PD-ABD-701
ISN 75517

UNITED STATES OF AMERICA
AGENCY FOR INTERNATIONAL DEVELOPMENT
U.S.A.I.D. MISSION TO KENYA

UNITED STATES POSTAL ADDRESS
US AID MISSION TO KENYA
BOX 201
APO NEW YORK, NEW YORK 09675

INTERNATIONAL POSTAL ADDRESS
POST OFFICE BOX 30261
NAIROBI, KENYA

Dr. James M. Onsando
Tea Research Foundation
Box 820
Kericho, Kenya

Subject: Grant No. 615-0262-G-SS-00-1069

Pursuant to the Authority contained in the Foreign Assistance Act of 1961, as amended, the Federal Grant and Cooperative Agreement Act of 1977, the Agency for International Development (hereinafter referred to as "A.I.D." or "Grantor") hereby grants to the Tea Research Foundation (hereinafter referred to as "Grantee") the sum of $150,000 to provide support for a research program entitled "Speciation of Armillaria spp. causing Root Rot Diseases of Tea and its Biological Control in Kenya", as described in Attachment 1 entitled "Schedule" of this grant; Attachment 2 entitled "Program Description", and the Grantee's proposal, as amended, which is made a part of the Grant and incorporated herein by reference.

This Grant is effective and obligation is made as of the date of this letter and shall apply to commitments made by the Grantee in furtherance of program objectives during the period beginning August 27, 1991 and ending August 26, 1996.

This Grant is made to the Grantee on condition that the funds will be administered in accordance with the terms and conditions as set forth in Attachment 1 entitled, the "Schedule", Attachment 2 entitled "Program Description", and Attachment 3 entitled "Standard Provisions", which have been agreed to by your organization.

Please sign all copies of this letter to acknowledge your receipt and acceptance of this Grant. Keep one copy for your files and return the original and all remaining copies to this office.

Sincerely,

Stafford Buker for

John R. Westley
Grant Officer
USAID/Nairobi

Attachments:

1. Schedule
2. Program Description
3. Standard Provisions
4. Proposal, amendments, and correspondence

ACKNOWLEDGED:

Grantee Name: Tea Research Foundation of Kenya

BY: Jamesdo.

TYPED NAME: Dr. James M. Onsando

TITLE : Plant Pathologist

Date: 16th Sept. 1991

Funds Available
CONT/KENYA
Initials
Date 8/30/91

FISCAL DATA

PIO/T No.	:	N/A
Appropriation No.	:	71-1111021.6
Budget Plan Code	:	DDSA-91-29615-KG11
Allotment No.	:	146-51-615-01-19-11
Project No.	:	936-5600
Proposal No.	:	11.135
Total Estimated Amount	:	$150,000
Total Obligated Amount	:	$150,000
Funding Source	:	USAID/Nairobi
Project Office	:	AID/SCI
Voucher Paying Office	:	RFMC-Nairobi
Voucher Approval Office	:	USAID/Nairobi

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SCHEDULE

A. Purpose of Grant No. 11.135

The purpose of this Grant is to provide support for the Tea Research Foundation of Kenya's proposal entitled "Speciation of *Armillaria* spp. causing Root Rot Diseases of Tea and its Biological Control in Kenya" (11.135) which is hereby incorporated by reference. This proposal was revised on May 28, 1991. In the event of conflict, the revised proposal takes precedence over the original.

B. Period of Grant

1. This is a five-year grant and the effective date is August 27, 1991. The expiration date of this Grant is August 26, 1996.

2. No Cost Extensions

Subject to the ceiling amount of this Grant (C.1. below) and with the prior written approval of the Project Officer (F.1. below) the grantee is authorized to extend the completion date stated above, provided that such extension:

a. Does not cause the elapsed time for the completion of the work, including the furnishing of all deliverables except for the Final Performance Report (E.1.b. below) to extend beyond 180 calendar days from the original specified completion date, or beyond the overall Project Activity Completion Date (PACD) of December 31, 1999; or

b. Does not cause the elapsed time for completion of the work (all deliverable except the Final Performance Report - as in Paragraph B., above) to extend beyond 365 calendar days from the original specified completion date, or beyond the overall PACD (December 31, 1999), provided the cause of the delay in completion of the research work is due to research requirements or circumstances beyond the control of the Principal Investigator (PI); e.g., weather, civil uprising, change in the PI or co-PI, etc.

In either of the above cases, the Grantee shall attach a copy of the Project Officer's approval of any extension of the term of this grant to the final voucher submitted for payment.

It is the Grantee's responsibility to ensure that the Project Officer's approved time extension do not result in costs incurred which exceed the total amount of this Grant. Under no circumstances shall time extensions authorized the Grantee to be paid any sum in excess of the amount of this grant.

C. Amount of Grant and Payment

1. AID hereby obligates the amount of $150,000 for the purposes of this Grant.

2. Payment shall be made to the Grantee in accordance with procedures set forth in Attachment 3, Optional Standard Provision No. 1 entitled "Payment - Cost Reimbursement."

3. All documentation required for payment under this provision shall be submitted to the Project Manager, Mr. James Dunn, USAID/Nairobi, Kenya where it will be reviewed and passed to the Controller for payment.

D. Grant Budget

The following Budget for this Grant includes local cost items, if authorized. Revisions to this Budget shall be made in accordance with Mandatory Standard Provision of this Grant entitled "Revision of Grant Budget." Within the total estimated cost of the Grant, the Grantee may adjust the line items as reasonably as necessary for the performance of the grant program.

"Reasonably as necessary" has been interpreted as up to 15 percent. Any amount greater than 15 percent would require the approval of the Project Officer.

Estimated Budget

Line Item	Amount	
Salaries	$ 32,000	
Equipment	31,500	(includes repair and Maintenance)
Training	42,500	
Consultation	21,000	
Regional Travel	12,500	
Reagents	8,000	(includes chemicals)
Contingencies	1,500	
Publications	1,000	
Total	$150,000	

A Line-Item Budget is attached as Attachment 2.A.

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Within the total estimated cost of the Grant, the Grantee may adjust the line items as reasonably as necessary for the performance of the Grant program.

E. Reporting and Evaluation

1. Technical Reports

Reports must be sufficiently detailed to substantiate the findings and to permit a scientific evaluation of the research. Overseas collaborators shall be given fair credit for their participation in the research and a chance to review and comment on the Final Report before it is submitted. The principal investigator will share a draft of the Final Report with the A.I.D. Project Officer for comments prior to the formal submission. Publication of results in scientific journals is encouraged. Additional guidance on report preparation is given in the "Interim Guidelines on Performance Report Preparation for PSTC and CDR Projects," available from AID/SCI.

a. Performance Reports: Performance reports are required every six months. The principal investigator will submit reports stating what has been accomplished to date and detailing project management issues. A Financial Status Report will be attached to each report. Reports are due within sixty (60) days after the end of each six-month period. Four copies of each report are to be submitted to the Office of the Science Advisor, Room 320 SA-18, Washington, D.C. 20523-1818; one copy to the AID/W Backstop Officer, Dr. Benjamin Waite, S&T/AGR/AP, Room 420E - SA-18, Agency for International Development, Washington, D.C. 20523-1809; one copy to the Project Manager, Mr. James Dunn, USAID/Nairobi, Nairobi, Kenya; one copy to Bureau for Program and Policy Coordination, Center for Development Information and Evaluation (PPC/CDIE), Room 215C, SA 18, Washington, D.C., 20523-1802; and two copies to the National Academy of Sciences, BOSTID, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

b. Final Performance Report: Within sixty (60) days after the termination of the Grant, the principal investigator will submit four copies of the Final Performance Report to the Office of the Science Advisor, A.I.D., Room 320 SA-18, Washington, D.C., 20523-1818; one copy to the AID/W Backstop Officer, Dr. Benjamin Waite, S&T/AGR/AP, Room 420E - SA-18, Agency for International Development, Washington, D.C. 20523-1809; one copy to the Project Manager, Mr. James Dunn, USAID/Nairobi, Nairobi, Kenya; one copy to Bureau for Program and Policy Coordination, Center for Development Information and Evaluation (PPC/CDIE), Room 215C, SA 18, Washington, D.C., 20523-1802; and two copies to the National Academy of Sciences, BOSTID, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

2. The Financial Status Report (SF-269) is required every six months and will be submitted as an attachment to the progress report.

a. Voucher Approving Office

Mr. James Dunn
Science Advisor
USAID/Nairobi
Nairobi, Kenya

b. Voucher Paying Office:

Office of the Controller
USAID/Nairobi
Nairobi, Kenya

c. Voucher Identification

Grant No:	615-0262-G-SS-00-1069
Project Number:	936-5600
SCI Grant No:	11.135
Project Office:	USAID/Nairobi
Budget Plan Code:	DDSA-91-29615-KG11

F. Project Officer and Investigators

1. Project Officer:

Mr. James Dunn
Science Advisor
USAID/Nairobi
Nairobi, Kenya

2. AID/W Technical Backstop Officer
Dr. Benjamin Waite
S&T/AGR/AP, Room 420E - SA-18
Agency for International Development
Washington, D.C. 20523-1809

3. Principal Investigators:

Dr. James M. Onsando, Plant Pathologist
Tea Research Foundation of Kenya
P.O. Box 820
Kericho, Kenya

4. Collaborating Institution and Co-PI

Dr. Philip M. Wargo
Research Plant Pathologist
51 Mill Pond Road
Hamden, CT 06514

G. Special Provisions

1. Compliance with Federal Guidelines and Regulatory Procedure:

a. The recipient will implement this research activity in accordance with all relevant guidelines for U.S. Government funded research, such as:

(1) Guidelines for the handling of radioactive materials;

(2) NIH and USDA guidelines for the handling of pathogenic microorganisms;

(3) USDA-APHIS procedures for animal and plant health inspection;

(4) Procedures issued by the USDA, EPA, or other appropriate Federal agency, regarding testing of genetically engineered organisms;

(5) A.I.D.'s environmental procedures; and

(6) Such other Federal guidelines and procedures as may apply during the course of research.

b. All existing comparable guidelines of the host country in which the research is actually located must be followed also.

c. Reports submitted under this activity to A.I.D. will address the cited regulatory issues. All modifications of protocols affecting these regulatory concerns must be reported. The investigators are responsible for reporting any difficulties encountered in implementing these protocols.

2. Containment and Safe Disposal of Animal or Plant Pathogens or Pests: Research will be conducted following the protocols described in attachment 4 (which is the original proposal, subsequent amendments, or letter(s) from the Principal Investigator), which insure the containment and safe disposal of animal or plant pathogens. Notwithstanding, the research must be conducted following procedures issued by the U.S. Government and those issued by the government of the Kenya for the containment of these pathogens or pests.

   If any protocol is revised, it must be re-reviewed by the investigator's institutional review committee(s) that approved the original protocol, and the Project Officer and Office of the Science Advisor must be informed in writing before the revised protocols are used. The revised procedures must be consonant with the guidelines of the country in which the laboratory is located, and of the United States. Copies of the approval of the revised protocols by the investigator's institutional review committees should also be provided the Project Officer and the Office of the Science Advisor.

   Similarly, the research will be conducted in the facilities described in Attachment 4. If the research is moved to new facilities, or the facilities are modified in such a way to affect safety or hazard containment, a description of the new facilities must be provided to the Project Officer and to the Office of the Science Advisor before the research is affected. Any applicable institutional reviews of the facilities should be repeated, and the re-certification should be provided to the Project Officer and the Office of the Science Advisor.

3. International Shipment of Organisms, Biologicals, or Controlled Materials or Equipment: Procedures for the international shipment of these materials must be in agreement with those approved in the permits cited in attachment 4 (which is the original proposal, subsequent amendments, or letter from the Principal Investigator). Notwithstanding, such shipments must be in compliance with International Import/Export Regulations for all countries to and from which regulated items are shipped. If the shipment procedures are varied from those specified for the permits, permits for the revised procedures must be provided to A.I.D., Office of the Science Advisor before shipping commences.

4. Environmental Hazards: Research will be conducted following the protocols described in attachment 4 (which is the original proposal or subsequent amendments, or letter from the Principal Investigator), which insure that there are no unacceptable environmental hazards incident to the research. Notwithstanding, the research must be conducted following A.I.D. environmental guidelines and those issued by the government of the host country.

   If any protocol involving environmental hazards is revised, it must be re-reviewed by the investigator's institutional review committee(s) that approved the original protocol, and the Project Officer and Office of the Science Advisor must be informed in writing before the revised protocols are used. The revised procedures must be consonant with the guidelines of the country in which the research is located, and of the United States. Copies of the approval of the revised protocols by the investigator's institutional review committee(s) must also be provided the Project Officer and the Office of the Science Advisor.

   In addition, however, and prior to commencement of any such activity which may create an environmental hazard, the grantee shall make a judgment and communicate the same to A.I.D. as to whether the regulations, procedures or facilities of the country in question are adequate to ensure the protection of the environment. In the event such judgment is that they are not, the grantee and A.I.D. will consult and agree on the conditions to be applied to the activity which will ensure environmental protection.

5. Intellectual Property Rights: Intellectual property rights stemming from the activities supported under this grant will be apportioned as described in attachment 4 (which is the original proposal or subsequent amendments, or letter from the Principal Investigator). If the participants chose to modify that apportionment, the revised apportionment must be submitted to the A.I.D. Project Officer for approval before it takes effect. Notwithstanding the intellectual property rights clauses provided in the following standard provisions apply.

H. STANDARD PROVISIONS

1. While in the field, the Grantee will keep the A.I.D. field mission(s) generally appraised of the research, but will not request administrative support except for the usual in-country introductions as may be appropriate. The Grantee will abide by Mission and host government regulations and customs as they apply to AID-supported in-country activities.

2. Project Officer approval required by paragraph (a) of optional Standard Provision 4 of this grant entitled "Air Travel and Transportation," is hereby granted for travel between the United States and all other countries described in the project description.

3. The principal investigator(s) and essential scientific staff of Grantee and co-principal investigator of the collaborating institutions may not be changed during the life of the research without prior written approval of the Office of the Science Advisor.

4. The Standard Provisions applicable to this grant are entitled "Mandatory Standard Provisions for U.S., Non-governmental Grantees" and "Optional Standard Provisions for U.S., Non-governmental Grantees" and are included herein as Attachment 3.

I. Title to Property

The title to property acquired under this Grant will vest in the Grantee in accordance with Optional Standard Provision No. 20 entitled "Title to and Use of Property (Tea Research Foundation)". When the principal Grantee makes a subagreement under this Grant Agreement, the principal Grantee will pass to the Subgrantee the provisions of the above cited reference, as appropriate. Equipment purchased on behalf of each collaborating institution shall remain the property of that institution.

J. Publications

Acknowledgment of A.I.D.'s contribution to any publication resulting from this Grant shall be made in accordance with procedures set forth in Attachment 3 - Optional Standard Provision No. 11 entitled "Publications".

