PN-ABD-575

AID - SCIENCE ADVISORY COUNCIL GRANT

LOCAL PLANTS USED BY RWANDANS FOR INSECT PROTECTION OF DRY EDIBLE BEANS

GRANT No. DPE-5542-G-SS-8047-00

PROJECT NUMBER 7.346

SIX MONTHS PROGRESS REPORT

PERIOD COVERED
JULY 1 TO DECEMBER 31, 1988

UNIVERSITY OF WISCONSIN

DEPARTMENT OF ENTOMOLOGY

MADISON, WISCONSIN 53706

TABLE OF CONTENTS

	PAGE
1.0. INTRODUCTION	1
2.0. RESEARCH ACCOMPLISHMENTS	1
2.1. Preliminary experiments with insects and plant parts	1
2.1.1. Behavioral response	2
2.1.2. Direct toxic response	2
2.2. Preliminary experiments with plant extracts	3
2.3. Gas chromatographic analysis of Ocimum canum sinus.....	4
2.3.1. Development of procedures.....	4
2.3.2 Results.....	4
2.3.3. Recommendations.....	5
2.4. Preliminary bioassay of linalool with Zabrotes subfasciatus..	5
2.5. Scanning electron microscopy of plant-insect interaction....	5
2.6. Summary.....	7
3.0. PLANNING WORKSHOP.....	9
3.1. Development of milestones, flowchart and critical path.....	9
3.2. Development of publication policy.....	10
3.3. Modifications requested in contract.....	10
3.4. Agenda.....	11
3.5. Conclusions.....	11
4.0. COLLABORATIVE RESEARCH VISITS.....	11
4.1. Mr. Joseph Kayitare.....	11
4.2. Mr. Aussumani Serugendo.....	12
4.3. Dr. Florence Dunkel.....	12
5.0. INTERACTION WITH RELATED RESEARCH PROJECTS.....	12

5.1. US-AID/HBCU Selma University - Insect resistance to insecticides and alternatives..... 12

5.2. Montana State University - Bioactive components of natural products..... 12

5.3. National University of Rwanda - Center for Research on Traditional Medicine (CURPHAMETRA)..... 13

6.0. VISITORS TO PROJECT..... 13

6.1. Dr. Donna Shalala, Chancellor of the University of Wisconsin 13

7.0. PLANS FOR THE NEXT SIX MONTHS..... 14

7.1. Visit of Dr. F. Dunkel to laboratories in Rwanda..... 14

7.2. Visit of Drs. Koval and Dunkel to Science Advisory Office Washington, D.C..... 14

7.3. Development of plant growth areas..... 14

7.4. Insect bioassay experiments..... 14

7.5. Preliminary experiments in human sensory evaluation..... 14

7.6. Completion of extensive literature review..... 15

8.0. REVISED GANTT CHART FOR PROJECT..... 16

LIST OF FIGURES

Figure 1. Plant species and their parts brought from Rwanda for preliminary studies at the University of Wisconsin a). list b). drawings of Ocimum c). drawings of Chenopodium.

Figure 2. Curves (+/- S.E.) toxic response of Acanthoscelides obtectus Say to plant products of a). Chenopodium spp.; b). Tetradentia riparia; and c). Ocimum spp.

Figure 3. Chromatographic scan of the components in Ocimum canum sinus leaves two months after drying and milling.

Figure 4. Scanning electron micrographs of a). dorsal view of Acanthoscelides obtectus Say exposed to freshly milled Ocimum canum leaves (25x X 5x7 size); b). elytra of A. obtectus (50x X 5x7) with close up of particles adhering to setae; c). abdominal view of Zabrotes subfasciatus exposed to freshly milled O. canum leaves with closeup of particle adhering to setae; d). O. canum leaf material immediately after milling; e). O. canum leaf material two months after drying and milling; f). O. canum leaf material after ethanol extraction two months after milling; g). intact dry leaf of O. canum (150x X5x7); h). intact dry leaf of O. canum (400x X5x7); i). intact dry leaf of O. canum (800x X5x7).

Figure 5. Milestones of project 1988-1991

Figure 6. Main categories of work breakdown structure (1988-1991)

Figure 7. Details of the work breakdown structure and estimated times for completion

Figure 8. Plan of insecticidal plant publications

Figure 9. Agenda of Workplan Meetings, Madison Wisconsin (August 20-25, 1988)

Figure 10. Description of Plant Growth Center (MSU) to be used for environmentally controlled studies of insecticidal plants

LIST OF TABLES

Table 1. Bioassay of milled plant parts a). behavioral response b). toxic response

Table 2. Preliminary observations of mortality and behavior in male Zabrotes subfasciatus Bohem exposed to Ocimum canum leaves.

Table 3. Toxicity of hexane and ethanol extracts of Ocimum canum sinus (Isongantoya) leaves to Zabrotes subfasciatus Bohem.

1.0. INTRODUCTION

1.1. Preliminary experiments, collections, discoveries, and discussions with the OPROVIA postharvest research team and at the National University of Rwanda

In the summer of 1984 a survey team of the US AID Rwanda Local Crop Storage Project consisting of Mr. Edouard Nizeryimana, Mr. Evariste Munyarshoka and Mr. Ted Wittenberger found a group of farmers in the Rukira commune of the Kibungo Prefecture using the leaves and stems of a plant in their stored beans for insect control (Dunkel et al. 1988). This plant was subsequently identified as Ocimum kilimandsharicum. Further information obtained in the survey indicated 5-10% of the Rwandans surveyed were using plants for postharvest insect control. In 1985 plans were made with the ISAR component of the project (Ms. Elizabeth Lamb and Mr. Leopold Gahamanyi) to partially support a M.S. student in Agronomy from the National University of Rwanda for his three month Stage and one year Memoir study. His work was to be a preliminary study of the insecticidal plants in Rwanda. In collaboration with CURPHAMETRA, the University of Minnesota, the National University of Rwanda, OPROVIA and ISAR, the project was completed in 1986 by Mr. Munyemana. In November 1986 a visit was made by Dr. Dunkel to the National Herbarium and CURPHAMETRA to introduce the new AID project which resulted in the present grant.

The main goal of this project was to understand the toxic and/or repellent properties of these plants used by Rwandan farmers so that they could be used in large scale Rwandan warehouses (OPROVIA).

1.2. Contract negotiations and signing of the contract

In May 1987 it was announced by AID Science Advisory Council that the grant had been awarded. AID-Kigali approval for the project, however, was granted only if the financial accounting for the project was done by a US university. Contract negotiations occurred during 1988, January to August. In February 1988 it was determined that the situation for administration of the grant was more favorable at the University of Wisconsin than at the University of Minnesota. On August 5, the contract was signed by AID and expenses were allowed to be incurred. Following August 5, the fiscal representative of the University of Wisconsin also signed the contract.

1.3. Commencement of work at Universities of Wisconsin and Minnesota

Immediately upon contract signature in Washington, the international tickets for the Rwandan team were prepared and plans began in earnest for the Workplan meeting to be held 20-25 August. Work began officially in the laboratories at both Universities on 29 August 1988.

2.0. RESEARCH ACCOMPLISHMENTS

2.1. Preliminary experiments with insects and plant parts

Portions of four plant species were brought from Rwanda by Mr. Kayitare. All specimens had been collected in August 1988 and air dried. They were transported in brown paper bags. In the laboratory in Wisconsin they were placed in airtight glass jars on the laboratory bench. The species and their parts brought from Rwanda are listed in Figure 1. Preliminary unreplicated studies indicated that there was a toxic and/or repellent response with both bruchid species, Acanthoscelides obtectus Say and Zabrotes subfasciatus Bohem. The following replicated studies were then designed and run to determine if the response had toxic or behavioral elements.

2.1.1. Behavioral response

Many types of repellent screening tests could be used. One of these is a two choice disk test in which extracts are placed on one disk and the other is immersed in only solvent. When dry, a disk of each type is placed in a petri dish arena. This may be used for future tests. For the tests done in this reporting period, a glass bucket pitfall trap was used. It consisted of a glass beaker filled with 400g treated beans (Phaseolus vulgaris) and a plastic pitfall trap in the center. Repellancy was measured in this apparatus by the number of insects which left the treated beans and fell into the trap (Table 1a).

Although there was good repellancy in the first, preliminary, unreplicated test with O. canum, subsequent tests with replicates and O. canum and other plants did not show any repellancy.

Following both this behavioral study and the direct toxic studies, a series of simple, but intense observations were made with Z. subfasciatus. Bruchids exposed to freshly milled O. canum leaves were immediately covered with a dust. Particles clung to antennae. Grooming activities of the antennae resulted in these particles being stuck on the tarsi. The insects then used their mouthparts to clean their tarsi and particles became stuck on their mouthparts. Insects exposed to O. canum which had been milled two months earlier caused no such clinging action of particles or grooming behavior. Bruchid behavior was also observed when they were exposed to O. canum leaves after ethanol extraction two months after milling. Similarly, no mortality, moribundity occurred. After 26 hours of exposure, however, some particles clinging to the exoskeleton and grooming activities were observed (Table 2).

Scanning electron microscopy (SEM) studies were initiated to further clarify this observation.

2.1.2. Direct toxic response

Based on the preliminary experiments, a larger experiment was conducted to test direct toxic effects of all plant parts brought from Rwanda. This experiment was, in some respects, a confirmation of Munyemana's studies in Rwanda (1986).

One g. plant material was placed on a #1 Whatman filter paper in a glass petri dish (9cm diameter). Ten adult A. obtectus and Z. subfasciatus, 3 +/- 3 days after adult eclosion, 5 males and 5 females, were placed in the petri dishes in a controlled environment room (28^{0} C; 16:8::L:D photoperiod; 60+/-10 %R.H.

Most noteworthy in the response of A. obtectus was its early mortality in day 3 and 6 and continued mortality higher than the controls until the end of the experiment at day 15 post adult eclosion when exposed to sun dried leaves of T. riparia. The effect of Chenopodium flowers and leaves, O. kilimandsharicum flowers, and O. canum leaves on A. obtectus was a 3-4 times higher mortality than the controls until day 15(Table 1b).

2.2. Preliminary experiments with plant extracts

2.2.1. Extraction procedure

Dried leaves of Ocimum canum sinus (the same lot as described above) were powdered in an electric blender and placed in a clear glass jar with a screwtop lid. Two months later (October 17, 1983) after storage on a laboratory shelf, two lots of 44g each were removed and placed in clean (RBS washed) 500 ml flasks. Absolute ethanol (120 ml) was added to one flask, and 120 ml hexane (HPLC grade) was added to the other. Each flask was swirled to expose the powdered leaves to the solvent. The flasks were then wrapped in foil (to prevent photodegradation), capped, and set aside at room temperature for 24 hours. After 24 hours, the ethanol and hexane supernatants (ca. 60 ml) were glass-wool filtered and each filtrate stored in clean (RBS) flasks under N2 at 5^{0} C.

2.2.2. Bioassay of plant extracts

2.2.2.1. Procedures

Plant extracts (1ml) were pipetted onto 9 cm #1 Whatman filter paper in a glass petri dish (9 cm diameter). After solvent had completely evaporated and filter paper was dry, 5 male and 5 female Z. subfasciatus 3+/- days post adult eclosion were placed on the filter paper in the controlled environment room described above. Observations were made at 1,15,24,48,72, and 96 hours. Ten replicates were used for each ethanol and hexane treatment and for the hexane and ethanol controls(Table 3).

No significant mortality was obtained. However, activity level was noticeably affected. At 15 hrs most insects were inactive in the treatments. In the controls, most insects were moving, ambulating. In the treatments, 6 of the 200 insects were walking on the sides of the petri dish. In the controls, 19 of 200 insects total, were walking on the sides or top of the dish. At 48 hrs, however, the color of the hexane extract had almost completely disappeared and 35 of 200 insects in the treatments were on the sides or on top of the dishes, and only 29 of the insects in the controls were similarly active.

It was concluded that the dosage of O. canum leaf extract was too low and there may be an anesthetic action due to volatiles of O. canum.

Using information from the capillary gas-liquid chromatography results, it was determined that the hexane extract of O. canum leaves resulted in 0.341 mg linalool per g. of dried leaves extracted. Therefore, the concentration of linalool in each test petri dish with hexane extract was 0.25 mg.

2.2.2.2. Results and Recommendations

Four days after inoculation, there were no statistically significant numbers of moribund individuals or mortality in either the hexane or ethanol extract test chambers or in the hexane and ethanol control chambers. There were, however, some obviously decreased ambulatory activity levels in test chambers in comparison to the controls. From unextracted studies, we know that freshly milled leaves of this plant are more toxic than milled preparations used two months after milling. The negative response may have been due to dose or the volatilization of associated active components.

2.3. Gas chromatographic analysis of Ocimum canum sinus

2.3.1. Preparative column chromatography

A clean (RBS washed) 24.0 x 0.6 cm (ID) glass column equipped with a Teflon stopcock and a glass wool plug was slurry-packed (1:1 hexane/diethyl ether; HPLC grade) with silica gel (70-230 mesh). Ten ml of the filtered hexane extract of O. canum sinus (from the preparation described above) was concentrated under a stream of purified N2 to ca. 500 ul. The concentrated hexane extract was then eluted through the above silica gel column with 1:1 hexane/diethyl ether at a flow rate of 1.0 ml per min. Sixteen labeled 4.0 ml fractions were collected in clean (RBS) vials and sealed with Teflon lined caps. The above fractions were stored at 5^{0} C to await further use.

2.3.2. Capillary gas-liquid chromatography

The instrument used was a HP-5890A equipped with flame ionization detector (FID) and a 25.0 m x 0.25 mm (ID) fused silica RSL-300 (= OV-17) capillary column. Column conditions used was a carrier gas flow of (Helium) 1.2 cc per min. Temperature program = 60^{0} C (held 0.5 min) raised to 250^{0} C. (held 5.0 min) at 10^{0} C per min. Injector temperature was 240^{0} C, and detector temperature was 255^{0} C. Injections were 0.5 ml of each (16) of the above preparative fractions co-injected with 0.5 ml of an internal standard (5-ethyl-2-heptanol; retention time (RT) = 5.18 min) (Figure 3),

2.3.3. Results

The target terpene, linalool (RT = 5.81 min determined by injection of a standard), first appeared in fraction #9-12 and was then detected in every fraction through #37-40. Apparently linalool was present in the O. canum leaves in great quantity. This accounts for its "tailing" through fractions #37-40. In fact, it is present in fractions #13-16, #17-20, and #21-24 in milligram quantities, with the most occurring in #17-20 (ca. 7.43 mg or 1.86 ug per ul).

Linalool occurs with a number of small "companion" peaks within 0.5 min of its 5.81 min RT; they could be related terpene compounds. Most of these fractions also have several sharply defined peaks between hydrocarbons with 10-20 carbon atoms (alkanes, alkenes, and dienenes). The final calculation from this procedure indicated there was 15 mg linalool in the extract of the 44g of milled leaves or 0.341 mg linalool per g leaves. Although linalool seems to be highly concentrated in the leaves even two months after drying and milling, bioassay procedures with both extracts and milled leaves, two months after milling, indicate this may be too low a percent a.i. (active ingredient) to be

effective. It is possible that there are other compounds present in much smaller concentrations that enhance the activity of linalool and these synergistic compounds do disappear during the two months following milling.

2.3.4. Recommendations

The next extractions should be done with a longer and larger diameter column for preparative column chromatography e.g. a 44 x 1 cm column. This is recommended because then linalool will come off more all at once and not tail through the other fractions. Then, a preparative HPLC could be done. There seemed to be many 10 - 20 carbon hydrocarbons. Some may be terpenes. If so, there are kits of standards for terpenes between 10 and 20 C. At CURPHAMETRA's pilot plant in Butare, soxalet columns with steam distillation are used. This is also useful for obtaining large quantities.

A definitive chromatography or mass spectrophotometry should be performed, and, most important, both bioassay and gas chromatography studies should be done with freshly milled leaves.

2.4. Preliminary bioassay of linalool with Zabrotes subfasciatus

Because the most abundant single chemical component of the hexane extract of milled O. canum leaves was linalool, pure (neat) linalool was assayed in 4 concentrations (100 ul, 10 ul, 1.0 ul, and 0.1 ul/ml hexane). 5 male and 5 female
Z. subfasciatus were placed in each test chamber (9cm diameter glass petri dish). Solution was allowed to dry. Insects were then added and covered with the upper petri dish. Four hours later (28^{0} C and continuous light) all insects were dead or moribund at the two highest concentrations.

It was concluded that a definitive linalool bioassay should be done with both Z. subfasciatus and A. obtectus.

2.5. Scanning electron microscopy of plant-insect interaction

A Hitachi S570 was used for these studies. Photos were taken with Polaroid film. Specimens were gold coated for 75 seconds (first set of specimens) and 90 seconds (second set of specimens) to a thickness of 200 to 300 angstroms with a sputter coater. The following specimens were examined:

-Z. subfasciatus (dorsal, ventral, lateral views) exposed to freshly milled O. canum leaves

-A. obtectus (dorsal, ventral, lateral views) exposed to freshly milled O. canum leaves

-O. canum sinus residue from ethanol extraction

-O. canum sinus residue from hexane extraction

-O. canum sinus freshly milled leaves (milled 22 October, prepared for SEM 26 October)

-O. canum sinus old milled leaves (2 months prior to SEM preparation)

-unmilled leaves of O. canum

-unmilled leaves of Tetradentia riparia

-flowers of Chenopodium schraderanem

The following scanning electron microscopic (SEM) observations were made with the bruchids exposed to freshly milled leaves of O. canum. SEM confirmed light microscopy observations that small particles were adhering to the entire insect body of both A. obtectus and Z. subfasciatus (Figures 4a,b,c). The particles ranged in size from 8 to 22μ. Most were 11 micra(μ) in diameter. All were an amorphous shape, subovoid or subspherical. Most of the body of these species of bruchids is covered with setae. The leaf particles adhered primarily to the shaft of the setae. In areas of the insect exoskeleton where setae were sparse, particles adhered directly to the exoskeleton itself.

Observations were then made of the leaf material immediately after milling (Figure 4d), two months after milling (Figure 4e), and after ethanol extraction two months after milling (Figure 3f). The main difference observable with SEM at 800x is the density of the subovoid, subspherical, amorphous particles. The main epidermal layer of the leaf and prominent vesicles were not visible in the freshly milled sample. The entire surface of the leaf particles seemed to be covered with a concretion of amorphous material.

Whereas with the O. canum leaves milled two months previously, the rugose layer of the leaf epidermis was clearly visible as were the vesicles (ca. 50 micra diameter). The only observable difference between these specimens and the same but ethanol extracted, was the more intensely rugose appearance of the leaf epidermis in the extracted leaf particles.

