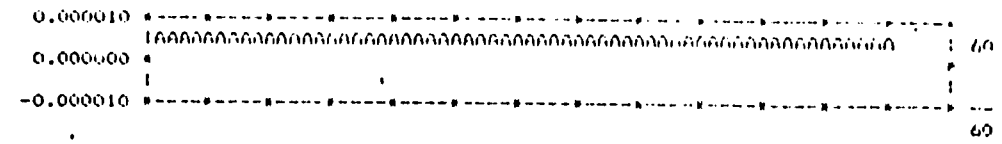
AMONG SEVERAL CHLOROTIC

SPOTS AGAINST TIME

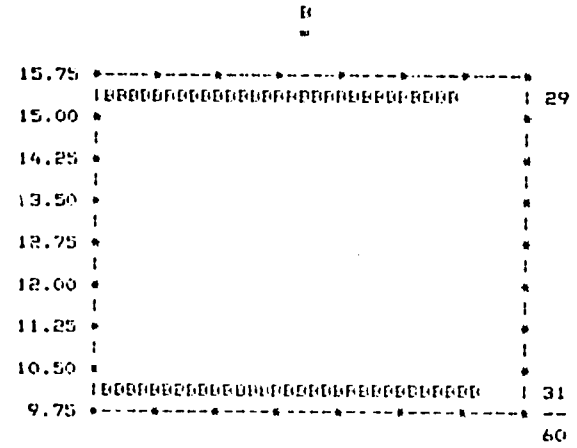
GRAPHS 4 & 5

GRAPH 4

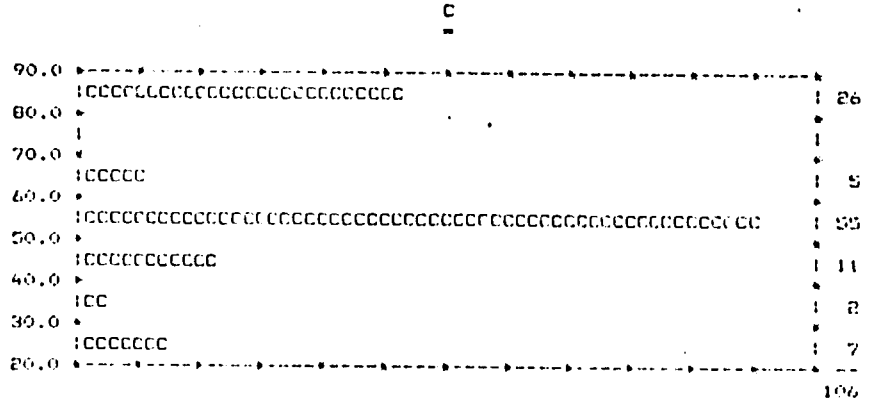
A


0.000010
0.000000
-0.000010
60

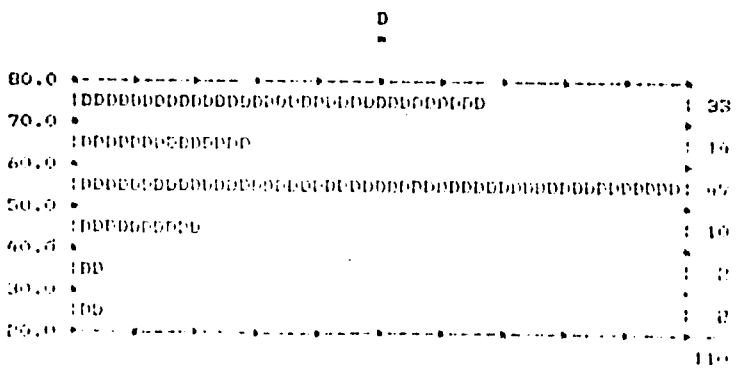
B


15.75
15.00
14.25
13.50
12.75
12.00
11.25
10.50
9.75
29
31
60

C

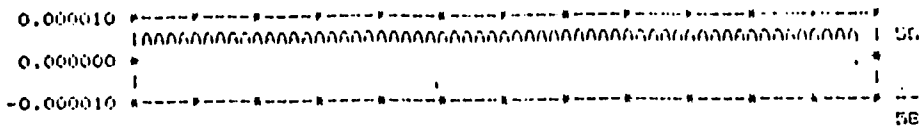

90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
26
5
55
11
2
7
106

D

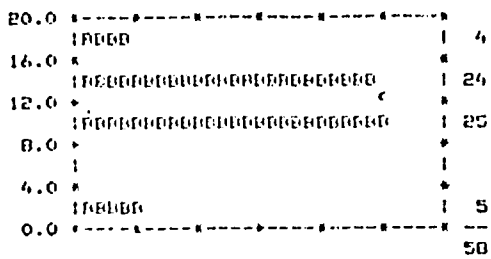

80.0
70.0
60.0
50.0
40.0
30.0
20.0
33
14
10
2
2
110

GRAPH 5

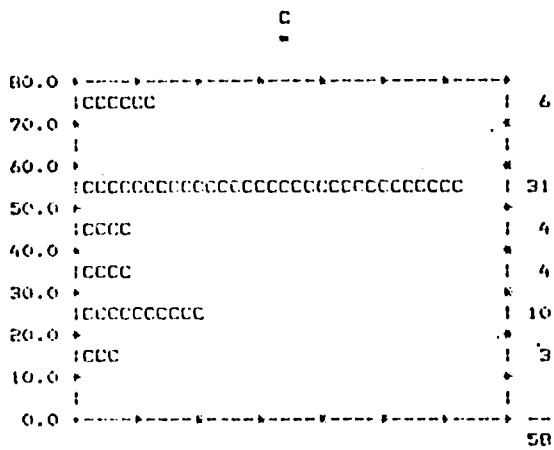
A


0.000010
0.000000
-0.000010
50
50

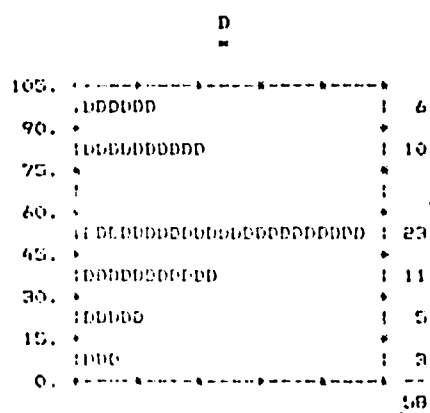
B


20.0
16.0
12.0
8.0
4.0
0.0
4
24
25
5
50

C


80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
6
31
4
4
10
3
50

D


105.
90.
75.
60.
45.
30.
15.
0.
6
10
23
11
5
3
50

FARM IDENTIFICATION

IDENTIFICATION NUMBER	IDENTIFICATION NAMES	FARMS NAME
2	C	CONCEPCION
3	P	PASTORIA
4	F	SAN FERNANDO
5	O	OFELIA
6	R	RODEO
7	E	EDEN
8	L	LORENA
9	Pa	PALMIRA
10	A	SAN ANTONIO LA PAZ
11	BA	SANTA BARBARA
12	S	SABANA GRANDE
13	V	VOLCAN
15	B	BELLA VISTA
16	SI	SAN IGNACIO
17	LE	LOS ENCUENTROS
19	PR	PARIS

20	Pach.	PACHILHA
22	CU	CUCHIL

Table 1

INDUCED RUST INFECTION IN COFFEE PLANTS

INOCULATION DATE	LOT	INOCULATED PLANTS	METHOD	TOTAL INFECTION
30/09/86	1	25	SPORE DISPERSION	60
27/10/86	2	50	SPORE DISPERSION	68
03/11/86	3	50	SPRAY	50
12/11/86	4	50	SPRAY	56
17/11/86	5	50	SPRAY	60
18/11/86	6	50	SPRAY	63
10/12/86	7	40	SPRAY	62
10/12/86	8	40	DIRECT LEAF TO LEAF CONTACT	53
10/12/86	9	66	SPORE DISPERSION	74
14/01/87	10	13	SPORE DISPERSION	74
15/01/87	11	181	SPRAY	65
19/01/87	12	50	DIRECT LEAF TO LEAF CONTACT	48
11/02/87	13	75	SPORE DISPERSION	78
25/02/87	14	75	SPORE DISPERSION	80
11/03/87	15	50	SPORE DISPERSION	84
25/03/87	16	75	SPORE DISPERSION	76
08/04/87	17	125	SPORE DISPERSION	78
22/04/87	18	75	SPORE DISPERSION	85
13/05/87	19	100	SPORE DISPERSION	80
27/05/87	20	100	SPORE DISPERSION	84
10/06/87	21	75	SPORE DISPERSION	88
24/06/87	22	75	SPORE DISPERSION	86
15/07/87	23	100	SPORE DISPERSION	83
29/07/87	24	50	SPORE DISPERSION	88
12/08/87	25	75	SPORE DISPERSION	84
26/08/87	26	75	SPORE DISPERSION	85
09/09/98	27	75	SPORE DISPERSION	89
23/09/87	28	100	SPORE DISPERSION	94

TABLE 2

RUST INDUCED INFECTION ON COFFEE LEAVES

INOCULATION DATE	LOT	GERMINATION %	APPEARENCE SMALL DECOLORED SPOTS (DAYS)	TOTAL INFECTION %	TOTAL INFECTION (DAYS)
17/02/87	1	23	26	37.00	44
07/03/87	2	43	17	88.00	46
11/03/87	3	38	19	59.70	41
13/03/87	4	35	28	94.33	48
17/03/87	5	40	18	86.00	44
27/03/87	6	42	13	60.90	35
09/04/87	7	40	18	93.78	36
30/04/87	8	39	13	90.10	39
05/05/87	9	41	15	92.30	42
08/05/87	10	42	17	94.50	42
14/05/87	11	42	15	93.10	40
20/05/87	12	43	14	95.18	37
22/05/87	13	40	15	91.30	38
26/05/87	14	43	15	94.60	41
28/05/87	15	42	14	93.80	40
08/06/87	16	43	18	90.10	42
26/05/87	17	42	16	92.40	39
26/05/87	18	41	16	90.00	43
22/07/87	19	38	19	88.50	40
02/09/87	20	34	16	100.00	47

TABLE 3
ISOLATED EPIPHYTE BACTERIA

COLLECTION TRIP	FARM	DILUTION	MEDIUM	IDENTIFICATION NUMBER
1	P	A-4	K	1
1	P	A-4	K	2
1	P	C-3	K	3
1	E	A-4	K	4
1	E	C-3	K	5
1	E	B-3	K	6
1	F	A-3	K	7
1	F	C-4	K	8
1	F	B-4	K	9
1	F	B-4	K	10
1	F	C-4	K	11
1	C	B-3	K	12
1	C	B-3	K	13
1	C	A-3	K	14
1	C	B-4	CH	15
1	C	B-4	CH	16
1	E	C-4	CH	17
1	C	C-4	CH	18
1	E	B-4	CH	19
1	F	B-4	CH	20
1	F	A-4	MRB	21
1	F	A-4	MRB	22
1	F	B-4	CH	23
1	P	B-4	CH	24
1	F	B-4	CH	25
1	E	A-4	CH	26
2	R	B-6	K	27
2	R	B-5	K	28
2	R	B-5	K	29
2	R	A-3	K	30
2	L	A-4	K	31
2	L	B-3	K	32
2	L	A-5	K	33
2	E	C-6	K	34
2	E	A-4	K	35
2	E	A-4	K	36
2	E	B-5	K	37
2	E	B-6	K	38
2	O	C-5	K	39
2	O	C-6	K	40
2	E	B-6	MRB	41
2	E	C-3	MRB	42
2	E	C-3	MRB	43
2	L	B-3	MRB	44
2	R	B-3	MRB	45
2	O	C-3	CH	46
2	O	C-3	CH	47
2	O	B-3	CH	48
2	O	A-3	CH	49
2	E	C-3	CH	50
2	E	C-3	CH	51

COLLECTION TRIP	FARM	DILUTION	MEDIUM	IDENTIFICATION ORDINAL NUMBER
2	L	B-3	CH	52
2	L	B-6	CH	53
2	L	A-3	CH	54
2	L	A-3	CH	55
2	L	A-3	CH	56
2	R	C-3	CH	57
2	R	A-6	CH	58
2	R	A-6	CH	59
2	R	A-3	CH	60
3	B	A-3	K	61
3	B	A-3	K	62
3	B	A-4	K	63
3	B	A-6	K	64
3	B	A-6	K	65
3	B	A-6	K	66
3	B	B-4	K	67
3	B	B-5	K	68
3	B	B-5	MRB	69
3	B	C-6	K	70
3	Pa	A-4	K	71
3	Pa	A-3	K	72
3	Pa	A-5	K	73
3	Pa	B-3	K	74
3	Pa	B-3	K	75
3	Pa	B-3	K	76
3	Pa	B-3	K	77
3	Pa	B-5	K	78
3	Pa	C-6	K	79
3	Pa	C-5	K	80
3	P	A-3	K	81
3	A	A-3	K	82
3	A	A-3	K	83
3	A	A-4	K	84
3	A	A-6	K	85
3	A	C-6	K	86
3	A	C-5	K	87
3	A	C-5	K	88
4	V	C-5	MRB	89
4	V	C-5	K	90
4	V	C-5	K	91
4	V	C-6	CH	92
4	V	C-3	CH	93
4	V	C-3	CH	94
4	V	B-6	CH	95
4	V	B-6	K	96
4	V	B-6	K	97
4	V	A-3	K	98
4	V	A-4	K	99
4	V	B-6	K	100
4	V	A-3	K	101
4	V	A-4	K	102
4	V	A-4	CH	103
4	V	C-5	CH	104
4	V	B-4	CH	105