K. Closeout Procedures

This section prescribes uniform closeout procedures for this Grant.

1. The following definitions shall apply for the purpose of this section:

   a. Closeout The closeout of a Grant is the process by which A.I.D. determines that all applicable administrative actions and all required work of the Grant have been completed by the Grantee and A.I.D.

   b. Date of Completion The date of completion is the date on which all work under the Grant is completed, or the date on the award document, or any supplement or amendment thereto, on which A.I.D. sponsorship ends.

   c. Disallowed Costs Disallowed costs are those charged to a Grant that A.I.D. or its representative determines to be unallowable, in accordance with the applicable Federal cost principles or other conditions contained in the Grant.

2. A.I.D. closeout procedures include the following requirements:

   a. Upon request, A.I.D. shall make prompt payments to a Grantee of allowable reimbursable costs under the Grant being closed out.

   b. The Grantee shall immediately refund any balance of unobligated (unencumbered) cash that A.I.D. has advanced or paid and that is not authorized to be retained by the Grantee for use in other Grants.

   c. A.I.D. shall obtain from the Grantee within 90 calendar days after the date of completion of the Grant all financial, performance, and other reports required as the condition of the Grant. A.I.D. may grant an extension when requested by the Grantee.

d. When authorized by the Grant, A.I.D. shall make a settlement for any upward or downward adjustments, to A.I.D.'s share of costs after these reports are received.

e. The Grantee shall account for any property acquired with A.I.D. funds, or received from the Government in accordance with the provisions of OMB Circular A-110, Attachment K.

f. In the event a final audit has not been performed prior to the closeout of the Grant, A.I.D. shall retain the right to recover an appropriate amount after fully considering the recommendations on questioned costs resulting from the final audit.

L. Source and Origin of Goods and Services

1. The country or countries where research or other scientific/technological cooperation takes place shall be deemed to be the cooperating country for the purpose of permitting local cost financing.

2. Goods and services, except for ocean shipping, financed by A.I.D. under the project shall have their source/origin in the Cooperating Country or the United States and Code 935 countries.

3. Ocean shipping financed by A.I.D. under the project shall, except as A.I.D. may otherwise agree in writing, be financed only on flag vessels of the United States.

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F. TECHNICAL WORK PLAN

1. Selective medium

1.1. Preferential carbon and nitrogen sources

Armillaria mellea for these studies will be grown on potato dextrose Agar (PDA). Richard's nutrient solution will be used as the medium for fungal growth. The medium consists of KNO_3, 10g; KH_2PO_4, 5.0g; $MgSO_4$, $7H_2O$, 2.5g; $Fecl_3$, 0.02g; Sucrose 50.0g and deionised water, 1000 ml. For the carbon sources, sucrose will be the standard and will be substituted by others in the wide range that will be tested. For the nitrogen sources, KNO_3 will be the standard and the other sources will be treated as above.

1.2. Preferential pH

Richard's nutrient solutions will be used as a medium for fungal growth. The pH will be varied by use of HCl and NaOH, respectively, to various values within the range of 3 to 9. The optimum pH level will be determined by a quadratic regression analysis.

1.3. Preferential temperature

For the temperature studies, a standard medium (Malt extract, 20g; Dextrose, 20g; Bacto peptone, 5g; Agar, 19g and 1,000 ml distilled water) will be used. Temperature will be varied by use of incubators and a refrigerator. The range will be 10°C to 35°C. The optimum temperature level will be determined by quadratic regression analysis.

1.4. Choice of selective antifungal agents

This aspect will take advantage of the fact that fungi, even related ones often differ in their sensitivity to various antifungal agents (Tsao, 1970). The fact that different species of the same fungal genus often differ in sensitivity to toxicant (Papavizas, 1967) will call for spectrum tests of a few representative species. Therefore, the screening will involve a

few known soil borne fungal pathogens and common fungal residents in the study which will have to be isolated. A wide range of fungicides with all the major chemical groups represented will be screened against *A. mellea* and all the other common soil fungal residents to be isolated along with the common soil borne fungal pathogens. The idea here will be to determine a fungicide with a wide spectrum of activity on soil borne fungal flora but least activity on *A. mellea*.

1.5. Choice of selective antibacterial agents

Unlike the search for suitable selective antifungal agents in which intra-fungal specificity is the key for success, the search for antibacterial agents for a fungal isolation medium will aim at finding materials with broadest possible spectrum against unwanted bacteria and actinomycetes. A similar medium like the one for antifungal agents will be used to grow *A. mellea*. A wide range of antibiotics will be screened to eliminate those with toxicity effects to the target fungus (*A, mellea*). Alongside *A. mellea*, common soil resident bacteria will be screened to be able to pick an antibiotic with a wide spectrum activity on the bacteria and actinomycetes and no activity or very little activity on the fungus (*A. mellea*).

1.6. Choice of a dye for pigmentation

A number of dyes will be screened for supplementary selective differentiation through pigmentation. As for the others, soil fungal isolates will be tested together with *A. mellea* against these dyes. This will be based on the knowledge that certain microorganisms have the ability to absorb selectively the pigment from the medium to induce specific secondary pigment formation or to reduce differentially the pigment intensity in the colony or the medium. A dye without antimicrobial ability on *A. mellea* but with the desired differential pigmentation properties will be included in the medium.

Because most of the experiments in this section will be done in the laboratory, a completely randomized block design with a minimum of four replicates for each treatment will be adopted. Due to possibilities of slow growth rate of A. mellea in the respective media, slight modifications of the media might initially be necessary. This might prolong the research duration for this aspect of the project to two years.

2. Determination of where *Armillaria* root rot disease on tea is severe in Kenya and where it is not

This will be carried out through district-by-district surveys and quantification of losses of tea bushes due to *Armillaria* root rot determined. The districts will then be rated on disease severity index which will be a computation of the following:

1. Number of farms with disease

2. Number of bushes that have died or are about to die due to *Armillaria* root rot.

3. Information on initial land preparation methods.

4. Information on the tree species that were cut prior to planting.

5. Cultural practices in operation.

Isolates of *Armillaria* will then be taken from very severe to moderate severity indices districts for species differentiation and characterization. At this stage the potential for use of cross-protection as management strategy will be assessed.

3. Characterization and differentiation of *Armillaria* species and clones and correlation of these to disease severity.

3.1. Species characterization and differentiation

Armillaria isolates originating from 10 different tea growing districts of Kenya will be used. Techniques such as mating tests, observation of vegetative mats in culture, the fruiting of isolates in culture, the comparison of those fruitbodies obtained with existing taxonomical descriptions, the study of monospore isolates from fruitbodies and microscopic examination of fruitbody tissue (hymenium) will be used (Anderson, 1986; Kile and Watling, 1983; Korkhonen, 1978; Guillaumin, 1986). It is important to note that Dr. Carolyn Mohammed of Oxford University in England is willing and able to help in species identification (Dr. Philip Wargo's letter enclosed).

3.2. Clonal characterization

The isolates will be collected as described under 3.1. and they will be subjected to Adam's method of clonal identification (Adams, 1974). The method (isolate interaction in paired culture), utilizes the criteria that pairing of isolates in which a dark line is formed along the common interface is considered dissimilar in parentage. Conversely isolates that freely intermingle when paired are considered compatible.

For this study, a basic medium (Malt extract, 3%; Glucose, 2% Peptone, 0.5%; Agar, 2%) will be used. The pH of the medium will be adjusted to 5.0 - 5.3.

The isolates will be in mycelial forms and whenever possible rhizomorphs and basidiospores will be used. The latter two are rarely present on infested tea areas in the region.

The paired isolates will be incubated for two to three weeks at 25°C in the dark (wrapped in aluminium foil).

The paired isolates will be replicated four times just to ascertain the consistency of the results.

3.3. Correlation of probable clonal differences to virulence

The mosts susceptible tea clone (11/4) will be subjected to artificial inoculum of the probable *A. mellea* clones or strains in the greenhouse. The virulence of the strain will be assessed on the basis of rate of kill of the young tea plants and extent of kill. The artificial inoculum will be prepared by cutting tea roots of uniform diameter and length (1.5 - 2.0 cm x 10 cm) and putting them in 2-litre jars containing 200 mls of water and autoclaving then inoculating them as outlined by Wilbur (Wilbur *et al*, 1971).

4. Determination of biological, chemical and physical properties of the rhizosphere, rhizoplane and soils in tea plantations where incidence and severity of root disease is high and where it is low or non-existent.

The areas in Kenya where *Armillaria* root rot disease is severe, moderate or non-existent would have been established by the time this aspect of the project is undertaken. A series of rhizosphere, rhizoplane and soil measurements will be carried out from the specific areas for comparison purposes. For the biological aspects which will mainly be microbial, the measurements will start with known antagonist(s).

4.1. Biological soil properties both rhizosphere and rhizoplane

4.1.1. Quantification of *Trichoderma, penicillium* and Mucor propagules from ten respective areas

This will be done by modified rose bengal-streptomycin agar medium (Martin, 1950). The modification is on the amount of streptomycin. To improve the selectivity of the medium mancozan will be added at the rate of 800 ppm per litre to selectively suppress Mucor spp. (Onsando, unpublished).

4.1.2. Quantification of other fungi and determination of diversity

A general purpose medium like potato dextrose Agar (PDA) will be used. Conventional fungal taxonomic procedures will be used for diversity determination.

4.1.3. Quantification of bacteria and determination of diversity

The bacteria will be isolated and quantified by weak peptone-dextrose agar consisting of $MgSO_4.7H_2O$, 0.2g; KH_2PO_4, 0.5g; $Fe_2(SO_4)_3.9H_2O$, Trace; Dextrose, 1.5g; Peptone, 0.5g; Agar 17g per litre of distilled water. Unless stated, quantification of the respective microorganisms will be by a ten-fold serial dilution technique (Taylor, 1962).

4.1.4. Quantification of actinomycetes and determination of diversity

Isolation, enumeration and species diversity of actinomycetes will be achieved by use of starch-casein medium with mystatin, 50µg/ml; actidione, 50µg/ml; polymyxin B sulphate, 5.0 µg/ml and sodium penicillin, 1.0 µg/ml (Williams and Davies, 1965).

4.2. Chemical soil properties (rhizosphere and rhizoplane)

The soil chemical properties that will be investigated in the respective areas will include pH, macronutrient composition (Nitrogen, phosphorus, potassium, calcium and magnesium), micronutrient composition (iron, manganese, copper, zinc, boron). The Tea Research Foundation soil chemistry department has kindly agreed to do the routine soil elemental analysis for us.

4.3. Physical soil properties (rhizosphere and rhizoplane)

Although the Kenyan tea growing soil tend to have very similar physical properties characteristics (Dr. Othieno, personal communication), there could be fine differences that might influence *Armillaria* root rot development. Therefore the following soil physical properties for the respective areas will be analysed by the soil physics department of the Tea Research Foundation of Kenya:

1. texture
2. structure
3. consistence
4. weight, pore space and air relationships
5. soil moisture regimes.

5. Manipulation of soil biological, physical and chemical properties to favour proliferation of *Trichoderma* spp. and other indigenous microbial antagonists to *Armillaria*

5.1. Use of organic amendments

A variety of locally available organic amendments will be screened for their ability to enhance the population of *Trichoderma* and other microbial antagonists to *A. mellea*. Initial work in this area has already shown great promise with coffee pulp (Onsando *et al*, 1989). The amendments will constitute main treatments while the rates will constitute subtreatments in a split plot design replicated 4 times.

The soils for this experiment will be collected from those tea areas with evidence of *Armillaria*. The environmental measurements in the greenhouse will be taken throughout the experimental durations.

Organic carbon in the amendments will be determined by a modified Walkey-Blackman method (Black, 1965) as stipulated in Onsando's paper (Onsando *et al*, 1989). Organic nitrogen on the other hand will be determined by Kjeldahl's method (Vogel, 1961).

The population of *Trichoderma* and other probable fungal antagonists will be monitored by modified rose bengal-streptomycin agar medium (Martin, 1950). The bacteria and actinomycetes will be isolated and quantified by weak peptone-dextrose agar consisting of $MgSO_4.7H_2O$, 0.2g; KH_2PO_4, 0.5g; $Fe_2(SO_4)_3.9H_2O$, Trace; Dextrose, 1.5g; Peptone, 0.5g; Agar, 17g and 1 litre of distilled water. To quantify the respective micro-organisms, a ten-fold serial dilution technique (Taylor, 1962) will be used.

The total activity of microbial biomass to give an indication of organic turnover will be determined by Fluorescein diacetate hydrolysis (Schnurer Johan and Thomas Rosswall, 1982).

The pre-determined soil moisture level will be kept constant as outlined in Onsando's paper (Onsando and Alvarez, 1987).

5.2. Establishment of the best method and time of application of organic amendment

Armillaria root rot in tea plantations is patchy depending on disease severity, the patches of which can be very many but small in size. The application of organic amendments therefore will be restricted to these patches. Before any elaborate experiments are undertaken to determine the best method and time of application of the organic amendments, the package usage will be confined to infilling those tea plants that have died due to *Armillaria* root rot. This is intended to involve digging a hole slightly larger than the standard (40cm x 25cm), mixing the soil thoroughly with an organic amendment at a rate to be determined and returning the same during infilling. This is anticipated to minimize subsequent deaths of infills and therefore diversion of useful labour from repeated infilling exercise.

Because of the nature of the disease pattern, the use of heavy machinery is not anticipated. Manual handling like in normal planting should be able to suffice.

To generate information however on the best method and time of application of the organic amendments, a long-term experiment will be established. In this experiment, the time of application will constitute main treatments while the methods will constitute subtreatments in a split plot design with 4 replications.

This aspect of the project will be tackled after establishing that a specific amendment does help in the management of the disease.

A location with very high natural inoculum potential of *Armillaria* will be selected for the work. The inoculum potential; will be assessed by the uniformity of dead tea bushes due to the disease.

The treatments will be as follows:

I. Main treatments

(i) No post-planting inter-row application of amendment.
(ii) One second-year inter-row application
(iii) Second and third-year inter-row application.
(iv) Second, third and fourth year inter-row application.

II. Sub-treatment

(i) A standard planting hole (40cm x 25cm), followed by amendment of the soil from the hole with an appropriate carbon rate of the amendment to 1 kg of dry soil.
ii) A planting hole of 60cm x 50cm treated as above.
(iii) A planting hole of 70cm x 65cm treated as above.
(iv) Control

5.3. Use of inorganic amendments

Four commercial fertilizers used in tea namely: Sulphate of ammonia, urea, Nitrogen, Phosphorus, Potassium and Sulphur (NPKS 25:5:5:5) and NPK 20:10:10 will be screened at the rate of 150 kg N/ha for their effect on proliferation of *Trichoderma* and other *A. mellea* antagonists. The rate of N/ha is the commercial recommendation for tea in Kenya.