Observations were then made of the unmilled leaf (Figure 4g,h,i). These specimens were characterized by a very rugose epidermis with regularly spaced (50-100 micra apart) spherical vesicles (ca. 50 micra diameter) inset in the epidermis. Between the vesicles were spicules. In the rugose epidermis, numerous stomata could be seen. No amorphous subsperical or subovid particles on the epidermis were observed as in the milled specimens.

It was concluded that the toxic effect of O. canum is related to the leaf concretions which adhere to the insect body.

Surfaces of other Rwandan plants were also made with the scanning electron microscope. The leaf surface of Tetradentia riparia also consists of a rugose epidermis with spherical vesicles (ca. 30 micra diameter). These vesicles, however, are stalked. The flower case of Chenopodium schraderanum has a smooth surface with spherical vesicles (ca. 60 micra diameter) not stalked and not inset. Numerous hollow spicules were attached between the vesicles to the flower case surface. The flower stalk surface was striated with only spicules, no vesicles.

2.6. Summary

Toxicity was observed with all plant preparations. O. canum was selected for a more intensive study of behavioral observations and chemical analyses. The O. canum leaf contains chemical compounds which are toxic to two species of bruchids. This effect seems to be direct toxicity and not repellency. One or more of the active chemicals may volatilize or degrade as time passes after the leaf is broken. This toxicity is related to particles which adhere to the entire insect body and which elicit intense grooming behavior.

Preliminary gas chromatographic (GC) analysis indicates O. canum leaves are ca. 0.034% linalool, a monoterpene. Linalool, in preliminary studies, was toxic to Z. subfasciatus adults. The toxic effect could be summarized as follows:

Pure linalool----------mortality

O. canum leaf

- freshly milled----------mortality
- old milled----------no mortality
- ethanol extract of old milled----------no mortality
- residue of ethanol extract of old milled----------no mortality
- hexane extract of old milled----------no mortality

LITERATURE CITED

Dunkel, F.V., T.P. Wittenberger, and N.R. Read. 1986. National storage survey of beans and sorghum in Rwanda. Miscellaneous Publication. Minnesota Experiment Station. No. 46. 184p.

Munyemana, P.C. 1986. Essai preservation du haricot (Phaseolus vulgaris L.) contre Acanthoscelides obtectus Say en utilisant les produits d'origine vegetales. Memoire. M.S. Agronomy. National University of Rwanda, Butare. 130p.

Ntezurubanza, L., J.J.C. Scheffer and A. Baerheim-Svendsen. 1988. Composition of the essential oils of Ocimum urticifolium (Lamiaceae) chemotypes grown in Rwanda. Bot. J. Linn. Soc. London. 96(2):97-104.

Ntezurubanza, L., editor. 1988. Journee nationale de concertation sur l'etude et l'exploitation des huiles essentielles au Rwanda. March 21, 1988. National University of Rwanda CURPHAMETRA. Butare, Rwanda. 65p.

3.0. PLANNING WORKSHOP

An initial planning workshop was held August 20-25 with all participants from Rwanda, the US and related projects present. The following members were present:

-Dr. Charles Koval, Co-Principal Investigator, Chairperson and Professor Department of Entomology University of Wisconsin, Madison.

-Dr. Florence Dunkel, Co-Principal Investigator, Adjunct Scientist University of Wisconsin, Madison (Associate Professor and Head Entomology Research Laboratory, Montana State University).

-Dr. Wendell Burkholder, Collaborator, Research Leader USDA-ARS Stored Product Insect Laboratory, Professor, Department of Entomology, University of Wisconsin.

-Dr. Lawrence Cutkomp, Collaborator, Professor Emeritus, Department of Entomology, University of Minnesota.

-Dr. William Breene, Collaborator, Professor, Department of Food Science, University of Minnesota.

-Mr. Ron Chastain, Interpreter, President Lingua Franca.

-Mr. Joseph Kayitare, Collaborator, Research Scientist, Plant Protection Division, ISAR, Rubona, Rwanda.

-Mr. Phocas Kayinamura, Collaborator, Manager, Postharvest research and extension Laboratory, OPROVIA, Kigali, Rwanda.

-Mr. Aussumani Serugendo, Collaborator, Leader of Food Science section of the Postharvest Research and Extension Laboratory, OPROVIA, Kigali, Rwanda.

Mr. Leopold Gahamanyi, Director of ISAR, was unable to come at the last moment due to family illness. Dr. T.P. Sriharen, Co-Principal Investigator of Postharvest Insect Resistance in Rwanda and Alternatives AID grant, Division of Natural Sciences, Selma University, Selma, Alabama was also present.

3.1. Development of milestones, flowchart, and critical path

Due to the large group of collaborators in 5 locations, a unified plan of work was developed together during the Workplan meetings. The milestones identified are in Figure 5. The areas of work are outlined in Figure 6. Details of these and the estimated time required for their completion were developed from the outline of work areas (Figure 7). The critical path was then determined.

3.2. Development of Publication Policy

Because the required conclusion of the project is publications, a plan with all participants was developed (Figure 8). It was determined that six publications would be anticipated during the three years. Each would begin with the senior author analyzing the data and drafting a working paper of 15 pages of text or less. This would begin immediately following the completion of one research section and be completed in 44 days. It would be reviewed by coauthors during the next 22 day period (using FAX and express mail) and be reviewed by external reviewers in the 44 days following. The paper would then be revised and submitted for publication.

The following refereed journals would be considered for submission:

-Journal of Stored Products Research

-Entomologica Applicata

-La Revue Scientifique du Rwanda

-Tropical Science

-Protection Ecology

-Journal of Chemical Ecology

-Environmental Entomology

-Canadian Journal of Food Science (French or English)

-Economic Entomology

-International Journal of Food Science and Technology

-Food Protection

Only after the scientific publication has been submitted will the extension articles be submitted. These will be primarily in the Bulletin Agricole du Rwanda. Publishing permission will be obtained from original journal for tables and figures which are duplicated.

3.3. Modifications Requested in Contract

-Addition of OPROVIA (Office National pour le Developpement et la Vulgarization de les Produits Viviers) to point 8. Collaborating Institutions.

-Addition of Drs. Breene and Vickers as collaborators and the approval for use of funds for reimbursement of their time.

-Addition of Dr. Innocent Butare as a collaborator and the approval for his travel to the U.S.A. for project consultation if funds are available within the budget toward the end of the project.

-In the Technical Workplan section g. substitution of pyrethrin flowers for Simno preparation and omission of Callosobruchus spp.

-Various additions to Program Description (i.e. Purpose of Grant and Specific Objectives).

3.4. Agenda of Planning Workshop

The agenda included both planning meeting with the large group and in small groups representing the various hypotheses to be tested (Figure 9). Tours were given of the facilities at the University of Wisconsin where this research will take place (e.g. the SEM and Stored Product Insect Facilities) and where similar research takes place (e.g. sensory laboratory in the Department of Food Science). The workshop was planned so that Mr. Kayitare and Mr. Serugendo would arrive in Madison 20 Auguse, one day prior to the commencement of formal meetings. Due to technicalities in Rwanda, they did not arrive until the evening prior to the last day of the meetings.

3.5. Conclusions

It was summarized that the budget for both Rwandan and US components was very small and that parallel projects could be funded to stretch the budget. Communication was of utmost importance. It was suggested that the four components (2 US and 2 Rwandan) submit one or two phrase weekly reports to Dr. Dunkel who would facilitate the coordination of activities. This would consist of the answer to three questions: are things going as scheduled?, any problems?, and what can be done to help alleviate the problem?

4.0. COLLABORATIVE RESEARCH VISITS

4.1. Mr. Joseph Kayitare

Mr. Kayitare arrived August 24, 1988 at the University of Wisconsin in Madison. Following the workplan meetings he began preliminary laboratory experiments with Dr. Burkholder in the Stored Product Insect Laboratory. Both behavioral and toxic responses of two species of bruchids were determined. A library search was initiated and English lessons were begun on a daily basis.

Mr Kayitare departed Wisconsin on November 16, 1988.

4.2. Mr Aussumani Serugendo

Mr. Serugendo arrived at the University of Wisconsin, Madison August 24, 1988. Following the workplan meetings he traveled to St. Paul Minnesota where he began collaborative studies with Dr. Breene and Dr. Vickers in cookability and sensory evaluation, respectively. Mr. Serugendo redesigned developed a detailed workplan for the sensory portion of this project, did a literature review and attended formal courses in food processing, sensory evaluation of food and statistical analysis. He also attended English classes on a regular basis. Mr Serugendo departed Minnesota on December 22, 1988.

4.3. Dr. Florence Dunkel

Dr. Dunkel joined Dr. Burkholder, Mr. Kayitare and the Stored Product Insect Laboratory personnel for collaborative research October 15-29, 1988. During this time, preliminary experiments were done with plant extracts and the GC. In depth discussions of the literature and preliminary behavioral and SEM observations caused the research to focus on linalool and time degradation problems with the milled product. During Dr. Dunkel's visit, she presented a seminar for the Department of Entomology on "Entomological Aspects of the Postharvest System in Rwanda (East Central Africa)." November 4 and 6 Dr. Dunkel returned with Mr. Serugendo to Wisconsin for additional SEM studies, discussions of research results, and planning.

5.0 INTERACTION WITH RELATED RESEARCH PROJECTS

5.1. US-AID/HBCU Selma University - Insect Resistance to Insecticides and Alternatives

Dr. T.P. Sriharen, Co-P.I. of the Selma Project attended workplan meetings of this project in Madison, Wisconsin. He introduced the Selma Project and we were able to coordinate visits of US scientists to Rwanda to make maximum use of overlapping visits. Large scale studies in both projects could also be coordinated. A visit of Dr. Dunkel to Selma University will be made in the next reporting period (February 1989). She will give a seminar on both the insecticidal plant project and the Selma insect resistance project.

5.2. Montana State University - Bioactive components of Natural Products

Commitments for collaborative studies were obtained from:

-Dr. John Cardellina (Department of Chemistry MSU) for bioassay studies of

natural plant products with microorganisms and other plants and for mass spectrophotometric analysis of active components.

-Dr. Larry Jackson (Department of Biochemistry and Molecular Biology MSU) for gas-liquid chromatographic analysis of natural plant products.

-Dr. Eloy Rodriquez (Department of Developmental and Cell Biology University of California, Irvine) for providing background in successful searches for insecticidal plant products.

-Dr. Tad Weaver (Department of Biology MSU) for providing background in taxonomic relations of plants and their allelochemical properties.

A proposal for MONTS-EPSCOR/NSF funds has been submitted. If awarded, this grant will provide for chemical analysis and non-target organism bioassay of Rwandan insecticidal plants as well as of related species grown in Montana in the family Labiatiae. This and the above assistance to the project will be coordinated with efforts at the University of Wisconsin and in Rwanda.

Another proposal will be made in April 1989 to MONTS-EPSCOR/NSF for a Center of Excellence in Natural Products. It will include our present Rwandan studies and the chemists mentioned above.

A grant was received in December 1988 by F. Dunkel from MONTS to bring to MSU for consultation and two public lectures an authority in the area of Natural Product Insecticides, Dr. Eloy Rodriquez. The Departments of Chemistry and Plant and Soil Science have also agreed to co-sponsor his four day visit.

5.3. National University of Rwanda - Center for Research on Traditional Medicine (CURPHAMETRA)

Dr. Dunkel visited CURPHAMETRA and the pilot extraction, chemical analysis facility on the UNR campus in 1986. Mr. Kayitare has made several visits to CURPHAMETRA also. They assisted him in obtaining symposium proceedings and plant material for this project.

6.0. VISITORS TO PROJECT

6.1. Dr. Donna Shalala, Chancellor of the University of Wisconsin

Dr. Shalala, Chancellor of the University of Wisconsin visited the Department of Entomology on October 25, 1988. One of five labs which she visited was the Stored Product Insect Laboratory. A brief presentation was made for her by Mr. Kayitare and Dr. Dunkel on the Rwandan Insecticidal Plant Project. The point was made that we are searchings for linking practical solutions of Rwandan farmers to large scale government storage practices in Rwanda as well as to low

input sustainable solutions for US farmers.

7.0. PLANS FOR THE NEXT SIX MONTHS

7.1. Visit of Dr. F. Dunkel to Laboratories in Rwanda

Dr. Dunkel will visit Rwanda April 4-11. During this time she will share research results and work plan changes with the ISAR and OPROVIA members of the team. Training necessary in bioassay procedures, mass cultures and standardization of plant storage procedures will be given. Meetings with CURPHAMETRA and the Botanical Museum on the University of Rwanda campus will also be held to coordinate research and to explore their possible involvement in this project. She will also confirm plans for obtaining and filing voucher specimens of plants. Plant material will be obtained for research in the US. Lab and field experiments in Rwanda will be discussed.

7.2. Visit of Drs. Koval and Dunkel to Science Advisory Office, Washington D.C.

April 13 and 14, Drs. Koval and Dunkel will meet with both the contract officer, Dr. Collier and the members of the Science Advisory Office, Drs. Ching and Asher. Details of requested workplan changes and a review of progress will be discussed.

7.3. Development of Plant Growth Areas

Field plots will be developed in Rwanda on the ISAR grounds and plans for controlled environment studies will be initiated by Dr. Dunkel in the Plant Growth Center at MSU (Figure 10).

7.4. Insect Bioassay Experiments

Bioassay studies with 2 species of bruchids will be conducted in the US and Rwanda to determine the time of peak plant production period for active ingredients. Other bioassay studies will be done with bruchid response to pure linalool.

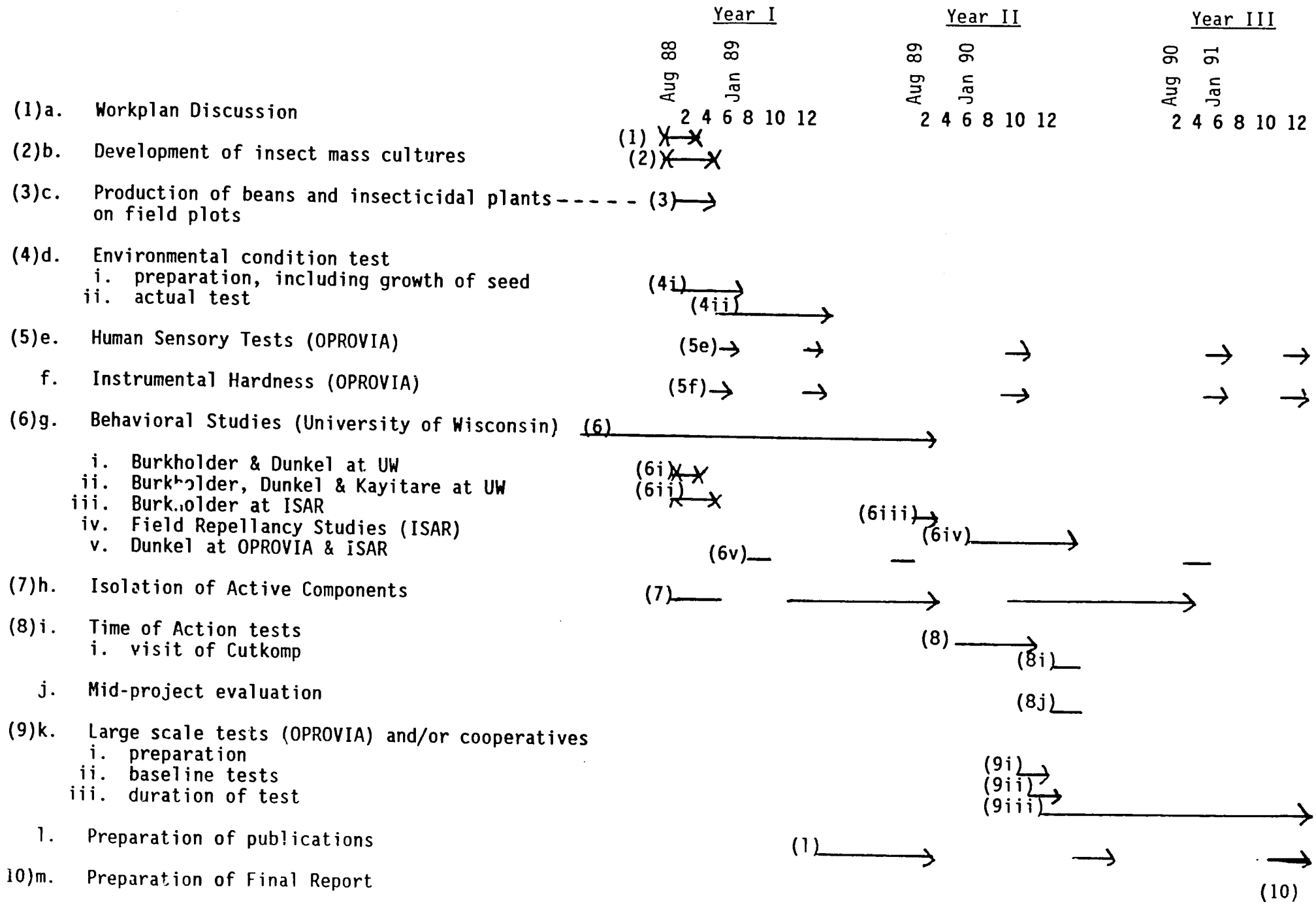
7.5. Preliminary Experiments in Human Sensory Evaluation

Preliminary studies will be begun to test the effect of O. canum on thermal softening of beans after storage and on human sensory acceptability of these beans.

7.6. Completion of Extensive Literature Review

Extensive computer-based literature review will be completed by Dr. Dunkel. It will especially focus on the Labiatiae and linalool. Over 103 references on Labiatiae and 3000 references on linalool will be examined. Funding for this was made available to Dr. Dunkel by the Montana Agricultural Experiment Station.

GANTT CHART



(Letters correspond to contract; numbers correspond to work breakdown structure; ✕—✕ indicates task is complete)

Figure 1. Plant species and their parts brought from Rwanda for preliminary studies at the University of Wisconsin a). list b). drawings of *Ocimum* c). drawings of *Chenopodium*.

FAMILY CHENOPODIACEAE:

Chenopodium schraderanum Roem. and Schult.	FLOWERS
	LEAVES
Chenopodium sp.	FLOWERS

FAMILY LABIATIAE:

Tetradentia riparia (Hochst) N. E. Br.	FLOWERS SUNDRIED
	LEAVES SUNDRIED
	LEAVES OVENDRIED
Ocimum kilimandsharicum Guerke	FLOWERS SUNDRIED
canum sinus	LEAVES

FAMILY SOLANACEAE:

Capsicum frutescens L.	LONG FRUITS
	SHORT FRUITS

Figure 1b. 1. Ocimum canum: 1a, rameau florifere (x1/2); 1b, calice fructifere (x2 1/2; 2, Ocimum trichodon; 2a, fragment de plante fructifere (x 1/2); 2b, calice fructifere (x2 1/2); 3. Ocimum basilicum; calice fructifere (x2 1/2); 4. Ocimum gratissimum; calice fructifere (x2 1/2); 5. Ocimum americanum; calice fructifere (x2 1/2). from Figure 104 Flore du Rwanda. Spermatophytes Vol. 3. 1985.