COLLECTION TRIP	FARM	DILUTION	MEDIUM	IDENTIFICATION ORDINAL NUMBER
16	P	C-5	PDA	269
16	P	C-5	PDA	270
16	P	C-5	PDA	271
16	P	C-5	PDA	272
16	P	C-5	PDA	273
17	LM	C-5	PDA	274
17	LM	C-5	PDA	275
17	LM	C-5	PDA	276
17	LM	C-5	PDA	277
17	LM	C-5	PDA	278
17	LM	C-5	PDA	279
17	LM	C-5	PDA	280
18	LM	C-5	PDA	281
18	LM	C-5	PDA	282
18	LM	C-5	PDA	283
18	LM	C-6	PDA	284
19	PACH	C-5	PDA	285
19	PACH	C-5	PDA	286
19	PACH	C-6	PDA	287
19	PACH	C-5	PDA	288
19	PACH	C-5	PDA	289
19	PACH	C-5	PDA	290
19	PACH	C-5	PDA	291
20	ST	C-4	PDA	292
20	ST	C-5	PDA	293
20	ST	C-5	PDA	294
20	ST	C-5	PDA	295
20	ST	C-5	PDA	296
20	CH	C-6	PDA	297
20	CH	C-5	PDA	298
20	CH	C-5	PDA	299
20	CH	C-6	PDA	300
21	CH	C-5	PDA	301
21	CH	C-5	PDA	302
21	CH	C-5	PDA	303
22	CH	C-5	PDA	304
22	CH	C-5	PDA	305
22	CH	C-6	PDA	306

MICROORGANISM FROM OTHERS COUNTRIES

FROM COLOMBIA	FROM BRAZIL	FROM COSTA RICA
5- PSHI	CNPDA- AP 3	ICRR
E - PSHI	CNPDA- AP 85	ICRA
16-PSHI	CNPDA- AP 150	
B- PSHI	CNPDA - AP 203	
2- PSHI	CNPDA- C 293	
9- PSHI	CNPDA- C 294	
D- PSHI		
4- PSHI		
C- PSHI		
A- PSHI		
F- PSHI		
13- PSHI		

TABLE 4

ISOLATED FUNGI

COLLECTION TRIP	FARM	DILUTION	MEDIUM	IDENTIFICATION ORDINAL NUMBER
1	P	A-4	MRB	1
1	P	A-4	MRB	2
1	P	B-3	MRB	3
1	P	C-4	MRB	4
1	P	A-4	MRB	5
1	F	A-4	MRB	6
1	F	C-4	MRB	7
1	F	B-4	MRB	8
1	F	B-4	MRB	9
1	C	A-3	MRB	10
1	C	A-4	MRB	11
1	C	A-3	MRB	12
1	C	A-4	MRB	13
1	C	A-4	MRB	14
1	C	B-4	MRB	15
1	C	C-4	MRB	16
1	E	C-4	MRB	17
1	E	A-4	MRB	18
1	E	C-4	MRB	19
1	E	A-2	MRB	20
1	P	B-4	MRB	21
1	P	C-4	MRB	22
1	P	A-4	MRB	23
1	P	B-4	MRB	24
1	P	A-4	MRB	25
1	O	A-5	MRB	26
2	R	A-6	MRB	27
2	R	A-6	TSM	28
2	O	C-3	MRB	29
2	O	A-3	MRB	30
2	O	A-5	MRB	31
2	O	B-6	MRB	32
2	L	B-6	MRB	33
2	L	C-6	MRB	34
2	L	A-6	MRB	35
2	R	A-6	MRB	36
2	R	B-3	MRB	37
2	E	C-6	CH	38
2	E	B-6	CH	39
2	E	A-6	CH	40
2	R	C-6	CH	41
2	R	B-6	CH	42
3	BA	A-4	MRB	43
3	BA	A-3	MRB	44
3	BA	A-5	MRB	45
3	BA	B-3	MRB	46
3	BA	B-6	MRB	47
3	BA	B-5	MRB	48
3	BA	B-5	MRB	49
3	BA	C-5	MRB	50

COLLECTION TRIP	FARM	DILUTION	MEDIUM	IDENTIFICATION ORDINAL NUMBER
3	PA	A-4	MRB	51
3	PA	A-5	MRB	52
3	PA	A-5	MRB	53
3	PA	B-5	MRB	54
3	A	A-4	MRB	55
3	A	A-3	MRB	56
3	A	A-5	MRB	57
4	V	C-4	CH	58
4	V	C-3	MRB	59
4	V	B-5	MRB	60
4	S	C-4	CH	61
4	S	A-5	MRB	62
4	B	C-6	MRB	63
4	B	B-4	MRB	64
4	B	A-4	MRB	65
5	PR	B-4	MRB	66
5	PR	A-4	MRB	67
5	PR	A-4	MRB	68
5	PR	A-3	CH	69
5	PR	C-3	MRB	70
5	PR	C-6	MRB	71
5	PR	C-5	MRB	72
5	PB	C-5	MRB	73
5	PB	B-5	CH	74
5	PB	A-3	MRB	75
5	PB	A-3	MRB	76
5	PB	C-3	MRB	77
5	PB	C-5	MRB	78
5	CH	A-6	MRB	79
5	CH	A-6	MRB	80
5	CH	B-6	MRB	81
5	CH	B-6	MRB	82
5	SI	C-6	MRB	83
5	SI	C-3	MRB	84
5	SI	A-4	MRB	85
5	SI	B-6	MRB	86
5	SI	B-6	MRB	87
6	PA	C-5	MRB	88
6	PA	C-4	MRB	89
6	PA	B-6	MRB	90
6	CA	B-5	MRB	91
6	CA	C-5	MRB	92
6	CA	C-5	MRB	93
6	CA	C-3	MRB	94
6	CA	C-3	MRB	95
6	CA	C-6	CH	96
6	BUA	A-6	CH	97
6	BUA	A-6	CH	98
6	BUA	A-5	CH	99
6	BUA	A-5	CH	100
6	BUA	C-3	MRB	101
6	BUA	C-5	MRB	102
6	BUA	C-6	MRB	103
6	BUA	C-3	CH	104
6	BUA	C-3	CH	105

COLLECTION TRIP	FARM	DILUTION	MEDIUM	IDENTIFICATION ORDINAL NUMBER
5	CH	B-5	TSM	106
4	B	B-2	TSM	107
1	P	A-6	PDA	108
1	P	C-5	PDA	109
1	P	C-3	MRB	110
1	P	C-4	MRB	111
1	P	C-6	MRB	112
1	P	C-5	MRB	113
1	P	C-3	MRB	114
1	P	C-3	MRB	115
1	P	C-6	MRB	116
3	Pa	C-5	TSM	118
3	Pa	C-4	TSM	119
3	Pa	A-3	TSM	120
3	A	A-3	TSM	121
5	A	A-6	TSM	122
6	BUA	B-5	TSM	123
5	A	B-5	TSM	124
5	V	B-5	PDA	125
5	V	C-5	PDA	126
5	V	C-5	PDA	127
5	V	C-5	PDA	128
5	V	C-5	PDA	129
5	V	C-5	PDA	130
5	V	C-5	PDA	131
5	V	C-5	PDA	132
5	V	C-5	PDA	133
5	V	C-5	PDA	134
5	V	C-5	PDA	135
5	V	C-5	PDA	136
5	V	C-5	PDA	137
5	V	C-5	PDA	138
5	V	C-5	PDA	139
5	V	C-5	PDA	130
6	P	C-5	PDA	141
6	P	C-5	PDA	142
6	P	C-3	PDA	143
6	P	C-4	PDA	144
6	P	C-5	PDA	145
6	B	C-6	PDA	146
6	B	C-7	PDA	147
6	SP	C-5	PDA	148
6	SP	C-5	PDA	149
6	SBA	C-5	PDA	150
6	P	C-4	PDA	151
6	P	C-5	PDA	152
6	P	C-3	PDA	153
6	P	C-4	PDA	154
6	P	C-5	PDA	155
6	P	C-5	PDA	156
7	P	C-4	PDA	157
7	P	C-5	PDA	158
7	P	C-5	PDA	159

COLLECTION TRIP	FARM	DILUTION	MEDIUM	IDENTIFICATION ORDINAL NUMBER
7	P	C-5	PDA	160
8	P	C-5	PDA	161
8	P	C-4	PDA	162
8	P	C-5	PDA	163
8	P	C-5	PDA	164
8	P	C-5	PDA	165
8	P	C-5	PDA	166
8	P	C-5	PDA	167
8	P	C-5	PDA	168
8	B	C-6	PDA	169
8	B	C-6	PDA	170
8	P	C-6	PDA	171
8	P	C-5	PDA	172
8	P	C-5	PDA	173
8	P	C-5	PDA	174
8	P	C-5	PDA	175
8	P	C-4	PDA	176
8	P	C-5	PDA	177
9	B	C-4	PDA	178
9	B	C-5	PDA	179
9	B	C-5	PDA	180
9	B	C-5	PDA	181
9	B	C-5	PDA	182
9	B	C-6	PDA	183
9	B	C-5	PDA	184
9	B	C-5	PDA	185
9	B	C-5	PDA	186
9	B	C-5	PDA	187
9	B	C-5	PDA	188
9	B	C-5	PDA	189
9	B	C-5	PDA	190
9	B	C-5	PDA	191
9	B	C-4	PDA	192
9	B	C-5	PDA	193
9	B	C-5	PDA	194
9	F	C-5	PDA	195
9	F	C-4	PDA	196
9	F	C-5	PDA	197
9	F	C-5	PDA	198
9	F	C-4	PDA	199
9	F	C-5	PDA	200
9	F	C-5	PDA	201
9	F	C-5	PDA	202
9	F	C-5	PDA	203
9	F	C-5	PDA	204
9	F	C-6	P A	205
9	F	C-5	PDA	206
9	F	C-5	PDA	207
9	F	C-5	PDA	208
10	PR	C-4	PDA	209
10	PR	C-4	PDA	210
10	PR	C-5	PDA	211
10	PR	C-5	PDA	212
11	L	C-5	PDA	213

COLLECTION TRIP	FARM	DILUTION	MEDIUM	IDENTIFICATION ORDINAL NUMBER
11	PR	C-5	PDA	214
11	SI	C-5	PDA	215
11	LE	C-5	PDA	216
12	SI	C-5	PDA	217
12	SI	C-5	PDA	218
12	SI	C-5	PDA	219
12	SI	C-5	PDA	220
12	SI	C-5	PDA	221
13	E	C-5	PDA	222
13	E	C-5	PDA	223
13	E	C-4	PDA	224
13	E	C-5	PDA	225
13	E	C-5	PDA	226
13	E	C-5	PDA	227
13	E	C-5	PDA	228
13	E	C-5	PDA	229
13	E	C-5	PDA	230
14	PB	C-6	PDA	231
14	R	C-5	PDA	232
14	R	C-5	PDA	233
14	PB	C-5	PDA	234
14	R	C-4	PDA	235
14	PB	C-5	PDA	236
14	PB	C-5	PDA	237
14	R	C-5	PDA	238
14	R	C-5	PDA	240
14	R	C-5	PDA	241
14	LE	C-6	PDA	242
14	LE	C-6	PDA	243
14	PB	C-5	PDA	244
14	PB	C-5	PDA	245
14	PB	C-5	PDA	246
14	PB	C-6	PDA	247
14	PB	C-5	PDA	248
14	PB	C-5	PDA	249
14	PB	C-5	PDA	250
14	PB	C-5	PDA	251
14	PB	C-4	PDA	252
14	PB	C-5	PDA	253
14	PB	C-5	PDA	254
15	SJL	C-4	PDA	255
15	SJL	C-5	PDA	256
15	SJL	C-4	PDA	257
15	SJL	C-5	PDA	258
15	SJL	C-5	PDA	259
15	SJL	C-5	PDA	260
15	SJL	C-5	PDA	261
15	SJL	C-5	PDA	262
15	SJL	C-5	PDA	263
15	SJL	C-5	PDA	264
16	P	C-4	PDA	265
16	P	C-4	PDA	266
16	P	C-5	PDA	267
16	P	C-5	PDA	268