The soil collection, treatment, moisture control will be carried out as outlined already.

The microbial population, activity and diversity will be determined as already outlined.

The pots in the greenhouse will be arranged in a randomized complete block design with four replications. Analysis of variance will be carried with the help of a computer.

5.4. Manipulation of soil pH

A soil survey will be carried out in old tea soils to locate a site with the lowest pH value. All the soils for the experiments will be taken from this site. The soil will be passed through a soil shredder fitted with a 1.5 cm screen to remove large particles (Weaver and Wehund, 1975). The finer soil will be homogenized and a sample taken for pH reading for confirmation purposes.

To determine the lime requirements to raise the soil pH to the desired levels, 10g samples of soil will each be put into 7 pairs of plastic beakers and treated in duplicate as tabulated

below. The Ca $(OH)_2$ stock solution used will be 2g in one litre of water.

Beaker number		1	2	3	4	5	6	7
Treatments-$Ca(OH)_2$	(Mls)	50	40	30	20	15	5	0
Water - H_2O	(Mls)	0	10	20	30	35	45	50

The beakers will be covered tightly and let stand for three days before the first pH reading is made. This will be continued until the pH change is less than 0.1 units between two successive readings in each case.

The weight of calcium/10g soil will be plotted against the resultant pH variables. This curve will be used to determine the amount of lime to be added to a given quantity of soil per pot i.e. 1000g to give the desired pH.

The other inorganic amendment i,e, $CaSO_4$ will be used on the basis of determined calcium weights to establish whether the observed effect is due to pH or just calcium and sulphur.

The substrate for microbial proliferations will be the best organic amendment that would have been determined. Therefore the organic amended and plain soil will be used in this experiment in a split plot design replicated four times. The type of soil will form the main treatment while pH values will constitute subtreatments.

The additives to the pot will be distributed uniformly by dumping the contents in a plastic bag and rolling the soil thoroughly and then placing the soil back into the pot.

The subtreatments will be five ranging from 4.0 to 7.5. The moisture content will be kept constant as already described.

All the microbial data will be taken as already discussed.

5.5. Soil moisture

Soil moisture will be varied by adding different amounts of water (0.5, 1.0, 2.0 mls etc) to samples of 10g air-dried soil and appropriate ratio of an organic amendment to be determined. The soil/organic amendment samples will be weighed and the weights recorded. The samples will be oven-dried at 110°C overnight to determine their dry weights. On the basis of the determined water content levels per mixture, a standard curve will be plotted on the amount of water added against the resulting moisture levels. The information will be extrapolated for the actual pot soils/organic amendment weights.

After each addition of the determined amounts of water, the weights of pots, soil organic amendment will be taken and recorded. The varied moisture levels will be kept constant. The pots and their contents will be weighed once a day and the lost

water added until the balance reads the original weight (Kendrick, 1948). The moisture levels variation will range from 11% to 60% which approximately will fall between (-5 to 0 bars).

The soil moisture levels will be correlated to soil potentials (-bars) by determining a moisture release curve for the site of soil sampling. The moisture release curve will be related to the moisture levels in the greenhouse pots as long as the soil bulk density in the pots will be adjusted roughly to that of the field by compressing the soil/organic amendment to take a specified volume.

The moisture levels will constitute five treatments in a complete randomized block design with four replications.

All the microbial data and other parameters will be taken as already outlined in section 5.1.

6. Assessment of antibiosis of soil antagonists to *Armillaria* root rot fungus

Paired colonies of antagonists and *A. mellea* will be done in Malt Extract Agar (MEA) and the zone of inhibition of *A. mellea* measured. The size of inhibition zone will reveal the level of antibiosis.

Filtrates from the probable antagonists will be subjected to *A. mellea* growing on Malt Extract and the radial growth of *A. mellea* determined. Millipore filters (cold sterilisation) will be used to sterilize the filtrates as outlined in Onsando's paper (Onsando and Waudo, 1991) (In press).

Phase contrast microscopy will also be used to establish the mode of parasitism between the probable antagonists and *A. mellea*. For the convenience of doing this, differential staining between the fungal antagonists and *A. mellea* has to be established. The microscopy techniques will be as outlined by Chet (Chet *et al*, 1981).

The identification of microbial antagonists to *A. mellea* will follow similar methods described above i.e. microscopy, inhibition zone measurements and effect of metabolites on filtrates.

7. Determination of whether the antibiosis of *Trichoderma* spp. and other soil antagonists to *Armillaria* will be influenced by specific species or clone of *Armillaria*

The same procedures as in 6 will be followed but this time different species/clones of *Armillaria* will be used as target organisms.

8. Determination of probable virulence differences within the *Armillaria* root rot fungus species/clones

The most susceptible tea clone (11/4) will be subjected to artificial inoculum of the probable *A. mellea* species/clones in the greenhouse. The virulence of the strain will be assessed on the basis of rate of kill of the young tea plants and extent of kill. The artificial inoculum will be prepared by cutting tea roots of uniform diameter and length (1.5 - 2.0 cm x 10 cm) and putting them in 2-litre jars containing 200 mls of water and autoclaving then inoculating them as outlined by Wilbur (Wilbur *et al*, 1971).

9. Testing of the package

Testing of the biocontrol package taking into account all the parameters that would have been worked out and established will be done both in the greenhouse and field.

The field experimental design will have to be determined when th factors under test are established. It will most likely be a factorial design.

C: BUDGET SUMMARY

	Year 1		Year 2		Year 3		Year 4		Year 5		Totals	
	AID	Match	AID	Match	AID	Match	AID	Match	AID	Match	Aid	Match
Salaries + Benefits												
Principal investigator (Kenya) 60%	0	11,000	0	11,000	0	11,000	0	12,000	0	12,000	0	57,000
Co-investigator (USA) 20%	0	-	0	-	0	-	0	-	0	-	0	-
Co-investigator (Kenya) 10%	0	10,000	0	10,000	0	10,000	0	11,000	0	11,000	0	52,000
Co-investigator (Kenya) 10%	0	15,000	0	15,000	0	16,000	0	16,000	0	18,000	0	80,000
Technician (Kenya)	0	4,000	0	4,000	0	4,000	0	5,000	0	5,000	0	22,000
A microbiologist (Kenya) 100%	6,000	0	6,000	0	6,000	0	7,000	0	7,000	0	32,000	0
Equipments + repair and maintenance	24,000	18,600	3,000	18,600	3,000	18,600	1,000	18,600	500	18,600	31,500	93,000
Training/Consultation												
Principal investigator	-	-	14,000	0	-	-	14,500	0	-	-	28,500	0
Co-investigator (Soil Scientist)	6,000	0	-	-	-	-	-	-	-	-	6,000	0
Technician	8,000	0	-	-	-	-	-	-	-	-	8,000	0
Consultation for the USA co-investigator	8,000	0	-	-	8,000	0	-	-	5,000	-	21,000	0
Regional/National travels												
Principal investigator Co-investigator, technician, microbiologist alternately/together depending on need	3,500	0	3,500	0	2,500	0	2,000	0	1,000	0	12,500	0
Reagents & chemicals	2,500	2,500	1,500	2,000	2,000	2,000	1,000	2,000	500	2,000	8,000	10,000
Contingencies	500	0	500	0	500	-	-	-	-	-	1,500	0
Publications					300	0	500		700	0	1,000	300
Total Grant (US Dollars)											150,000	314,300

International travels for the principal investigator, soil scientist and co-investigator have been worked out at $150-180 per day for subsistence.

Air fares, medical cover and life insurance covers have been included in the budget totals shown.

OMB Control No. 0412-0510
Expiration Date: 12/31/89

APPENDIX 4D
MANDATORY STANDARD PROVISIONS FOR NON-U.S., NONGOVERNMENTAL GRANTEES[2]

1. Allowable Costs
2. Accounting, Audit, and Records
3. Refunds
4. Revision of Grant Budget
5. Termination and Suspension
6. Disputes
7. Ineligible Countries
8. Debarment, Suspension, and Other Responsibility Matters
9. U.S. Officials Not to Benefit
10. Nonliability
11. Amendment
12. Notices

1. ALLOWABLE COSTS (MAY 1986)

(a) The grantee shall be reimbursed for costs incurred in carrying out the purposes of this grant which are determined by the grant officer to be reasonable, allocable, and allowable in accordance with the terms of this grant and the applicable* cost principles in effect on the date of this grant, which are attached.

(1) Reasonable. Shall mean those costs that do not exceed those which would be incurred by an ordinarily prudent person in the conduct of normal business.

(2) Allocable Costs. Shall mean those costs which must are necessary to the grant.

(3) Allowable Costs. Shall mean those costs which must conform to any limitations set forth in this grant.

*NOTE: For educational institutions use OMB Circular A-21; for all other non-profit organizations use OMB Circular A-122; and for profit making firms use Federal Acquisition Regulation 31.2 and AID Acquisition Regulation 731.2.

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[2]When these Standard Provisions are used for cooperative agreements, the following terms apply: "Grantee" means "Recipient" "Grant" means "Coopertive Agreement," and "AID Grant Officer" means "AID Agreement Officer."

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(4) Unallowable costs, direct or indirect, include but are not limited to the following examples: Advertising, bad debts, contingencies, entertainment, fines and penalties, interest, fund raising, investment management costs, losses on other awards, taxes, first class air fare unless specifically approved. Additionally, public information service costs are unallowable as indirect costs.

(b) Prior to incurring a questionable or unique cost, the grantee should obtain the grant officer's written determination as to whether the cost will be allowable.

* 2. ACCOUNTING, AUDIT, AND RECORDS (MAY 1991)

(a) The grantee shall maintain books, records, documents, and other evidence relating to the AID-sponsored project or program in accordance with generally accepted accounting principles formally prescribed by the U.S., the cooperating country, or the International Accounting Standards Committee (an affiliate of the International Federation of Accountants) to sufficiently substantive charges to this grant. Accounting records that are supported by documentation will as a minimum be adequate to show all costs incurred under the grant, receipt and use of goods and services acquired under the grant, the costs of the program supplied from other sources, and the overall progress of the program. The grantee records and subgrantee records which pertain to this grant shall be retained for a period of three years from the date of expiration of this grants and may be audited by AID and/or its representatives. The grantee shall insert this paragraph (a) in all subgrants valued in excess of $10,000.

(b) If the grantee receives $25,000 per year or more under this grant, the grantee agrees that it shall have an audit made of the funds provided under this grant and of the financial statements of the organization as a whole. The grantee shall select an independent auditor in accordance with the "Guidelines for Financial Audits Contracted by Foreign Recipients" issued by the AID Inspector General. The audit shall be a financial audit performed in accordance with such guidelines and in accordance with generally accepted government auditing standards issued by the Comptroller General of the United States. Audits shall be performed annually.

(c) The audit report shall be submitted to AID within 30 days after completion of the audit, but the audit shall be completed and the report submitted not later that 13 months after the close of the grantee's fiscal year. The AID Inspector General will review this report to determine whether it complies with the audit requirements of this grant. No audit costs may be charged to this grant if audits have not been made in accordance with the terms of this provision. In cases of continued inability or unwillingness to have an audit performed in accordance with the terms of this provision, AID will consider appropriate sanctions which may include suspension of all or a percentage of disbursements until the audit is satisfactorily completed. *

(d) The grantee shall require subgrantees that receive $25,000 or more per year under this grant to have audits performed in accordance with the requirements of this provision. The subgrantee's audit report shall be submitted to the grantee within 30 days after completion of the audit, but the audit shall be completed and the report submitted not later than 13 months after the close of the subgrantee's fiscal year. The grantee shall ensure that appropriate corrective actions are taken on the recommendations contained in subgrantees' audit reports; consider whether subgrantees' audits necessitate adjustment of its own records; and require each subgrantee to permit independent auditors to have access to records and financial statements as necessary. *

3. REFUNDS (SEPTEMBER 1990)

(a) The grantee shall remit to AID all interest earned on funds provided by AID.

(b) Funds obligated by AID but not disbursed to the grantee at the time the grant expires or is terminated shall revert to AID, except for such funds encumbered by the grantee by a legally binding transaction applicable to this grant. Any funds advanced to but not expended by the grantee at the time of expiration or termination of the grant shall be refunded to AID except for such funds encumbered by the grantee by a legally binding transaction applicable to this grant.

(c) AID reserves the right to require refund by the grantee of any amount which AID determines to have been expended for purposes not in accordance with the terms and conditions of this grant, including but not limited to costs which are not allowable in accordance with the applicable Federal cost principles or other terms and conditions of this grant. In the event that a final audit has not been performed prior to the closeout of this grant, AID retains the refund right until all claims which may result from the final audit have been resolved between AID and the grantee.

4. REVISION OF GRANT BUDGET (MAY 1986)

(a) The approved grant budget is the financial expression of the grantee's program as approved during the grant award process.

(b) The grantee shall immediately request approval from the grant officer when there is reason to believe that within the next 30 calendar days a revision of the approved grant budget will be necessary for any of the following reasons:

(1) To change the scope or the objectives of the project and/or revise the funding allocated among project objectives.

(2) Additional funding is needed.

(3) The grantee expects the amount of AID authorized funds to exceed its needs by more than $5,000 or five percent of the AID award, whichever is greater.

* Revised

(4) The grantee plans to transfer funds budgeted for indirect costs to absorb increases in direct costs or vice versa.

(5) The grantee intends to contract or subgrant any of the work under this grant, and such contracts or subgrants were not included in the approved grant budget.

(c) Except as required by other provisions of this grant specifically stated to be an exception from this provision, the Government shall not be obligated to reimburse the grantee for costs incurred in excess of the total amount obligated under the grant. The grantee shall not be obligated to continue performance under the grant (including action under the "Termination and Suspension" provision) or otherwise to incur costs in excess of the amount obligated under the grant, unless and until the grant officer has notified the grantee in writing that such obligated amount has been increased and has specified the new grant total amount.

5. TERMINATION AND SUSPENSION (MAY 1986)

(a) For Cause. This grant may be terminated for cause at any time, in whole or in part, by the grant officer upon written notice to the grantee, whenever it is determined that the grantee has failed to comply with the conditions of the grant.

(b) For Convenience. This grant may be terminated for convenience at any time by either party, in whole or in part, if both parties agree that the continuation of the grant would not produce beneficial results commensurate with the further expenditure of funds. Both parties shall agree upon termination conditions, including the effective date and, in the case of partial terminations, the portion to be terminated. The agreement to terminate shall be set forth in a letter from the grant officer to the grantee.

(c) Suspension: Termination for Changed Circumstances. If at any time AID determines that continuation of funding for a program should be suspended or terminated because such assistance is not in the national interest of the United States or that it would be in violation of an applicable law, then AID may, following notice to the grantee, suspend this grant and prohibit the grantee from incurring additional obligations chargeable to this grant other than necessary and proper costs in accordance with the terms of this grant during the period of suspension. If the situation causing the suspension continues for 60 days or more, then AID may terminate this grant on written notice to the grantee and cancel that portion of this grant which has not been disbursed or irrevocably committed to third parties.