Figure 1c. 1. Chenopodium procerum: 1A, 1B et 1C, types de feuilles (x 1/2); 1D, face inferieure du limbe foliaire, avec glandes stipitees (x 5); 1E, inflorescence generale (x 1/2); 1F, fleur (x 20); 1G, akene entoure du perigone (x 20), 2. Chenopodium schraderanum: 2A, feuille (x 1); 2B, face inferieure du limbe foliaire, avec glandes sessiles (x 5), 3. Chenopodium ugandae: 3A a 3F, types de feuilles (x 1/2); 4B, feuille do la partie moyenne de la tige (x 1/2); 4C, fleur (x 25), 5. Chenopodium pumilio: 5A, rameau florifere (x 1/2); 5B, feuille (x 1), 1 (E-G), d"apres F.C.B.R.U.; 4(c), d'apres F.Gabon. from Flore du Rwanda. Spermaphytes. Vol. 1. 1978.

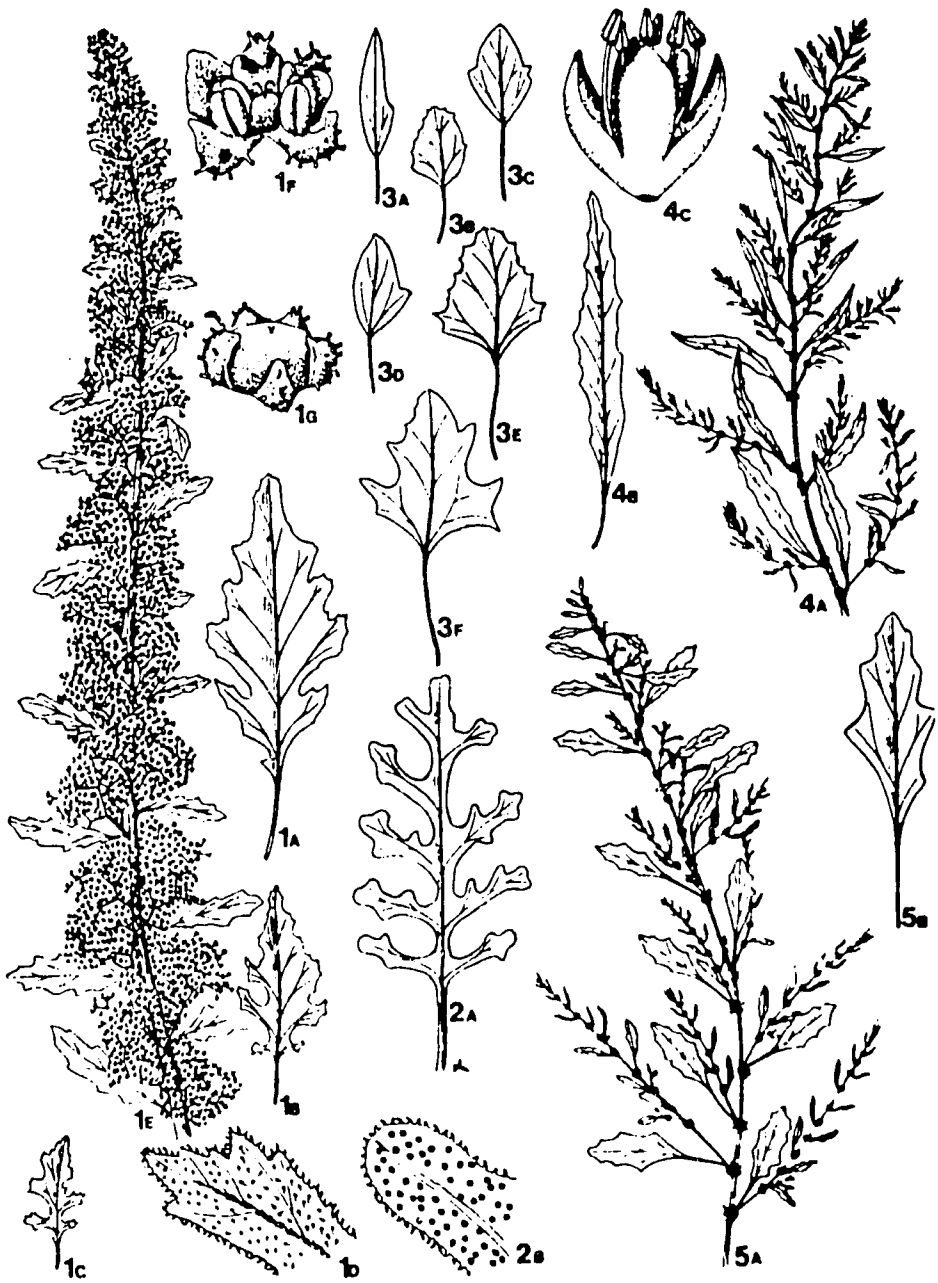
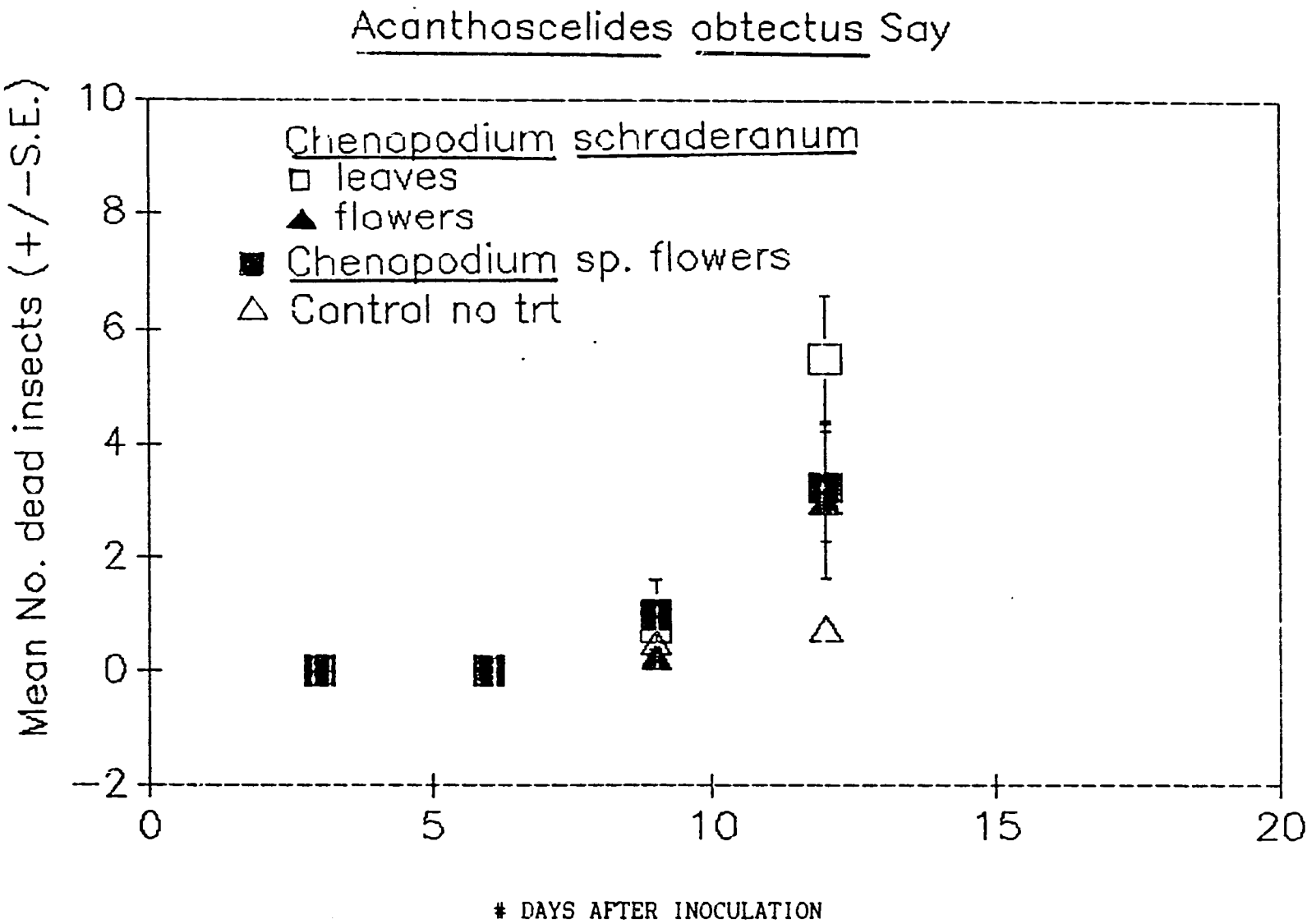
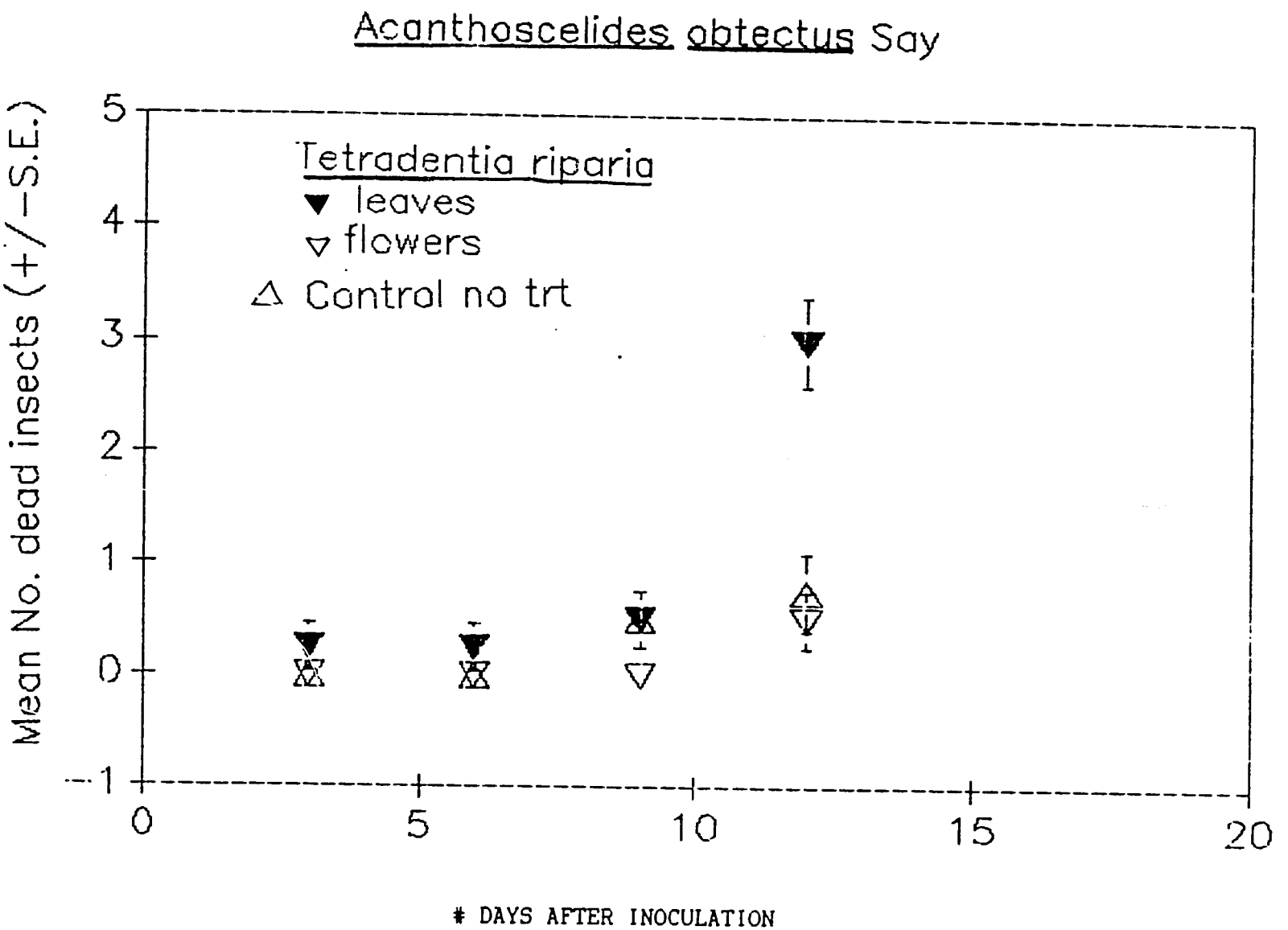
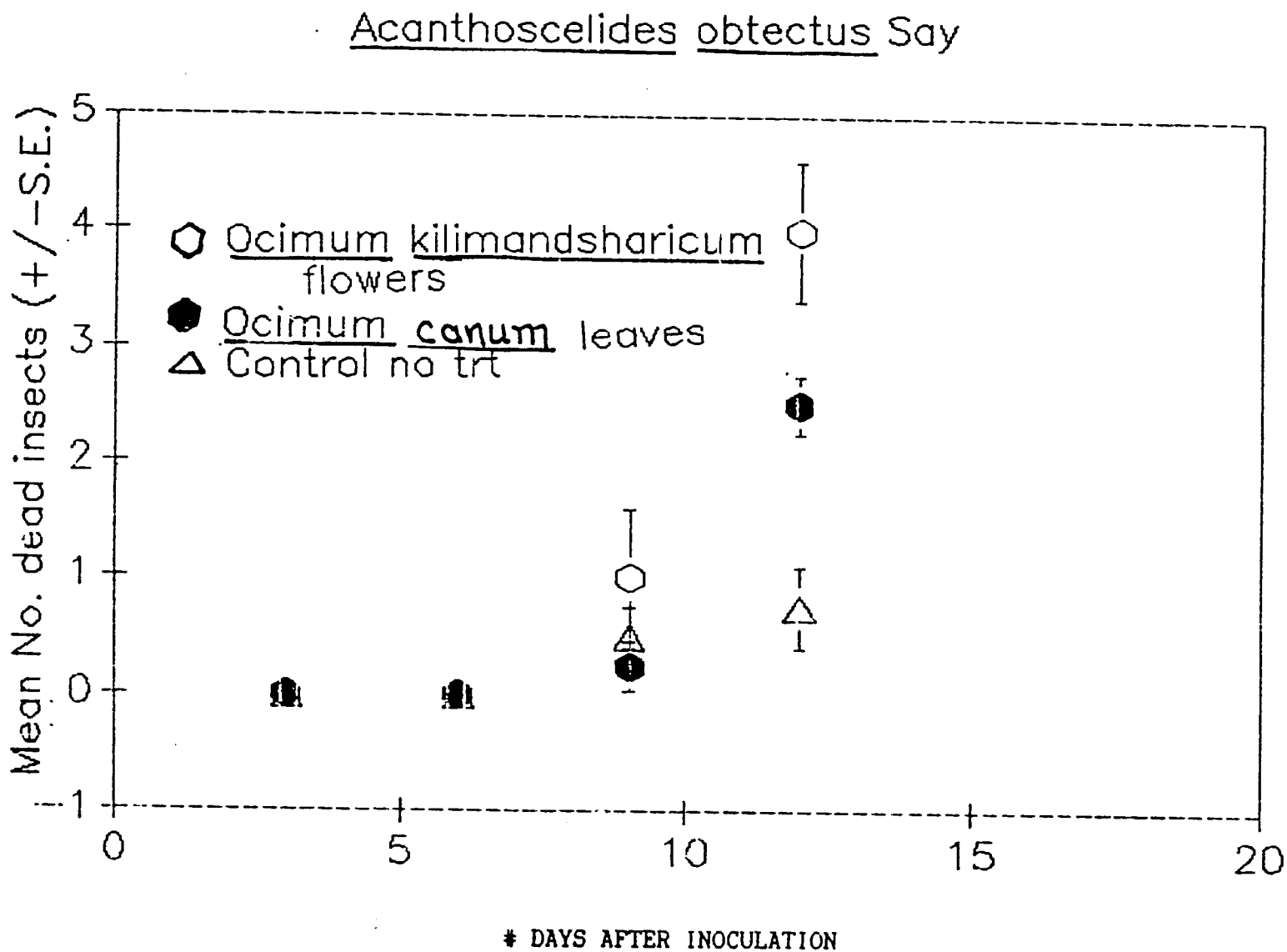
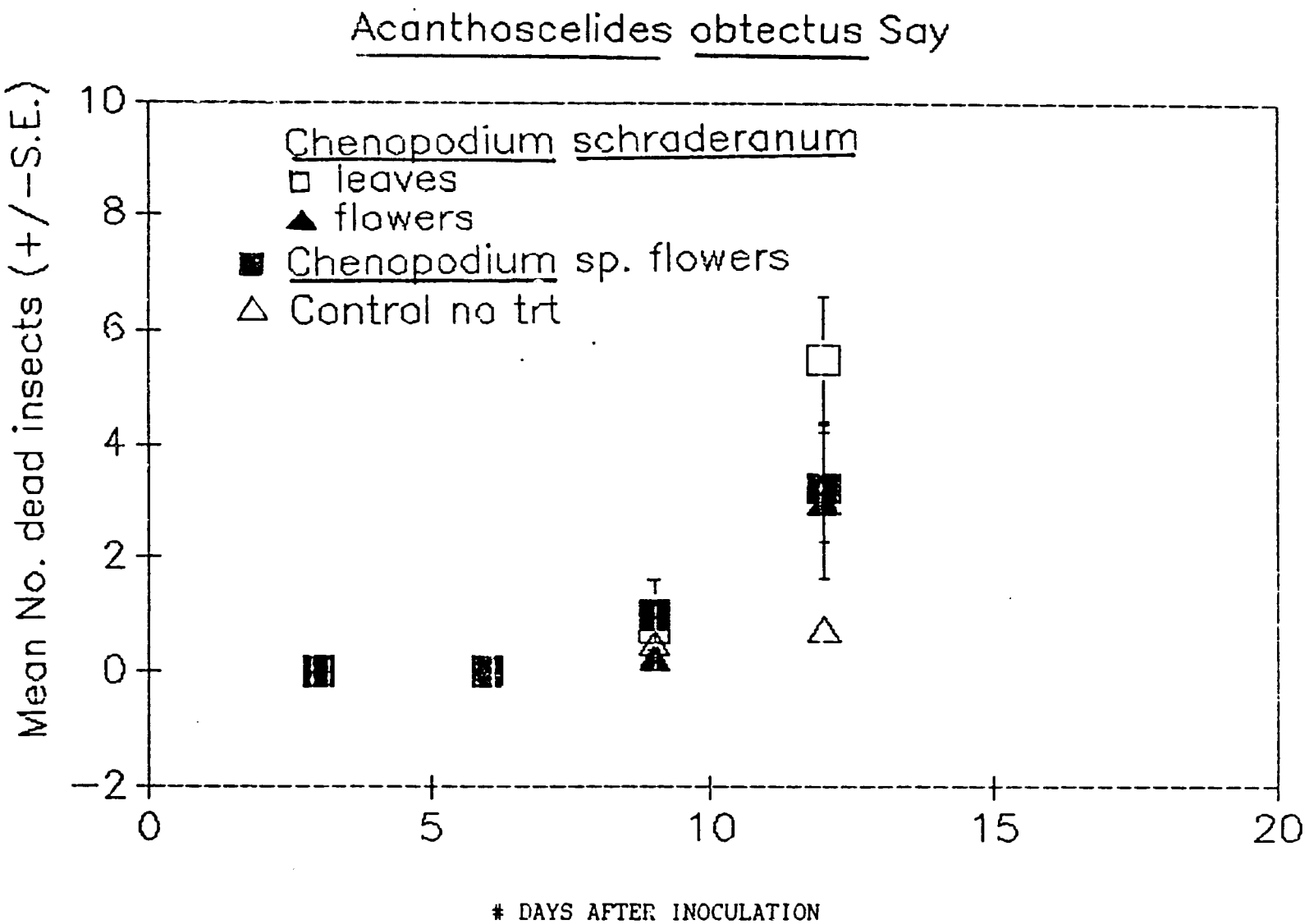