TABLE 5

ISOLATED MYCOPARASITES

COLLECTION TRIP	FARM	MEDIUM	IDENTIFICATION ORDINAL NUMBER
5	V	PDA	213
17	V	PDA	214
13	V	PDA	215
15	V	PDA	216
18	V	PDA	217
20	V	PDA	218
5	V	PDA	219
7	V	PDA	220
9	V	PDA	221
11	V	PDA	222
18	V	PDA	223
21	V	PDA	224
23	V	PDA	225
1	Pa	PDA	226
4	Ba	PDA	227
4	Ba	PDA	228
2	Pa	PDA	229
3	A	PDA	230
4	Ba	PDA	231
4	Ba	PDA	232
4	Ba	PDA	233
4	Ba	PDA	234
4	Ba	PDA	235
2	A	PDA	236
2	Pa	PDA	238
2	A	PDA	239
2	Pa	PDA	240
2	Pa	PDA	241
2	A	PDA	242
2	Pa	PDA	243
2	Pa	PDA	244
2	Pa	PDA	245
2	Pa	PDA	246
2	Pa	PDA	247
2	Pa	PDA	248
2	Pa	PDA	249
11	R	PDA	250
7	LE	PDA	251
9	Pb	PDA	252
10	SI	PDA	253
6	SI	PDA	254
6	SI	PDA	255
6	SI	PDA	256
6	SI	PDA	257
6	SI	PDA	259
8	PR	PDA	260
13	L	PDA	261
12	E	PDA	262

TABLE 5

ISOLATED MYCOPARASITES

COLLECTION TRIP	FARM	MEDIUM	IDENTIFICATION ORDINAL NUMBER
5	V	PDA	213
17	V	PDA	214
13	V	PDA	215
15	V	PDA	216
18	V	PDA	217
20	V	PDA	218
5	V	PDA	219
7	V	PDA	220
9	V	PDA	221
11	V	PDA	222
18	V	PDA	223
21	V	PDA	224
23	V	PDA	225
1	Pa	PDA	226
4	Ba	PDA	227
4	Ba	PDA	228
2	Pa	PDA	229
3	A	PDA	230
4	Ba	PDA	231
4	Ba	PDA	232
4	Ba	PDA	233
4	Ba	PDA	234
4	Ba	PDA	235
2	A	PDA	236
2	Pa	PDA	238
2	A	PDA	239
2	Pa	PDA	240
2	Pa	PDA	241
2	A	PDA	242
2	Pa	PDA	243
2	Pa	PDA	244
2	Pa	PDA	245
2	Pa	PDA	246
2	Pa	PDA	247
2	Pa	PDA	248
2	Pa	PDA	249
11	R	PDA	250
7	LE	PDA	251
9	Pb	PDA	252
10	SI	PDA	253
6	SI	PDA	254
6	SI	PDA	255
6	SI	PDA	256
6	SI	PDA	257
6	SI	PDA	259
8	PR	PDA	260
13	L	PDA	261
12	E	PDA	262

COLLECTION TRIP	FARM	MEDIUM	IDENTIFICATION ORDINAL NUMBER
11	R	PDA	263
12	SI	PDA	264
13	PA	PDA	265
13	L	PDA	266
5	F	PDA	267
9	PB	PDA	268
9	PB	PDA	269
5	SF	PDA	270
13	L	PDA	271
13	L	PDA	272
5	F	PDA	273
13	L	PDA	274
8	PR	PDA	275
5	F	PDA	276
13	L	PDA	277
13	L	PDA	278
6	SI	PDA	279
8	PR	PDA	280
19	SJL	PDA	281
19	SJL	PDA	282
19	SJL	PDA	283
19	SJL	PDA	284
19	SJL	PDA	285
19	SJL	PDA	286
19	SJL	PDA	287
18	M	PDA	288
18	M	PDA	289
16	CH	PDA	290
17	P	PDA	291
16	M	PDA	292
16	M	PDA	293
16	M	PDA	294
17	M	PDA	295
17	M	PDA	296
17	P	PDA	297
17	P	PDA	298
17	P	PDA	299
19	SJL	PDA	300
14	SJL	PDA	301
14	PA	PDA	302
15	ST	PDA	303
15	ST	PDA	304
16	CH	PDA	305
17	P	PDA	306
17	P	PDA	307
19	SJL	PDA	308

BACTERIA FROM OTHRES COUNTRIES

FROM BRAZIL

AP -3
AP -85
AP -150
AP -203
LEC -2

TABLE 6

T S M (MODIFIED)

g P/L

A. $Mg_2SO_4.7H_2O$.....0.29

B.-K_2HPO_4.....0.90

C.-KCl.....0.15

D.-NH_4NO_3.....1.00

E.-GLUCOSE.....3.00

F.-Microelements.....1 ml stock solution

from:

$FeSO_4.7H_2O$.....1.00

$MnSO_4.H_2O$.....0.065

$ZnSO_4.H_2O$.....0.90

G.- AGAR.....15.00

TABLE 7

K I N G (MODIFIED)

	g P/L
A.- ASPARAGINA	2.5
B.- BEEF EXTRACT	2.0
C.- PEPTON	5.0
D.-K_2HPO_4	1.5
E.-$MgSO_4$	1.5

TABLE 8

POLL OF ISOLATED FUNGI

GROUP A

1	77	61	125	66	129	75	36
2	67	89	84	65	96	76	106
3	121	105	79	88	95	71	85
4	102	126	108	86	120	92	83
5	104	78	100	93	112	128	119
6	80	73	115	103	63	87	70
7	68	81	72	253	90	66	4
	8	9	10	11	12	13	14

GROUP C

15	158	168	296	136	177	148
16	145	149	209	116	189	148
17	203	174	132	255	173	171
18	160	206	170	155	190	157
19	163	138	179	142	165	147
20	169	204	119	192	134	154
	21	22	23	24	25	26

GROUP D

27	211	243	270	249	229	232	261
28	230	343	224	235	189	258	207
29	140	260	227	236	265	217	241
30	255	256	234	244	219	221	266
	31	32	33	34	35	36	37

GROUP E

38	280	144	295	160	138	177	276
39	131	133	300	273	278	182	198
40	274	140	174	134	197	215	237
41	165	203	303	292	286	255	212
42	306	192	239	283	81	211	242
	43	44	45	46	47	48	49

GROUP H

50	31	9	43	14	1	23	49
51	7	18	38	26	10	13	39
52	15	41	8	33	30	5	20
53	45	6	32	12	29	4	16
54	19	44	11	37	3	34	28
55	27	47	35	2	50	25	49
56	40	21	42	17	25	46	22
	57	58	59	60	61	62	63

GROUP I

64	55	123	74	143	107	159	164
65	101	156	139	64	54	122	111
66	51	114	65	141	99	155	161
67	91	150	135	59	117	52	109
68	124	56	97	151	153	98	82
69	53	118	127	58	130	152	94
70	146	57	60	137	110	162	113
	71	72	73	74	75	76	77

GROUP J

78	289	272	263	304	294	281
79	264	282	291	299	98	269
80	262	298	284	271	301	287
81	286	268	305	267	293	275
82	279	302	297	285	290	277
	83	84	85	86	87	88

GROUP K

89	167	186	233	201	253	187	238
90	214	172	216	188	240	256	205
91	185	231	176	220	254	202	213
92	245	180	223	248	195	259	210
93	191	257	208	178	222	196	250
94	228	184	175	218	200	252	181
95	194	247	199	251	226	183	225
	96	97	98	99	100	101	102

TABLE 9

POOL OF ISOLATED BACTERIA

GROUP B

1	257	233	182	269	104	293	219
2	270	258	249	256	259	200	195
3	288	296	241	185	238	239	262
4	129	78	131	197	157	130	286
5	144	198	243	292	273	201	305
6	207	230	261	115	169	242	260
7	235	247	297	298	246	199	237
	8	9	10	11	.2	13	14

GROUP E

15	298	267	287	252	229	147	176
16	72P	216	72	236	303	304	251
17	290	225	267	174	146	301	251
18	244	255	245	271	213	284	268
	19	20	21	22	23	24	25

GROUP F

26	294	120	302	279
27	275	299	208	222
28	218	221	178	219
	29	30	31	32

GROUP G

33	1	36	38	19	20	13	25
34	32	5	105	41	64	111	8
35	141	107	59	75	88	77	87
36	45	80	97	116	128	136	148
37	155	110	53	56	81	49	119
38	62	122	132	65	153	85	68
39	134	125	152	138	145	94	90
	40	41	42	43	44	45	46

GROUP H

47	180	263	2	60	101	149	196
48	23	73	240	139	28	51	93
49	10	277	171	40	69	162	285
50	55	86	154	18	203	121	7
51	12	300	108	[illegible]	79	15	234
52	22	67	189	226	46	133	37
53	50	112	42	127	219	76	253
	54	55	56	57	58	59	60

GROUP I

61	232	54	164	159	66	190	113
62	4	170	43	193	220	17	156
63	99	248	103	26	84	142	272
64	307	167	95	14	123	202	71
65	16	181	58	177	3	47	118
66	114	35	188	11	210	291	21
67	29	98	172	133	283	93	264
	68	69	70	71	72	73	74

GROUP J

75	209	143	33	52	160	82	254
76	44	213	206	140	12	124	70
77	89	265	175	274	100	184	48
78	166	74	39	211	96	183	281
79	186	109	191	214	157	30	137
80	31	106	282	150	27	135	204
81	266	91	163	65	224	158	278
	82	83	84	85	86	87	88

GROUP K

89	161	63	24	126	228
90	250	165	61	223	151
91	205	92	280	187	102
92	117	308	173	227	169
93	168	194	215	295	192
	94	95	96	97	98

TABLE 10

ISOLATED MICROORGANISM SCREENING RESULTS FOR BACTERIA

POOL	RUST IMPLANTATION EFFEC-TIVITY %	RUST IMPLANTATION ANTAGONIC EFFECT	SPORULATION INHIBITION EFFECT-TIVITY %	SPORULATION INHIBITION ANTAGONIC EFFECT	EFFECT OVER FULL DEVELOPED LESIONS EFFEC TIVITY %	EFFECT OVER FULL DEVELOPED LESIONS ANTAGONIC EFFECT
1	10	N	0	N	0	N
2	0	N	70	I	44	N
3	0	N	10	N	50	N
4	10	N	0	N	30	N
5	0	N	86	P	44	N
6	14	N	20	N	25	N
7	0	N	0	N	60	I
8	10	N	40	N	50	N
9	0	N	64	I	43	N
10	0	N	0	N	50	N
11	20	N	10	N	50	N
12	0	N	82	P	70	I
13	10	N	20	N	60	I
14	20	N	0	N	44	N
15	20	N	25	N	55	N
16	0	N	0	N	55	N
17	10	N	25	N	0	N
18	0	N	20	N	40	N
19	0	N	14	N	46	N
20	40	N	44	N	33	N
21	0	N	0	N	64	I
22	0	N	0	N	75	I
23	0	N	12	N	50	N
24	20	N	0	N	50	N
25	0	N	0	N	58	N
26	22	N	68	I	30	N
27	12	N	22	N	12	N
28	62	I	10	N	40	N
29	66	I	20	N	58	N
30	10	N	11	N	10	N
31	57	N	65	N	0	N
32	10	N	40	N	20	N
33	10	N	50	N	38	N
34	0	N	67	I	12	N
35	0	N	0	N	14	N
36	40	N	40	N	0	N
37	0	N	25	N	40	N
38	25	N	0	N	0	N
39	30	N	50	N	0	N
40	0	N	50	N	0	N
41	10	N	67	I	12	N
42	50	N	50	N	14	N
43	20	N	0	N	38	N

	RUST IMPLANTATION		SPORULATION INHIBITION		EFFECT OVER FULL DEVELOPED LESIONS	
POLL	EFFEC TIVITY %	ANTAGONIC EFFECT	EFFEC TIVITY %	ANTAGONIC EFFECT	EFFEC TIVITY %	ANTAGONIC EFFECT
44	40	N	0	N	0	N
45	0	N	10	N	0	N
46	58	N	50	N	25	N
47	0	N	67	I	50	N
48	33	N	25	N	0	N
49	20	N	0	N	0	N
50	10	N	67	I	50	N
51	10	N	33	N	0	N
52	0	N	75	I	28	N
53	38	N	40	N	25	N
54	0	N	75	I	20	N
55	28	N	40	N	0	N
56	50	N	67	I	25	N
57	0	N	0	N	0	N
58	10	N	10	N	50	N
59	40	N	50	N	0	N
60	28	N	67	I	50	N
61	33	N	20	N	0	N
62	10	N	25	N	38	N
63	40	N	50	N	50	N
64	28	N	10	N	25	N
65	0	N	75	I	0	N
66	0	N	75	I	71	I
67	30	N	75	I	25	N
68	50	N	75	I	25	N
69	14	N	75	I	71	I
70	25	N	25	N	20	N
71	0	N	40	N	0	N
72	0	N	75	I	0	N
73	33	N	10	N	33	N
74	40	N	0	N	38	N
75	0	N	25	N	33	N
76	40	N	0	N	25	N
77	0	N	33	N	0	N
78	10	N	40	N	25	N
79	40	N	25	N	25	N
80	25	N	0	N	38	N
81	50	N	50	N	0	N
82	33	N	0	N	33	N
83	0	N	0	N	0	N
84	0	N	33	N	25	N
85	10	N	0	N	50	N
86	28	N	0	N	25	N
87	33	N	40	N	25	N
88	0	N	20	N	0	N
89	40	N	50	N	0	N
90	0	N	25	N	0	N
91	50	N	83	P	20	N