(d) Termination Procedures. Upon receipt of and in accordance with a termination notice as specified in either paragraph (a) or (b) above, the grantee shall take immediate action to minimize all expenditures and obligations financed by this grant and shall cancel such unliquidated obligations whenever possible. Except as provided below, no further reimbursement shall be made after the effective date of termination. The grantee shall within 30 calendar days after the effective date of such termination repay to the Government all unexpended AID funds which are not otherwise obligated by a legally binding transaction applicable to this grant. Should the funds paid by the Government to the grantee prior to the effective date of the termination of this grant be insufficient to cover the grantee's obligations in the legally binding transaction, the grantee may submit to the Government within 90 calendar days after the effective date of such termination a written claim covering such obligations. The grant officer shall determine the amount(s) to be paid by the Government to the grantee under such claim in accordance with the applicable cost principles.

6. DISPUTES (NOVEMBER 1985)

(a) Any dispute under this grant shall be decided by the AID grant officer. The grant officer shall furnish the grantee a written copy of the decision.

(b) Decisions of the AID grant officer shall be final unless, within 30 days of receipt of the decision of the grant officer, the grantee appeals the decision to AID's Deputy Assistant to the Administrator for Management Services. Any appeal made under this provision shall be in writing and addressed to the Deputy Assistant to the Administrator for Management Services, Agency for International Development, Washington, D.C. 20523. A copy of the appeal shall be concurrently furnished to the grant officer.

(c) In order to facilitate review on the record by the Deputy Assistant to the Administrator for Management Services, the grantee shall be given an opportunity to submit written evidence in support of its appeal. No hearing will be provided.

(d) A decision under this provision by the Deputy Assistant to the Administrator for Management services shall be final.

7. INELIGIBLE COUNTRIES (MAY 1986)

Unless otherwise approved by the AID grant officer, funds will only be expended for assistance to countries eligible for assistance under the Foreign Assistance Act of 1961, as amended, or under acts appropriating funds for foreign assistance.

8. DEBARMENT, SUSPENSION, AND OTHER RESPONSIBILITY MATTERS (MARCH 1989)

(1) The grantee certifies to the best of its knowledge and belief, that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;

(b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and

(d) Have not within a three-year period preceding this application/propsal had one or more public transactions (Federal, State or local) terminated for cause or default.

(2) The grantee agrees that, unless authorized by the Grant Officer, it will not knowingly enter into any subagreements or contracts under this grant with a person or entity that is included on the "Lists of Parties Excluded from Federal Procurement or Nonprocurement Programs". The grantee further agrees to include the following provision in any subagreements or contracts entered into under this grant:

DEBARMENT, SUSPENSION, INELIGIBILITY, AND VOLUNTARY EXCLUSION (MARCH 1989)

The recipient/contractor certifies that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.

(3) The policies and procedures applicable to debarment, suspension and ineligiblity under AID-financed transactions are set forth in 22 CFR Part 208.

9. U.S. OFFICIALS NOT TO BENEFIT (NOVEMBER 1985)

No member of or delegate to the U.S. Congress or resident U.S. Commissioner shall be admitted to any share or part of this grant or to any benefit that may arise therefrom; but this provision shall not be construed to extend to this grant if made with a corporation for its general benefit.

10. NONLIABILITY (NOVEMBER 1985)

AID does not assume liability for any third party claims for damages arising out of this grant.

11. AMENDMENT (NOVEMBER 1985)

The grant may be amended by formal modifications to the basic grant document or by means of an exchange of letters between the grant officer and an appropriate official of the grantee.

12. NOTICES (NOVEMBER 1985)

Any notice given by AID or the grantee shall be sufficient only if in writing and delivered in person, mailed, or cabled as follows:

To the AID grant officer, at the address specified in the grant.

To grantee, at grantee's address shown in the grant or to such other address designated within the grant.

Notices shall be effective when delivered in accordance with this provision, or on effective date of the notice, whichever is later.

(END OF MANDATORY STANDARD PROVISIONS)

The Tea Research Foundation of Kenya

ALL COMMUNICATIONS SHOULD BE ADDRESSED TO THE DIRECTOR

P.O. BOX 820
KERICHO
KENYA

Please quote reference: OS/A/2

Tel. KERICHO 20598/20599

FAX: 254-0361-20575

10th May, 1991

Miloslav Rechcigl Jr.,
Research Review Director,
Office of the Science Advisor,
AID/SCI. Room 320 SA-18,
Agency for International Development,
WASHINGTON D.C. 20523-1818,
U.S.A.

Dear Dr. Miloslav,

Thanks for your letter of the 26th November, 1990 and the good news concerning the favourable response of the review panel to our project [redacted]

[redacted] areas the reviewers thought should be emphasized more. The [redacted] title therefore reads "Speciation of Armillaria spp. causing root rot disease of tea and its biological control in Kenya".

As per the recommendation of the review panel, I had to consult with Dr. Wargo the USA Co-investigator to further focus the objectives and narrow the scope of the project. This did take a lot of time hence the delay. It is my sincere hope that you will bear with us.

The following is an outline of the concern and provisos and our reactions to them.

1. The scope of the project

The reviewers thought the scope of the project in relation to host/parasite relations was overstated. We have now restricted the study to Armillaria/Camellia sinensis pathosystem. Because of the different governmental restrictions on movement of pathogens and entry procedures for non-citizens, the project initially has been restricted to Kenya thus giving us only two collaborating countries - Kenya and U.S.A.

2. Selective media studies

This aspect will concentrate on modification of the existing media rather than starting from scratch. According to Dr. Wargo (letter enclosed), the literature on selective media for Armillaria is not vast, so the phenol-benomyl and streptomycin medium will be tried and modification experiments tried to improve its recovery efficiency and selectivity for the Kenyan Armillaria isolates.

MAY 29 1991

3. Characterization of Armillaria populations before employing biological control

The revised project has given this aspect more attention. Dr. Wargo has consulted Dr. Carolynn Mohammed of Oxford University in England to help in this regard and she is willing (Dr. Wargo's letter to me, enclosed). Sections 3.1. and 3.2. cover this.

4. Cross protection

The revised project has included the aspect of exploring possibilities of using non-pathogenic Armillaria to manage pathogenic strains of the fungus (see section 3.3).

5. Identity of A. mellea

No specific work has been carried out by the principal investigator on the taxonomy of the root rot fungus of tea. But according to David Pegler of the Royal Botanic Gardens, Kew (personal communication) the tea root rot fungus is A. mellea. Since this project will address to species characterization, this aspect will be sorted out with certainity (see sections 3.1 and 3.2).

6. Does the author have suitable antagonistic isolates to Armillaria?

Yes the author has 11 isolates of Trichoderma which have shown varied antibiotic properties to Armillaria root rot fungus of tea. This work has been written up and submitted to plant pathology. The paper is entitled "Antibiosis of Kenyan Trichoderma isolates to Armillaria mellea a root rotter of tea".

7. Tests for antibiosis of antagonists

The tests described in the project (11.135) section 6 will be used. The detailed methods are contained in the "Antibiosis of Kenyan Trichoderma isolates" Paper.

8. Educational problems on intricate amendment programme of organic materials

This aspect has been included in the revised version of the project section 5.2.

9. Application methods in respect to heavy machinery to be addressed

This aspect is tackled under section 5.2 of the revised version of the project.

10. Isozyme studies

Isozyme studies have not been included in the revised project as per the recommendation of one of the reviewers. This has subsequently resulted into elimination of an electrophoretic machine from the budget.

11. Soil moisture studies

This aspect was questioned by one of the reviewers. It has however been left intact because it will answer one very practical question. The tea growing areas are high rainfall areas with a short spell of drought of about 3 months. So this aspect will help to determine whether organic material amendment will be more beneficial if applied during the wet or dry seasons. The benefits referred to here are the proliferation of antagonists. So the fine soil moisture level variations are not critical in this study.

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12. Disease loss assessment information

This has been tackled on page 7 under Background and rationale in the revised project.

13. Why is tea expanding to forest areas

This has been tackled on page 4 under the potential benefits of this research project. But just to mention it again, it is a Kenyan government policy to plant tea along the forest belts so that it acts as a barrier against illegal entrants into forest reserves.

Visits by the U.S. Cooperator

These were tagged at U.S.$36,000 but have been scaled down to $21,000 (see summary of the budget). This modification was prompted by the feeling by one of the reviewers that the cost was a bit on the higher side.

Handling of rights

The rights or any patents as a result of this work will be shared by the principal investigator and the U.S. Co-investigator.

I have tried to respond as much as possible to the comments and suggestions by the reviewers: This has resulted into a modified version of the proposal which I now forward to you for re-evaluation.

Enclosed please find the following:-

1. The revised version of the proposal.
2. Three years audited statements of accounts.
3. A financial information sheet from the Finance Manager.
4. Assurance of compliance with laws and regulations.
5. Certification regarding Drug-free workplace requirements.
6. Certification regarding lobbying.
7. A. C.V. for Dr. Caleb Othieno, a soil scientist co-investigator.

Yours sincerely,

James M. Onsando,
Pathologist.

JMO/jkm.

Encls:

MAY 28 1991

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Review pol process

SPECIATION OF *ARMILLARIA* SPP. CAUSING ROOT ROT DISEASES OF TEA AND ITS BIOLOGICAL CONTROL IN KENYA

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I. a. SPECIATION OF *ARMILLARIA* spp. CAUSING ROOT ROT DISEASES OF TEA AND ITS BIOLOGICAL CONTROL IN KENYA

b. Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya.

c. Proposal number 11.135

d. (i) Principal investigator - James M. Onsando, Plant Pathologist, Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya. Tel. 20598/20599, Kericho. Fax 254-0361-20575.

(ii) Co-investigators - Philip M. Wargo, Research Plant Pathologist, North-eastern Forest Expt. Station, 51 Mill Pond Road, Hamden CT 06514, U.S.A. Tel. 203-773-2030; S.W. Waudo, Senior Lecturer of Plant Pathology, Botany Dept., Kenyatta University, P.O. Box 43844, Nairobi, Kenya; Caleb O. Othieno, Soil Scientist and Director, Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya. Tel.20598/20599, Kericho, Kenya. Fax 254-0361-20575.

e. (i) Dr. Caleb Othieno, Director, Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya. Tel. 20598/20599, Kericho, Kenya. Fax 254-0361-20575.

Signature/Date..... .....

(ii) Dr. Denver P. Burns, Director, North-eastern Forest Experimental Station, 5 Radnor Corporate Center, 100 Matisonford Rd, Suite 200, Radnor, PA, 19087 Tel. 215-975-4017.

Signature/Date..... .....

f. (i) Proposed total cost - US$ 150,000
(ii) Project duration - 5 years starting may 1992.

g. Collaborating countries - U.S.A.

- Kenya

h. Speciation ecology management of *Armillaria mellea* by indigenous antagonists.

II. SCIENTIFIC SUMMARY

Tea is a very important crop in the eastern, southern and central African regions where the economy is predominantly agricultural based. In Kenya it ranks second to coffee as a foreign exchange earner and provides a livelihood to more than a million people. *Armillaria* root rot is the most significant tea disease in the regions in terms of yield reductions particularly for smallholders.

The root disease fungus *Armillaria* is one of the most prominent killers and decayers of deciduous and coniferous trees and shrubs in natural forest stands, plantations, orchards and gardens throughout the world. Because of its destructive nature, it is a global tea disease of major concern

It is now generally accepted that *Amillaria* root rot found in different parts of the world is in fact a complex of similar diseases caused by distinct *Armillaria* species which differ in their ecology and pathogenicity. Even within *Armillaria mellea*, the potential for sub-terranean growth creates the possibility for development of clones. Clones of *A. mellea*, collected from areas remote from each other have shown remarkable differences in virulence and disease epidemiology. Whereas species differentiation, characterisation and clonal variability has been documented for other parts of the world, the same is lacking for the African continent, Kenya being no exception. Since these genetic differences within this fungus exhibit different virulence and pathogenicity characteristics, then the need to carry out speciation and clonal differentiation and characterisation in the growing areas in Kenya as pre-requisite to management studies of the fungus is necessary. This is particularly so because the probable management package will be influenced by the mentioned genetic differences of the fungus.

Being a soil -borne pathogen, any sound management package will have to depend on a thorough understanding of the ecology of the fungus. The understanding however has not been possible without a technique to selectively recognize *Armillaria* amidst a myriad of diversified microorganisms in the soil.

A few management strategies against *Armillaria* root rot of tea are in force, but none of them is practical considering the local tea growing conditions in the region. For example, soil fumigants like carbon disulphide, methyl bromide, Shell D.D. proved effective in India, but the hazards, logistic problems of application and costs has made adoption of the strategy impossible. Complete removal of all substrate sources in the soil by uprooting and destroying all root remnants has been advocated. This method however is machinery and labour intensive given that the current expansion of tea is taking place on former forest reserves, because as a matter of policy, the Kenya government has established a tea belt along forest areas to check movement of people into government forest reserves. The heavy machinery

element also has a financial implication on the growers who are basically very poor. Ring-barking of forest trees for a period of 1 to 2 years to reduce starch reserves in the roots and hence minimize food source for the fungus is a very delicate method in itself, in that if the cut is made too deep, the tree dies back rapidly as if it was directly felled. On the other hand if the cut is shallow, regeneration of the bark takes place. In practice therefore this method could be carried out wrongly, going by the literacy level of the tea smallholders in Kenya. This therefore calls for a more practical, cheap and relevant strategy of management of root rot of tea. The strategy the investigators have in mind is biological control of *A. mellea* by enhancing indigenous soil antagonists to the fungus. The project objectives therefore are:

1. Develop a selective medium for *Armillaria* root rot fungus of tea .

2. Determine where *Armillaria* root disease on tea is severe in Kenya and where it is not.

3. Determine what species of *Armillaria* are causing root disease of tea and if different species of the fungus are related to differences in disease severity.

4. Determine the spatial relationships of genotypes within and between species in diseased plantations and if they are mutually exclusive.

5. Determine the biological, chemical and physical properties of the rhizosphere, rhizoplane and soils in tea plantations where incidences and severity of root rot disease is high and where it is low or non-existent.

6. Manipulate soil biological, physical and chemical properties to favour proliferation of *Trichoderma* spp. and other indigenous microbial antagonists to *Armillaria*.

7. Determine whether the antibiosis of *Trichoderma* spp. and other antagonists to be established will be influenced by the specific species or clone of *A. mellea*.

The uniqueness of this project is that we shall endeavour to establish innate kenyan soil factors that are antagonistic to *Armillaria*. Secondly, the project will attempt to enhance the proliferation of indigenous microbial antagonists to *Armillaria* fungus.