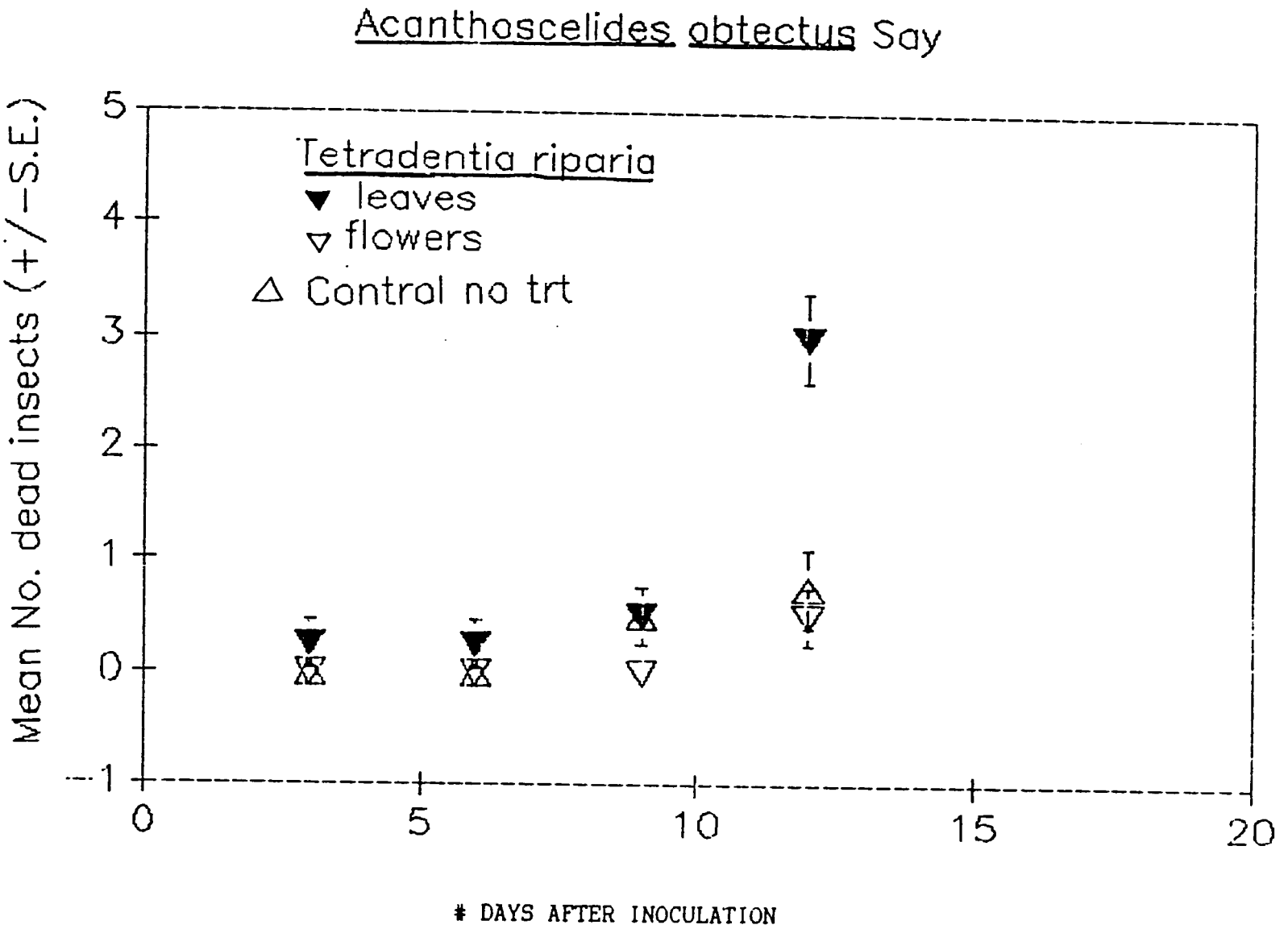
Figure 2. Curves (+/- S.E.) toxic response of *Acanthoscelides obtectus* Say to plant products of a). *Chenopodium* spp.; b). *Tetradentia riparia*; and c). *Ocimum* spp.


Acanthoscelides obtectus Say
Chenopodium schraderanum
leaves
flowers
Chenopodium sp. flowers
Control no trt
Mean No. dead insects (+/-S.E.)
10
8
6
4
2
0
-2
0
5
10
15
20
DAYS AFTER INOCULATION


Acanthoscelides obtectus Say
Tetradentia riparia
leaves
flowers
Control no trt
Mean No. dead insects (+/-S.E.)
5
4
3
2
1
0
-1
0
5
10
15
20
DAYS AFTER INOCULATION


Acanthoscelides obtectus Say
Ocimum kilimandsharicum flowers
Ocimum canum leaves
Control no trt
Mean No. dead insects (+/-S.E.)
5
4
3
2
1
0
-1
0
5
10
15
20
DAYS AFTER INOCULATION


Acanthoscelides obtectus Say
Chenopodium schraderanum
leaves
flowers
Chenopodium sp. flowers
Control no trt
Mean No. dead insects (+/-S.E.)
10
8
6
4
2
0
-2
0
5
10
15
20
DAYS AFTER INOCULATION


Acanthoscelides obtectus Say
Tetradentia riparia
leaves
flowers
Control no trt
Mean No. dead insects (+/-S.E.)
5
4
3
2
1
0
-1
0
5
10
15
20
DAYS AFTER INOCULATION

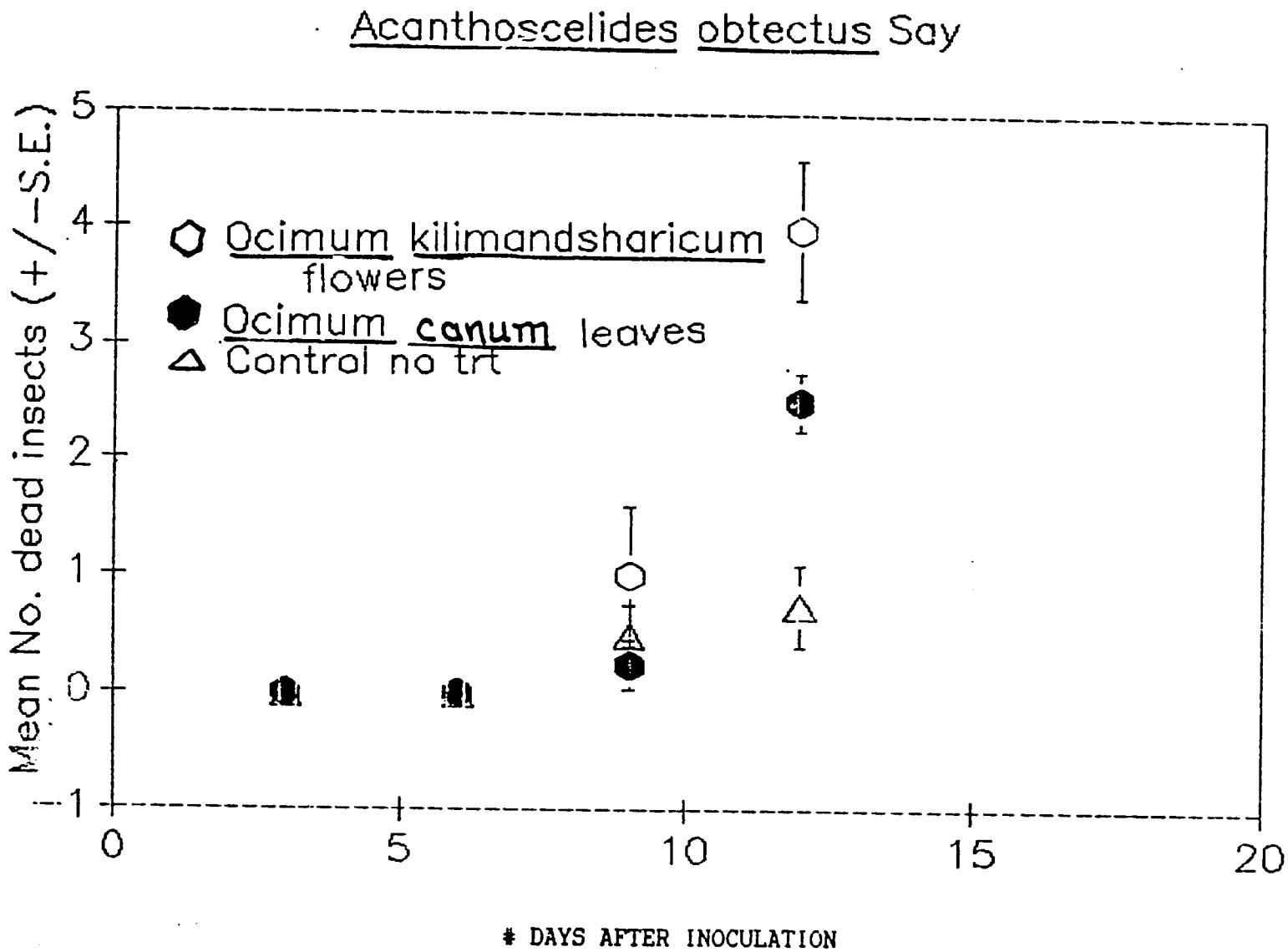
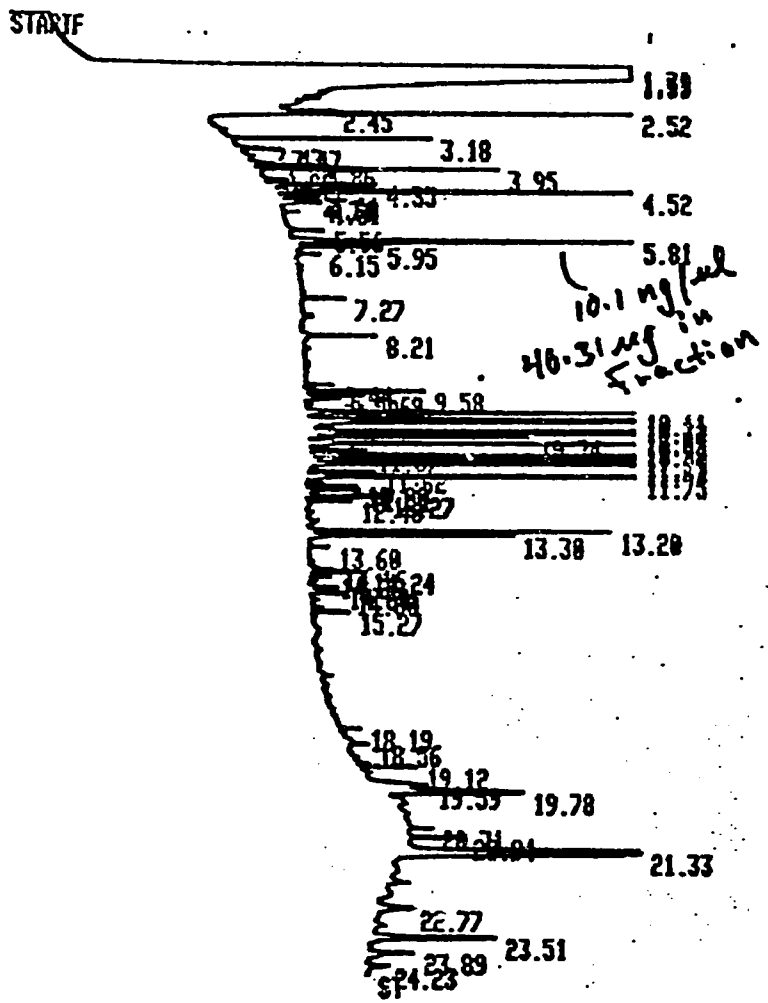

Acanthoscelides obtectus Say
Mean No. dead insects (+/-S.E.)
5
4
3
2
1
0
-1
Ocimum kilimandsharicum flowers
Ocimum canum leaves
Control no trt
0
5
10
15
20
DAYS AFTER INOCULATION

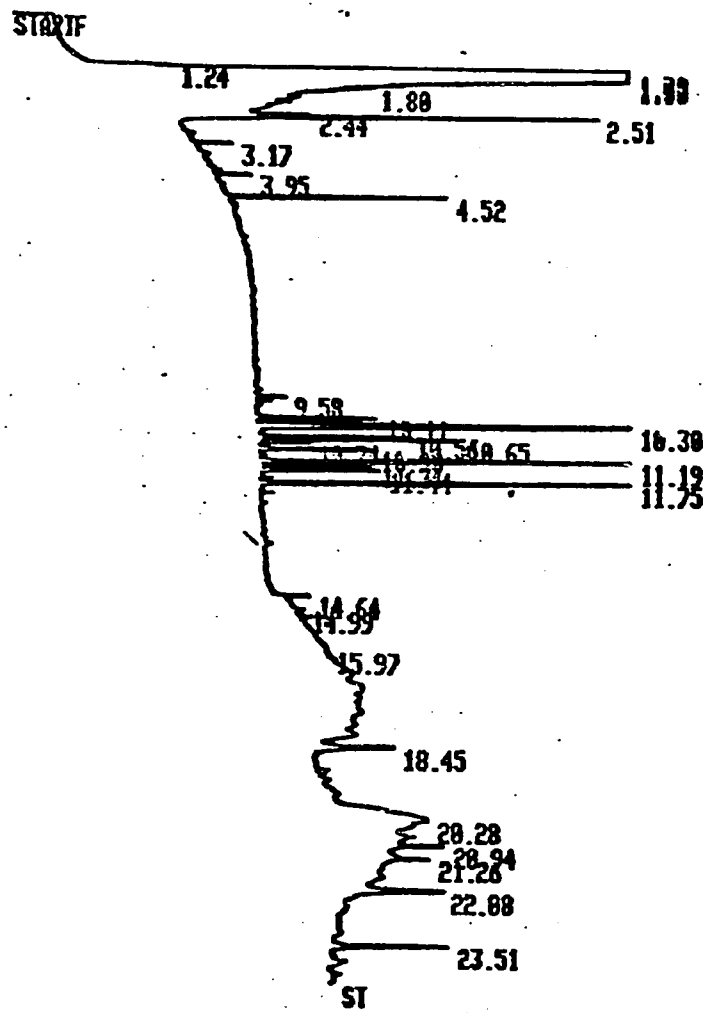
Figure 3. Chromatographic scan of the components in Ocimum canum sinus leaves two months after drying and milling.

Ocimum canum hexane extract. Silica gel fractions. 60 degrees (.5)---10---> 250(5). Internal standard = 5 ethyl-2 heptanol (retention time = 5.17). Injection prior to run indicated linalool retention time = 5.85. This varied from 5.84 to 5.86 due to computer system in the gas chromatograph. Numbers on the scans correspond to the milliliter in which those compounds exited e.g. #5-8 means that fraction came off in the fifth to eighth milliliter from the sample that passed through the column.