	RUST IMPLANTATION		SPORULATION INHIBITION		EFFECT FULL DEVELOPED LESIONS	
POOL	EFFEC TIVITY %	ANTAGONIC EFFECT	EFFEC TIVITY %	ANTAGONIC EFFECT	EFFEC TIVITY %	ANTAGONIC EFFECT
93	20	N	33	N	0	N
94	0	N	40	N	25	N
95	0	N	25	N	25	N
96	10	N	0	N	38	N
97	0	N	50	N	0	N
98	30	N	80	P	33	N

TABLE 11
EVALUATION OF ANTAGONIC ACTIVITY FOR FUNGI

POOL	RUST IMPLANTATION EFFECTIVITY %	RUST IMPLANTATION ANTAGONIC EFFECT	SPORULATION INHIBITION EFFECTIVITY %	SPORULATION INHIBITION ANTAGONIC EFFECT	EFFECT OVER FULL DEVELOPED LESIONS EFFECTIVITY %	EFFECT OVER FULL DEVELOPED LESIONS ANTAGONIC EFFECT
1	57	N	38	N	54	N
2	50	N	50	N	60	I
3	62	I	14	N	54	N
4	50	N	0	N	84	P
5	66	I	60	I	67	I
6	60	I	44	N	50	N
7	50	N	28	N	50	N
8	50	N	50	N	90	P
9	55	N	12	N	75	I
10	40	N	0	N	76	I
11	44	N	62	I	67	I
12	56	N	25	N	71	I
13	50	N	55	N	75	I
14	71	I	40	N	50	N
15	14	N	0	N	10	N
16	0	N	28	N	0	N
17	0	N	60	I	75	I
18	12	N	20	N	7	N
19	0	N	33	N	50	N
20	12	N	0	N	10	N
21	10	N	0	N	25	N
22	0	N	0	N	36	N
23	12	N	67	I	42	N
24	12	N	0	N	43	N
25	10	N	33	N	45	N
26	0	N	11	N	44	N
27	28	N	75	I	40	N
28	50	N	50	N	70	I
29	14	N	50	N	61	I
30	25	N	25	N	82	P
31	11	N	50	N	30	N
32	0	N	50	N	30	N
33	28	N	0	N	75	I
34	0	N	50	N	50	N
35	0	N	60	I	83	P
36	12	N	25	N	70	I
37	50	N	75	I	65	I
38	20	N	43	N	50	N
39	12	N	0	N	80	P
40	44	N	22	N	50	N
41	28	N	0	N	50	P
42	62	I	12	N	50	N
43	40	N	0	N	55	N
44	10	N	40	N	55	N
45	0	N	20	N	55	N
46	24	N	40	N	80	P
47	18	N	22	N	55	N
48	70	I	30	N	50	N

POOL	RUST IMPLANTATION EFFECTIVITY %	RUST IMPLANTATION ANTAGONIC EFFECT	SPORULATION INHIBITION EFFECTIVITY %	SPORULATION INHIBITION ANTAGONIC EFFECT	EFFECT OVER FULL DEVELOPED LESIONS EFFECTIVITY %	EFFECT OVER FULL DEVELOPED LESIONS ANTAGONIC EFFECT
49	48	N	0	N	75	I
50	0	N	60	I	60	I
51	0	N	33	N	0	N
52	33	N	60	I	67	I
53	0	N	50	N	50	N
54	25	N	80	P	25	N
55	0	N	0	N	33	N
56	0	N	0	N	0	N
57	0	N	25	N	0	N
58	33	N	67	I	75	I
59	25	N	50	N	60	I
60	0	N	90	P	50	N
61	50	N	60	I	67	I
62	33	N	50	N	75	I
63	33	N	0	N	0	N
64	0	N	40	N	50	N
65	25	N	33	N	0	N
66	50	N	50	N	0	N
67	0	N	0	N	25	N
68	0	N	25	N	0	N
69	10	N	0	N	33	N
70	0	N	40	N	0	N
71	20	N	33	N	0	N
72	10	N	33	N	0	N
73	0	N	50	N	0	N
74	0	N	25	N	33	N
75	40	N	0	N	0	N
76	0	N	4	N	25	N
77	50	N	25	N	0	N
78	25	N	50	N	0	N
79	0	N	33	N	50	N
80	10	N	0	N	85	P
81	0	N	0	N	33	N
82	40	N	25	N	25	N
83	10	N	25	N	0	N
84	0	N	0	N	80	P
85	33	N	40	N	25	N
86	6	N	0	N	0	N
87	0	N	33	N	33	N
88	30	N	40	N	0	N
89	20	N	50	N	0	N
91	0	N	0	N	0	N
91	25	N	25	N	50	N
92	0	N	0	N	33	N
92	0	N	0	N	0	N
94	10	N	33	N	25	N
95	0	N	50	N	0	N
96	30	N	0	N	0	N
97	0	N	25	N	33	N

	RUST IMPLANTATION		SPORULATION INHIBITION		EFFECTO OVER FULL DEVELOPED LESIONS	
POOL	EFFEC TIVITY %	ANTAGONIC EFFECT	EFFEC TIVITY %	ANTAGONIC EFFECT	EFFEC TIVITY %	ANTAGONIC EFFECT
98	25	N	0	N	0	N
99	0	N	40	N	25	N
100	33	N	50	N	0	N
101	40	N	0	N	0	N
102	0	N	33	N	0	N

TABLE 12

MICROORGANISM FROM OTHERS COUNTRIES

FUNGI FROM COLOMBIA

	EFFECT OVER FULL DEVELOPED LESIONS	
	EFECTIVITY %	ANTAGONIC EFFECT
5- PSHI	40	N
E- PSHI	25	N
16- PSHI	25	N
B- PSHI	25	N
2- PSHI	50	N
9- PSHI	50	N
D- PSHI	37	N
4- PSHI	37	N
C- PSHI	25	N
A- PSHI	62	N
F- PSHI	37	N
13- PSHI	25	N

MICROORGANISM FROM BRAZIL

CNPDA-AP-3	25	N
CNPDA-AP-85	25	N
CNPDA-AP-150	25	N
CNPDA AP-203	25	N
CNPDA-C-293	25	N
CNPDA-C-294	25	N

MICROORGANISM FROM COSTA RICA

ICRRA	37	N
ICRRA	37	N

TABLE 13

IDENTIFICATION OF ANTAGONISTS

MICROORGANISM	ISOLATION	IDENTIFICATION	EVALUATION OF ANTAGONIC ACTIVITY	
			INHIBITION OF SPORULATION	FULL DEVELOPED LESIONS
Bacteria	4-V-A-4,CH-102	Pseudomonas sp	Pool 91=83% Pool 98=80%	
Fungus	2-R-B-3,PDA-37	Cladosporium sp	Pool 54=80% Pool [illegible]	
Fungus	6-BUA-C-5,MRB-102	Trichoderma like verticillium		Pool 4=84% Pool 8=90%
Fungus	12-ST-C-5,PDA-219	Verticillium leptobactrum		Pool 30=82% Pool 35=83%
Fungus	16-P-C-5,PDA-273	Paecilomyces lilacinus		Pool 39=80% Pool 46=80%
Fungus	20-CH-C-5,PDA-298	Aphanocladium aeliolae		Pool 80=85% Pool 84=80%

TABLE 14

MEDIUM USING FOR GROWTH CURVES

	g/l
GLUCOSE.....	2.00
AMONIUM SULPHATE.....	1.00
yeast....1 g	
peptone..4 g	
DIPOTASIUM PHOSPHATE.....	1.00
SALT SOLUTION A.....	10 ml
MGSO4.7H2O.....25.00 g	
FESO4.7H2O.....2.8 g	
MGSO4.H2O.....1.70 g	
NACL .....0.60 g	
CACL2.2H2O.....0.10 g	
NAMO.2H2O.....0.10 g	
ZNSO4.7H2O.....0.06 g	
HCL 0.1 M.....1.00 l	

TABLE 15

MEDIUM FOR ENZIMATIC ACTIVITY ASSAY

A. CARBON SOURCE

LAMINARIN
CELULOSE
QUITINE
GLUCOSE

B.-YEAST.....1g/lt

C.-PEPTONE.....4g/lt

D.-K2HPO4.....1g/lt

E.-SAL SOLUTION A.....0.10 mlOF A STOCK SOLUTION

PREPARED AS FOLLOWS:

MGSO4.7H2O.....0.2 g/lt

FESO4.7H2O.....2.8 g/lt

MNSO4.H2O.....0.1 g/lt

NAMOO.2H2O.....0.1 g/lt

CACL2.....0.03g/lt

ZNSO4.7H2O.....0.03g/lt

HCLO.1 M.....50 ml

TABLE 16

ENZIMATIC ACTIVITY OF ANTAGONIST

EXPRESSED IN uMOLES GLUCOSE/mg PROTEIN

	LAMINARASE	CELULASE	CHITINASE
PAECILOMYCES LILACINUS	1.45	0.26	0.00
TRICHODERMA LIKE VERTI-CILLIUM	0.53	0.06	0.00
APHANOCLADIUM LILACINUS	0.05	0.01	0.00
VERTICILLIUM LEPTOBACTRUM	0.00	0.00	0.00

TABLE 17

EFFECT OF GERMINATION BY ANTAGONIST OF COFFEE RUST

MICROORGANISM	GERMINATION %
CONTROL	5
VERTICILLIUM LEPTOBACTRUM	3.50
APHANOCLADIUM MELIOLAE	3.50
TRICHODERMA LIKE VERTICILLIUM	2.00
PAECILOMYCES LILACINUS	4.50
CLADOSPORIUM SP	3.50
PSEUDOMONA SP	2.22

FIGURES DESCRIPTION

VIRULENCE OF THE FOUR MYCOPARASITES FUNGI OVER COFFEE RUST

(FIGURE 1-4)

ANTAGONIST GROWTH CURVES

(FIGURE 5-8)

EFFECT OF *Hemileiae vastatrix* ON CHLOROPHYLL CONTENT OF LEAVES

(FIGURE 9)

ANTAGONIST GREENHOUSE EXPERIMENTS

(FIGURE 10-13)

ANTAGONIST FIELD EXPERIMENTS

(FIGURE 14-17)