The potential benefits of the research would include:

(i) Easy adoption of the technology due to use of non-toxic materials.
(ii) Avoidance of areas whose soil properties favour the inoculum build-up.

(iii) Tea expansion into forest reserves as a result of the Kenyan government policy to use tea as a barrier against illegal entrants into forest reserves, will go on with minimal risks.

(iv) The bio-control package could be adopted by other tea growing countries in the region with minimal modifications.
(v) The package will save Kenya and other would-be users foreign exchange that they so much need,

BODY OF THE PROPOSAL

A. OVERALL AIM AND SPECIFIC OBJECTIVES

The project aims at differentiating and characterising of the tea root rot fungus, and developing a cheap and easily adaptable biological control package for use by tea farmers in Kenya and adjacent areas against root rot, *A. mellea*.

The specific objectives of the project are:

(i) Development of a selective technique to study the ecology of *A. mellea* in the soil.

(ii) Determination of where the *Armillaria* root rot is severe and where it isn't.

(iii) Determination of what species of *Armillaria* are causing root disease of tea and if different species of the fungus are related to differences in disease severity.

(iv) Determination of the spatial relationships of genotypes within and between species in diseased plantations and if they are mutually exclusive..

(v) Determination of the biological, chemical and physical proprties of the rhizosphere, rhizoplane and soils in the plantations where incidence and severity of root rot disease is high and where it is low or non-existent.

(vi) Manipulation of soil biological, physical and chemical properties to favour proliferation of *Trichoderma* spp. and other indigenous microbial antagonists to *Armillaria* root rot fungus.

B. RELEVANCE OF THE PROJECT TO DEVELOPMENT

Agency for International Development (A.I.D.), as the name suggests is a body whose basic objective is to promote economic development internationally. Development of technologies geared towards solving problems facing certain facets of the international community or just the entire international community and will have a major economic implication in the beneficiaries will be of interest to A.I.D.

The root rot fungus *A. mellea* is a very cosmopolitan pathogen with an extremely wide host range. In the developing and even developed countries, the fungus attacks crops of major economic importance e.g. fruit trees, a wide range of forest trees and for the Eastern Africa region tea, *Camellia sinensis*. For the developing countries the cost of a technology to a farmer is the most important aspect. This is more so, because the farmers involved are of low literacy level to appreciate sophisticated technologies and are very poor even to afford them. If this biological control package is fully developed for the tea and indications from preliminary work are promising, then the same can be adopted with modifications for other tea growing countries in the East Africa region. For the developing countries and particularly the Eastern Africa region, some of the organic amendments that have shown positive preliminary indications are easily available. This will enhance their use in the effort to try to keep *Armillaria* under manageable levels.

No likely side effects are anticipated because each region will have to promote or enhance existing soil microbial antagonists to *Armillaria*. The substrates for the antagonists will be those that are locally available and might have to vary from region to region, but for the East African region, the tea soils are to a lot of extent similar, and the agricultural organic wastes tend to be common.

The cost effectiveness of the technology anticipated to be developed has been mentioned or implied. The target country/countries intended to immediately benefit are economically troubled countries with no sufficient foreign exchange reserves to be able to afford the alternatives of management of *A. mellea* that exist. IF forced by circumstances to afford, the profit margins will be reduced drastically. So any reduction of costs of production of tea whose importance has been discussed elsewhere in this project will go along way in enhancing the respective economies of the country/countries affected and hence their development.

C. INNOVATIVE ASPECTS

The uniqueness of the proposed research is that it will attempt to investigate probable innate potential of certain Kenyan tea soils to suppress *Armillaria*. It will also try to identify the probable source of suppressiveness and explore possibilities of harnessing the same for management of the disease.

The project will also attempt to explore the possibilities of using less virulent species of *Armillaria* to discourage infection by the more virulent species (cross protection).

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The other uniqueness is that in biological control of plant pathogens, the tendency has been importation of biological control agents, developed in first world countries by third world countries. This has tended to result in ecological imbalances in certain cases due to introduction of an organism whose behaviour in the new environment cannot be ascertained immediately. The emphasis in this project however is to enhance multiplication and general growth of the probable indigenous soil microbial antagonists by identifying locally available substrate(s) for them.

There is also a chance that the technology developed for tea could be useful with modifications to other pathosystems.

D. STRENGTHENING INNOVATIVE RESEARCH CAPACITY OF DEVELOPING COUNTRY INSTITUTIONS

The Tea Research Foundation of Kenya where most laboratory work will be carried out, has a department of plant pathology. The department has a laboratory with basic equipment, reagents, chemicals, glassware for pathological work. As an appendage to this the department has a greenhouse that is operational. The department is manned by a Plant Pathologist with more than a decade post-college experience. Assisting the plant Pathologist are four technicians.

The project proposed here, is on-going but on a narrower scope. The initial results are encouraging. The published initial results are enclosed.

To facilitate the project, the following capital equipment will be necessary as shown in the budget justification.

1. Two incubators

2. Laminar flow cabinet

3. A sterilizer.

4. A phase contrast microscope with camera facility.

The equipments will supplement the existing facilities and will cost approximately $30,000.

Since th project has started in a small way and is wholly funded by the tea industry, Kenya, there is a lot of possibilities that it will continue after the five-year duration. The assistance will definitely enhance other aspects like speciation of *Armillaria* and exploration into possibilities of cross protection as a management strategy.

The capabilities of the principal investigator could be assessed by a list of his publications which will appear in his curriculum vitae.

The U.S.A. collaborating scientist (Dr. Phillip Wargo) has worked with *Armillaria* for over 25 years. His participation in the project will not only be of phenomenal contribution but will act as a training and enhancement of the existing expertise on *Armillaria* for the personnel from this part of the world.

The collaborating Soil SCientist (Dr. Caleb Othieno) has a working experience of over 20 years on Kenyan tea soils. Because non-biological soil aspects as they relate to tea root rot fungus will be tackled as well, his participation in the project will be an immense asset. His C.V. has been enclosed for evaluation.

E. BACKGROUND AND RATIONALE

Tea is a very important crop in th eastern/southern and central African region where the economy is predominantly agricultural based. In Kenya it ranks second to coffee as an earner of foreign exchange and provides a livelihood to more than a million people (anon, 1987). For the last five years tea has provided Kenya with an average of over two hundred and twenty million US dollars (US$220 million) per year (Anon, 1989). Tea diseases are a major limiting factor in the region and other tea growing areas of the world (Carr, 1972; Venkata Ram, 1970 and Sudoi, 1984). Of these diseases, root rot, *A. mellea* is the most important in Kenya. For *Armillaria*, disease incidence is equivalent to severity. Once a tea disease is infected it dies. Countrywide yield loss studies have not been carried out but it is not uncommon to see losses of up to 50% among the smallholder tea farmers. The potential for crop loss is anticipated to even increase in the newly-established tea belt along the forest reserves for reasons that have been explained elsewhere.

The root disease fungus *A. mellea* is one of the most important killers and decayers of deciduous and coniferous trees and shrubs in natural forest stands, plantations, orchards and gardens throughout the world (Wargo and Shaw III, 1985). Because of its destructive nature, *Armillaria* root rot is a disease of major concern in all tea growing areas of the world (Leach, 1939; Hulbert, 1957; Gibson and Goodchild, 1960; Venkata Ram and Davasayam, 1974; Onsando, 1986; onsando *et al*, 1989). Being a soil-borne pathogen, any sound management package will have to depend on a thorough understanding of the ecology of the fungus. The said understanding however has not been possible due to lack of a selective technique to selectively recognize *Armillaria mellea* amidst other soil microbial residents.

Selective media

Soil-borne pathogenic fungi are a distinct minority amidst a myriad of diversified microorganisms (Tsao, 1970). Understanding aspects of these pathogens such as inoculum density, population dynamics, saprophytic behaviour and survival has a tremendous bearing on their management. The development of a selective medium is based on the principle of selective exclusion of undesired microorganisms, thus permitting the preferential establishment of the desired fungi on the isolation medium. This in most cases is achieved by use of selective antimicrobial chemical agents (Tsao, 1970; Elad, 1981; Vaartaja, 1960; Farley, 1972), narrowing nutritional requirements to the specific fungus (Flowers and Hendrix, 1969; Lingappa and Lockwood, 1962), use of dyes as selective differentiation tools through pigmentation (Fung and Miller, 1973; Tsao, 1970).

Basidiomycetes, in which *A. mellea* falls are taxonomically and to a great extent ecologically distinct from members of other classes of fungi and as such should differ also in some physiological traits and to toxicological reactions to inhibitors (Tsao, 1970). But reports on the selective media for basidiomycetes are few. One of the early ones was developed by Russell in 1956 and its selectivity was based on orthophenylphenol. The literature on selective media for *A. mellea* is not vast. A combination of phenol-benomyl and streptomycin seems to be "best" (Philip Wargo, personal communication). This project will attempt to ascertain the level of selectivity and recovery efficiency of phenol-benomyl and streptomycin on Kenyan *Armillaria* isolates and establish whether the medium could be used for ecological studies of the fungus. As a safety valve all the other protocols on development of a selective medium will be left intact in case a modification needs to be done.

Speciation of *Armillaria* and clonal existence within *Armillaria mellea*

The speciation of *Armillaria* spp. from the continent of Africa is very scanty. This has been attributed in part to be due to scarcity of basidiosomes (Mohammed *et al*, 1990). Only two species have been described in any detail by the classical taxonomists from the continent; *A. heimii* (*=clitocybe elegans*) and *A. mellea sensu stricto* (Pegler, 1977 and 1986; Kile and Watling, 1987). In Kenya *A. heimii* and *A. mellea* have been described (Pegler, personal communication). It is now generally accepted that the *Armillaria* root rot found in different parts of the world is in fact a complex of similar diseases caused by distinct *Armillaria* species which differ in the ecology and pathogenecity (Mohammed, *et al*, 1990). In Europe, Australia and North America, differentiation and characterization of the *Armillaria* species present has been done (Anderson, 1986; Kile and Watling, 1983; Korhonen, 1978; Guillaumin, 1986). For Africa and indeed Kenya this needs to be done because the inevitable species pathogenicity differences will influence management of

the disease.

The potential for sub-terranean growth by *Armillaria mellea* creates the possibility for development of clones that traverse multiple hosts (Anderson *et al*, 1979). Clonal identification of *Armillaria mellea* was first demonstrated in a ponderosa pine forest in south Central Washington (Adams, 1974). In this study out of 243 isolates of the fungus, three clones emerged. Persistence and clonal distribution studies in the same forest indicated that all isolates from within a 600-hectares area, which included several root rot infection centres, belonged to a single clone. Continuity of the clone over the areas suggested that fungal distribution has been by vegetative growth from a single point of origin rather that by spores (Shaw III and Roth, 1976). Clones of *A. mellea* collected from areas remote from each other have shown remarkable differences in virulence and disease epidemiology (Wilbur *et al*, 1971). This aspect has made the study of clonal or genetic differences within the *Armillaria mellea* fungus very important. In Kenya and the adjacent areas, tea is grown in different agro-ecological zones and *Armillaria mellea* is a very important pathogen. The mode of spread of this disease traversing all the mentioned zones is not understood. Secondly, whether the fungus is of one genetic origin regionally and/or nationally is equally unknown. Whether clonal types of *A. mellea* of tea exist and their possible significance in the epidemiology and management of the disease in the region and/or the nation are unknown.

Control and management

Control and management of *Armillaria* root rot is made difficult due to the fact that the fungus is soil-borne and soil environment is complex. In forestry, where most management studies have been carried out, economic constraints have tended to hamper the application of some of the management strategies (Shaw III and Roth, 1977; Wargo and Shaw III, 1985).

Use of resistant sources was tried for pine but none of the pine trees showed high enough levels of resistance to be utilized commercially (Morquer and Touvet, 1972; Risbeth, 1972). Although some clones of tea (6/8 and 11/40) have tended to exhibit higher susceptibility to *Armillaria* root rot, no specific commercial clone has shown high levels of resistance (Onsando, unpublished).

Avoidance of sites with high disease hazard when establishing new plantations has been suggested (Gibson, 1975). This approach is not practical because the tea areas and forest areas in Eastern Africa region are high potential areas for agriculture and therefore are highly populated thus making alternative sites rare.

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Complete removal of all substrate sources in the soil by removing diseased trees, uprooting and destroying stumps and root remnants (Horne 1974; Sokolov, 1964) sounds attractive but expensive for low value crops like forest trees. The method is equally expensive for the small tea holders in the East African region due to the heavy machinery required.

In East Africa, indigenous forest trees form an alternate host for the tea root rot fungus. Ring barking of forest trees for a period of 1 to 2 year, prior to tea planting has been the recommendation (Leach, 1939; Gibson and Goodchild, 1960; Goodchild, 1959). This recommendation is meant to deplete starch rserves and hence food source for the fungus that would otherwise be locked in the roots of the trees. Although ring barking is the only preventive measure available against *Armillaria*, it is very technical in itself in that if the cut is too deep, the tree dies back rapidly as if it was felled. On the other hand, if the cut is shallow, regeneration of the bark takes place (Leach, 1939). This therefore calls for a more practical method that takes the literacy level of the users into consideration.

Management of *Armillaria* by chemical means has not made a lot of progress mainly due to lack of s soil fungicide with high activity against soil-borne basidomycetes. The other constraint is the fact that levels of chemical efficacy against soil-borne fungi are higher with soil fumigants which are very expensive. Most soil fumigants are also pre-plant and a lot of tea growers will prefer a product which will kill the fungus *in situ* and spare the crop. Despite the mentioned drawbacks chemical management of *A. mellea* especially in forest trees has been tried but with low to medium success (Pawsey and Rahman, 1974 and 1976; Rykowski, 1974; Filip an Roth, 1977).

In citrus, carbon disulphate (CS_2) controlled *A. mellea*, as long as the fumes penetrated all infested soil in sufficient concentration to allow *Trichoderma* to become dormant in the soil microflora (Blis, 1951). In this work, it so happened that carbon disulphide favoured the colonization of citrus roots rhizosphere and rhizoplane with *Trichoderma*. a common soil mould which had antibiotic activity against *A. mellea*. Hence the control was not entirely due to fumigant action. In India, an experiment initiated in 1972 (Anon, 1974) against *A. mellea* of tea indicated that carbon disulphide, Methyl bromide, Shell D-D and Metham sodium killed the fungus *in situ*. The antibiosis phenomenon by *Trichoderma viride* was observed too (Anon, 1974). The potential of *Trichoderma* spp. as an antagonist to soil-borne fungal plant pathogen has been extensively documented (Sivan *et al* 1987; Liu and Baker, 1980; Rodriguez-Kabana *et al*, 1978; Biles and Hill 1988; Hader et al 1979; Elad and Hadar, 1981; Elad *et al*, 1980; Cheng *et al*, 1986; Chet *et al*, 1981; Tronsmo and Dennis, 1977; Nobuo and Kilachiro, 1968). Artoun, 1952 demonstrated in vitro parasitism between ten *Trichoderma* isolates and *Armillaria mellea*. In his studies the type of parasitism was pH dependent.