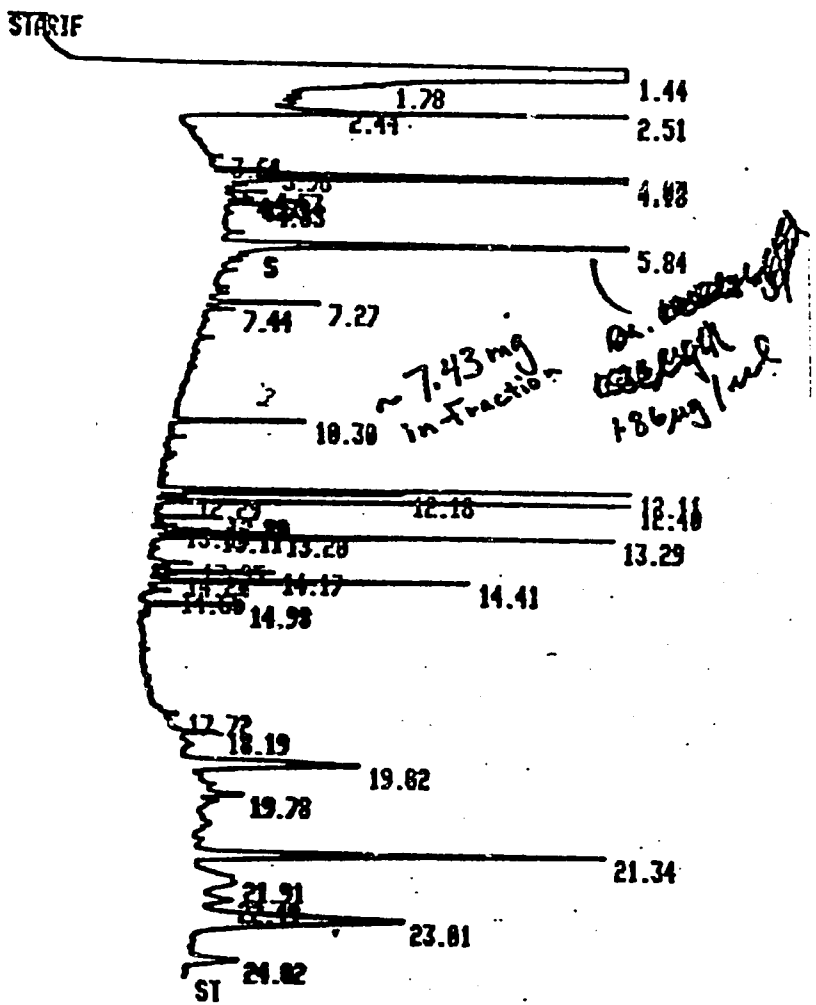
#9-13 0.5 µl



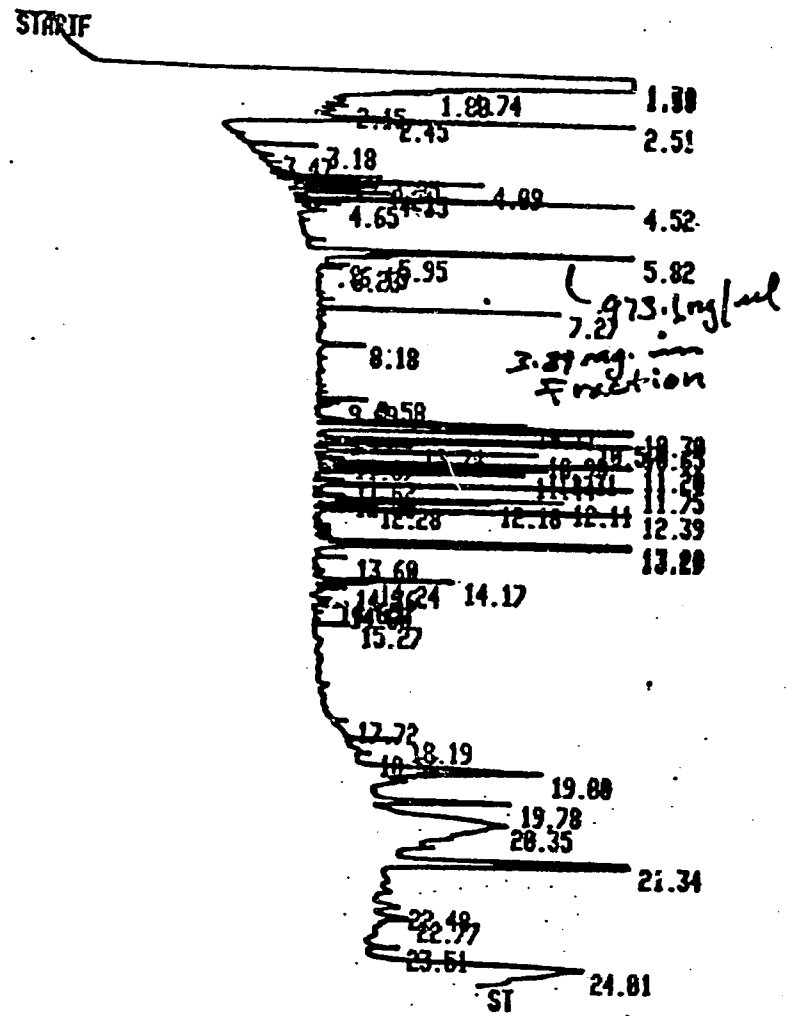
#5-8 0.5 µl

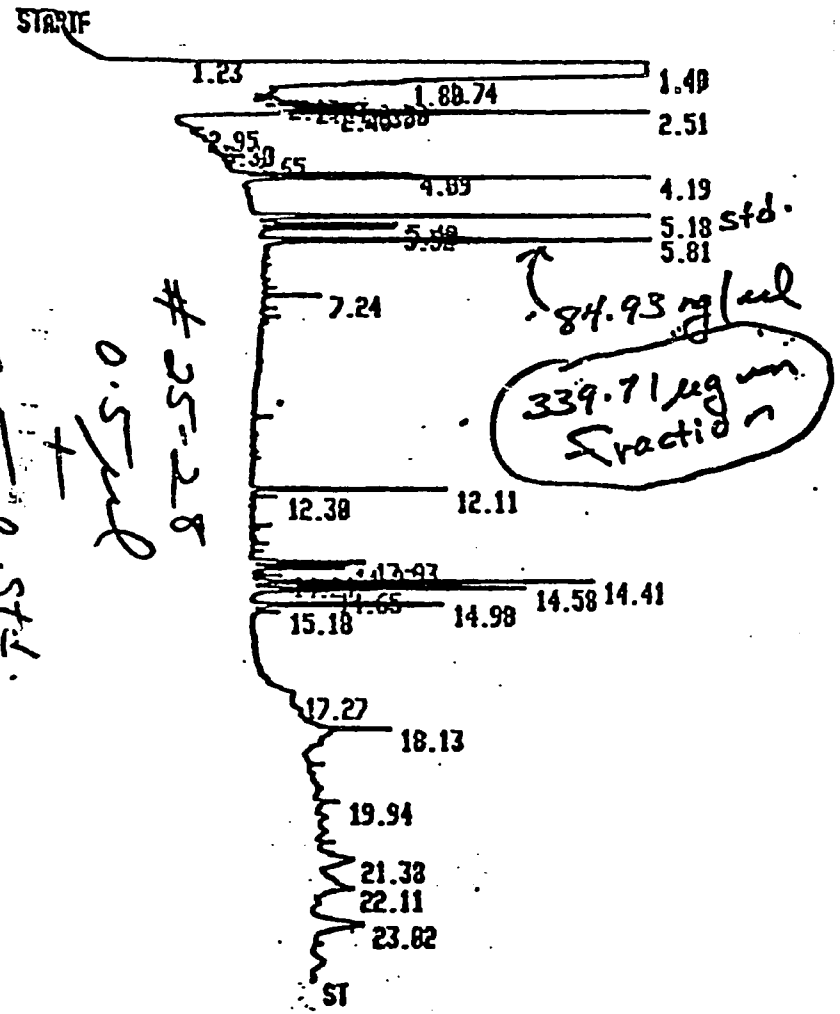


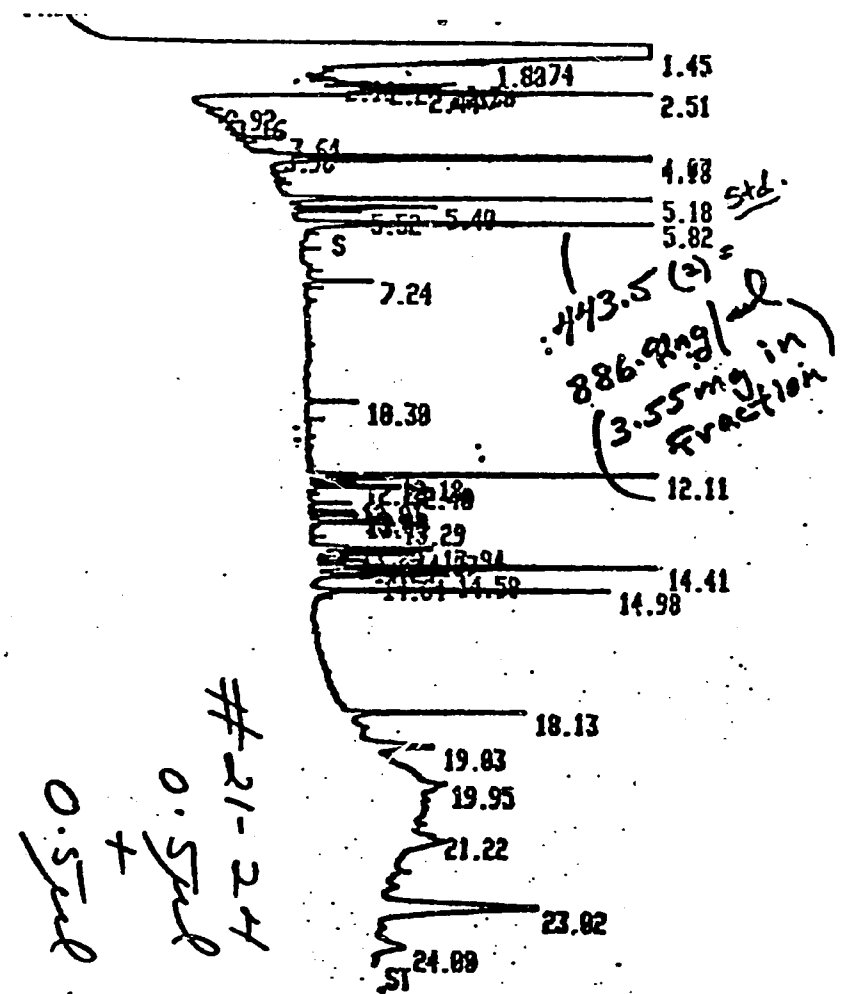
#17-20
0.5 ml

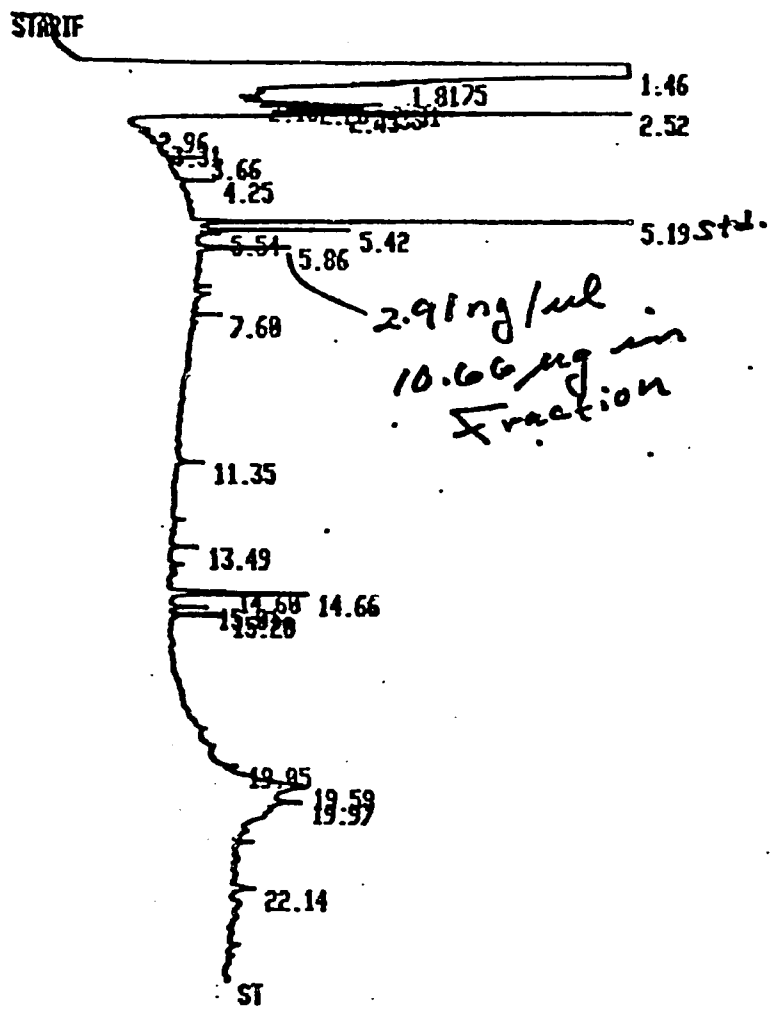


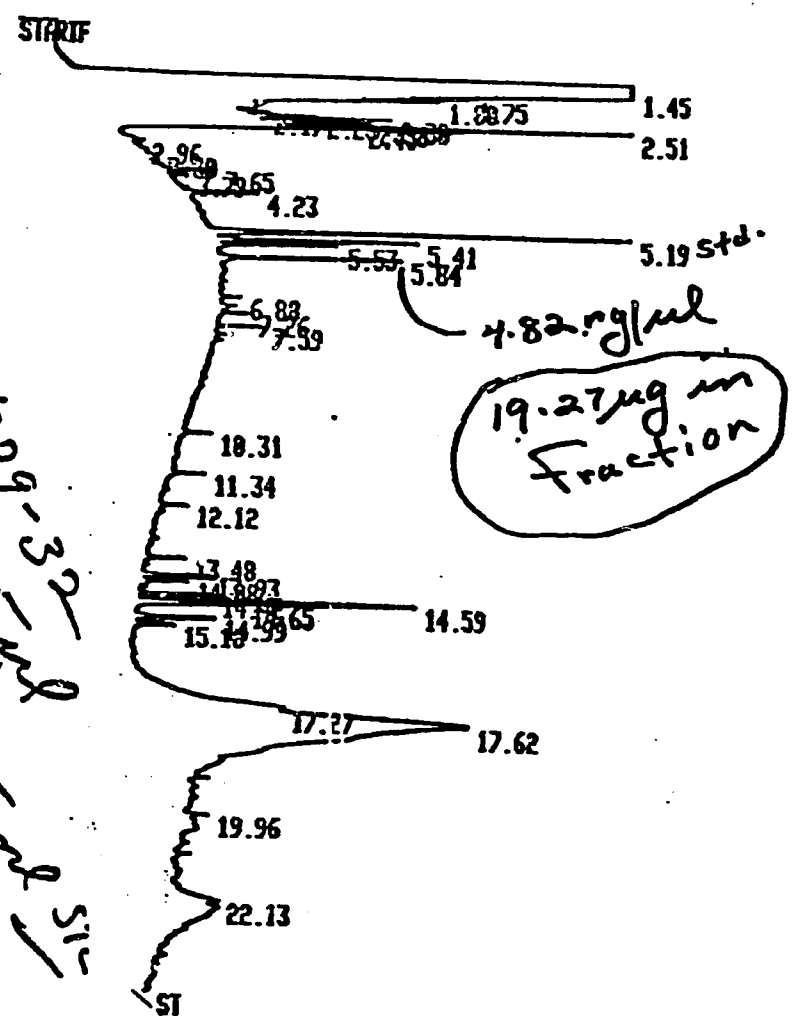
#13-16
0.5 µl




STARIF
1.23
1.40
1.88.74
2.51
2.95
4.19
5.18 std.
5.81
7.24
84.93 ng/ul
339.7 µg in fraction
12.38
12.11
14.58
14.41
15.18
14.98
17.27
18.13
19.94
21.38
22.11
23.82
ST
#25-28
0.5 ul
+
0.5 ul std.


1.45
1.8374
2.51
4.18
5.18 std.
5.82
S
7.24
443.5 (2)
886.9 ng/ul
3.55 mg in fraction
18.38
12.11
14.41
14.98
18.13
19.03
19.95
21.22
23.82
24.09
ST
#21-24
0.5 ul
+
0.5 ul std.

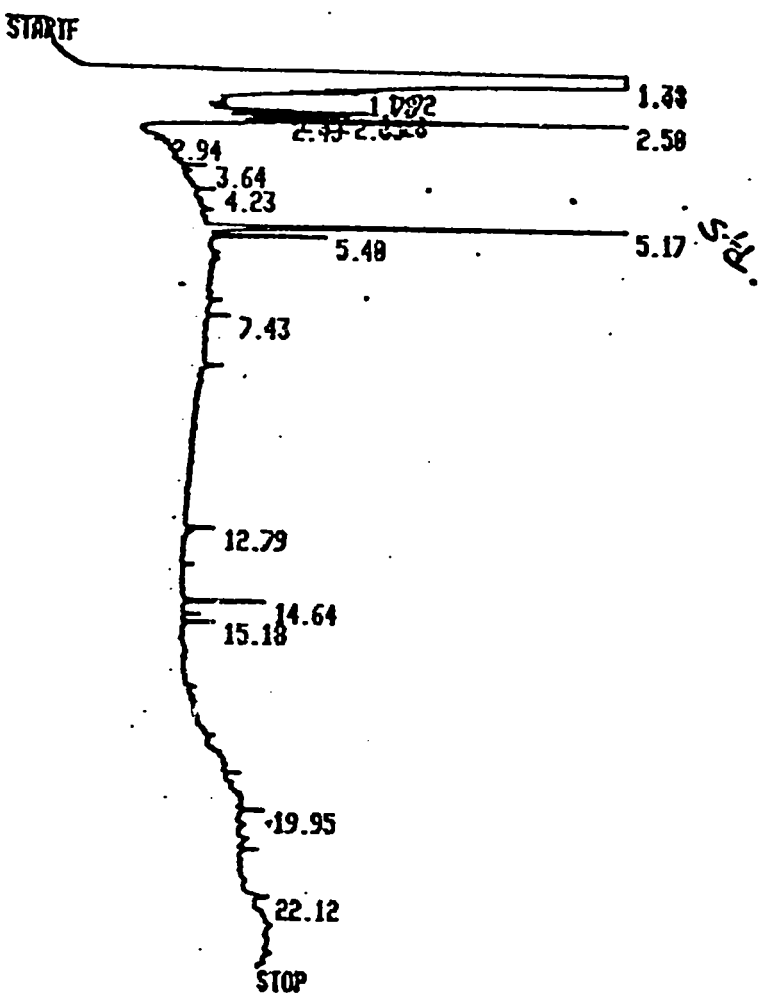




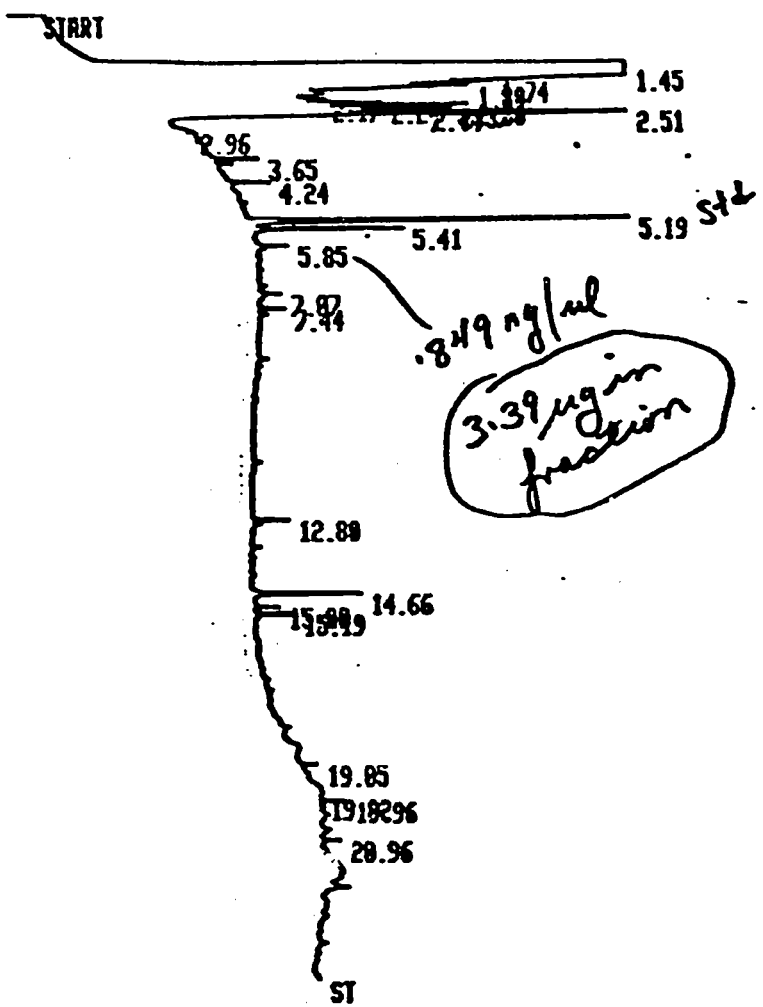
#29-32
0.5 µg
+ 0.5 ml Sil

ESCAPE

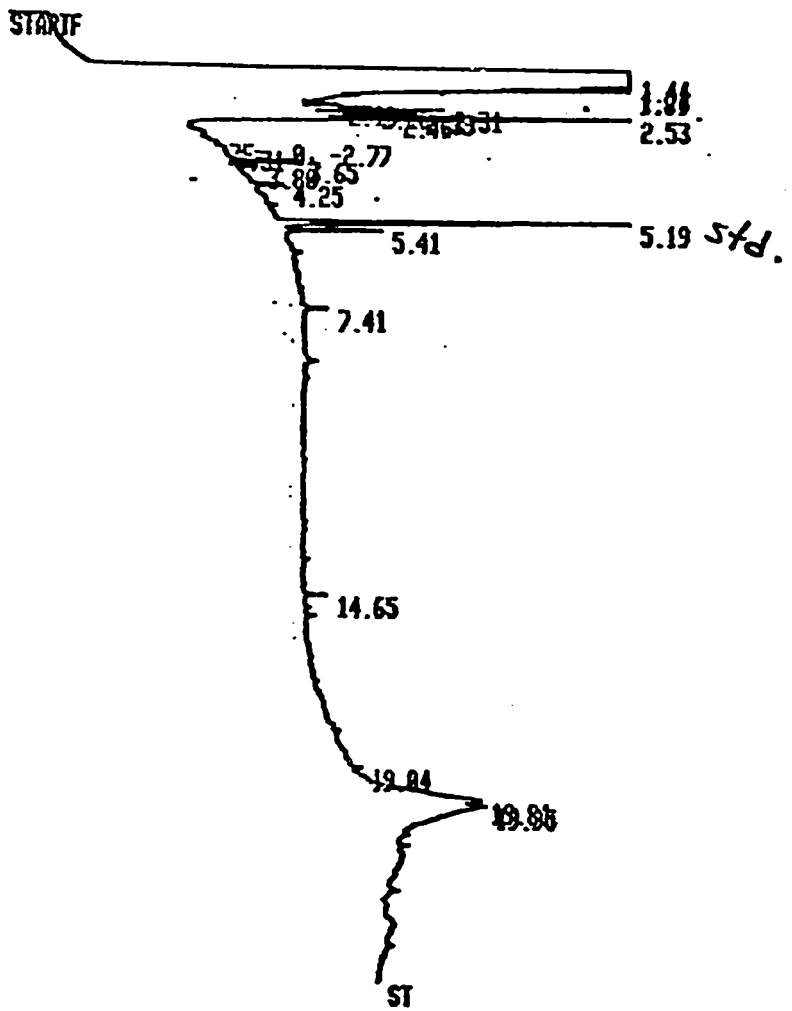
#41-44
0.5 µl
+ 6.5 µl std.