FIGURE 1 TRICHODERMA likeVERTICILLIUM

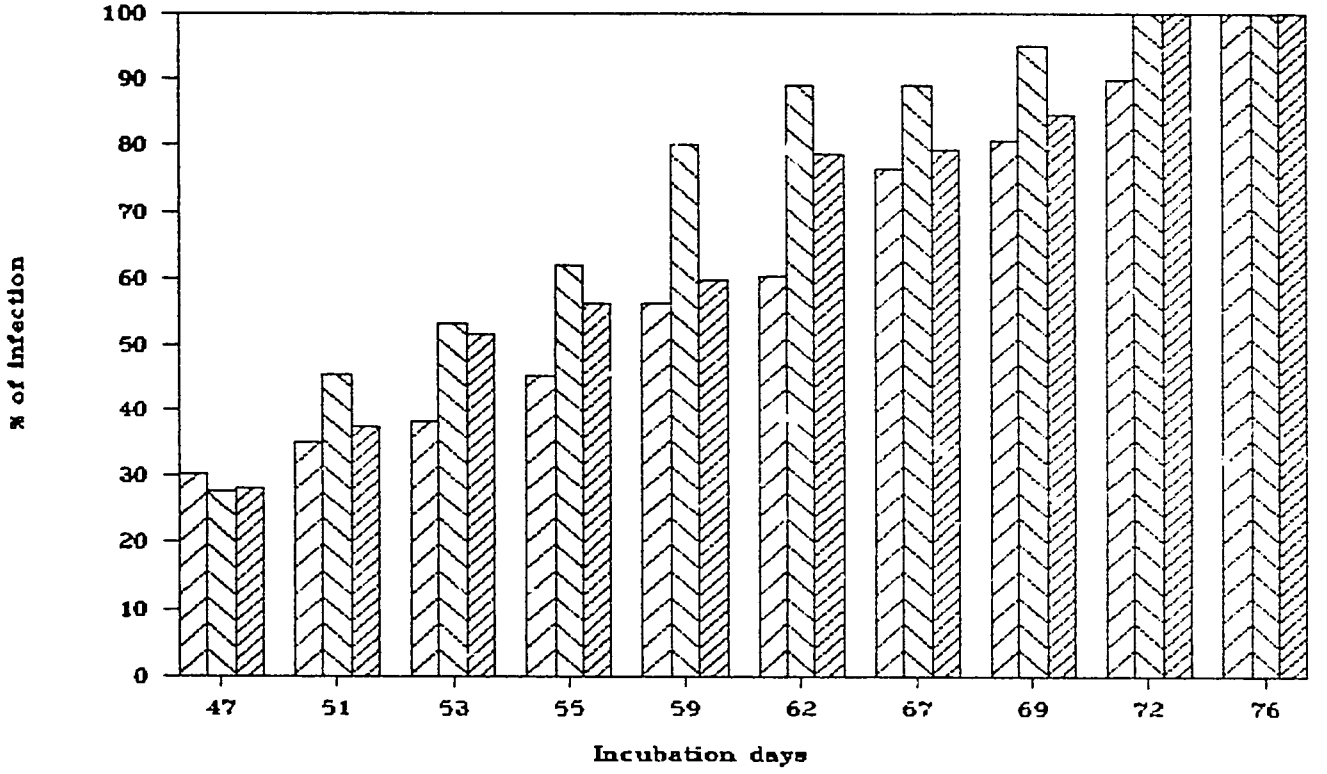


FIGURE 2 APHANOCLADIUM MELIOLAE

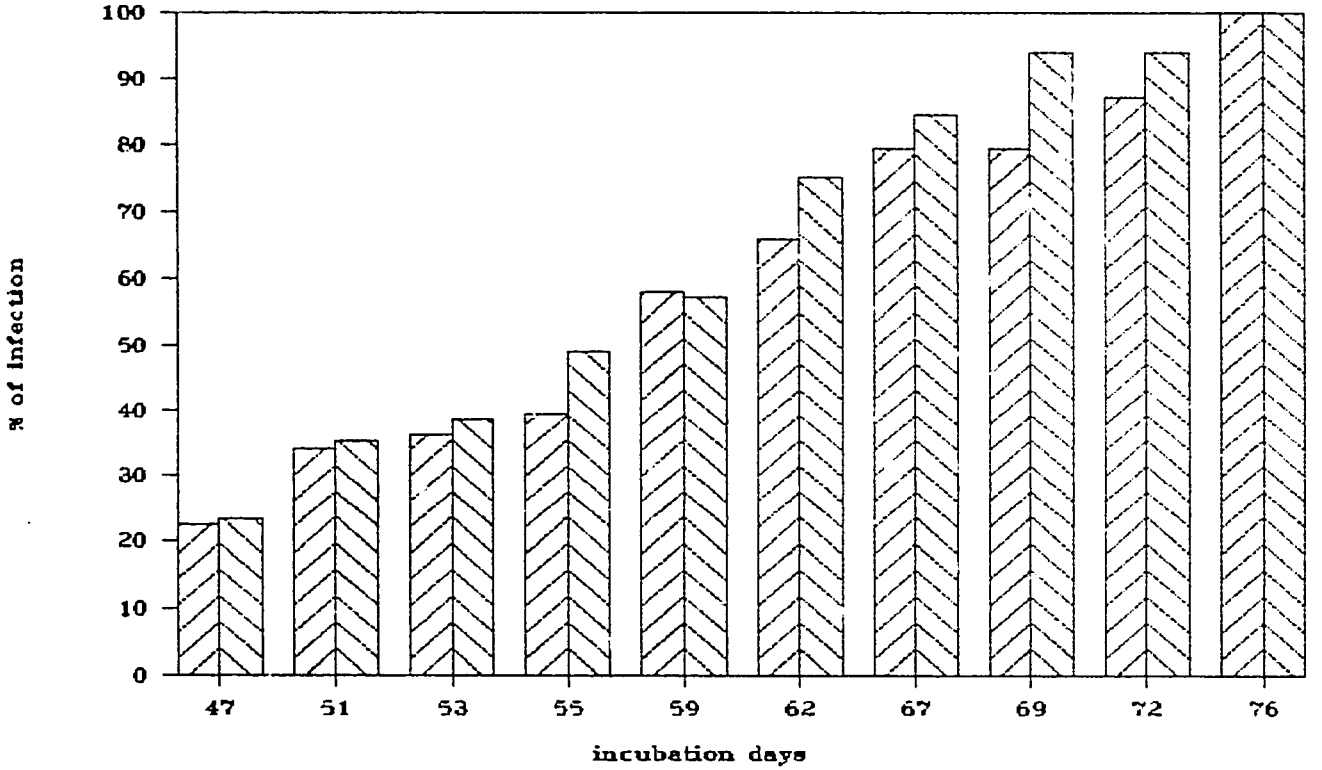


FIGURE 3 PAECILOMYCES LILACINUS

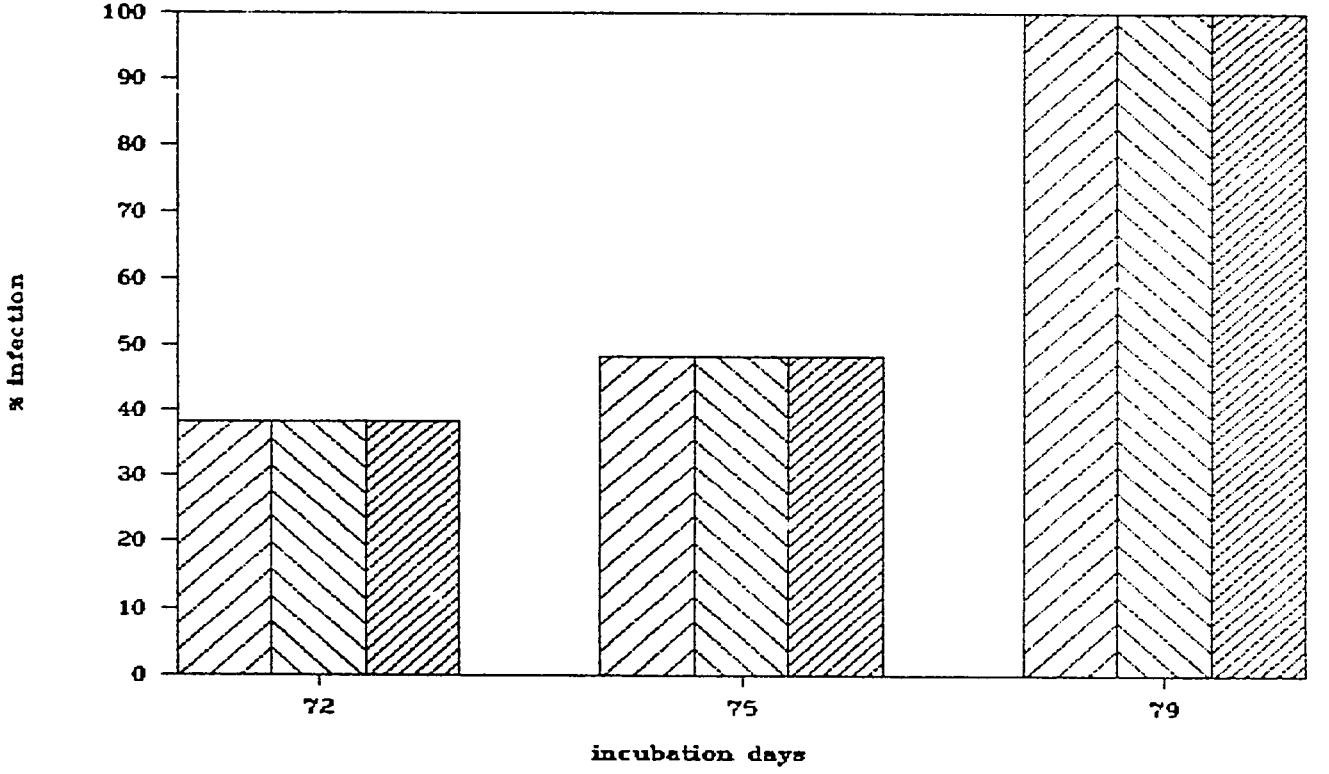


FIGURE 4 VERTICILLIUM LEPTOBACTRUM

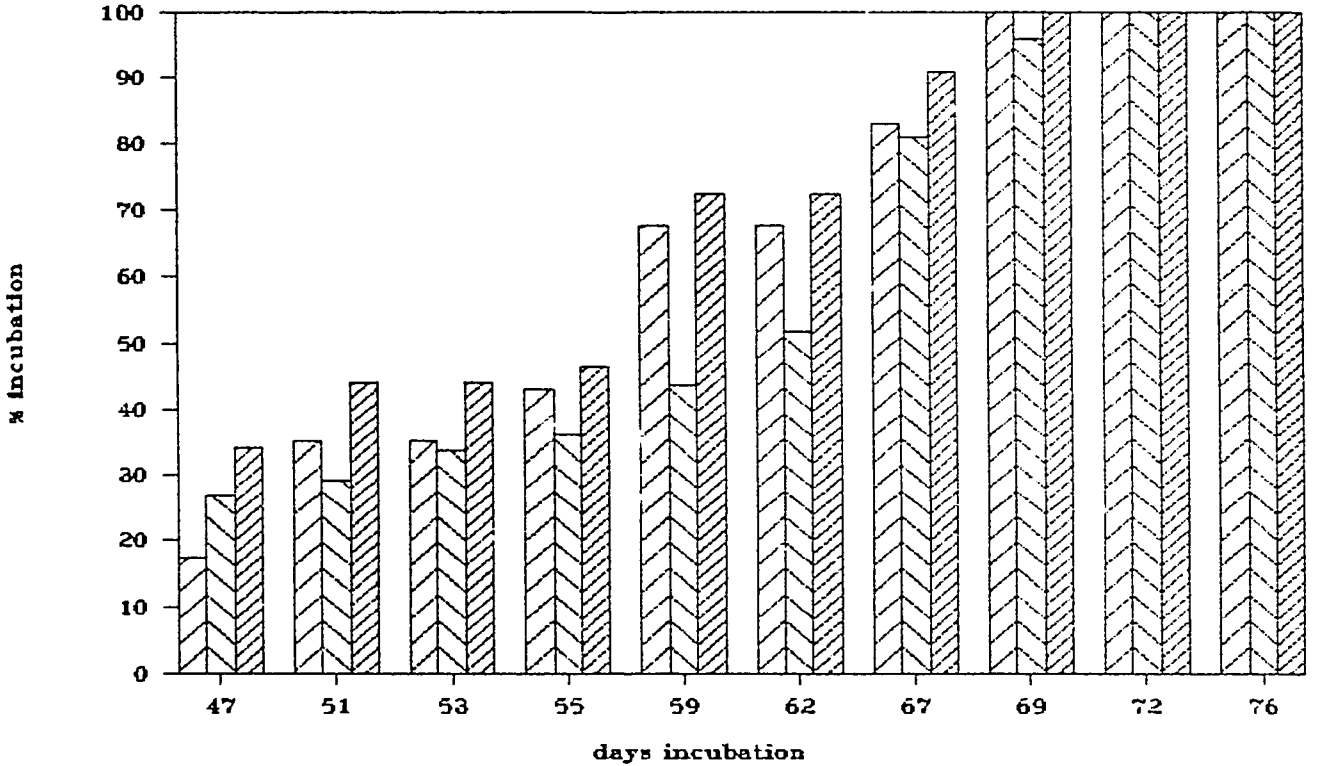


FIGURE 5

Verticillium leptobactrum