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Considering the tea situation in East African region and particularly in Kenya and in view of the constraints of the existing management strategies of *A. mellea* that have already been discussed, biological control of the disease will be most ideal. While the ideal nature of biocontrol is well recognized, control of *Armillaria* by introducing organisms is difficult (Risbeth, 1976). This has been attributed to the positional advantage of *A. mellea* over the introduced biocontrol agents. Despite this, a few cases of biocontrol of *A. mellea* have been documented for forest trees (Peno et al, 1975; Eghbaltalab, 1975; Rifle, 1973). None of these cases however is of commercial significance. *Trichoderma virde* has been implicated although without direct evidence, to be a possible contributor to reduction of *Armillaria* on tea (Anon, 1974). The difficulty in maintenance of effective populations of antagonists to *Armillaria* under field conditions has been noted as a main factor limiting successful biological control (Shaw III and Roth, 1978). This therefore calls for the need to manipulate soil properties to favour the proliferation of *Trichoderma* spp. and other probable microbial antagonists to *Armillaria*.

Kenyan tea soils have low levels of indigenous *Trichoderma* spp. (an approximate average of $1x10^4$ propagules/g of soil) (Onsando, unpublished). We know that soil microbial population and activity is dependent on factors such as, organic matter status, inorganic salts, pH, moisture, etc. (Alexander, 1976). Therefore manipulation of any of the mentioned factors should alter the population and activity of *Trichoderma* spp. and other microbial antagonists to *Armillaria* to be determined in the course of the study.

Organic amendments

Organic amendments have been used in the management of soil-borne plant pathogens. Although the physical chemical and biological interactions in soil are so complex and varied for easy determination of specific effects responsible for disease control (Huber and Watson, 1970), there is every evidence implicating microbial participation (Burke, 1954; Williams and Hacks, 1957). The mechanism of biological control as enhanced by organic amendments, involves specific microbial interactions and host physiology. The microbial interactions referred to here include competition for nutrients, space, microbial antibiosis, hyperparasitism and lysis. Greenhouse studies on Avocado root rot, *Phytophthora cinnamomi* indicated that soil amendments of alfalfa meal and cotton waste controlled avocado root rot (Zentimeyer, 1963; Gilpatrick, 1969). this was attributed to microbial antagonism. Composts prepared from agricultural and industrial organic wastes have been recently shown to have significant suppressive properties to many soil -borne pathogens (Chung, 1989). The suppressiveness is mainly microbial in origin. Work done in Kenya on six different locally available organic amendments revealed significant enhancement of *Trichoderma* spp. in the soil by coffee pulp (Onsando *et al*, 1989). Further work

showed antibiosis of the *Trichoderma* isolates to *Armillaria* isolates from two regions in Kenya. The antibiosis varied from isolate to isolate even within the same species (Onsando and Waudo, 1991).

The hypothesis here is that determination of a suitable substrate (organic amendment) for *Trichoderma* and other microbial antagonists against *A. mellea* will keep the pathogen under check.

Inorganic amendments and soil microbial ecology

The biological availability of nitrogen, phosphorus and potassium is of considerable economic importance because they are the major nutrients derived from the soil. Of the three, nitrogen stands out as the most susceptible to microbial transformations (Alexander, 1977). This element is a key building block of the protein molecule upon which all life is based.

Inorganic soil amendments are widely used in agriculture as fertilizers and to change the phytochemical properties of the soil. The long and short term effects of these minerals frequently influence the microorganisms present in the environment (Henis and Katan, 1974). Inorganic amendments may affect the interaction between soil-borne pathogens and saprophytic soil residents in plant debris and in the rhizosphere. This subsequently affects the survival ability of the pathogen (Garret, 1970). Addition of nitrogenous fertilizers to the soil for example, decreased the population of antibiotic producing organisms in corn but not in wheat soil. The form of N i.e. NH4-N or NO3-N indirectly influenced the disease dynamics of take-all of wheat *Gaeumannomyces graminis* var. *tritici* by altering the number of bacteria and Streptomyctes (Smiley, 1977). In this study the NH4-N was more effective and the effect was indirect, i.e. through antagonists.

The literature dealing with the effect of inorganic amendments on soil-borne diseases is abundant and the subject is covered in reviews and books (Chapman, 1965; Davey and Danielson, 1968; Garret, 1956; Garret, 1970; Sadasivan, 1965; Christensen and Brett, 1985; Huber, *et al* 1968; Huber *et al* 1968; Huber *et al* 1980; Taylor *et al*, 1983; Trolldenier, 1981; Engel, 1968). The suppression of disease in certain cases due to source of N, has been attributed to pH changes in the rhizoshpere (Semiley and Cook, 1973). The pH changes have in turn been thought to stimulate the activity of antagonists (Smiley, 1978). Since the inoculation of the soil with antagonists is generally ineffective as a biological control method, unless the environmental conditions are conducive (Wiendling and Fawcett, 1936), the project will address the antagonists of *Armillaria* directly.

The requirement of Nitrogen by the tea plant far exceeds that of Phosphorus and Potassium due to the regular plucking (Beevers, 1967, Willson, 1975). In Kenya nitrogen is applied to tea in form of compound fertilizer NPKS 25:5:5:5, sulphate of

ammonia and urea (Wanyoko, 1983). The compound fertilizer avails nitrogen in both NO_3 and NH_4 form. Whereas the yield responses to these fertilizers are documented severally (Willson, 1967; Othieno, 1983; Wanyoko, 1983), no effort has been made to address the effects of these on the microbial ecology of tea soils and the probable microbial antagonists that could emerge and help in management of *Armillaria*. Since no yield advantage has been attributed to any of the fertilizers (Wanyoko, 1988), the probable microbial benefits should be addressed. The hypothesis here is that if the type of N fertilizer that promotes soil antagonists to *Armillaria* like *Trichoderma* spp. and others is determined, then this would contribute to reduction of *Armillaria* root rot in tea soils. This project will therefore screen the effects of types of N fertilizers on the proliferation of *Trichoderma* spp. and other soil microbial antagonists to be determined later to *Armillaria*.

pH and soil microbial ecology

Soil acidity is an extremely complex phenomenon, that is influenced by many factors such as concentration of salts and carbon dioxide in the solution, microbial activity and the exchangeable cations present (Jenny, 1961; Williams *et al*, 1970). It is one of the important factors that affect the population and activity of soil microorganisms. Of the major microbial groups in the soil, fungi are more tolerant of acidity whereas most bacteria and actinomycetes are relatively intolerant (Alexander, 1976). In highly acidic soils, fungi are regarded as the major component of the microbial population. This makes them particularly important in acid forest soils (Foth, 1978).

The effect of soil pH on soil-borne plant pathogens has received preferential attention over the years. This has been due to the management benefits accrued as a result of determining pH levels that are conducive to the respective crops but adverse to their corresponding pathogens (Bateman, 1961; Smiley and Cook, 1973; Jones and Woltz, 1972; Gillespie and Hurst, 1981; Baard and Pauer, 1982). For example manipulation of soil pH has been used successfully for many years in the control of potato scab, club root of crucifiers and black rot of tobacco (Bateman, 1961).

It is evident from the review that most works on soil pH manipulation for management benefits have addressed the direct effect of pH on known pathogens. The fact that very few management packages utilizing pH manipulations are of commercial value, might imply need for change of strategy to pH effects on known antagonists. In fact the inoculation of the soil with antagonists is generally ineffective as a biological control method, unless the environmental conditions are also modified to favour the antagonist (Weindling and Fawcett, 1936). This leads to the hypothesis that determination of an optimal soil pH for ascertain antagonist/s will result into the proliferation of the antagonist at the expense of the pathogen.

Moisture and soil microbial ecology

Moisture governs microbial activity because water is the major component of protoplasm and therefore an adequate supply must be available for vegetative development (Alexander, 1976). The said importance however, is thought to be indirect i.e. through moisture effects on solute diffusion and continuity of water pathways (Griffin, 1969). Considering bacteria, actinomycetes and fungi, bacteria are more prone to low moisture levels. This is thought to be due to their dependence upon continuous water pathways for their movement (Greenwood, 1967). Actinomycetes on the other hand prefer drier conditions compared to bacteria (Alexander, 1976). Fungi have been shown to have a wider soil moisture tolerance limits than all the other groups of microorganisms mentioned (Cheu and Griffin, 1966). Despite the ability of soil fungi to tolerate a wider range of moisture levels, there are several cases of soil-borne fungal plant pathogens that flourish or cause disease at specific or optimal soil moisture levels. For example, *Fusarium oxysporum* F. sp. *Cucumerium* is most favoured at 20-50% moisture content in sterilized soil and 20% in non-sterilized soil (Iida *et al*, 1982). Colonization of bean and wheat stems by *Microphomina phaseolina*, occurred at 15-25% moisture holding capacity and there was a decrease with increasing moisture (Dhingra an Chagas, 1981). Hyphal lysis of *Phytophthora cinnamomi* was most rapid in soils at or below field capacity when the temperature of incubation was 25-27°C (Nesbitt *et al*, 1979). Survival of *Rhizoctonia solani* in a natural soil was greater at intermediate water potentials (-2 to -15 bars) than in moister or drier soils (Ploetz and Mitchell, 1985). Disease incidence of cephalosporium stripe of winter wheat caused by *Cephalosporium gramineum* increased in response to increasing soil matric potential (Anderegg and Murray, 1988). While the vegetative forms of soil-borne fungal pathogens seem to have specific optimal soil moisture levels as already indicated, the resting stages seem to survive better at low moisture levels (Sheikh and Gaffer, 1980; Mukherjee *et al*, 1983; Rana and Gupta, 1983).

From th foregoing literature review, it is evident that most of the studies involving soil borne-fungi have tended to concentrate on the plant pathogens. This is so because most of the studies involving soil moisture effects have had management benefits accruing from the manipulations at the background. But one of the very few cases known, where soil moisture manipulation is a practical management strategy of a soil borne plant pathogen is that one of potato common scab *Streptomyces scabies*. For this disease use of irrigation at certain critical dry periods during the cycle of the crop, keeps it under check (Lapwood, 1966; Lapwood *et al*, 1973). This perhaps might imply that direct effects of moisture manipulations on soil borne fungal pathogens might not be a very promising area. This could therefore necessitate a shift of emphasis to indirect moisture effects of

the pathogens through the soil microbial antagonists of *Armillaria*. Hence the need to look into the effect of soil moisture on *Trichoderma* spp. and other antagonists. Soil moisture has been indicated to play an important role in survival of *Trichoderma* spp. irrespective of the soil type (Wu, 1986). In this study however, types of *Trichoderma* spp. involved are not specified. This further signifies the importance of soil moisture on the population dynamics of *Trichoderma*. The hypothesis for this project is that, if the soil moisture levels are optimal for survival of *Trichoderma* and other microbial antagonists, then the other soil manipulations like organic amendments, inorganic amendments and soil pH will further enhance the proliferation.

F. TECHNICAL WORK PLAN

1. Selective medium

1.1. Preferential carbon and nitrogen sources

Armillaria mellea for these studies will be grown on potato dextrose Agar (PDA). Richard's nutrient solution will be used as the medium for fungal growth. The medium consists of KNO_3, 10g; KH_2PO_4, 5.0g; $MgSO_4$, $7H_2O$, 2.5g; $Fecl_3$, 0.02g; Sucrose 50.0g and deionised water, 1000 ml. For the carbon sources, sucrose will be the standard and will be substituted by others in the wide range that will be tested. For the nitrogen sources, KNO_3 will be the standard and the other sources will be treated as above.

1.2. Preferential pH

Richard's nutrient solutions will be used as a medium for fungal growth. The pH will be varied by use of HCl and NaOH, respectively, to various values within the range of 3 to 9. The optimum pH level will be determined by a quadratic regression analysis.

1.3. Preferential temperature

For the temperature studies, a standard medium (Malt extract, 20g; Dextrose, 20g; Bacto peptone, 5g; Agar, 19g and 1,000 ml distilled water) will be used. Temperature will be varied by use of incubators and a refrigerator. The range will be 10°C to 35°C. The optimum temperature level will be determined by quadratic regression analysis.

1.4. Choice of selective antifungal agents

This aspect will take advantage of the fact that fungi, even related ones often differ in their sensitivity to various antifungal agents (Tsao, 1970). The fact that different species of the same fungal genus often differ in sensitivity to toxicant (Papavizas, 1967) will call for spectrum tests of a few representative species. Therefore, the screening will involve a

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few known soil borne fungal pathogens and common fungal residents in the study which will have to be isolated. A wide range of fungicides with all the major chemical groups represented will be screened against *A. mellea* and all the other common soil fungal residents to be isolated along with the common soil borne fungal pathogens. The idea here will be to determine a fungicide with a wide spectrum of activity on soil borne fungal flora but least activity on *A. mellea*.

1.5. Choice of selective antibacterial agents

Unlike the search for suitable selective antifungal agents in which intra-fungal specificity is the key for success, the search for antibacterial agents for a fungal isolation medium will aim at finding materials with broadest possible spectrum against unwanted bacteria and actinomycetes. A similar medium like the one for antifungal agents will be used to grow *A. mellea*. A wide range of antibiotics will be screened to eliminate those with toxicity effects to the target fungus *(A, mellea)*. Alongside *A. mellea*, common soil resident bacteria will be screened to be able to pick an antibiotic with a wide spectrum activity on the bacteria and actinomycetes and no activity or very little activity on the fungus (*A. mellea*).

1.6. Choice of a dye for pigmentation

A number of dyes will be screened for supplementary selective differentiation through pigmentation. As for the others, soil fungal isolates will be tested together with *A. mellea* against these dyes. This will be based on the knowledge that certain microorganisms have the ability to absorb selectively the pigment from the medium to induce specific secondary pigment formation or to reduce differentially the pigment intensity in the colony or the medium. A dye without antimicrobial ability on *A. mellea* but with the desired differential pigmentation properties will be included in the medium.

Because most of the experiments in this section will be done in the laboratory, a completely randomized block design with a minimum of four replicates for each treatment will be adopted. Due to possibilities of slow growth rate of A. mellea in the respective media, slight modifications of the media might initially be necessary. This might prolong the research duration for this aspect of the project to two years.

2. Determination of where *Armillaria* root rot disease on tea is severe in Kenya and where it is not

This will be carried out through district-by-district surveys and quantification of losses of tea bushes due to *Armillaria* root rot determined. The districts will then be rated on disease severity index which will be a computation of the following:

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1. Number of farms with disease

2. Number of bushes that have died or are about to die due to *Armillaria* root rot.

3. Information on initial land preparation methods.

4. Information on the tree species that were cut prior to planting.

5. Cultural practices in operation.

Isolates of *Armillaria* will then be taken from very severe to moderate severity indices districts for species differentiation and characterization. At this stage the potential for use of cross-protection as management strategy will be assessed.