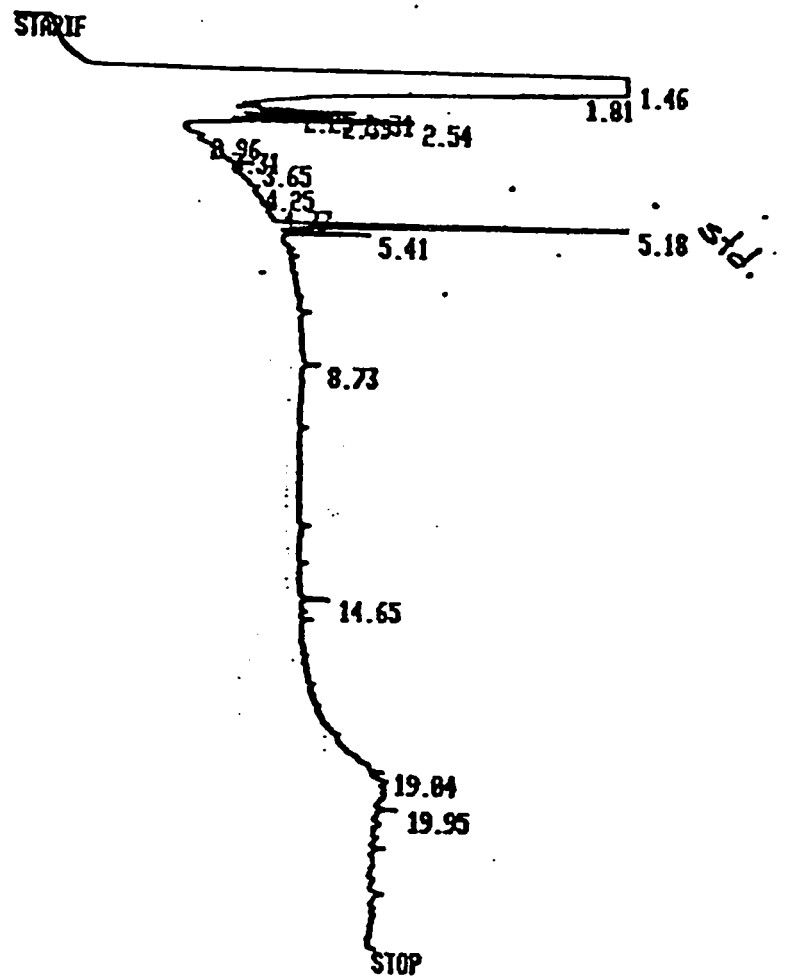
#37-40
0.5 µl
+ 0.5 µl std.



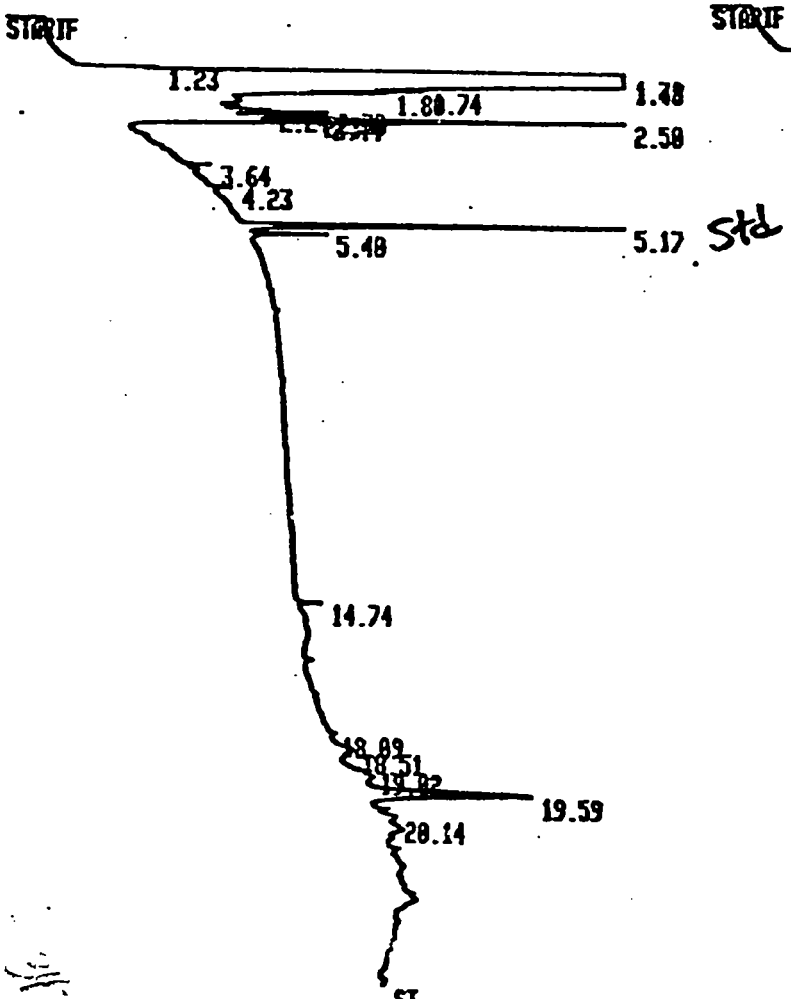
#49-52 0.5 µl + 0.5 std



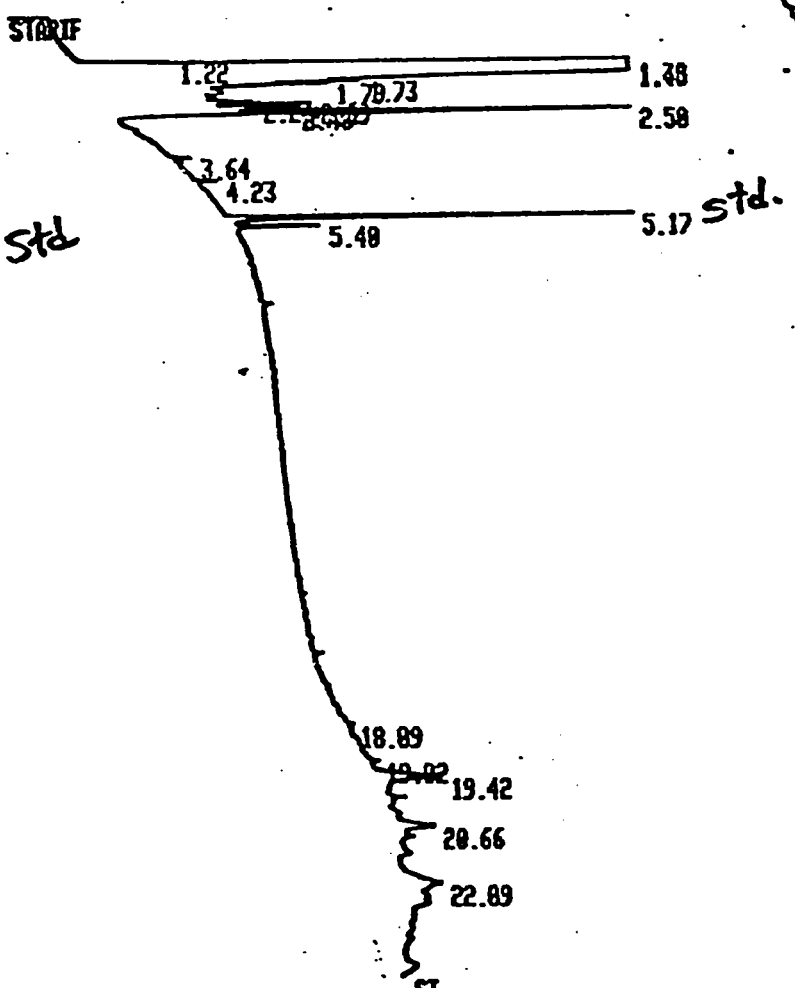
#45-48 0.5 µl + 0.5 std



#61-64 0.5 µl + 0.5 µl std



#57-60 0.5 µl + 0.5 µl std



#53-56 0.5 µl + 0.5 std

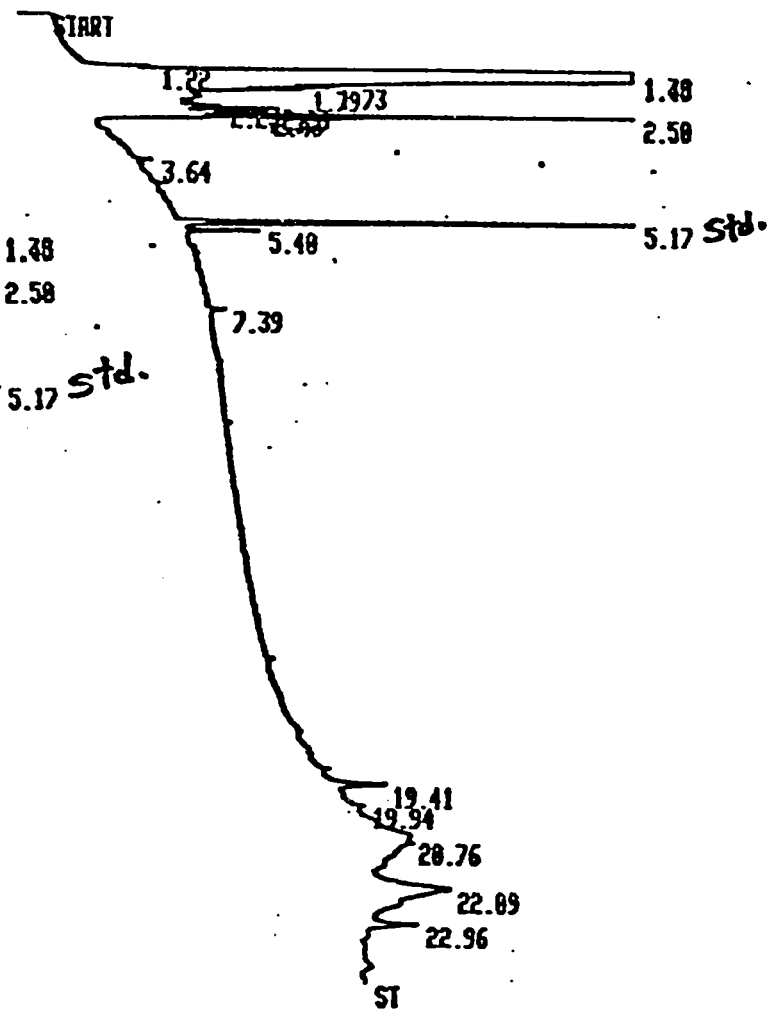


Figure 4. Scanning electron micrographs of a). dorsal view of Acanthoscelides obtectus Say exposed to freshly milled Ocimum canum leaves (25x X 5x7 size); b). elytra of A. obtectus (50x X 5x7) with close up of particles adhering to setae; c). abdominal view of Zabrotes subfasciatus exposed to freshly milled O. canum leaves with closeup of particle adhering to setae; d). O. canum leaf material immediately after milling; e). O. canum leaf material two months after drying and milling; f). O. canum leaf material after ethanol extraction two months after milling; g). intact dry leaf of O. canum (150x X5x7); h). intact dry leaf of O. canum (400x X5x7); i). intact dry leaf of O. canum (800x X5x7).

Figure 4b

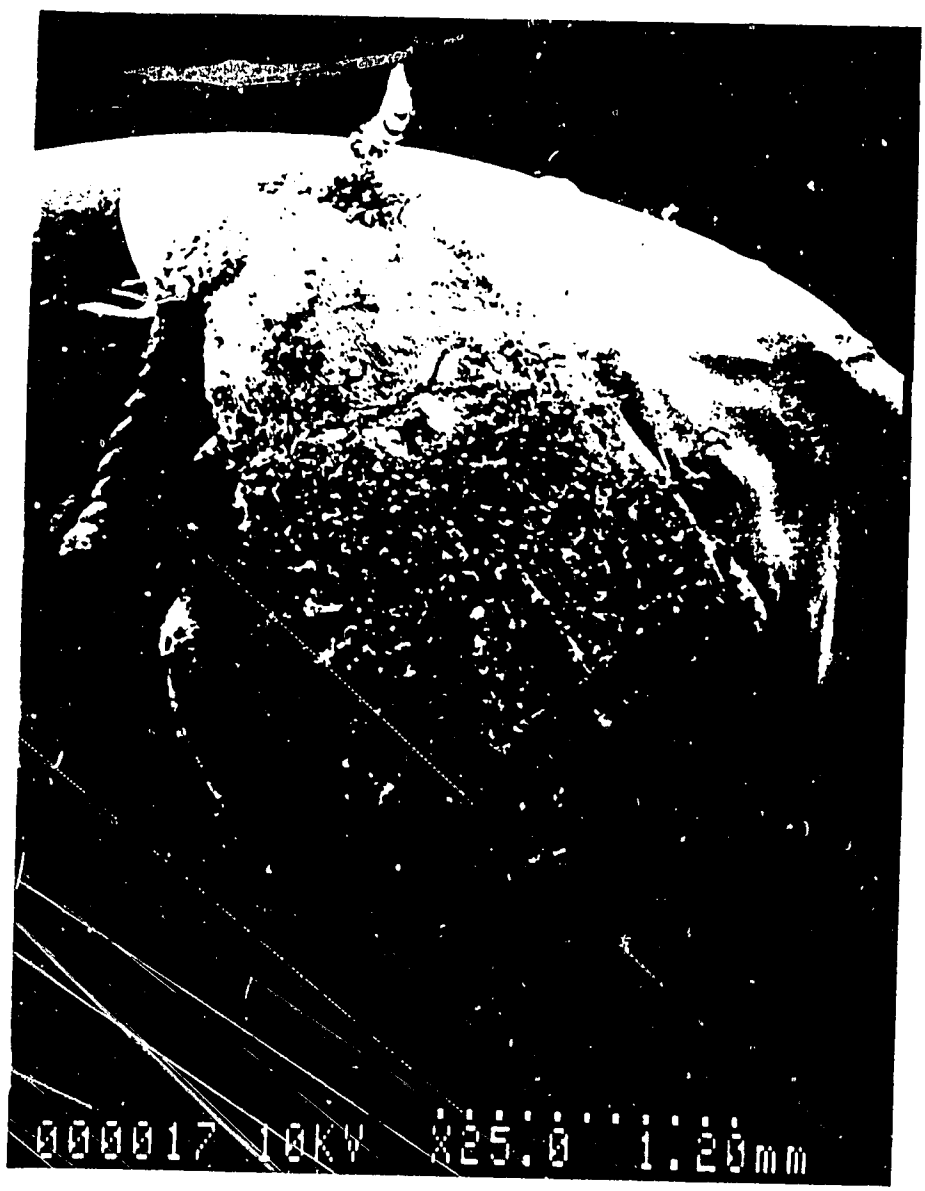
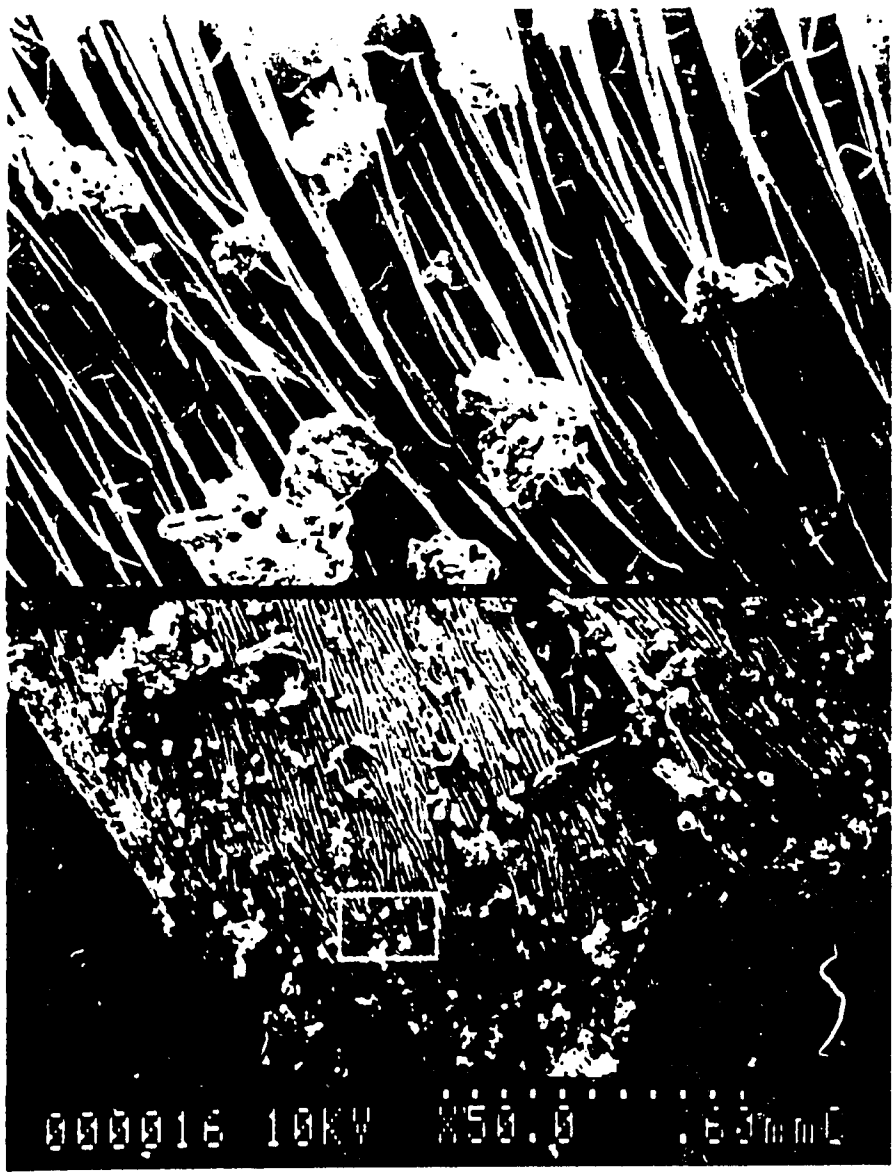