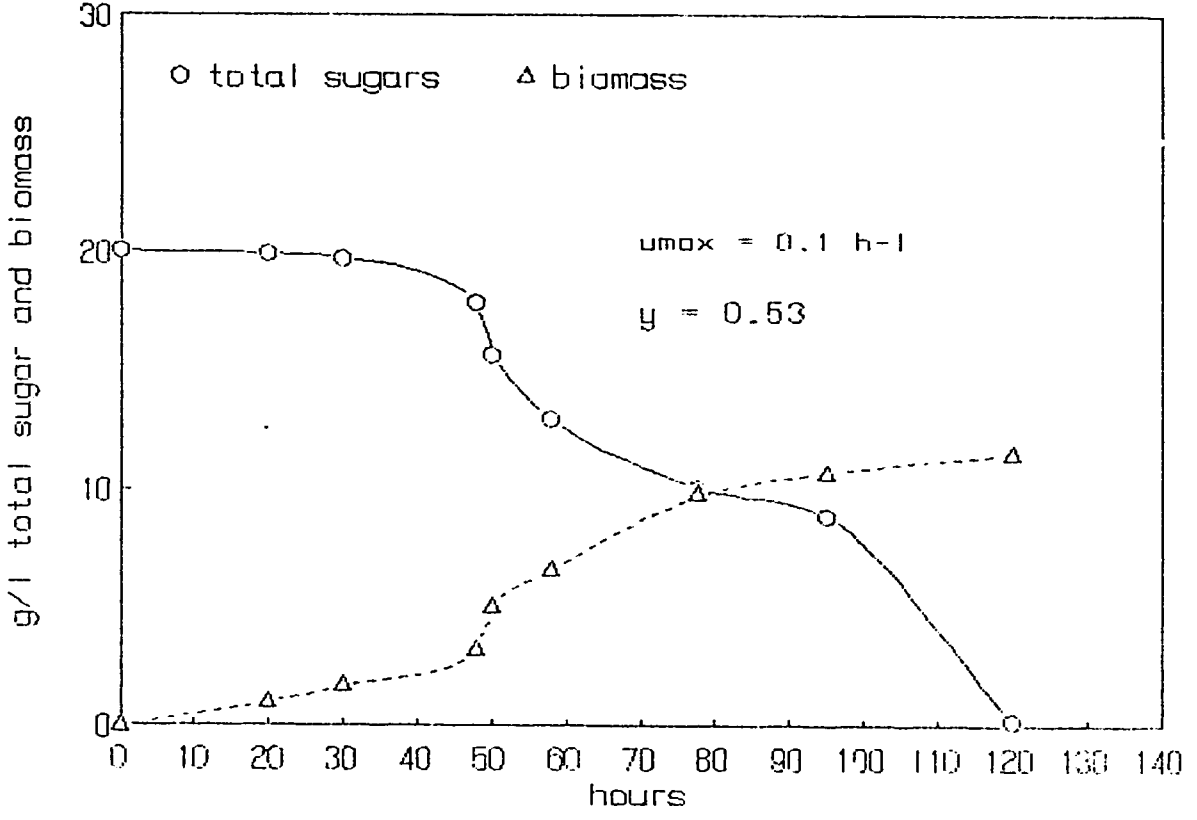


FIGURE 6

Phaecilomyces lilacinus

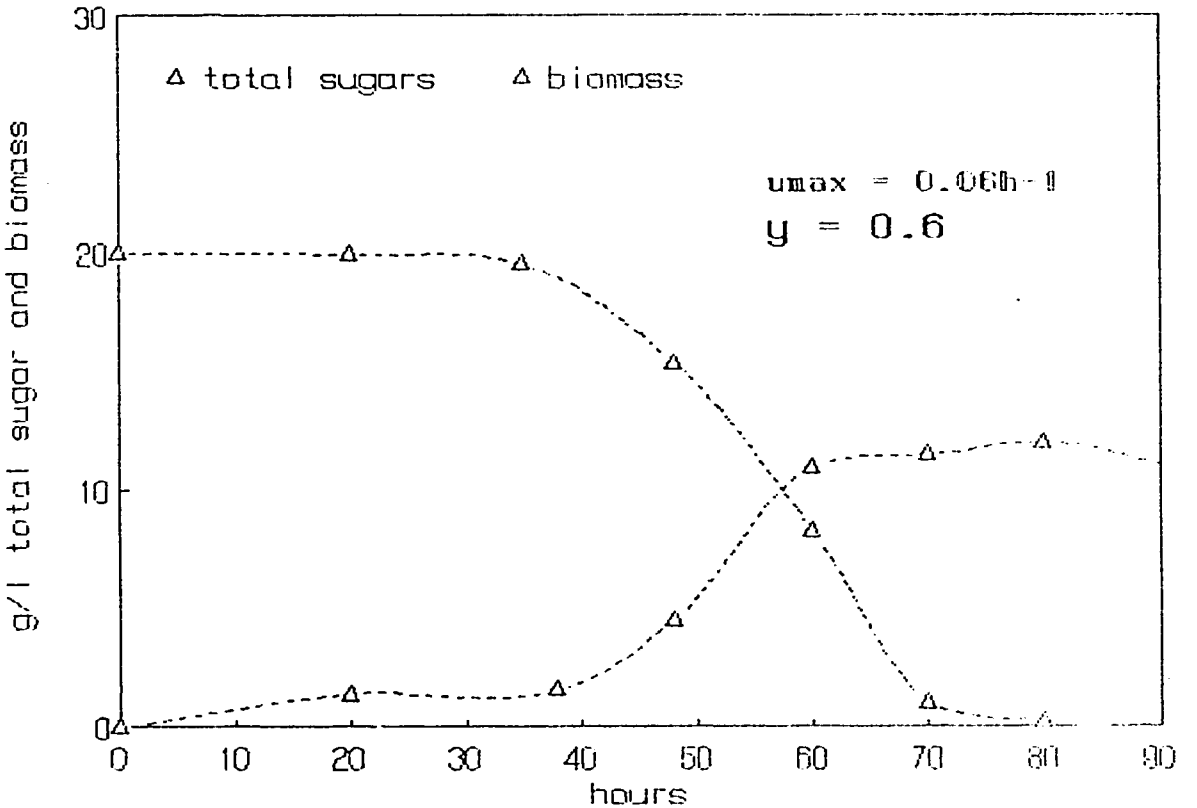


FIGURE 7

Trichoderma like verticillium

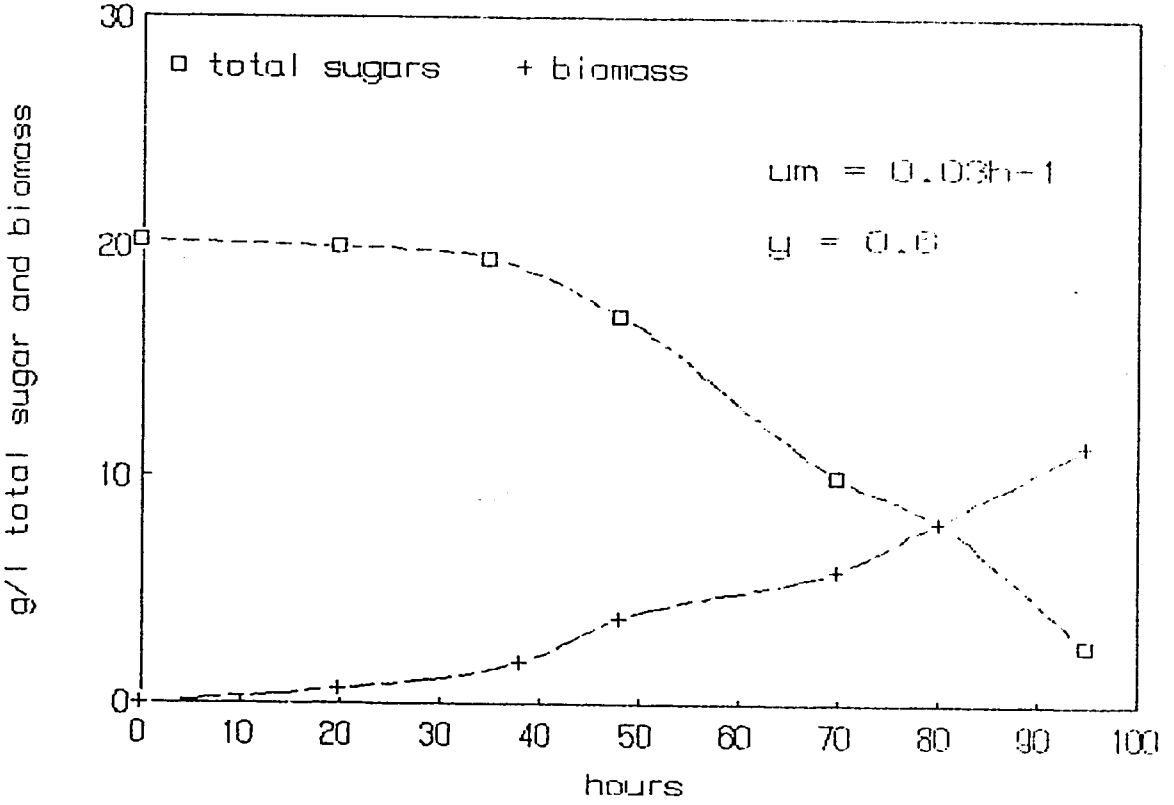


FIGURE 8

Aphanocladium meliolae

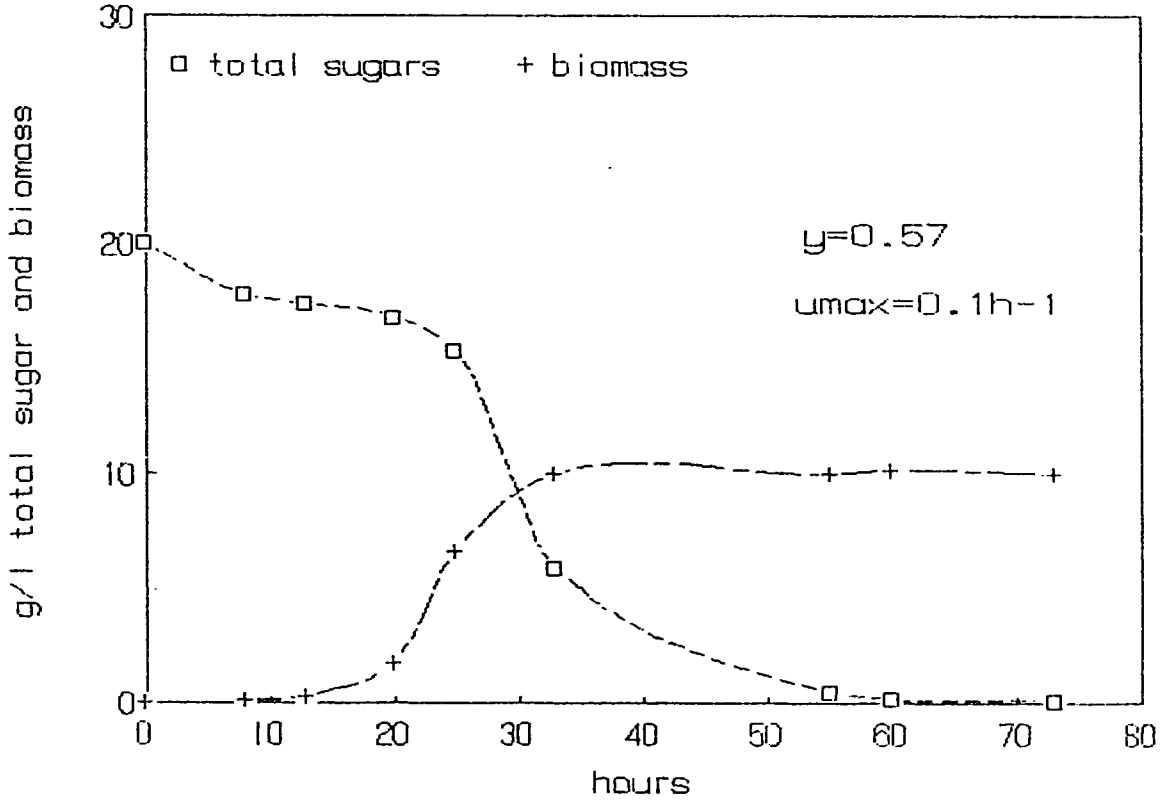


FIGURE 9
CHLOROPHYLL

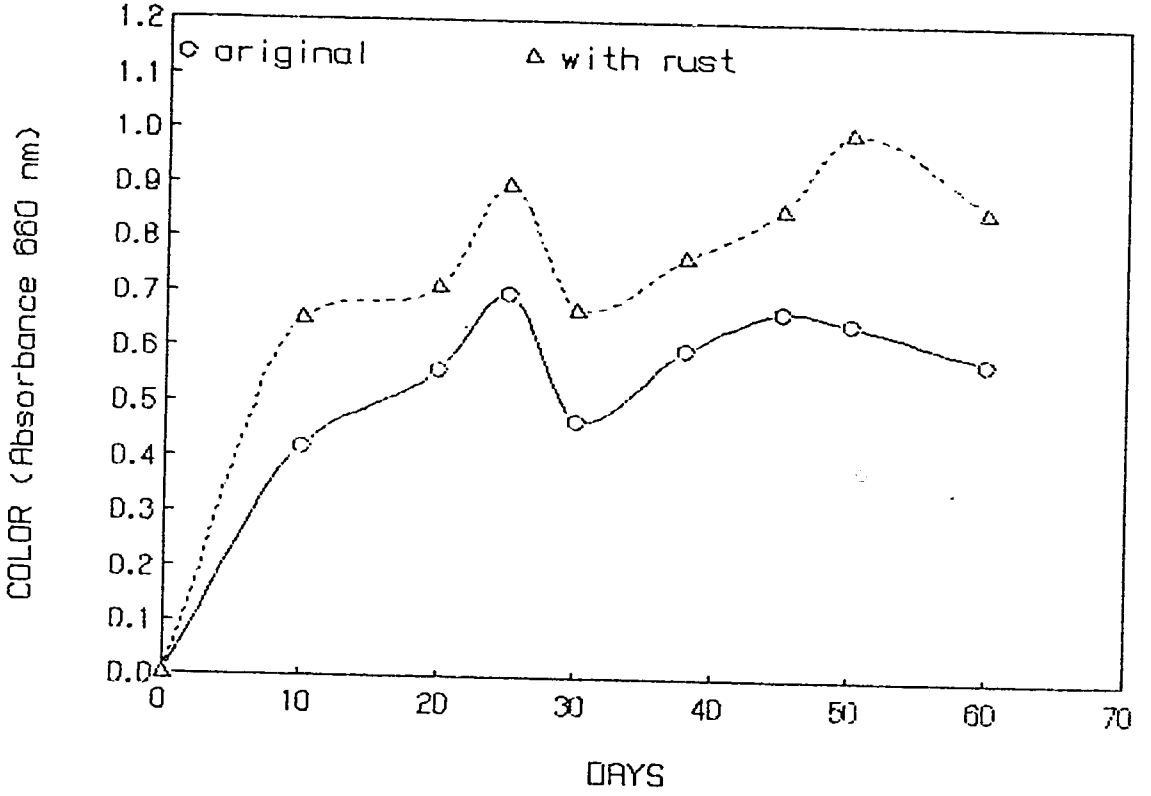