3. Characterization and differentiation of *Armillaria* species and clones and correlation of these to disease severity.

3.1. Species characterization and differentiation

Armillaria isolates originating from 10 different tea growing districts of Kenya will be used. Techniques such as mating tests, observation of vegetative mats in culture, the fruiting of isolates in culture, the comparison of those fruitbodies obtained with existing taxonomical descriptions, the study of monospore isolates from fruitbodies and microscopic examination of fruitbody tissue (hymenium) will be used (Anderson, 1936; Kile and Watling, 1983; Korkhonen, 1978; Guillaumin, 1985). It is important to note that Dr. Carolyn Mohammed of Oxford University in England is willing and able to help in species identification (Dr. Philip Wargo's letter enclosed).

3.2. Clonal characterization

The isolates will be collected as described under 3.1. and they will be subjected to Adam's method of clonal identification (Adams, 1974). The method (isolate interaction in paired culture), utilizes the criteria that pairing of isolates in which a dark line is formed along the common interface is considered dissimilar in parentage. Conversely isolates that freely intermingle when paired are considered compatible.

For this study, a basic medium (Malt extract, 3%; Glucose, 2% Peptone, 0.5%; Agar, 2%) will be used. The pH of the medium will be adjusted to 5.0 - 5.3.

The isolates will be in mycelial forms and whenever possible rhizomorphs and basidiospores will be used. The latter two are rarely present on infested tea areas in the region.

The paired isolates will be incubated for two to three weeks at 25°C in the dark (wrapped in aluminium foil).

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The paired isolates will be replicated four times just to ascertain the consistency of the results.

3.3. Correlation of probable clonal differences to virulence

The mosts susceptible tea clone (11/4) will be subjected to artificial inoculum of the probable *A. mellea* clones or strains in the greenhouse. The virulence of the strain will be assessed on the basis of rate of kill of the young tea plants and extent of kill. The artificial inoculum will be prepared by cutting tea roots of uniform diameter and length (1.5 - 2.0 cm x 10 cm) and putting them in 2-litre jars containing 200 mls of water and autoclaving then inoculating them as outlined by Wilbur (Wilbur *et al*, 1971).

4. Determination of biological, chemical and physical properties of the rhizosphere, rhizoplane and soils in tea plantations where incidence and severity of root disease is high and where it is low or non-existent.

The areas in Kenya where *Armillaria* root rot disease is severe, moderate or non-existent would have been established by the time this aspect of the project is undertaken. A series of rhizosphere, rhizoplane and soil measurements will be carried out from the specific areas for comparison purposes. For the biological aspects which will mainly be microbial, the measurements will start with known antagonist(s).

4.1. Biological soil properties both rhizosphere and rhizoplane

4.1.1. Quantification of *Trichoderma, penicillium* and Mucor propagules from ten respective areas

This will be done by modified rose bengal-streptomycin agar medium (Martin, 1950). The modification is on the amount of streptomycin. To improve the selectivity of the medium mancozan will be added at the rate of 800 ppm per litre to selectively suppress Mucor spp. (Onsando, unpublished).

4.1.2. Quantification of other fungi and determination of diversity

A general purpose medium like potato dextrose Agar (PDA) will be used. Conventional fungal taxonomic procedures will be used for diversity determination.

4.1.3. Quantification of bacteria and determination of diversity

The bacteria will be isolated and quantified by weak peptone-dextrose agar consisting of $MgSO_4.7H_2O$, 0.2g; KH_2PO_4, 0.5g; $Fe_2(SO_4)_3.9H_2O$, Trace; Dextrose, 1.5g; Peptone, 0.5g; Agar 17g per litre of distilled water. Unless stated, quantification of the respective microorganisms will be by a ten-fold serial dilution technique (Taylor, 1982).

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4.1.4. Quantification of actinomycetes and determination of diversity

Isolation, enumeration and species diversity of actinomycetes will be achieved by use of starch-casein medium with mystatin, 50µg/ml; actidione, 50µg/ml; polymyxin B sulphate, 5.0 µg/ml and sodium penicillin, 1.0 µg/ml (Williams and Davies, 1965).

4.2. Chemical soil properties (rhizosphere and rhizoplane)

The soil chemical properties that will be investigated in the respective areas will include pH, macronutrient composition (Nitrogen, phosphorus, potassium, calcium and magnesium), micronutrient composition (iron, manganese, copper, zinc, boron). The Tea Research Foundation soil chemistry department has kindly agreed to do the routine soil elemental analysis for us.

4.3. Physical soil properties (rhizosphere and rhizoplane)

Although the Kenyan tea growing soil tend to have very similar physical properties characteristics (Dr. Othieno, personal communication), there could be fine differences that might influence *Armillaria* root rot development. Therefore the following soil physical properties for the respective areas will be analysed by the soil physics department of the Tea Research Foundation of Kenya:

1. texture
2. structure
3. consistence
4. weight, pore space and air relationships
5. soil moisture regimes.

5. Manipulation of soil biological, physical and chemical properties to favour proliferation of *Trichoderma* spp. and other indigenous microbial antagonists to *Armillaria*

5.1. Use of organic amendments

A variety of locally available organic amendments will be screened for their ability to enhance the population of *Trichoderma* and other microbial antagonists to *A. mellea*. Initial work in this area has already shown great promise with coffee pulp (Onsando *et al*, 1989). The amendments will constitute main treatments while the rates will constitute subtreatments in a split plot design replicated 4 times.

The soils for this experiment will be collected from those tea areas with evidence of *Armillaria*. The environmental measurements in the greenhouse will be taken throughout the experimental durations.

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Organic carbon in the amendments will be determined by a modified Walkey-Blackman method (Black, 1965) as stipulated in Onsando's paper (Onsando *et al*, 1989). Organic nitrogen on the other hand will be determined by Kjeldahl's method (Vogel, 1961).

The population of *Trichoderma* and other probable fungal antagonists will be monitored by modified rose bengal-streptomycin agar medium (Martin, 1950). The bacteria and actinomycetes will be isolated and quantified by weak peptone-dextrose agar consisting of $MgSO_4.7H_2O$, 0.2g; KH_2PO_4, 0.5g; $Fe_2(SO_4)_3.9H_2O$, Trace; Dextrose, 1.5g; Peptone, 0.5g; Agar, 17g and 1 litre of distilled water. To quantify the respective micro-organisms, a ten-fold serial dilution technique (Taylor, 1962) will be used.

The total activity of microbial biomass to give an indication of organic turnover will be determined by Fluorescein diacetate hydrolysis (Schnurer Johan and Thomas Rosswall, 1982).

The pre-determined soil moisture level will be kept constant as outlined in Onsando's paper (Onsando and Alvarez, 1987).

5.2. Establishment of the best method and time of application of organic amendment

Armillaria root rot in tea plantations is patchy depending on disease severity, the patches of which can be very many but small in size. The application of organic amendments therefore will be restricted to these patches. Before any elaborate experiments are undertaken to determine the best method and time of application of the organic amendments, the package usage will be confined to infilling those tea plants that have died due to *Armillaria* root rot. This is intended to involve digging a hole slightly larger than the standard (40cm x 25cm), mixing the soil thoroughly with an organic amendment at a rate to be determined and returning the same during infilling. This is anticipated to minimize subsequent deaths of infills and therefore diversion of useful labour from repeated infilling exercise.

Because of the nature of the disease pattern, the use of heavy machinery is not anticipated. Manual handling like in normal planting should be able to suffice.

To generate information however on the best method and time of application of the organic amendments, a long-term experiment will be established. In this experiment, the time of application will constitute main treatments while the methods will constitute subtreatments in a split plot design with 4 replications.

This aspect of the project will be tackled after establishing that a specific amendment does help in the management of the disease.

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A location with very high natural inoculum potential of *Armillaria* will be selected for the work. The inoculum potential; will be assessed by the uniformity of dead tea bushes due to the disease.

The treatments will be as follows:

I. Main treatments

(i) No post-planting inter-row application of amendment.
(ii) One second-year inter-row application
(iii) Second and third-year inter-row application.
(iv) Second, third and fourth year inter-row application.

II. Sub-treatment

(i) A standard planting hole (40cm x 25cm), followed by amendment of the soil from the hole with an appropriate carbon rate of the amendment to 1 kg of dry soil.
ii) A planting hole of 60cm x 50cm treated as above.
(iii) A planting hole of 70cm x 65cm treated as above.
(iv) Control

5.3. Use of inorganic amendments

Four commercial fertilizers used in tea namely: Sulphate of ammonia, urea, Nitrogen, Phosphorus, Potassium and Sulphur (NPKS 25:5:5:5) and NPK 20:10:10 will be screened at the rate of 150 kg N/ha for their effect on proliferation of *Trichoderma* and other *A. mellea* antagonists. The rate of N/ha is the commercial recommendation for tea in Kenya.

The soil collection, treatment, moisture control will be carried out as outlined already.

The microbial population, activity and diversity will be determined as already outlined.

The pots in the greenhouse will be arranged in a randomized complete block design with four replications. Analysis of variance will be carried with the help of a computer.

5.4. Manipulation of soil pH

A soil survey will be carried out in old tea soils to locate a site with the lowest pH value. All the soils for the experiments will be taken from this site. The soil will be passed through a soil shredder fitted with a 1.5 cm screen to remove large particles (Weaver and Wehund, 1975). The finer soil will be homogenized and a sample taken for pH reading for confirmation purposes.

To determine the lime requirements to raise the soil pH to the desired levels, 10g samples of soil will each be put into 7 pairs of plastic beakers and treated in duplicate as tabulated

below. The Ca $(OH)_2$ stock solution used will be 2g in one litre of water.

Beaker number		1	2	3	4	5	6	7
Treatments-$Ca(OH)_2$	(Mls)	50	40	30	20	15	5	0
Water - H_2O	(Mls)	0	10	20	30	35	45	50

The beakers will be covered tightly and let stand for three days before the first pH reading is made. This will be continued until the pH change is less than 0.1 units between two successive readings in each case.

The weight of calcium/10g soil will be plotted against the resultant pH variables. This curve will be used to determine the amount of lime to be added to a given quantity of soil per pot i.e. 1000g to give the desired pH.

The other inorganic amendment i,e, $CaSO_4$ will be used on the basis of determined calcium weights to establish whether the observed effect is due to pH or just calcium and sulphur.

The substrate for microbial proliferations will be the best organic amendment that would have been determined. Therefore the organic amended and plain soil will be used in this experiment in a split plot design replicated four times. The type of soil will form the main treatment while pH values will constitute subtreatments.

The additives to the pot will be distributed uniformly by dumping the contents in a plastic bag and rolling the soil thoroughly and then placing the soil back into the pot.

The subtreatments will be five ranging from 4.0 to 7.5. The moisture content will be kept constant as already described.

All the microbial data will be taken as already discussed.

5.5. Soil moisture

Soil moisture will be varied by adding different amounts of water (0.5, 1.0, 2.0 mls etc) to samples of 10g air-dried soil and appropriate ratio of an organic amendment to be determined. The soil/organic amendment samples will be weighed and the weights recorded. The samples will be oven-dried at 110°C overnight to determine their dry weights. On the basis of the determined water content levels per mixture, a standard curve will be plotted on the amount of water added against the resulting moisture levels. The information will be extrapolated for the actual pot soils/organic amendment weights.

After each addition of the determined amounts of water, the weights of pots, soil organic amendment will be taken and recorded. The varied moisture levels will be kept constant. The pots and their contents will be weighed once a day and the lost

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water added until the balance reads the original weight (Kendrick, 1948). The moisture levels variation will range from 11% to 60% which approximately will fall between (-5 to 0 bars).

The soil moisture levels will be correlated to soil potentials (-bars) by determining a moisture release curve for the site of soil sampling. The moisture release curve will be related to the moisture levels in the greenhouse pots as long as the soil bulk density in the pots will be adjusted roughly to that of the field by compressing the soil/organic amendment to take a specified volume.

The moisture levels will constitute five treatments in a complete randomized block design with four replications.

All the microbial data and other parameters will be taken as already outlined in section 5.1.

6. Assessment of antibiosis of soil antagonists to *Armillaria* root rot fungus

Paired colonies of antagonists and *A. mellea* will be done in Malt Extract Agar (MEA) and the zone of inhibition of *A. mellea* measured. The size of inhibition zone will reveal the level of antibiosis.

Filtrates from the probable antagonists will be subjected to *A. mellea* growing on Malt Extract and the radial growth of *A. mellea* determined. Millipore filters (cold sterilisation) will be used to sterilize the filtrates as outlined in Onsando's paper (Onsando and Waudo, 1991) (In press).

Phase contrast microscopy will also be used to establish the mode of parasitism between the probable antagonists and *A. mellea*. For the convenience of doing this, differential staining between the fungal antagonists and *A. mellea* has to be established. The microscopy techniques will be as outlined by Chet (Chet *et al*, 1981).

The identification of microbial antagonists to *A. mellea* will follow similar methods described above i.e. microscopy, inhibition zone measurements and effect of metabolites on filtrates.

7. Determination of whether the antibiosis of *Trichoderma* spp. and other soil antagonists to *Armillaria* will be influenced by specific species or clone of *Armillaria*

The same procedures as in 6 will be followed but this time different species/clones of *Armillaria* will be used as target organisms.

8. Determination of probable virulence differences within the *Armillaria* root rot fungus species/clones

The most susceptible tea clone (11/4) will be subjected to artificial inoculum of the probable *A. mellea* species/clones in the greenhouse. The virulence of the strain will be assessed on the basis of rate of kill of the young tea plants and extent of kill. The artificial inoculum will be prepared by cutting tea roots of uniform diameter and length (1.5 - 2.0 cm x 10 cm) and putting them in 2-litre jars containing 200 mls of water and autoclaving then inoculating them as outlined by Wilbur (Wilbur *et al*, 19[illegible]).

9. Testing of the package

Testing of the biocontrol package taking into account all the parameters that would have been worked out and established will be done both in the greenhouse and field.

The field experimental design will have to be determined when th factors under test are established. It will most likely be a factorial design.

G. STAFF AND RESOURCES

The curicula vitae and publication lists for all investigators essential to the project are enclosed.

The facilities at the Tea Research Foundation of Kenya relevant to the project have been briefly mentioned under D, but the following is an outline:

Plant Pathology laboratory

1. An autoclave
2. 2 refrigerators
3. A microscope
4. 2 ovens
5. 1 incubator
6. A range of chemicals for media
7. Basic glassware
8. Spectrophotometer
9. Magnetic stirrer
10. Electronic balances
11. Orbital shaker
12. Hot plates, haemocytometer
13. Dissecting microscope, etc

Soil Physics and Chemistry labs

1. Atomic absorption Spectrophotometer
2. Moisture analyser
3. Flame photometer
4. Kjeldahl apparatus for nitrogen analysis
5. 2 muffle furnaces each handling 20 samples.
6. HPLC and GLC
7. Soil moisture extractor with accessories.
8. Tensiometers.
9. Double ring infiltrometer
10. Penetrometer

H. RELATION TO PREVIOUS A.I.D. FUNDED WORK

This is the first project to A.I.D. for funding from Plant Pathology department of the Tea Research Foundation, of Kenya.