Figure 4b



000004 10KV X350 86umC

Figure 1:

000012 10KV X800 38um

000010 10KV X800 38 μm

Figure 4g

Figure 4h

Figure 41

Figure 5. Milestones of project 1988-1991

Project start - August 5, 1988

Serugendo Training in U.S. - Arrival August 24, 1988
- Departure December 22, 1989

Kayitare U.S. research - Arrival August 24, 1988
- Departure November 16, 1988

Financial and Progress Reports

First - December 31, 1988
Second - June 30, 1989
Third - December 31, 1989
Fourth - June 30, 1990
Fifth - December 31, 1990
Sixth - June 30, 1991

Final Progress Report - June 30, 1992

Completion of Publications

No. 1
No. 2
No. 3
No. 4
No. 5
No. 6

Final Financial report - November 4, 1992

Burkholder visit to Rwanda - Arrival November, 1989
- Departure November, 1989

In conjunction with the related Insecticidal Plant Project, another visit will be made later in 1989 (ca. September)

Dunkel visits to Rwanda - Arrival April 4, 1989
- Departure April 11, 1989
- Arrival January, 1991
- Departure January, 1991

Cutcomp visit to Rwanda - Arrival 1990
- Departure 1990

Breene visit to Rwanda

Project end August 4, 1990

Figure 6. Main categories of work breakdown structure (1988-1991)

RWANDA INDIGENOUS INSECTICIDAL PLANT PROJECT

WORK BREAKDOWN STRUCTURE

1.0. WORKPLAN MEETINGS (UNIVERSITY OF WISCONSIN)

2.0. DEVELOPMENT OF INSECT MASS CULTURES

3.0. PRODUCTION OF TEST PLANTS (BEANS AND INSECTICIDAL PLANTS)

4.0. TEST OF EFFICACY IN SELECTED ENVIRONMENTAL CONDITIONS*

5.0. HUMAN CONSUMER PREFERENCE AND BEAN COOKABILITY TESTS*

6.0. INSECT BEHAVIORAL RESPONSE STUDIES (FIELD AND LAB)*

7.0. ISOLATION OF ACTIVE COMPONENTS*

8.0. TIME OF ACTION STUDIES*

9.0. LARGE SCALE (WAREHOUSE) TEST*

10.0. PREPARATION OF PUBLICATIONS

11.0 PREPARATION OF PROGRESS AND FINAL REPORTS

12.0. EVALUATION

* = HYPOTHESIS TESTING ACTIVITIES

Figure 7. Details of the workbreakdown structure and estimated times for completion

* Start at harvest *

4.0 TEST OF EFFICACY IN SELECTED ENVIRONMENTAL CONDITIONS*

4.1. Set up incubators or environmental chambers and beans

4.1.1. Room arrangement (fans, space heaters, room dividers). 22 da

4.1.2. Arrange equillibrium relative humidity (eRH) chambers and saturated salt solutions. 5 da.

4.1.3. Treat beans (use only the more effective plants).

4.1.3.1. Plant 1. 3 da.
4.1.3.2. Plant 2. 3 da.
4.1.3.3. Plant 3. 3 da.
4.1.3.4. Plant 4. 3 da.
4.1.3.5. Plant 5. 3 da.

4.2. Incubation of beans with plant preparation

4.2.1. Plant 1. 265 da.
4.2.2. Plant 2. 265 da.
4.2.3. Plant 3. 265 da.
4.2.4. Plant 4. 265 da.
4.2.5. Plant 5. 265 da.

4.3. Large bean chamber repellency tests with A. obtectus and Z. subfasciatus.

4.3.1. Insect behavioral runs after 10 da exposure. 44 da.
4.3.2. Insect behavioral runs after 30 da exposure. 44 da.
4.3.3. Insect behavioral runs after 90 da exposure. 44 da.
4.3.4. Insect behavioral runs after 6 mo exposure. 44 da.
4.3.5. Insect behavioral runs after 9 mo exposure. 44 da.
4.3.6. Insect behavioral runs after 12 mo exposure. 44 da.

4.4 Writing of working paper (the effect of selected environmental conditions on efficacy of plant preparations) (15 p. or less)

4.4.1. Analysis of data. 22 da.
4.4.2. Drafting manuscript. 22 da.
4.4.3. Review by coauthors. 22 da.
4.4.4. Review by external reviewers. 44 da.

6.0 INSECT BEHAVIORAL RESPONSE STUDIES (FIELD AND LAB)*

6.1 Lab study (in Madison)

6.1.1. Preliminary tests

6.1.1.1. Bean variety tests (US pinto vs red kidney) 45 da.
6.1.1.2. Concentration of plant preparation (includes preliminary test of plant parts) 10 da.
6.1.1.3. Timing of repellency assay. 10 da.

6.1.2. Assembly of materials

6.1.2.1. Beans. 2 da.
6.1.2.2. Buckets and traps. 5 da.
6.1.2.3. Plant material. 5 da.

6.1.3. Repellency test using large chambers with beans

6.1.3.1. A. obtectus run. 5 da.
6.1.3.2. Z. subfasciatus run. 5 da.

6.1.4. Writing of working paper (15 p. or less)

6.1.4.1. Analysis of data. 22 da.
6.1.4.2. Drafting of manuscript. 22 da.
6.1.4.3. Review by coauthors. 22 da.
6.1.4.4. Review by scientists outside of project. 44 da.

6.2. Field study (in Rwanda)

7.0 ISOLATION OF ACTIVE COMPONENTS* (all preliminary studies to be used as basis for follow on grant.)

7.1 Literature review and interviews at CURPHAMETRA (UNR) (Dr. Luc Van Puyvelde)

7.1.1. Literature review. 10 da.
7.1.2. Interviews. 5 da.

7.2 Extraction and purification

7.2.1. Solvent extraction. 22 da.
7.2.2. Column purification. 22 da.

7.3 Two-choice bioassay extract test

7.3.1. A. obtectus runs. 44 da.
7.3.2. Z. subfasciatus runs. 44 da.

7.4 Writing of working paper (10 p. or less)

7.4.1. Analysis of data. 22 da.
7.4.2. Drafting of manuscript. 22 da.
7.4.3. Review by coauthors. 22 da.
7.4.4. Send out manuscript to scientists in other countries for review. 44 da.

8.0 TIME OF ACTION STUDIES*

8.1. Tests with unprocessed dry plant materials

8.1.1. Set up experimental chambers (units) (e.g. lucite rings with screens) 10 da.

8.1.2. Incubation and reading of tests (# bruchids/g. beans specified)

8.1.2.1. Fecundity. 22 da.

8.1.2.2. % hatch. 26 da.

8.1.2.3. First instar prior to penetration and after. 45 da.

8.1.2.3. Emergent adults. 50 da.

8.2. Tests with extracts of the one most promising plant preparation and the most vulnerable insect life stage

8.2.1. Set up of chambers and materials. 10 da.

8.2.2. Incubation and reading of tests. 50 da.

8.3. Writing of working paper (15 or less pages)

8.3.1. Analysis of data. 22 da.

8.3.2. Drafting of manuscript.

8.3.3. Review by coauthors. 22 da.

8.3.4. Review by scientists outside of project in other countries. 44 da.

9.0 LARGE SCALE (WAREHOUSE) TEST*

9.1. Preliminary bag test

9.1.1. Jute bag treatments

9.1.1.1. Timing test

9.1.2. Woven plastic bag treatment

9.1.2.1. Timing test.

9.2. Warehouse (Kicukiro) test

9.2.1. Assembly of materials

9.2.1.1. Bags and pallets
9.2.1.2. Beans (a single variety from increase plots at ISAR or a mixture).
9.2.1.3. Selected plant preparation.

9.2.2. Set up of experiment

9.2.2.1. Drying of beans.
9.2.2.2. Mixing of beans and plant material.
9.2.2.3. Filling and placing of bags on pallets.

9.2.3. Baseline samples

9.2.3.1. General quality analysis (% MC, test weight, damage, insect # and species).
9.2.3.2. General quality data analysis.
9.2.3.3. Sensory evaluation by trained panel or an nutritional centers.
9.2.3.4. Sensory data analysis.
9.2.3.5. Instrumental hardness.
9.2.3.6. Analysis of instrumental hardness data.
9.2.3.7. Insect behavioral assay (Note - exact interval of tests will be determined by results of the lab study completed in 1990).
9.2.3.8. Analysis of insect behvioral data.

9.2.4. Four month sampling

9.2.4.1. General quality analysis.
9.2.4.2. Analysis of general quality data.
9.2.4.3. Sensory evaluation.
9.2.4.4. Analysis of sensory data.
9.2.4.5. Instrumental hardness.
9.2.4.6. Analysis of instrumental hardness.
9.2.4.7. Insect behavioral assay.
9.2.4.8. Analysis of insect behavior data.

9.2.5. Eight month sampling

9.2.5.1. General quality analysis.
9.2.5.2. Analysis of general quality data.
9.2.5.3. Sensory evaluation.
9.2.5.4. Analysis of sensory data.
9.2.5.5. Instrumental hardness.
9.2.5.6. Analysis of instrumental hardness.
9.2.5.7. Insect behavioral assay.
9.2.5.8. Analysis of insect behavior data.

9.2.6. Twelve month sampling

9.2.6.1. General quality analysis.
9.2.6.2. Sensory evaluation.
9.2.6.3. Instrumental hardness.
9.2.6.4. Insect behavioral assay.

9.3. Writing of working paper (20 p. or less)

9.3.1. Summary of data. 44 da.
9.3.2. Drafting of manuscript. 22 da.
9.3.3. Review by coauthors. 44 da.
9.4.4. Review by external reviewers. 44 da.

Figure 8. Plan of insecticidal plant publications

1. Efficacy of insecticidal Rwandan plants in controlling bean weevils under selected environmental conditions.

Literature Review - Kayitare/Burkholder/Dunkel Fall '88

Data Collection - Kayitare

Data Analysis - Kayitare

Drafting of Working Paper - Kayitare

Senior Author - Kayitare

Coauthors - Burkholder
Dunkel
Cutkomp

2. Effect of local insecticidal/repellent plants on consumer acceptance and cookability (thermal softening) of Rwandan mixed beans (*Phaseolus vulgaris*).

Literature Review - Serugendo/Breene Fall '88

Data Collection - Serugendo/Kayinamura

Data Analysis - Serugendo

Drafting of Working Paper - Serugendo/Kayinamura

Senior Author - Serugendo

Coauthors - Breene
Kayinamura

3. Behavioral response of bruchids *A. obtectus* Say, *Z.* _____ to local insecticidal plants in Rwanda. I. Laboratory studies.

(may include the extract studies)

Literature Review - Kayitare/Burkholder/Dunkel Fall '88

Data Collection - Kayitare/Burkholder/Dunkel

Data Analysis - Kayitare/Burkholder/Dunkel

Drafting of Working Paper - Kayitare

Senior Author - Kayitare

Coauthors - Burkholder
Dunkel
Cutkomp
Koval

4. Field response of Acanthosclides obtectus to Phaseolus vulgaris treated with Rwandan insecticidal/repellent plants before harvest.

Literature Review - Kayitare/Dunkel/Cutkomp Fall '88

Data Collection - Kayitare

Data Analysis - Kayitare

Drafting of Working Paper - Kayitare

Senior Author - Kayitare

Coauthors - Burkholder
Dunkel
Cutkomp
Butare

5. Effect of local Rwandan insecticidal plants on life stage of A. _____.

Literature Review - Cutkomp/Kayitare Fall '88

Data Collection - Kayitare

Data Analysis - Kayitare/Cutkomp

Drafting of Working Paper - Kayitare/Cutkomp

Senior Author - Kayitare

Coauthors - Cutkomp
Dunkel

6. Efficacy of insecticidal Rwandan plants in controlling bean weevils during large scale bag storage of beans.

Literature Review - all previous manuscript reviews for this project

Data Collection - Munyarshoka/Nizeyimana/Serugendo/food science technicians

Data Analysis - Munyarshoka/Serugendo/Nizeyimana

Drafting of Working Paper - Kayinamura

Senior Author - Kayinamura

Coauthors - Munyarshoka
Serugendo
Breene
Koval
Dunkel
Nizeyimana
Mukakunzi

36

Figure 9. Agenda of Workplan Meetings, Madison Wisconsin (August 20-25, 1988)

RWANDAN INSECTICIDAL PLANT PROJECT

Workplan Meeting

University of Wisconsin

Madison, Wisconsin

August 20-25, 1988

Saturday, August 20, 1988

6:00 p.m. arrival of Dunkel, Diggs, and Kayinamura in Madison. Check in at the Madison Inn, Langdon St, on northwest edge of campus.

8:32 p.m. Arrival of Kayitare and Serugendo at the Madison airport (Northwest Airlines 1143). Met by Dunkel, Diggs, and Kayinamura.

10:00 p.m. Check in at Madison Inn.

Sunday, August 21, 1988

(Informal tour, walking or riding, through campus area)

Informal meetings of team members if desired.

The Breenes and Cutkomp will arrive at the Madison Inn in early afternoon.

2:00 p.m. Chastain arrives at Madison Inn by taxi from airport.

3:00 p.m. Meeting regarding logistics and agenda. (ca. 30 minutes).

4:30 p.m. Boat tour of Lake Monona (possibly Lake Mendota). Optional.

6:30 p.m. Dinner and theater. Optional.

Monday, August 22, 1988

8:15 a.m. Depart for Department of Entomology.

8:30 a.m. Introduction Meeting
University of Wisconsin
(Dr. Charles Koval, Department Chairperson, Department of Entomology)

(Dr. Niel Jorgensen, Associate Dean and Director of the College of Agriculture)

Project Team (U.S. and Rwandans)
(Dr. Florence Dunkel, Co-investigator)

ISAR (Description of programs and affiliations)
(Mr. Joseph Kayitare, Plant Protection section leader)

OPROVIA and OPROVIA/GRENARWA II Recherches
(Mr. Phocas Kayinamura, Director of Laboratory)

10:00 a.m. Break.

10:15 a.m. Introduction to Project (Dunkel).

10:30 a.m. Tour of Stored Product Insect Laboratories (behavioral, biologic, and molecular control/management programs)
(Dr. Wendell Burkholder, Director; Dr. Michael Strand and Dr. Nancy Beckage, faculty).

12:00-1:00 p.m. Lunch in the Union South. Obtain food in second floor carosel and proceed to designated private dining room.

1:00 p.m. Completion of tour of Stored Product Insect Laboratories

2:30 p.m. Babcock Break.

3:00 p.m. Tour of Food Science and Sensory Lab facilities (Dr. Robert Lindsay).

5:00 p.m. Return to Madison Inn for light refreshments.

6:00 p.m. Depart for boat.

6:30 p.m. Boat tour of Lake Mendota.

9:00 p.m. Return to hotel from boat. Late dinner in local restaurants.

<u>Tuesday, August 23, 1988</u>

(All meetings at the Madison Inn Conference Rooms, top floor)

8:30 a.m. Development of schedule for research activities for 3 years of Project. Introduction to critical path management by using work breakdown structure, milestones and flow (Gantt) and bar charts. (Dunkel)

9:15 a.m. Individual Research Group Meetings

9:15 - 11:15 a.m. Insect Behavioral Group (Burkholder, Kayitare, Dunkel, Cutkomp ad hoc, Gahamanyi ad hoc)

11:15 - 2:15 p.m. (one hour break for lunch when appropriate) Insect Toxicology Group (Cutkomp, Kayitare, Dunkel, Burkholder, ad hoc, Gahamanyi ad hoc)

9:15 - 2:15 p.m. (one hour break for lunch when appropriate) Food Science Group (Breene, Serugendo, Kayinamura, ad hoc)

2:30 p.m. Large Scale Bag Study (all of the above participants)

5:30 p.m. Turn in workbreakdown structure WBS and bar charts for Individual Research Sections.

6:30 p.m. Dinner and social gathering at the Kovals.

(Prior to Wednesday morning, Dunkel will combine WSB and bar charts to determine the critical path).

<u>Wednesday, August 24, 1988</u>

9:00 a.m. Conference Room, Department of Entomology. Presentation of entire WBS and the critical path. (Dunkel)

9:30 a.m. Questions.

10:00 a.m. Determination of informal reporting process and description of formal reporting process. Assignment of responsibilities.

10:45 a.m. Break.

11:00 a.m. Introduction to companion project, Evaluation of Insect Resistance to Postharvest Insecticides used in Rwanda and development of Alternatives. (Dr. Shobha Sriharan, principal investigator).

12:00 - 1:00 p.m. Lunch.

1:00 p.m. Tour of Electron Microscopy facilities in Entomology Department. (Stan Carlson).

1:45 p.m. Tour of Steenbock Agricultural Library and computer search capabilities

2:15 p.m. Meet in Steenbock library conference room. Determination of tentative publication list and procedures:

--titles agreement
--authorship
--literature review timetable

--suggested journals
--language

5:30 p.m. return to Madison Inn

(evening reserved for meetings of Rwandans and Americans regarding administrative details of the institutions involved)

Thursday, August 25, 1988

9:00 a.m. Summary of decisions.