FIGURE 10 Phaecilomyces lilacinus

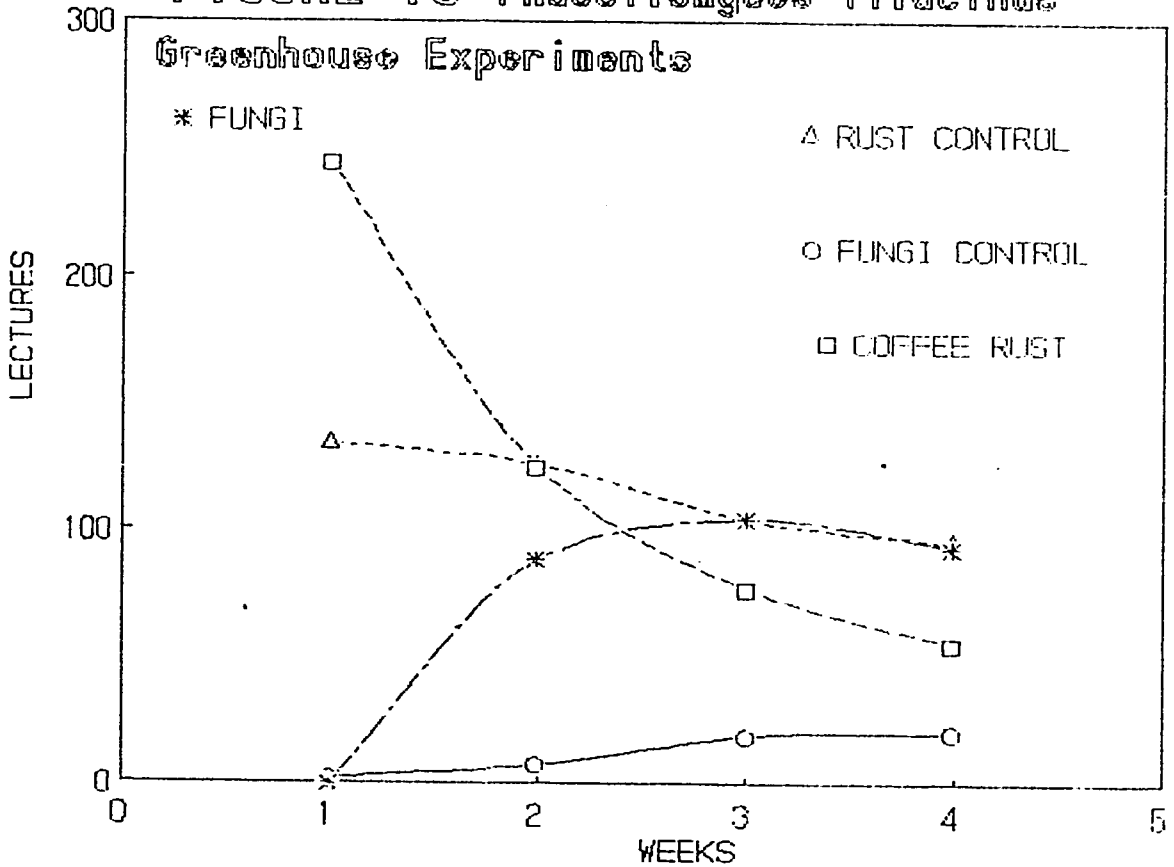


FIGURE 11 Trichoderma like verticillium

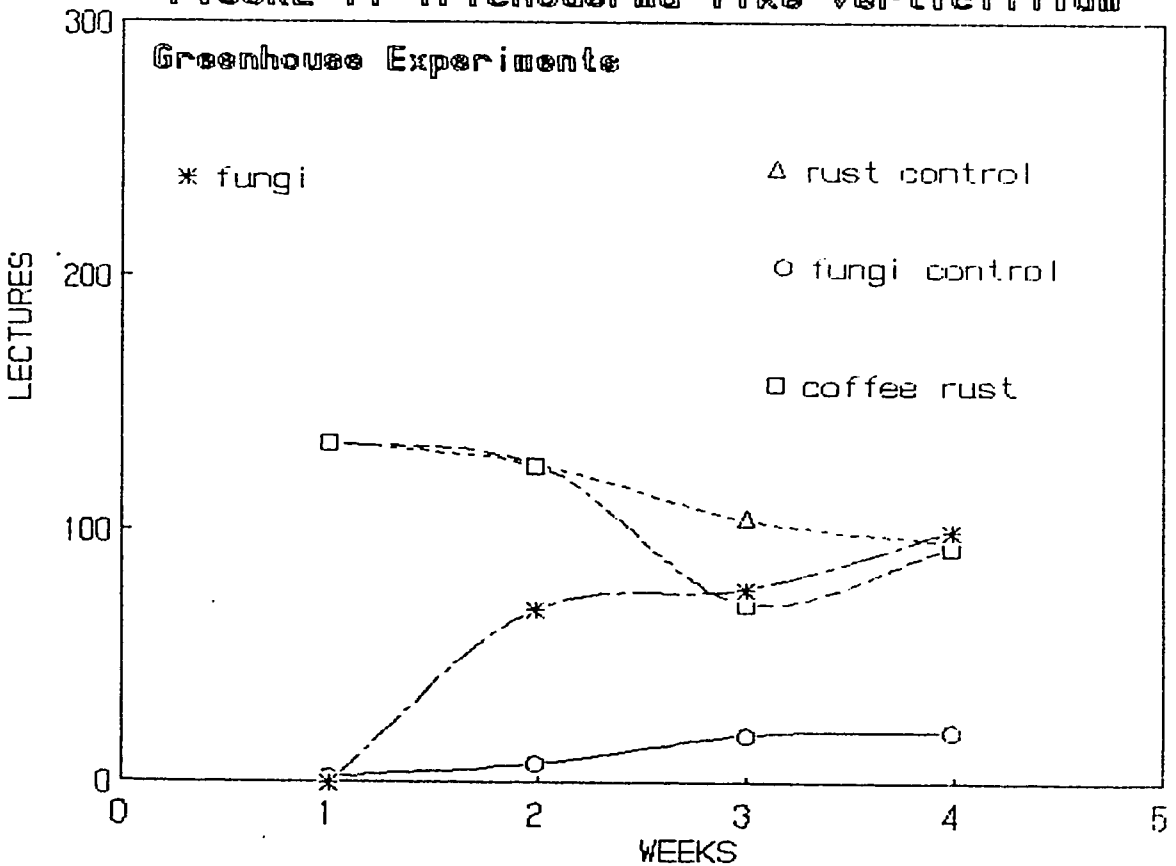


FIGURE 12 Verticillium leptobactrum

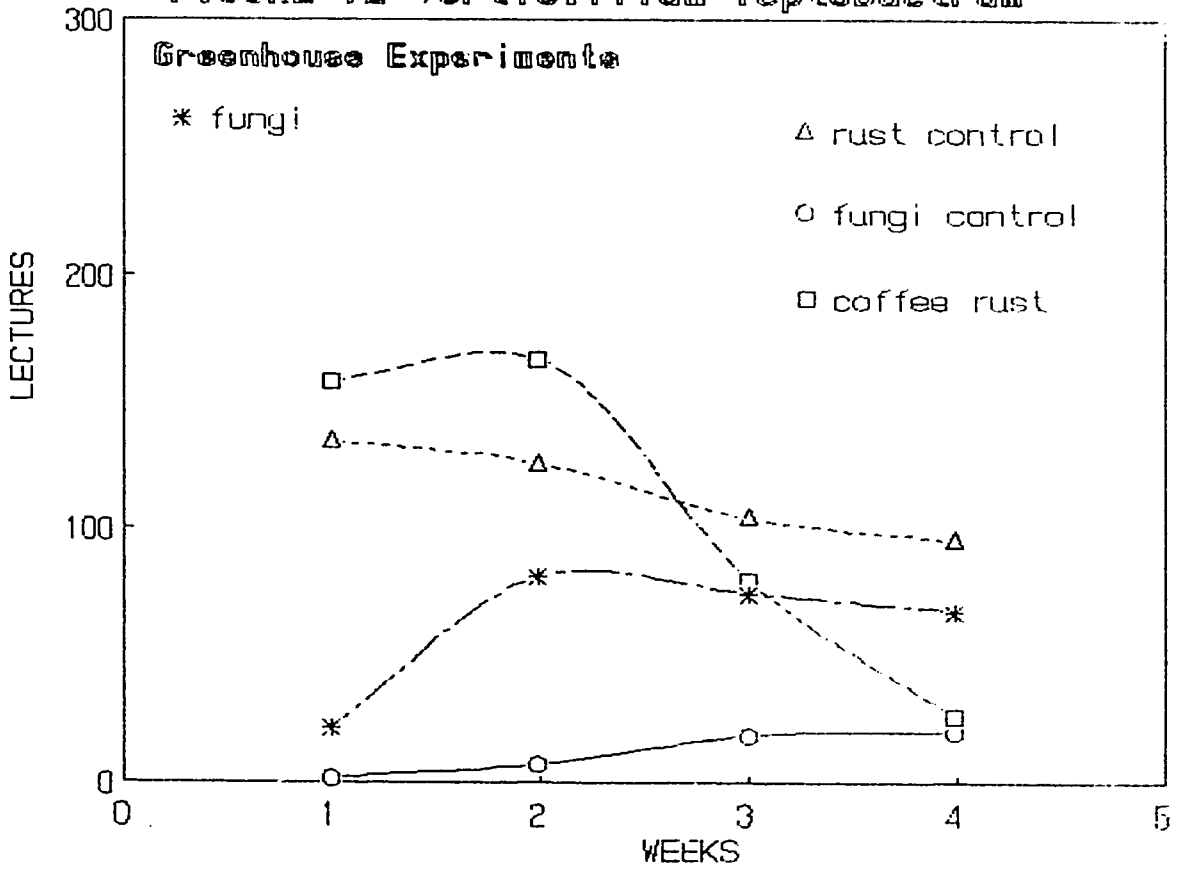


FIGURE 13 Aphanocladium meliolae

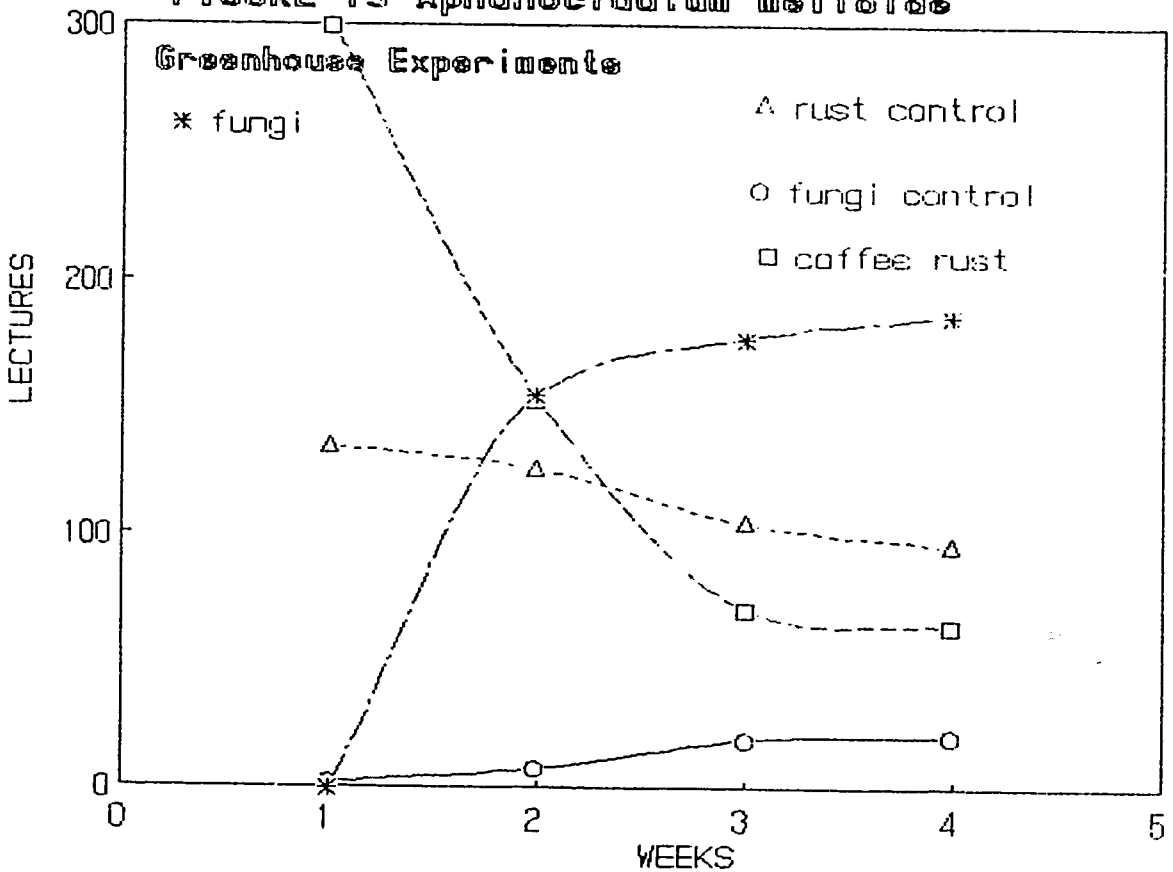


FIGURE 14 Phaecilomyces lilacinus