I. SCIENTIFIC COLLABORATION

The principal investigator has not worked with *Armillaria* for as long as the USA collaborating scientist. Therefore the experience the latter has gathered over the years will be shared to the benefit of the principal investigator and hence this part of the world. The local collaborating scientist from Kenyatta University has been working with the principal investigator on the same project but at a smaller scale. Therefore due to his awareness of the project, he has been included in this bigger project. The Director of the Tea Research Foundation of Kenya (Dr. Caleb Othieno) who is a Soil SCientist has been included because of the need to address probable effects of soil chemical and physical properties on the tea root rot development.

J. GRANT(S) HISTORY

The national project which has been going on for about three years has been supported wholly by the kenyan Tea Industry. This project therefore has not been submitted elsewhere for funding.

SPECIAL CONCERNS

1. Safety protocols for working with *Armillaria*

Since the project has now been revised to a national one from regional, the danger of introducing more virulent strains of *Armillaria* does not arise. All the *Armillaria* isolates for the project will be from Kenyan tea growing areas. The normal safety precautions of handling plant pathogens will however be in force.

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2. Shipment of pathogens

Shipment of *Armillaria* isolate for species differentiation and characterization to the UK (Oxford Forestry Institute) is contemplated. The permits will be submitted to AID/SCI as soon as they are finalized.

3. Handling of Intellectual Property Rights

The technologies that would accrue from this project will be patented in the joint names of the principal investigator and the American collaborating scientist.

K. BUDGET

1. Salaries

A microbiologist	$ 6,400 per year
A technician*	$ 4,400 per year
A plant pathologist*	$11,400 per year
A plant pathologist*	$10,400 " "
A soil Scientist*	$16,500 " "

2. Equipments

2 incubators	$ 8,000
1 cold sterilizer	$ 3,000
Powerful microscope with phase contrast & camera	$ 4,000
Laminar flow cabinet	$ 9,500
A pH meter	$ 1,500

3. Training/consultation

Principal investigator	$28,500
A soil scientist	$ 6,000
A technician	$ 8,000

4. International consultation

Visits for the USA collaborator three times for one month each time,	$21,000

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5. Regional/national travels

Subsistence for the principal investigator, co-investigator and a technician, alternately or together depending on need	$12,000
6. Reagents and chemicals - For media, petri dishes and other consumables	$ 8,500
7. Repairs of equipment	$ 5,000
8. Unforeseen costs	$1,500
9. Publications	$1,000

* Are employees of their respective institutions, therefore will not draw salaries.

International travels for the principal investigator and the American co-investigator have been worked out at $150-180 per day.

B. BUDGET JUSTIFICATION

1. Salaries

Salaries were computed by summing up the monthly basic salaries plus housing, medical benefits, contribution towards retirement scheme and other domestic allowances x 12 months divided by 27 Ksh, to convert everything to US dollars.

2. Equipments

The current costs of the items in Kenya plus an escalation of 10% divided by 27 Ksh to get the dollar equivalent.

pH meter

The effect of soil pH on *A. mellea* antagonists will be investigated. This will therefore require a pH meter. The existing one is being used for routine media pH determinations.

Incubators

Most of the *in vitro A. mellea* studies ranging from antibiosis to species determination will be carried out at specified temperatures hence the need for incubators.

Cold sterilizers

Some of the antibiotic metabolites of the antagonists to *A. mellea* might be heat-sensitive. For their activity to be established, cold sterilization will be necessary.

A powerful microscope with phase contrast facility

To determine the mechanism of antagonism between *A. mellea* and the would-be soil antagonists microscopic interactions will have to be studied. This will need a phase contrast microscope with a fitted camera.

Laminar flow cabinet

Most of the *in vitro* studies involving *A mellea* and antagonists will have to be carried out aseptically. A laminar flow cabinet will help the realization of this goal.

3. Training/consultation

Two visitations by the principal investigator to Northeastern forest Experiment Station, Hamden, and University of Toronto, are envisaged in the course of the project. Both are anticipated to last two months each. The first one to take place in the second year of the project and the second in the fourth year. This will give the principal investigator the opportunity to get more exposure on speciation of *A. mellea* complex and some aspects of biological control under the guidance of Dr. Philip Wargo. The second visit to the University of Toronto under Dr. Jim Anderson is anticipated to strengthen aspects of species identification of *A. mellea*. Dr. Jim Anderson was recommended by one of the reviewers as a resourceful person in *A. mellea* research. The soil scientist is anticipated to visit University of California. Riverside, Department of Soil Science, mainly to check on instrumentation and latest soil analytical techniques. This will reinforce the soils aspects of the project. The visitation is anticipated to last 14 days.

The subsistence for the visits has been worked out at the rate of $140-180 per day. The other costs such as bench fees, life insurance cover and air fares have been included in the estimate totals.

The visitation by the technician in the project has been arranged to a university in USA to be determined by Dr. Philip Wargo. The visit lasting one month is intended to familiarize the technician on available mating techniques for distinguishing *A. mellea* clones. For this attachment to be useful, it is intended to take place in the initial stages of the project, most preferably during the first year of the project. The other aspect of the attachment will involve methods of induction of fruiting body formation (basidiomes) for *Armillaria* taxonomic work in Kenya.

4. Consultation by Dr. Philip Wargo

Three visits have been budgeted for to enable Dr. Wargo to assess the laboratory facilities and participate in the initial

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detailed laying of strategies to achieve the desired objectives, to assess the progress on the ground and to discuss with the principal investigator on the modalities of publications of the results and probable patents of the findings if any. For him to effectively tackle the above, the visits have been scheduled for the first, third and fifth years of the project.

Only air fare, subsistence, insurance have been taken into account in computing the costs involved. Since Dr. Wargo will work at the Tea Research Foundation laboratory, bench fees will not be required.

Regional/national travels

The regional/national travels will mainly be made to collect *A. mellea* isolates for species identification, setting up field trials on management of *Armillaria* root rot throughout the tea growing areas within Kenya, and the routine soil sampling for assessment of microbial antagonists, disease and other data parameters. Such visits are undertaken but on a much smaller scale. The vehicle and a driver will be provided by the Tea Research Foundation but the per diem and fuel plus vehicle repairs while on these trials will be borne from the project money.

The initial stages of the project will require more frequent travelling to set up the trials. The calculations are based on $50 per day per person. It is anticipated that three persons will travel at a time. Petrol has been worked out at at $0.6 per litre which is the current running cost. The petrol consumption has been extrapolated from the previous national trips on the same project which has been going on at a marginal level due to costs.

Reagents and chemicals

The consumption of petri dishes, salts and other chemical for media preparation costs about $2,000 per year for the project. The scale of operation is anticipated to be about two times. This is how an additional cost of $1,600 per year has been worked out.

Publications

It is anticipated that at the end of the project 4-5 papers will have been written up. The $1,000 will therefore cover the page charges which are inevitable with journals like plant disease and phytopathology. Report writing will also use the same vote.

Contingencies

A contingency vote of $1,5000 has been provided for to subsidize any shortfall in the other votes.

C: BUDGET SUMMARY

	Year 1		Year 2		Year 3		Year 4		Year 5		Totals	
	AID	Match	AID	Match	AID	Match	AID	Match	AID	Match	Aid	Match
Salaries + Benefits												
Principal investigator (Kenya) 60%	0	11,000	0	11,000	0	11,000	0	12,000	0	12,000	0	57,000
Co-investigator (USA) 20%	0	-	0	-	0	-	0	-	0	-	0	-
Co-investigator (Kenya) 10%	0	10,000	0	10,000	0	10,000	0	11,000	0	11,000	0	52,000
Co-investigator (Kenya) 10%	0	15,000	0	15,000	0	16,000	0	16,000	0	18,000	0	80,000
Technician (Kenya)	0	4,000	0	4,000	0	4,000	0	5,000	0	5,000	0	22,000
A microbiologist (Kenya) 100%	6,000	0	6,000	0	6,000	0	7,000	0	7,000	0	32,000	0
Equipments + repair and maintenance	24,000	18,600	3,000	18,600	3,000	18,600	1,000	18,600	500	18,600	31,500	93,000
Training/Consultation												
Principal investigator	-	-	14,000	0	-	-	14,500	0	-	-	28,500	0
Co-investigator (Soil Scientist)	6,000	0	-	-	-	-	-	-	-	-	6,000	0
Technician	8,000	0	-	-	-	-	-	-	-	-	8,000	0
Consultation for the USA co-investigator	8,000	0	-	-	8,000	0	-	-	5,000	-	21,000	0
Regional/National travels												
Principal investigator Co-investigator, technician, microbiologist alternately/together depending on need	3,500	0	3,500	0	2,500	0	2,000	0	1,000	0	12,500	0
Reagents & chemicals	2,500	2,500	1,500	2,000	2,000	2,000	1,000	2,000	500	2,000	8,000	10,000
Contingencies	500	0	500	0	500	-	-	-	-	-	1,500	0
Publications					300	0	500		700	0	1,000	300
Total Grant (US Dollars)											150,000	314,300

International travels for the principal investigator, soil scientist and co-investigator have been worked out at $150-180 per day for subsistence.

Air fares, medical cover and life insurance covers have been included in the budget totals shown.

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C: BUDGET SUMMARY

	Year 1 AID	Year 1 Match	AI
Salaries + Benefits			
Principal investigator (Kenya) 60%	0	11,000	C
Co-investigator (USA) 20%	0	-	C
Co-investigator (Kenya) 10%	0	10,000	C
Co-investigator (Kenya) 10%	0	15,000	C
Technician (Kenya)	0	4,000	C
A microbiologist (Kenya) 100%	6,000	0	C
Equipments + repair and maintenance	24,000	18,600	:
Training/Consultation			
Principal investigator	-	-	1
Co-investigator (Soil Scientist)	6,000	0	
Technician	8,000	0	
Consultation for the USA co-investigator	8,000	0	
Regional/National travels			
Principal investigator Co-investigator, technician, microbiologist alternately/together	[illegible]	0	

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CURRICULUM VITAE

PERSONAL PARTICULARS

NAME	Caleb O. Othieno, A.A., B.Sc., M.Sc., PhD
DATE OF BIRTH	3rd March 1939
MARITAL STATUS	Married with three children
NATIONALITY	Kenyan
ADDRESS	P.O. Box 820, Kericho, Kenya

EDUCATION AND TRAINING

a) Primary: CMS Pumwani, Nairobi, Kenya 1948-1950.

b) Primary Intermediate: Ng'iya Intermediate, Siaya, Kenya 1951-1955.

c) Secondary: St. George's Secondary School, Mukono, Uganda 1956-1958.

Undergraduate

a) Imperial Valley Junior College, Imperial, California, U.S.A. 1960-1962. A.A. (Associate in Arts)

b) California State Polytechnique University, San Luis Obispo, California, U.S.A. 1962-1965. (B.Sc. Soil Science, Soil Physics/Chemistry).

Postgraduate:

a) University of Illinois, Urbana, Illinois, U.S.A. (Special Training in Soil Testing and Fertility Practices. June-September, 1965)

b) University of Manitoba, Winnipeg, Manitoba, Canada 1969-1971. (M.Sc., Soil Chemistry and Plant Nutrition).

c) University of Nairobi, Nairobi, Kenya, 1978 (PhD., Soil Science).

EMPLOYMENT RECORD

1. October 1965 - June 1966: National Agricultural Laboratories, Kabete, Kenya.

Position: Soil Chemist

Duties:
a) In-charge of soil-testing laboratory
b) Advisory serices
c) Research into fertilizer response.

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2. July - Septem..r 1966 Shell Chemicals of E Africa.

Position: Technical Representative

Duties: a) Advisory Services
b) Conducting Trials
c) Product Evaluation

3. October 1966 to present: Tea Research Institute of East Africa/ Tea Research Foundation of Kenya

a) October 1966 - September 1969

Position Assistant Chemist

Duties a) In-Charge of Institute's Chemistry Laboratory
b) Leaf Analysis Advisory Services
c) Research into Fertilizer Responses
d) Economics of Fertilizer use in tea
e) Research into Plant/Soil/Water Relationships.

b) October 1969 - May 1971

Position: Assistant Chemist

Duties: Undergoing Postgraduate training in Soil Chemistry and Plant Nutrition.

c) June 1971 - June 1981

Position: Hydrologist and Head of Hydrology Department (Crop Environment Department).

Duties: a) Departmental Administration and research management.

b) Planning and estimating for all departmental research projects, including irrigation projects.

c) Carrying out experiments, analysing and interpreting results for use by tea growers.

d) Carrying out advisory visits to farmers with special problems.

e) Responsible for the departmental contributions to all TRIEA publications.

f) Responsible for the departmental contributions to all TRIEA courses and training held at at the institute.

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d) July 1981 to present

Position: Director, Tea Research Foundation of Kenya

Duties: a) Executive Director of the Foundation

b) Institutional management:
i) Formulation and implementation of research programmes.

ii) Control and management of the Foundation's affairs.

iii) Editor of all the Foundation's publications.

INTERNATIONAL MEETINGS PARTICIPATED IN

1. December 1981: A key member of the Kenya's delegation to the ISO meeting on black tea standards, in Nairobi.

2. October 1982: A key member of the Kenyan delegation to UNCTAD organised meeting on Minimum Export Standards for Black tea, in Geneva.

3. May/June 1983: A key member of the Kenyan delegation to the ISO meeting on black tea standards held in Budapest, Hungary.

4. December 1984: A key member of the Kenyan delegation to the ISO meeting on black tea standards held in New York, U.S.A.

5. June 1986: Conference on Drought Desertification Food Deficits in Africa, ICIPE, Nairobi, Kenya.

6. June 1986: International Conference on tea production and marketing, Rize, Turkey.

7. July 1986: Symposium on pesticides use in tea plantations, London, U.K.

8. October/ November 1986: Leader of Kenya delegation to the ISO meeting on black tea standards held in Hangzhou, China.

9. October 1989 Leader of Kenya delegation to the ISO meeting on black tea standards held in Hangzhou, China.

10. March 1991 One-man Kenya delegation to an "International symposium on physiological and Pharmacological effects of tea (Camellia sinensis) on a human being, New York, U.S.A.

CONSULTANCY:

1. Uganda Government/ World Bank - December 1983: Agricultural Rehabilitation Project. Study of tea production in Uganda - Uganda Government/World Bank short-term consultancy as a

part-time member of the Mwenge International Associates.

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(d) South Nyanza District, 1985
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