10:30 a.m. Informal meeting re: insect resistance project with Kayinamura, Cutkomp, Sriharens, and Dunkel.

10:30 a.m. Tours as desired of University Biotron and Forest Products Laboratory.

1:00 p.m. Reserved for small group conferences as needed.

4:00 p.m. Departure of Dunkel, Kayinamura, Serugendo, others (?) to Minneapolis.

Friday, August 26, 1988

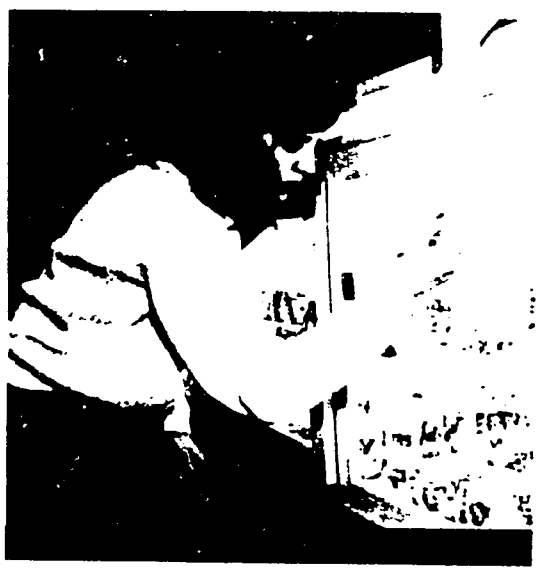
no defined schedule (Greenes, Kayitare remaining in Madison)

Figure 10. Description of Plant Growth Center (MSU) to be used for environmentally controlled studies of insecticidal plants

Support Facilities

Research and teaching in the five major areas of the Plant Growth Center are backed by the necessary space and equipment for a variety of supporting tasks. These include:

- **Soils**—Storage and preparation (cleaning, drying, steam-aerating and mixing)
- **Plant Materials**—Storage (cool and room temperature), wet lab, handling and drying
- **Seeds**—Storage (cool temperature)
- **Chemical Storage**—Storage of toxic and non-toxic substances



Research Opportunities

The Plant Growth Center is designed to meet the needs of scientists in a wide variety of research areas:

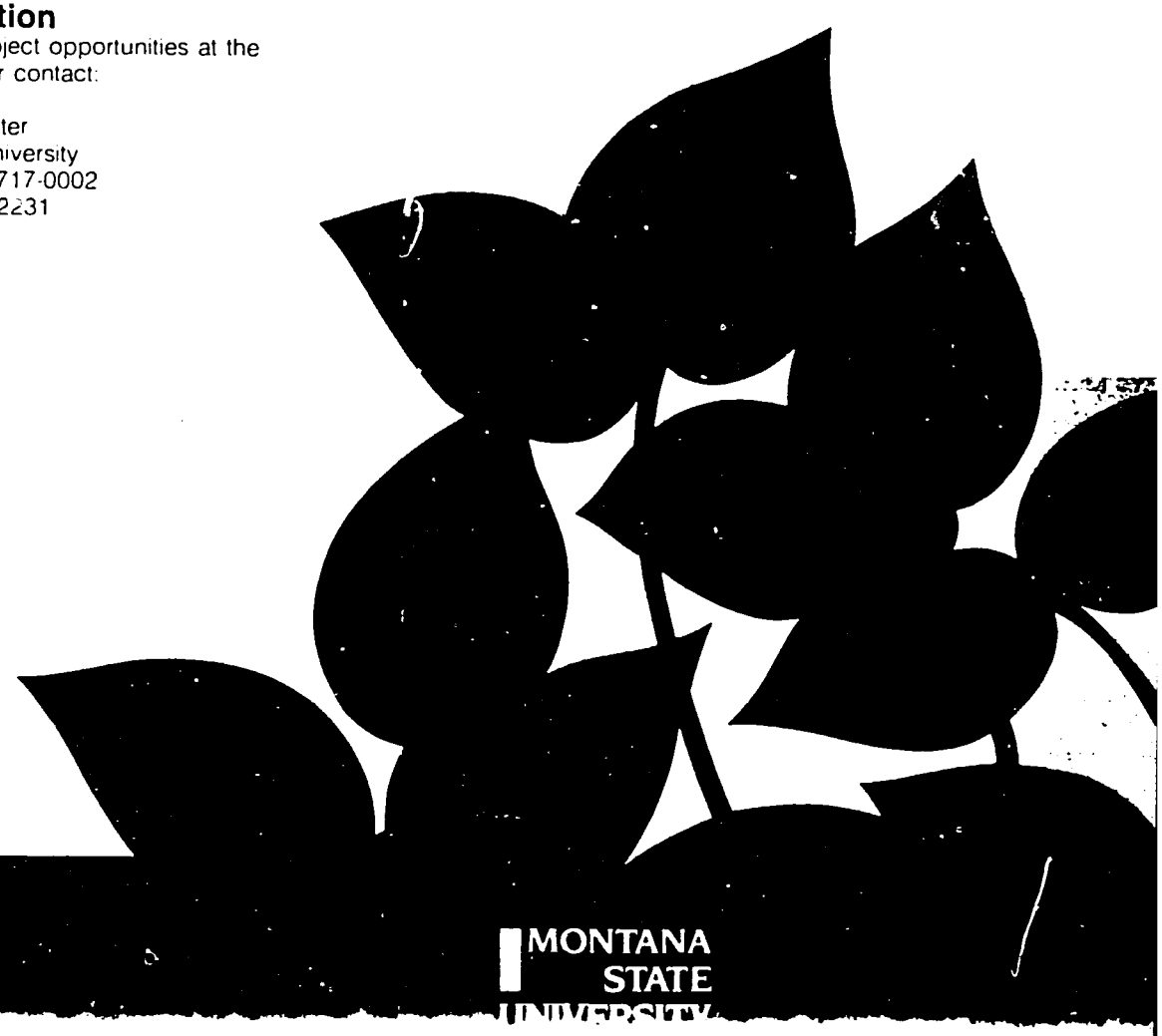
Crops
breeding
ecology
genetics
pathology
physiology
virology
Forages
Horticulture
Insects
Integrated Pest Management
Plant Biology
Range Ecology and Improvement
Seeds
Soils
chemistry
fertility
physics
Turf and Nursery
Weeds
biological control
herbicides

More Information

For details on project opportunities at the Plant Growth Center contact:

Manager
Plant Growth Center
Montana State University
Bozeman, MT 59717-0002
Phone (406) 994-2231

QUALITY RESEARCH THROUGH QUALITY CONTROL



MONTANA STATE UNIVERSITY

MONTANA STATE UNIVERSITY
Bozeman

FIVE ENVIR

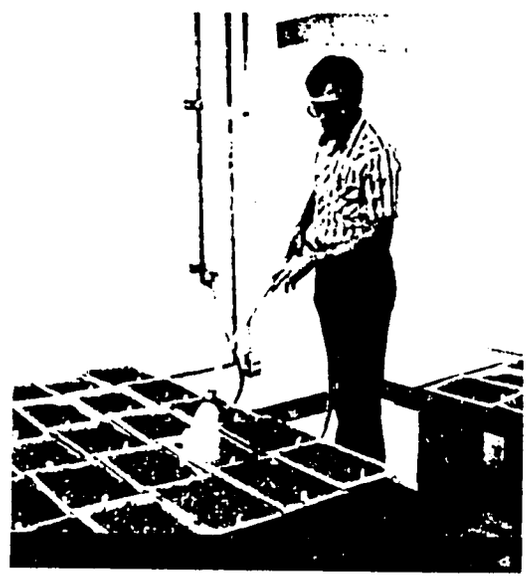
Greenhouse

It is divided into 28 sections. Each has 288 square feet of bench space along with separate light and temperature controls.

Temperature is normally maintained at 70-80 degrees F but can be controlled from 40-100 degrees F.

As in all the plant growing areas, the intensity and duration of light can be adjusted to produce desired diurnal cycles. Rolling shade screens can be used to reduce intense summer sun radiation.

Two 6 x 24-foot rolling-top benches in each section give scientists easy access to plants and minimize wasted space.



Growth Rooms

The facility has space for 13 walk-in rooms with more complete control of light and temperature than the greenhouse. These rooms are ideal for biotechnology-related research.

Temperature can be controlled within the range of 50-100 degrees F.

Light intensity can be set as high as 8,000 foot candles. Special fluorescent tubes simulate the spectrum of sunlight much more closely than incandescent and cool-white fluorescent lighting systems.

Smaller growth rooms are 11 feet wide and 16 feet long. The larger ones are 15 x 20 feet.

A central computer board provides current environmental information on each greenhouse section and growth room.

Insect

This u
and rea
weeds a

- Two
  chan
- Proc
  disea
- Cont
  withc
- Two
  squa
  as h
  contr
- Secu
  entry
  air fil
  cedu
  para
  inse

IRONMENTS

rooms
d
ese
ted

n the

8,000
simulate
losely
escent

de and
20 feet.
current
enhouse

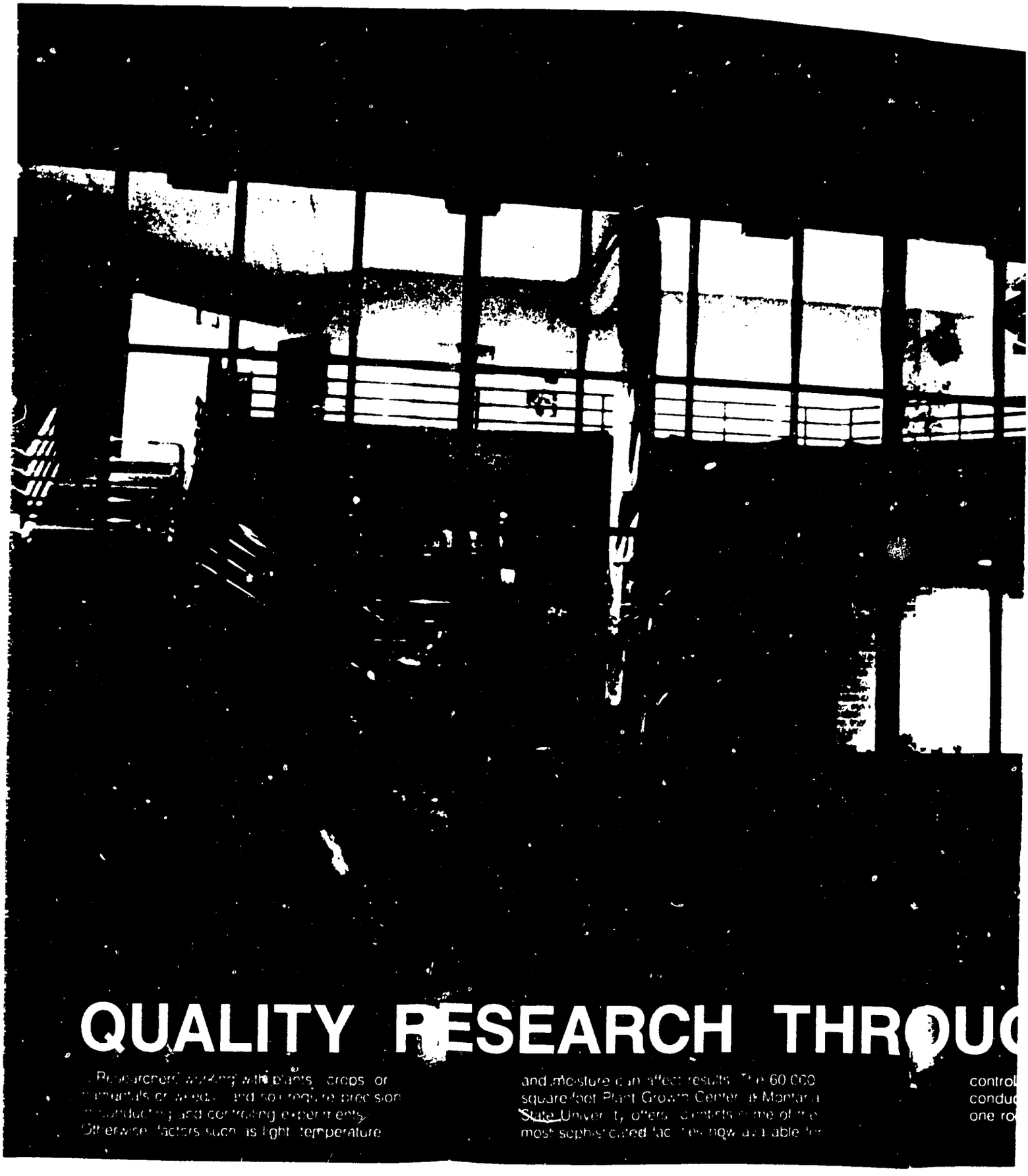
Insect Quarantine Lab

This unit is designed for isolating, studying and rearing insects used in biological control of weeds and insects. Features include:

- Two growth rooms and several growth chambers
- Processing room to screen insects for diseases and parasites
- Controlled-humidity room for growing plants without soil to study root-attacking insects
- Two glass houses, with a total of 576 square feet of bench space, to test plants as hosts for insects and rear weed-controlling insects for release in the field
- Security measures include double-door entry, a personnel alarm system, light traps, air filter and waste water sterilization procedures to control people, diseases and parasites and prevent release of uncleared insects and other biological agents.

Growth Chambers

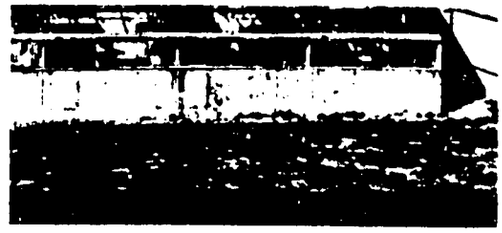
These units offer the ultimate in controlling light and temperature. Space is available for about 35 chambers, each measuring about 4 feet wide and 6 feet long.



QUALITY RESEARCH THROUG

Researchers working with plants, crops or animals or people, and so require precision in conducting and controlling experiments. Otherwise factors such as light, temperature and moisture can affect results. The 60,000 square-foot Plant Growth Center at Montana State University offers scientists some of the most sophisticated facilities now available for

control
conduc
one ro



Plant Disease Isolation Area

This 1,280-square foot facility has four greenhouses, each with attached lab. This is a medium level containment area to prevent spread of disease.

THROUGH QUALITY CONTROL

0,000
ont
of the
ble for

Table 1a. Repellent behavior of *Zabrotes subfaciatus* exposed to various Rwandan plant products (50 inscects/replicate)

Test #	Treatment	# of insects repelled (i.e. choosing to exit) Day 1	2	3	4	5	6	7
1	*Ocimum canum* sinus	7	9	10	11	12	13	13
(rep = 1)	Control	3	4	5	5	6	7	8
2	*O. canum* s.	1,4,0,1	4,11,3,2	9,18,8,3	17,20,14,2	21,21,15,2	24,22,18,4	24,23,24,4
(rep = 4)	Control	2,1,4,4	8,7,12,6	12,13,15,12	17,17,19,19	12,21,24,23	15,22,24,25	15,22,24,25
3	*O. canum* s.	3,1,1,3	7,4,8,6	10,9,11,10	15,13,15,16	17,15,18,17		
	Chenopodium schradaranum	6,6,4,6	12,12,7,9	16,14,7,11	19,18,7,16	21,21,7,18		
	Control	0,7,4,10	3,14,10,14	3,18,18,15	3,23,21,19	3,25,21,22		
4	*Tetradentia riparia*	5,7,5,0	8,12,8,9	13 14,10,17	16,17,15,22	16,19,19,24		
	Capsicium frutescens	4,3,0,10	12,8,13,15	15,11,19,18	16,15,19,20	19,17,21,22		
(rep = 4)	Control	0,2,2,0	10,15,14,9	14,19,17,12	16,22,20,14	20,22,20,20		

Table 1b. Mortality of storage bruchids, *Acanthoscilides obtectus* Say and *Zabrotes subfasciatus* Bohem. exposed to Rwandan plant materials (n=4).

Plant	*A. obtectus* Days after inoculation 3	6	9	12	15	*Z. subfasciatus* Days after inoculation 3	6	9	12
Chenopodium schradericum flowers	0.00	0.00	0.25	3.00	7.00	0.00	0.75	3.50	7.00
Chenopodium schradericum leaves	0.00	0.00	0.75	5.50	9.25	0.50	1.25	7.00	9.50
Chenopodium sp. flowers	0.00	0.00	1.00	3.25	7.50	0.00	1.50	5.25	9.25
Tetradentia riparia flowers, sundried	0.00	0.00	0.00	0.50	4.75	0.00	1.25	2.75	6.00
Tetradentia riparia leaves, sundried	0.25	0.25	0.50	3.00	5.25	0.25	0.75	2.50	4.75
Tetradentia riparia ovendried	0.00	0.00	0.25	2.00	4.00	0.25	0.75	2.00	5.00
Ocimun kilimandsharicum flowers, sundried	0.00	0.00	1.00	4.00	8.75	0.00	1.00	2.50	7.25
Ocimun canum sinus leaves	0.00	0.00	0.25	2.50	7.25	1.25	1.75	4.25	7.25
Capsicum frutescens long fruits	0.00	0.00	0.00	0.75	3.00	0.75	1.50	3.50	5.75
C. frutescens short fruits	0.00	0.00	0.75	2.25	5.00	0.25	1.00	3.25	6.00
Control	0.00	0.00	0.50	0.75	2.25	0.00	0.25	1.75	4.50

Table 2: Preliminary observations of mortality and behavior in male *Z. subfasciatus* exposed to *O. canum* leaves

Treatment	Observations (hrs. after inoculum) 2.5	26
1g. freshly milled *O. canum* leaves	10 moribund; covered with green particles intensely grooming	8 lying on backs; 2 ambulating slowly: all covered with green particles
1g. residue of ETOH extract of old milled *O. canum* leaves	10 active; no coating of green particles no grooming	10 active; some had some particles on exoskeleton and were grooming

1. exposure chamber = 9cm glass petri dish lined with whitman filter paper and liquid teflon.

2. 10 male *Z. subfasciatus* 1 trt.

Table 3. Toxicity of hexane and ethanol extracts of Ocimum canum sinus (Isongantoya) leaves to Zabrotes subfasciatus (5 female and 5 male/replicate; 10 replicates/treatment) (28° C. 60±10 % RH. 16:8: :L:D photoperiod)

	Mean number insects dead/moribund hrs. after inoculation					
	1	15	24	48	72	96
Ocimum canum						
Ethanol Extract	0	0	0	0	0.1	0.1 *
Hexane Extract	0	0	0 *	0.1	0	0
Control						
Ethanol	0	0	0	0	0	0
Hexane	0	0	0	0	0	0

* moribund