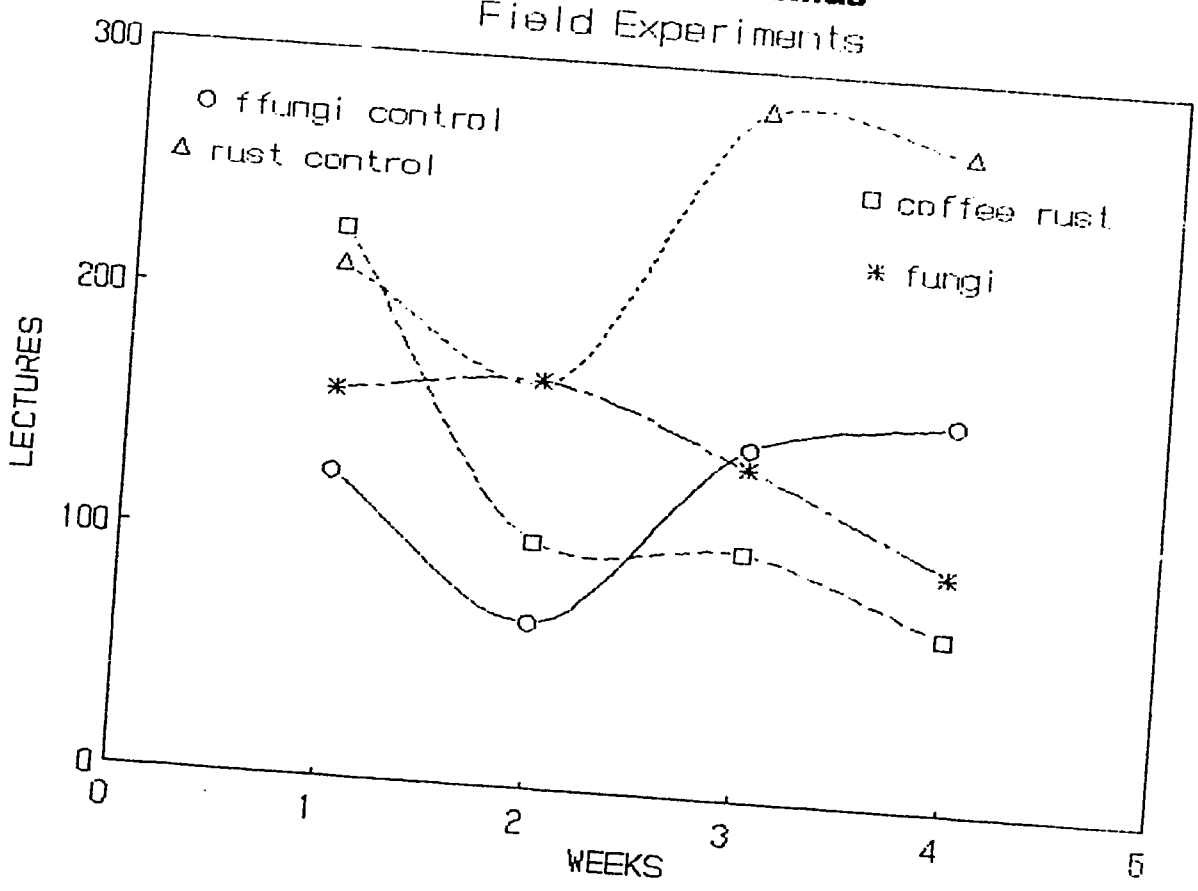


FIGURE 15 Aphanocladium meliolae

Field Experiments

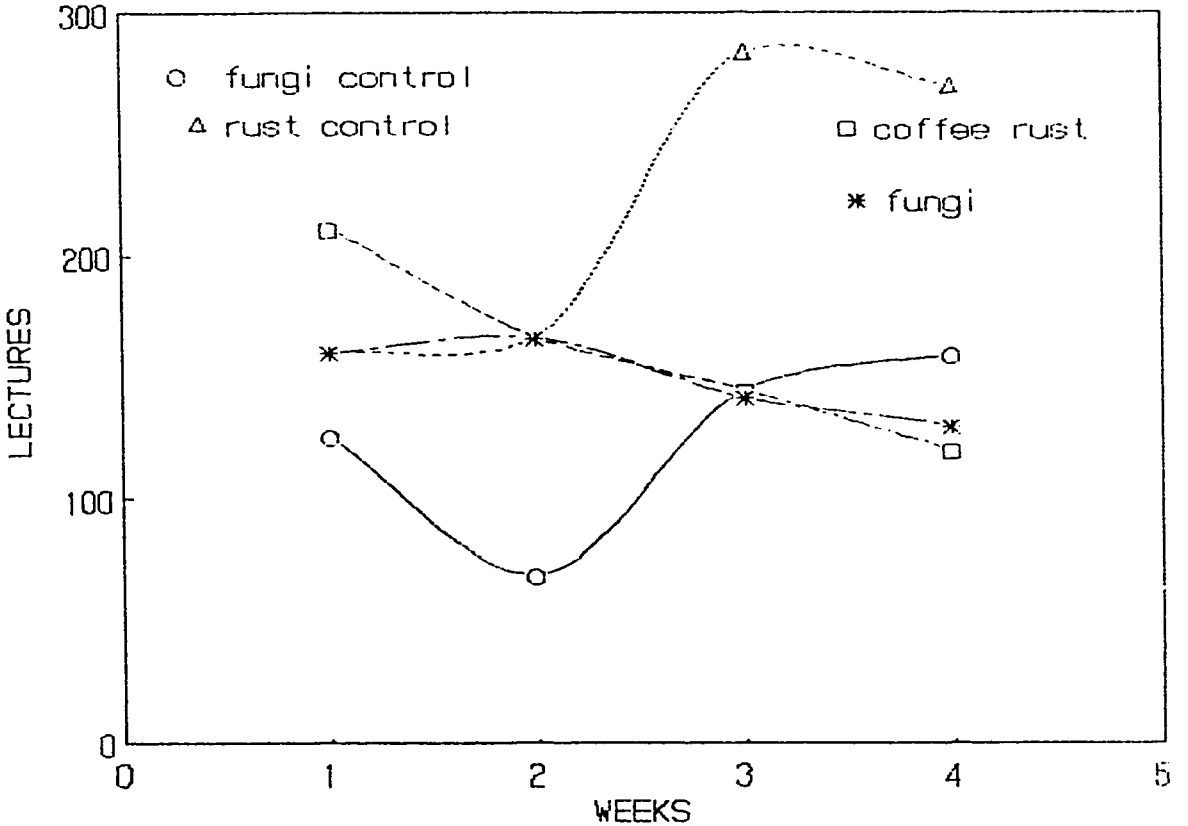


FIGURE 16 Verticillium leptobactrum

Field Experiments

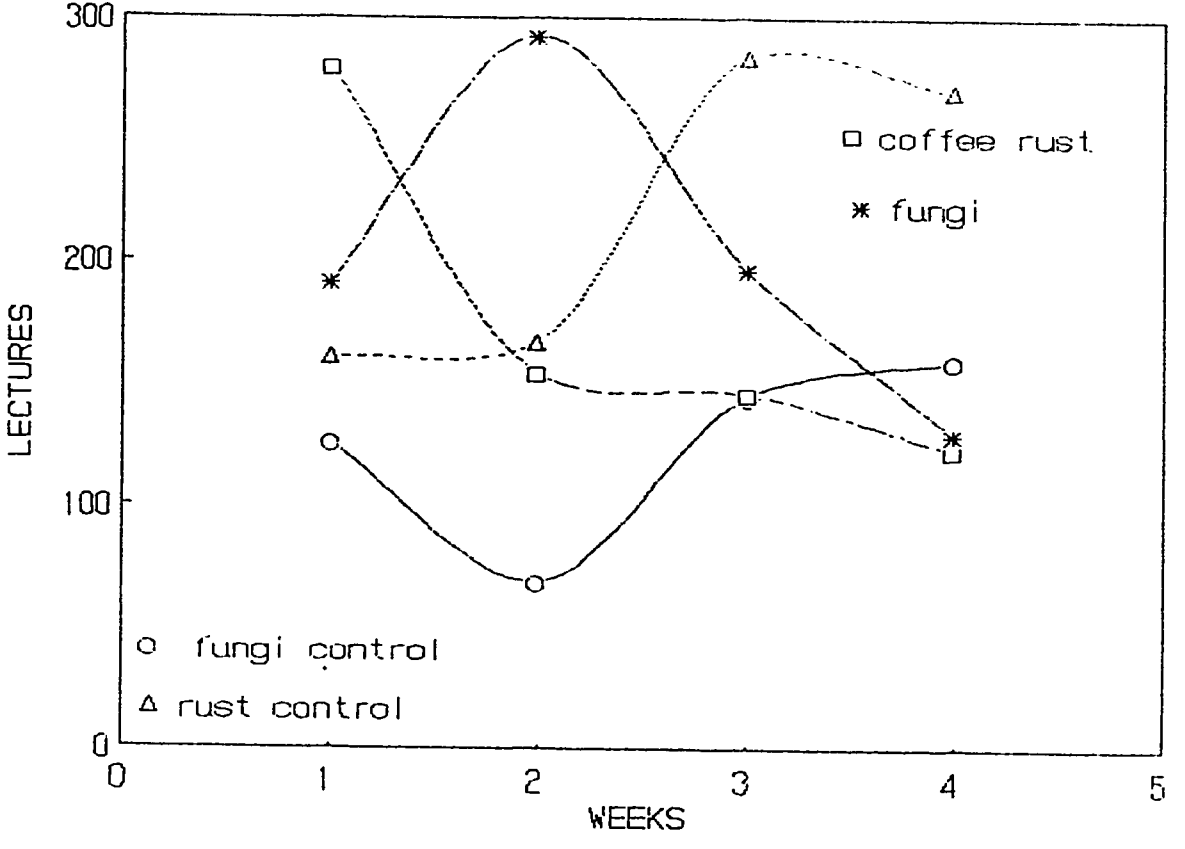
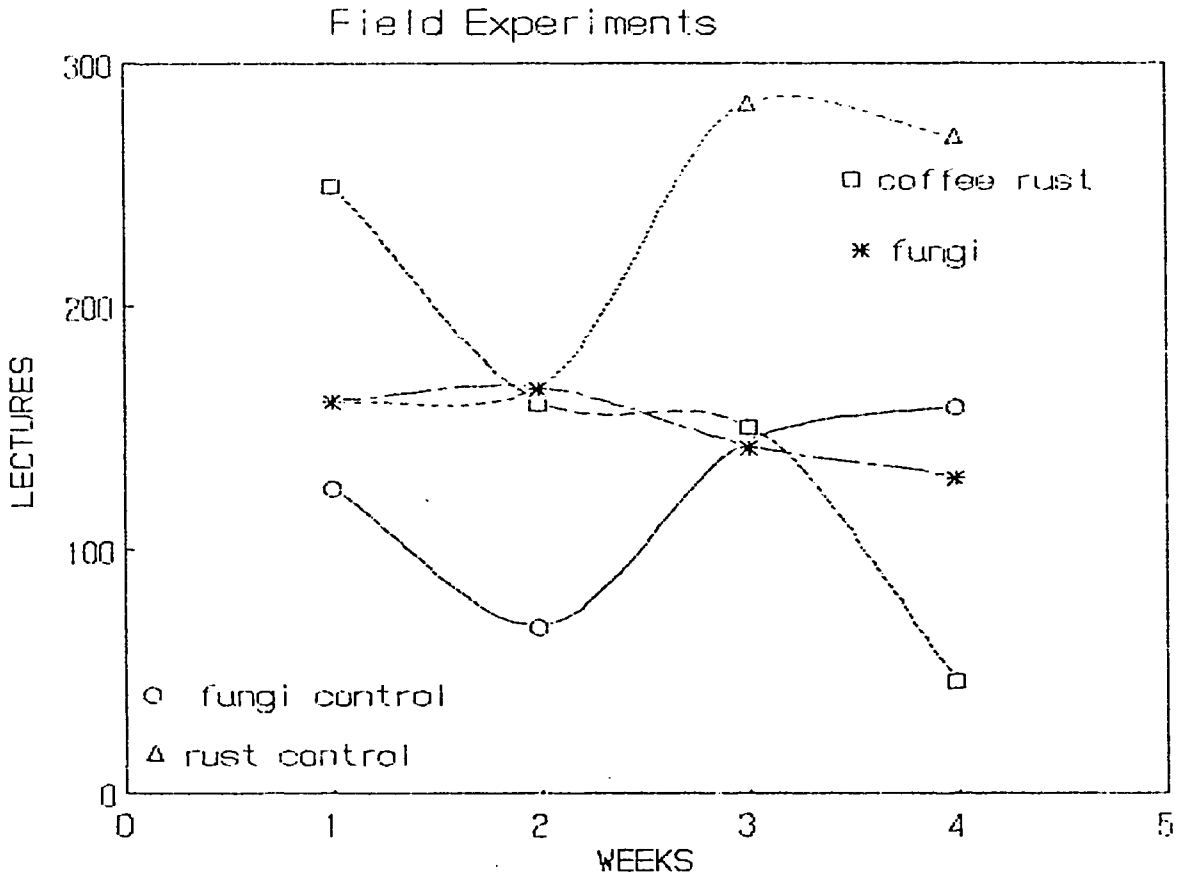


FIGURE 17 Trichoderma like verticillium



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